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ORIGINAL ARTICLE

Patch test results with the European baseline series, 2019/20—Joint European results of the ESSCA and the EBS working groups of the ESCD, and the GEIDAC

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Members of the ESSCA and EBS Working Groups, and GEIDAC are provided in Appendix section.

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Abstract

Background: Continual analyses of patch test results with the European baseline series (EBS) serve both contact allergy surveillance and auditing the value of included allergens.

Objectives: To present results of current EBS patch testing, obtained in 53 departments in 13 European countries during 2019 and 2020.

Methods: Anonymised or pseudonymised individual data and partly aggregated data on demographic/clinical characteristics and patch test results with the EBS were prospectively collected and centrally pooled and analysed.

Results: In 2019 and 2020, 22 581 patients were patch tested with the EBS. Sensitization to nickel remained most common (19.8 [19.2–20.4]% positivity [95% confidence interval]). Fragrance mix I and *Myroxylon pereirae* yielded very similar results with 6.80 (6.43–7.19)% and 6.62 (6.25–7.00)% positivity, respectively. Formaldehyde at 2% aq. yielded almost one percentage point more positive reactions than 1% concentration (2.49 [2.16–2.85]% vs. 1.59 [1.33–1.88]); methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) and MI alone up to around 5% positives. Among the new additions, propolis was most commonly positive (3.48 [3.16–3.82]%), followed by 2-hydroxyethyl methacrylate (2.32 [2.0–2.68]%).

Conclusion: Ongoing surveillance on the prevalence of contact sensitization contributes to an up-to-date baseline series containing the most frequent and/or relevant contact sensitizers for routine patch testing in Europe.

KEYWORDS

baseline series, contact allergy, clinical epidemiology, surveillance, patch testing, RRID: SCR_001905

1 | INTRODUCTION

A 'baseline' series should comprise those contact allergens which are of greatest importance and relevance for the majority of patients. It is regularly patch tested in all patients presenting with suspected allergic contact dermatitis. Its composition varies in time and with a geographical region, reflecting changes and differences in exposure, respectively. Following fundamental conceptual thoughts delineating the objectives of a baseline series published two decades ago,¹ the criteria for inclusion have recently been revisited.² The 2019 version of the European baseline series (EBS)³ has been used since then, partly with modifications by national contact dermatitis groups or single departments. The present paper summarizes results obtained in the years 2019/20 by members of the European Surveillance System on Contact Allergies (ESSCA), a working group of the European Society of Contact Dermatitis (ESCD; <https://www.escd.org>), by members of the EBS taskforce of the ESCD, and by the Spanish 'Grupo Español de Investigación en Dermatitis de Contacto y Alergia Cutánea'

(GEIDAC)/'Registro Español de Dermatitis de Contacto' (REIDAC) surveillance network described in the study by Hernández-Fernández et al.⁴ Moreover, several departments prospectively tested the recommended additions in their baseline series ('audit allergens'). Results with these have been published separately.⁵ This article collates prospective audit data with the EBS from a wide spread of departments with a view to informing a further revision of the EBS. Thereby, similar reports on previous EBS results are continued; regarding the last two periods, see.^{6,7}

2 | METHODS

Across Europe, different national contact dermatitis groups are active, many of these using electronic data collection of patch test results.⁸ Moreover, a high degree of methodological standardization had been achieved, as documented by the ESCD patch test guidelines.⁹ The value of networking has been multiply demonstrated.¹⁰ The present

analysis combines, for the first time, individual, anonymized/pseudonymized data and aggregated data (results) from three different sources, the methods of which are briefly outlined below, covering the period January 2019 to December 2020. All groups adhered to the ESCD patch test guideline.⁹ As the final reading the maximum reaction between Day (D) 3 and D5 (inclusive) was used, following current ESSCA standards (see Section 4).

2.1 | ESSCA working group of the ESCD

The objective of the ESSCA is the clinical surveillance of contact allergy.^{10,11} To this end, contributing departments (Table S1) submit either all patch test results, or just patch test results obtained with the European baseline series (or national or local adaptations thereof, as evident from Table S2), obtained following ESCD standards,⁹ to the data centre in Erlangen. This is accompanied by important demographic and clinical information, ranging from 'MOAHLFA index'¹² characteristics to a wider range of information according to the ESSCA 'minimal dataset' definition.^{10,13} Data from contributing departments are delivered in an anonymous format or partly, following national network standards, in a pseudonymized format, where the pseudonym cannot be related to actual personal data except in the contributing department itself. This difference is of importance, as only with pseudonymized data can re-investigations of patients be identified and eliminated, to avoid duplication of entries. However, in view of the short study period, the effect of re-consultation appeared negligible. Data were quality checked, providing an 'internal report' for each contributing department for scrutiny and approval before pooling the respective data.¹¹ Data management and analysis were

performed with the R software package (<https://www.r-project.org>; RRID:SCR_001905), version 4.0.3. For the calculation of 95% confidence intervals (CIs) to zero proportions an approximation to an exact CI was used.¹⁴

2.2 | EBS working group of the ESCD

To enable the contribution of individual, anonymized data, an online documentation was set up in 2018, based on a local academic implementation of a SoSci server (<https://www.soscisurvey.de/>). Subsequently, two departments, namely Coimbra, Portugal and Antwerp, Belgium, used this for data contribution. Budapest, Hungary contributed individual data using a spreadsheet, with a defined set of 12 of the EBS allergens related to the 'audit allergens' tested in the same period.⁵

2.3 | GEIDAC

In Spain, all the participating centres are members of the Spanish Contact Dermatitis and Skin Allergy Research Group (GEIDAC). Data were collected prospectively in the Spanish Contact Dermatitis Registry (REIDAC). This is an online-based multicentre registry that uses the OpenClinica platform (OpenClinica LLC and collaborators). Data were anonymized at the source and the registry complies with all ethical standards in terms of informed consent and data protection legislation. Clinical data match exactly with those set out in the minimal data set of the ESSCA, which allows them to be exported to other databases with identical categories. Ideally, the centres systematically

TABLE 1 Demographic and clinical characteristics according to the MOAHLFA index¹² with 'P-measure',¹⁵ that is, the proportion of patients with at least one positive reaction to a baseline series allergen per country

Country	M	O	A	H	L	F	A ⁽²⁾	P
AT	24.8	15.0	26.1	22.1	5.8	19.0	61.9	75.7
BE	32.3	13.0	38.4	19.7	10.9	20.6	58.4	66.1
CH	38.1	13.5	22.1	25.0	6.0	18.2	68.6	59.0
DE	38.0	31.8	28.2	43.8	5.3	9.6	73.1	54.9
ES	34.0	8.4	17.0	22.5	6.1	15.6	70.7	46.8
FI	49.5	76.3	23.7	80.4	1.0	9.3	44.3	49.5
HU	24.1	0.8	13.0	35.4	7.4	17.0	69.7	34.8
IT	30.8	7.2	16.6	19.9	3.5	11.6	62.2	40.1
LT	24.6	27.4	5.2	33.0	9.8	25.6	60.6	47.8
NL	33.3	16.3	38.8	13.5	0.9	4.5	59.8	69.9
PT	29.4	32.8	18.0	43.3	7.1	14.2	57.0	51.7
SI	29.4	3.9	7.9	47.1	16.7	21.0	61.5	44.4
UK	30.4	5.8	46.2	24.7	2.6	33.5	53.9	40.4

Abbreviations: A, % patients with atopic dermatitis; A⁽²⁾, % patients age 40 and above; F, % patients with face dermatitis; H, % patients with hand dermatitis; L, % patients with leg dermatitis; M, % male patients; O, % patients with occupational dermatitis; P, share of patients with at least one positive reaction to a baseline series allergen.

TABLE 2 Patch test results (days 3–5) with the European baseline series in consecutive patients in the 53 active departments of the European Surveillance System on Contact Allergies (ESSCA), additional contributors from the EBS working group, and the contributing 'Grupo Español de Investigación en Dermatitis de Contacto y Alergia Cutánea' (GEIDAC) members (TRUE Test results see Table 3)

Allergen	Conc.	tested	+ / ++ / +++	?+ / IR	% pos. (95% CI)
Metals					
Potassium dichromate	0.5	16 296	712	332	4.37 (4.06–4.69)
Cobalt chloride	1.0	16 608	1027	396	6.18 (5.82–6.56)
Nickel sulfate	5.0	16 540	3274	263	19.8 (19.2–20.4)
Fragrances					
Fragrance mix I	8.0	16 928	1151	302	6.80 (6.43–7.19)
Fragrance mix II	14.0	17 519	660	193	3.77 (3.49–4.06)
HICC	5.0	15 191	201	43	1.32 (1.15–1.52)
<i>Myroxylon pereirae</i> (balsam of Peru)	25	16 980	1124	370	6.62 (6.25–7.00)
Preservatives					
Formaldehyde	1.0 ^a	8315	132	48	1.59 (1.33–1.88)
Formaldehyde	2.0 ^a	8193	204	48	2.49 (2.16–2.85)
MCI/MI 3:1	0.01 ^a	5627	192	25	3.41 (2.95–3.92)
MCI/MI 3:1	0.02 ^a	8871	613	133	6.91 (6.39–7.46)
Methylisothiazolinone	0.05 ^a	4863	192	33	3.95 (3.42–4.53)
Methylisothiazolinone	0.20 ^a	11 950	905	214	7.57 (7.11–8.06)
Paraben mix	16	13 525	76	98	0.56 (0.44–0.70)
Quaternium-15	1.0	10 156	62	12	0.61 (0.47–0.78)
Methyldibromo glutaronitrile	0.3	533 125	227	165	4.26 (3.73–4.84)
Methyldibromo glutaronitrile	0.5	6596	372	102	5.64 (5.1–6.22)
Medicaments, excipients					
Caine mix III (benzo-, cincho-, tetracaine)	10.0	6592	103	80	1.56 (1.28–1.89)
Budesonide	0.01	9438	46	43	0.49 (0.36–0.65)
Budesonide	0.1	1863	0	0	0 (0–0.19)
Tixocortol pivalate	0.1	7541	26	28	0.34 (0.23–0.5)
Tixocortol pivalate	1.0	3532	21	8	0.59 (0.37–0.91)
Neomycin sulfate	20	11 737	97	18	0.83 (0.67–1.01)
Lanolin (wool alcohols)	30	14 947	206	92	1.38 (1.20–1.58)
Rubber additives					
Thiuram mix	1.0	16 632	389	49	2.34 (2.12–2.58)
N-Isopropyl-N'-phenyl-p-phenylenediamine	0.1	16 866	133	47	0.79 (0.66–0.94)
Mercapto mix ⁽ⁱ⁾	1.0	5454	39	27	0.72 (0.51–0.98)
Mercapto mix ⁽ⁱⁱ⁾	2.0	9510	39	7	0.41 (0.29–0.57)
Mercaptobenzothiazole	2.0	16 657	83	35	0.50 (0.40–0.62)
Resins/glues					
Colophonium	20.0	16 994	564	75	3.32 (3.06–3.60)
Epoxy resin	1.0	16 214	209	41	1.29 (1.12–1.48)
p-tert-Butylphenol formaldehyde resin	1.0	11 753	55	14	0.47 (0.35–0.61)
2-Hydroxyethyl methacrylate	2.0	7675	178	15	2.32 (2.00–2.68)
Other					
p-Phenylenediamine	1.0	11 173	402	28	3.60 (3.26–3.96)
Sesquiterpene lactone mix	0.1	8658	71	19	0.82 (0.64–1.03)
Propolis	10	11 952	416	292	3.48 (3.16–3.82)
Textile dye mix	6.6	10 323	370	82	3.58 (3.23–3.96)

Note: Conc., concentration in %, tested in petrolatum, except where indicated otherwise. For composition of mixes see Wilkinson et al.³ Abbreviations: CI, confidence interval; epoxy resin, diglycidyl ether of bisphenol A; HICC, hydroxyisohexyl 3-cyclohexene carboxaldehyde; mercapto mix⁽ⁱ⁾, containing N-cyclohexylbenzothiazyl sulfenamide, dibenzothiazyl disulfide, and morpholinylmercaptobenzothiazole; mercapto mix⁽ⁱⁱ⁾, containing N-cyclohexylbenzothiazyl sulfenamide, mercaptobenzothiazole, dibenzothiazyl disulfide, and morpholinylmercaptobenzothiazole; MCI, methylchloroisothiazolinone; MI, methylisothiazolinone.

^aAqua.

upload clinical data and results of patch tests on the day of the last reading, thus providing epidemiological centralized data in real-time.

The GEIDAC is responsible for the definition and publication of the Spanish Baseline Series, the use of which is recommended in all patients patch tested in Spain. During the study period, a particular form with an 'Extended Spanish Baseline Series' which includes the European allergens not included in the Spanish series was temporally added to the registry (REIDAC).⁴

3 | RESULTS

In total, 22 474 patients were patch tested with the EBS in 2019–2020 (inclusive). The individual contribution by country and department is shown in Table S1. Population characteristics according to the MOAHLFA index,¹² extended by the 'P-measure', that is, the proportion of patients positive to at least one allergen from the baseline series used,¹⁵ are illustrated in Table 1.

TABLE 3 Patch test results obtained with the TRUE Test used as baseline series (supplemented with other, pet.- or aq.-based allergens) in 12 Spanish departments and in Groningen, The Netherlands

Allergen	mg/cm ²	Tested	+ / ++ / +++	% pos. (95% CI)
Potassium dichromate	0.054	3620	114	3.15 (2.6–3.77)
Cobalt (II)-chloride, 6*H ₂ O	0.02	3619	178	4.92 (4.24–5.67)
Nickel (II)-sulfate hexahydrate	0.2	3615	867	23.98 (22.6–25.41)
Gold sodium thiosulfate	0.075	3063	152	4.96 (4.22–5.79)
Fragrance mix	0.5	3620	123	3.4 (2.83–4.04)
Balsam of Peru (<i>Myroxolon pereirae</i>)	0.8	3619	60	1.66 (1.27–2.13)
Formaldehyde	0.18	3620	38	1.05 (0.74–1.44)
Methylchloroisothiazolinone methylisothiazolinone (MCI/MI)	0.004	3620	250	6.91 (6.1–7.78)
Paraben mix	1	3620	11	0.3 (0.15–0.54)
Quaternium 15	0.1	3620	47	1.3 (0.96–1.72)
Diazolidinyl urea	0.55	3472	16	0.46 (0.26–0.75)
Imidazolidinyl Urea (Germall 115)	0.6	3472	17	0.49 (0.29–0.78)
Thiomersal (Thimerosal)	0.007	3620	113	3.12 (2.58–3.74)
2-Bromo-2-nitro-1,3-propanediol	0.25	3065	20	0.65 (0.4–1.01)
Methyldibromoglutaronitrile	0.005	3065	20	0.65 (0.4–1.01)
Caine Mix III (benzo-, cincho-, tetracaine)	0.63	3620	33	0.91 (0.63–1.28)
Budesonide	0.001	3471	27	0.78 (0.51–1.13)
Tixocortol-pivalate	0.002	3471	13	0.37 (0.2–0.64)
Hydrocortisone-17-butyrate	0.02	3471	15	0.43 (0.24–0.71)
Neomycin sulfate	0.6	3619	28	0.77 (0.51–1.12)
Ethylene diamine-di-HCl	0.05	3619	32	0.88 (0.61–1.25)
Quinoline mix	0.19	3064	12	0.39 (0.2–0.68)
Bacitracine	0.6	3063	2	0.07 (0.01–0.24)
Lanolin (wool fat) alcohols	1	3619	25	0.69 (0.45–1.02)
Thiuram mix	0.027	3619	59	1.63 (1.24–2.1)
PPD (black rubber) mix	0.075	3619	32	0.88 (0.61–1.25)
Mercapto mix (CBS, MBTS, MOR)	0.075	3471	15	0.43 (0.24–0.71)
Mercaptobenzothiazole	0.075	3619	18	0.5 (0.3–0.78)
Carba mix	0.25	3619	81	2.24 (1.78–2.77)
Colophony (Rosin)	1.2	3619	79	2.18 (1.73–2.71)
Epoxy resin	0.05	3619	58	1.6 (1.22–2.07)
<i>p</i> -tert-Butylphenol formaldehyde resin (PTBFR)	0.045	3618	81	2.24 (1.78–2.78)
<i>p</i> -Phenylenediamine (PPD)	0.08	3619	140	3.87 (3.26–4.55)
Parthenolide	0.003	3064	8	0.26 (0.11–0.51)
2-Bromo-2-nitro-1,3-propanediol	0.25	3065	20	0.65 (0.4–1.01)

Patch test results with the EBS are shown in Table 2, grouped for allergen classes. A supplemental analysis stratified for the contributing countries can be found in Table S2. As the composition of the TRUE Test (Panels 1–3) partly differs from the EBS, and as concentrations (effectively: doses per area) are partly different, the results obtained in 12 Spanish and the one Dutch department are presented separately in Table 3, with a breakdown by country offered in Table S3.

Positive reactions to nickel were most common (19.8 [19.2–20.4]% positivity [95% CI in parentheses]), see Table 2; the higher share seen with the TRUE Test can be attributed to the Spanish results (Table 3), but also the Italian prevalences are high (Table S2) reflecting well-known regional differences.^{16,17} Among the fragrances and related substances tested in the EBS, fragrance mix (FM) I and *Myroxylon pereirae* yielded very similar results with 6.80 (6.43–7.19)% and 6.62 (6.25–7.00)% pos., respectively.

Biocides (preservatives) comprise the largest number of allergens in the EBS ($n = 6$). Formaldehyde at 2% aq. yielded almost one percentage point more positive reactions than the 1% concentration (2.49 [2.16–2.85]% vs. 1.59 [1.33–1.88]); the pooled prevalence of both concentrations being 2.04 (95% CI: 1.83–2.26%). However, the results are difficult to compare, as they were obtained in different patients. The higher concentration of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) and MI alone, respectively, elicited positive reactions more frequently than the lower concentration still in use (Table 2), namely, in 6.91 (6.39–7.46)% to MCI/MI and 7.57 (7.11–8.06)% to MI, respectively. MCI/MI tested in the TRUE Test yielded a very similar result with 6.91 (6.1–7.78)% positive reactions (Table 3). While positive reactions to paraben mix and quaternium 15 are (notoriously) rare, the prevalence seen of methyldibromoglutaronitrile (MDBGN) sensitization is still high, with 4.26 (3.73–4.84)% at 0.3% pet. and 5.64 (5.1–6.22)% at 0.5% pet., while only 0.65 (0.4–1.01)% positive reactions were observed when MDBGN was tested in Spain using the TRUE Test.

Contact allergy to rubber allergens is not common, the frequency of positive reactions being below 1% in the overall group of consecutive patch-tested patients. An exception is the thiuram mix, with positive reactions in 2.34 (2.12–2.58)% and 1.63 (1.24–2.1)% , respectively, when tested in the EBS and the TRUE Test, respectively. Positive patch test reactions to the three resins included in the EBS, namely, colophonium, epoxy resin, and *p*-tert-butylphenol formaldehyde resin (PTBFR), were seen in 3.32 (3.06–3.60)%, 1.29 (1.12–1.48)%, and 0.47 (0.35–0.61)%, respectively. Positive reactions to the corticosteroid screening allergens and neomycin sulfate were rare in both EBS and TRUE Test results. Patch tests with both dye-related allergens, *p*-phenylenediamine (PPD) and textile dye mix (TDM), yielded very similar results, with 3.60 (3.26–3.96)% and 3.58 (3.23–3.96)% positive reactions, respectively (Table 2). Among 5403 patients simultaneously tested with both PPD and TDM, coupled reactivity was marked, with $n = 110$ patients reacting to both allergen preparations, $n = 86$ only to PPD, and $n = 95$ only to TDM (odds ratio [OR]: 68.8 [95% CI: 48.6–97.4]).

Among the substances added last to the EBS, propolis was most frequently positive (3.48 [3.16–3.82]%), followed by 2-hydroxyethyl

methacrylate (HEMA; 2.32 [2.0–2.68]%), see Table 2. Coupled reactivity between propolis and *M. pereirae* resin was evaluated based on 7879 patients tested with both; 116 patients reacted to both, 480 only to *M. pereirae*, and 179 only to propolis (OR: 9.6 [95% CI: 7.5–12.3]). Co-reactivity between propolis and FM I was in a similar range, with 92 of 7821 patients reacting to both allergens, 200 only to propolis, and 450 only to FM I (OR: 7.2 [95% CI: 5.5–9.4]). Concerning colophonium, 65 patients reacted to both natural mixtures, 228 only to propolis, and 237 only to colophonium (OR: 8.9 [95% CI: 6.6–12.1]). A positive patch test reaction to FM I, FM II, hydroxyisohexyl 3-cyclohexene carboxaldehyde (HICC), and/or *M. pereirae* resin was seen in 10.3% (95% CI: 9.8%–10.7%) of patients tested with these ($n = 15\,071$ for whom individual data were available for analysis). Furthermore, caine mix III 10% pet. elicited 1.56 (1.28–1.89)% pos.; in the TRUE Test, the frequency of positive reactions was 0.91 (0.63–1.28)%.

4 | DISCUSSION

This update of previous similar ESSCA reports on EBS patch test results^{6,7} is novel in that it not only includes ESSCA data but also additional data from the EBS working group and results obtained by the GEIDAC previously not included in the analyses. Before the results with different allergens are discussed, methodological issues shall be addressed.

4.1 | Methodology

This study includes data collected in 2019 and 2020. Owing to the coronavirus disease (COVID-19) pandemic, many dermatology departments experienced extreme variations in working conditions and patient selection, with the contact dermatitis units sometimes completely closed, and at other times trying to recover the patch testing backlog.¹⁸ Therefore, the number of cases included is less than expected, also owing to the recommendations by authorities to use hospitals only when strictly necessary. At this time, it is unknown whether and how this has impacted the global results. Considerable variation of the percentage of patients with positive reactions to at least one baseline series allergen (the ‘P-measure’) has been noted before and to a similar extent,¹⁵ thus likely not being indicative of COVID-19-related effects.

It has been repeatedly demonstrated that D7 readings improve the accuracy of patch test results, as a certain share of sensitized patients will exhibit a clear (be it weak) positive reaction only at a late reading, while having negative or doubtful reactions at earlier readings. The share of such late positive readings differs vastly between contact allergens and is highest in corticosteroids and, for example, neomycin sulfate.¹⁹ Moreover, patient characteristics and patch test exposure time may have an impact on the share of reactions which are appearing as positive only on D7.²⁰ Accordingly, a D7 reading combined with an earlier reading (D2 and D3 or D4) is recommended

as 'optimum' schedule in the ESCD patch test guideline.⁹ However, in practice many national networks deviate from this recommendation, usually owing to logistical reasons. In the part of the data documenting the full matrix of D1 to D7 readings (i.e., departments using the WinAlldat software),¹³ 208 instances of positive reactions which had been non-positive at earlier readings were identified, ranging from single events to a maximum of 72 reactions; these were observed in Groningen, which appears to be the only department regularly reading D3 and D7. Three German departments assess 60%–77% of their patients with a D7 reading, but in the remainder D7 readings are probably only used selectively. Thereby, some underestimation of true sensitization prevalences may be anticipated when relying on the conventional D3-D5 reading outcome (implemented some 20 years ago to cover most reading schedules), at least concerning those allergens which are known to cause late reactions.

Some well-known characteristics of the TRUE Test, such as poor sensitivity of FM I,²¹ and better detection of the MCI/MI preparation than with MCI/MI 100 ppm aq.²² should be taken into account when comparing these results with other results obtained with investigator-loaded, pet.-/aq.-based allergens. Currently, the sensitization prevalence to MCI/MI 200 ppm aq. is very close to that obtained with the TRUE Test, possibly supporting the recommendation to use the 200 ppm concentration when patch testing MCI/MI aq. in investigator-loaded chambers.

4.2 | Metals

Compared to the previous reporting period,⁶ the share of positive patch test reactions was slightly higher in the present analysis. However—also for other allergens subsequently discussed—some impact of the limitations imposed by the COVID-19 pandemic in terms of potentially patch testing in a more selective way in 2020 could play a role. Previous analyses pointed to a decrease of nickel contact allergy in younger patients, while a 'cohort effect' can be observed in those older females already sensitized before nickel regulation came into effect.²³ Moreover, the prevalence of nickel sensitization varies greatly between countries with, for example, higher crude estimates in Austria (30.32 [24.33–36.84]%) and Italy (26.79 [25.02–28.62]%), and a lower prevalence in Finland (14.77 [8.11–23.94]%) and the United Kingdom (13.1 [11.6–14.8]%), which can partially be explained by the late introduction of EU nickel regulation in Italy.¹⁷

Concerning chromium, a clear effect of regulating the chromium-VI content in leather to <3 ppm/kg leather (EU 301/2014)²⁴ has probably yet to be demonstrated although a recent Danish analysis identified a significantly lower prevalence in recent years.²⁵ The clinical relevance of cobalt contact allergy is often elusive, for example, in as much as 80% of consecutive patients testing positive.²⁶ Leather exposure seems to be increasingly important,²⁶ and the role of cobalt liberation from jewellery should probably not be underestimated,^{27,28} although both exposures are not easy to ascertain in clinical practice, as the cobalt spot test is often negative, even if potentially relevant metal liberation is identified by chemical analysis along EN1811:2011 + AC:2012.²⁸

4.3 | Fragrances and plant materials

The percentage of positive reactions to the fragrance markers FM I, FM II, and *M. pereirae* (balsam of Peru) is slightly higher than in 2015–2018.⁶ However, the above-mentioned putative selection effects hamper a direct interpretation of time trends. Conversely, HICC contact allergy has declined further, as compared to 2013/14 (1.7%; 95% CI: 1.5%–1.8%).⁷ This confirms a downward trend following a ban by the European Commission in 2017 during, or even before, a 4-year transition period ending in August 2021 owing to preemptive adaptation by industry.²⁹ New fragrance compounds introduced to the market, or general changes in exposure, will affect the screening abilities of the above-mentioned markers, as illustrated by considerable added reactivity to, for example, limonene or linalool hydroperoxides, which has been confirmed by the analysis of 'audit allergens' during the same study period.⁵ Moreover, the mixes themselves are insufficiently sensitive to diagnose contact allergy to their constituents, so patch testing with individual components such as the 26 fragrances to be labelled in the EU has been recommended.³⁰

Propolis, a newly introduced part of the EBS, has been reported to often cross-react with *M. pereirae* resin.³¹ In the present results, the OR of 9.6 (95% CI: 7.5–12.3) indicates only moderate concomitant reactivity, which is moreover asymmetrical: while 39.3% of propolis-positive patients also reacted to *M. pereirae*, only 19.5% of *M. pereirae*-positive patients also reacted to propolis. Interestingly, the increase of gross usage data of propolis in Germany has been found to concur with the increase of positive patch test reactions observed in that country,³² providing supporting evidence of a real increase in sensitization prevalence. However, the prevalence of propolis contact allergy vastly differs between European countries, with a higher share of contact allergy in the centre and East, and a lower in the South(west), see Table S2. Moreover, the different geographical origin (and thereby, different chemical composition) of propolis,³³ renders the interpretation of patch test results complex. Co-reactivity between propolis and *M. pereirae*, FM I, and colophonium, respectively, which had been reported earlier as significant,³³ was significant in our results, too, albeit on a comparatively moderate level. The role of sesquiterpene lactone mix and the Compositae mix in diagnosing contact allergy to this important plant family has been discussed in the study by Uter et al.⁵

4.4 | Biocides (preservatives)

The current figures on MCI/MI and MI are slightly higher than those in the previous analysis,⁶ despite a presumptive further decline after MI contact allergy has peaked in 2013/14. Again, the putatively different patient selection makes it difficult to interpret these data. Probably, pre-epidemic levels which have been reported by some groups,³⁴ but not by others,³⁵ will not be reached soon, in view of the vast number of MI-sensitized patients, who might present with other current problems but still be MI-allergic. MI contact allergy might not be clinically relevant in many cases, as allergen avoidance should be

(relatively) easy owing to (i) cosmetic restrictions with a ban in leave-on cosmetics and a restriction to 15 ppm in leave-on cosmetics and (ii) classification as CLP/GHS 'H 317: I A' contact allergen under REACH regulation in Europe, with a requirement to disclose the presence of MI in all products if this exceeds 1.5 ppm. However, less easily avoidable occupational or hidden exposures such as MI emanating from freshly painted walls, causing airborne allergic contact dermatitis, may still pose difficult to avoid problems for the MI sensitized.³⁶

One puzzling finding in the present data is the frequency of positive reactions to MDBGN: the prevalences seen in 2019/20 are markedly higher—also considering some often-cited selection effects during the COVID-19 pandemic—than in the years 2015–2018. In that preceding study period, MDBGN 0.3% pet. had caused 3.29% (95% CI: 3.09%–3.51%) and MDBGN 0.5% pet. 3.89% (95% CI: 3.58%–4.23%) positive reactions,⁶ compared to 4.26 (3.73%–4.84)% pos. to 0.3% pet. and particularly 5.64 (5.1–6.22)% pos. to MDBGN 0.5% pet. (Table 2). Of note, positive reactions to MDBGN tested as part of the TRUE Test in Spain are much lower, at 0.65 (0.4%–1.01)% pos. (Tables 3 and S3). However, if the test concentration used in the TRUE Test (5 µg/cm²) is compared to the dose/area of MDBGN 0.5% pet. (200 µg/cm²),³⁷ a difference by a factor of 40 is apparent. Vehicle effects may differentially affect allergen bioavailability, but it appears unlikely that equivalence can be assumed. As it has been noted that the increase of the MDBGN patch test concentration from 0.2 to 0.3% pet.—corresponding to a factor of just 1.5, albeit presumably in a different area of the dose-effect curve—has led to an almost doubled share of positive reactions,³⁸ it appears very likely that the seemingly low prevalence in Spain (when relying on TRUE Test data) is due to a presumably unduly low patch test dose. A suitable patch test concentration of 0.2% pet., and potentially false-positive reactions to 0.3% and particularly to 0.5% pet. have been identified in a previous study of the German Contact Dermatitis Research Group (DKG) gauging the diagnostic properties of 0.2, 0.3, and 0.5% pet. against repeated open application test results with MDBGN.³⁹ The concentration of 0.2% would correspond to a dose/area of 80 µg/cm²; this could probably be regarded as a target, or as the lower floor, for an amendment of the TRUE Test MDBGN preparation, taking possible vehicle effects into account.

MDBGN has been banned from use in cosmetics more than 10 years ago, and other exposures to MDBGN are often elusive. Therefore, it has been suggested that MDBGN contact allergy nowadays is largely irrelevant and that MDBGN can safely be withdrawn from the baseline series.⁴⁰ However, under the synonym 2-bromo-2-(bromomethyl)pentanedinitrile (DBDCB) MDBGN still is one of the permitted preservatives in the EU for 'product type 6', that is, for preservation during storage of products other than foodstuffs, feeding stuff, cosmetics or medicinal products or medical devices.⁴¹ Examples of non-cosmetic exposures associated with MDBGN have been reviewed recently,⁴² and hence it may be premature to regard the still high number of patients sensitized to MDBGN as an entirely 'historical' problem. Hence, in addition to optimizing the MDBGN TRUE Test concentration, research efforts should be put into a better delineation of its currently most relevant exposure sources.

Contact allergy to formaldehyde and formaldehyde releasers, including quaternium 15 included in the EBS, has recently been analysed in detail and presented in a separate paper⁴³ and will thus not be addressed here. Benzisothiazolinone and octyl isothiazolinones have been evaluated as 'audit' or 'candidate' allergens for inclusion in the EBS, as presented and discussed in the study by Uter et al.⁵ Positive reactions to paraben mix are very infrequent, both in the EBS and in the TRUE Test. This may argue for removal from the EBS.⁴⁴

4.5 | Rubber, plastic and glues

With 2.34 (2.12–2.58)% positive reactions, thiuram mix (still) is the most important rubber allergen in the EBS. In the TRUE Test, the yield is just slightly lower, with 1.63 (1.24–2.1)% positive reactions. Importantly, the thiurams included in the mix also represent the corresponding dithiocarbamates, which are also used as accelerators in vulcanization, as both form a redox pair. Thiurams may be better screening substances for dithiocarbamate contact allergy than the carbamates themselves, as discussed previously.⁴⁵ By contrast, sensitization prevalences observed with other rubber compounds, that is, the benzothiazoles and *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine (IPPD) as representative of PPD-derivatives used as antioxidants and -ozonants, are low. Although difficult to compare, the yield with IPPD 0.1% pet. in the EBS, with 0.79 (0.66–0.94)% positive reactions, and with black rubber mix 75 µg/cm² eliciting 0.88 (0.61–1.25) % positive reactions is similar. However, previous results seem to indicate that patch testing with IPPD alone will under-diagnose contact allergy to this class of antidegradants.^{45,46} The discussion of the best patch testing strategy for benzothiazoles—mercaptobenzothiazole with either the four-component mercapto mix 2% pet. or, preferably, the three-component mercapto mix 1% pet.⁴⁵—shall not be re-iterated here. All these allergens appear to be borderline for inclusion in the EBS, with an upper 95% CI between 0.5% and 1%; a discussion on whether rubber allergens beyond thiuram mix qualify for inclusion for other reasons (such as 'often unsuspected, but retrospectively often relevant') seems warranted.

Colophonium is a relatively common allergen with a multitude of sources of exposure. Co-reactivity to *Evernia furfuracea* (tree moss) is common,⁵ owing to the (oxidized) resin acids present in the extract resulting from a rather robust collection process of this lichen from conifer bark.⁴⁷ Therefore colophonium-allergic patients should be advised to avoid fragrances containing this compound, and vice versa. Concerning epoxy resin, in terms of the diglycidyl ether of bisphenol A as tested in the EBS, it could be argued that (occupational) exposure can usually be traced relatively well. However, the sheer frequency of reactions along with the growing use of epoxy resin systems, also beyond occupational exposure (and thus perhaps often ill-recognized) argues in favour of this allergen being part of the EBS. Conversely, contact allergy to PTBFR appears to be rare. It should, however, be noted that the performance of commercially available patch test preparations containing this allergen may vary.⁴⁸ Besides glues used in shoe manufacture, other relevant exposures to this complex mixture

include, for example, textiles, leather and rubber items, adhesives, domestic glues and even medical devices.^{49–52} Although it does not detect contact allergy to all resins based on phenol and formaldehyde,⁵³ PTBFR is currently the sole representative of this class of compounds routinely tested, and thus should probably be maintained in the EBS for the time being. The recent addition of 2-HEMA has certainly proven to be well-justified, in terms of the overall frequency of sensitization, probably driven mainly by the current fashion trend of artificial nails, but also relevant in the field of medical devices, dental materials, and industrial applications.⁵⁴ In November 2020, the use of 2-HEMA in nail cosmetics has been restricted in the context of the EU Cosmetics Regulation (EC 1223/2009), permitting only professional use.⁵⁵ Time will tell whether this restriction has an effect on the ongoing 'nail cosmetics epidemic'.

4.6 | Medicaments, excipients and dyes

In the last revision of the EBS in 2019, caine mix III 10% pet. has been included to replace benzocaine 5% pet. Comparing just the sensitization frequencies, this has resulted in a more than doubled detection: from 0.69 (0.58–0.82)% to benzocaine seen during 2015–2018 to 1.56 (1.28–1.89)% positive reactions to caine mix III in the present analysis. While the sensitivity of this mix diagnosing benzocaine allergy appears acceptable, if concomitant reactivity is analysed, this is not the case regarding cinchocaine and tetracaine, the other two constituents of the mix.⁵⁶

At present, two representatives of corticosteroid allergens are included in the EBS: tixocortol pivalate and budesonide. Both concentrations of the corticosteroid markers yielded low prevalences of contact allergy, the 95% CI (by far) not exceeding 1%, except for budesonide tested in the TRUE Test (Table 3). The vastly differing yield of positive reactions, even significant in the case of budesonide, is difficult to interpret, as different patients (with a differing exposure background) have been tested. A previous study comparing budesonide 0.01 and 0.1% pet. by synchronous application found these to be equivalent.⁵⁷ The low sensitization prevalence to corticosteroids is striking since exposure to topical corticosteroids in the treatment of many dermatoses is very high. The addition of a Group 3 corticosteroid allergen (such as clobetasol propionate) has been suggested to improve the diagnosis of allergy to topical corticosteroids⁵⁸; the usefulness seems to depend on (national) prescription habits and thus differences in exposure. Lanolin alcohols are a natural mixture of emulsifying alcohols of various chain lengths and structures. They are weak contact allergens contained in wool fat or wool wax (lanolin) produced from sheeps' wool.⁵⁹ Considering the broad use of lanolin (alcohols), a frequency of sensitization of 1.38 (1.20–1.58)% is relatively low. In contrast to the United States, the importance of neomycin sulfate as a contact allergen seems to be diminishing, for example, from 1.24 (1.11–1.37)%⁵⁹ in 2009–2012 or 1.23 (1.11–1.35)%⁶ in 2015–2018 to 0.83 (0.67–1.01)% at present. This decline has been found to be linked to decreasing sales/prescriptions.⁵⁹

The EBS includes two allergen preparations used to diagnose contact allergy to dyes (oxidative hair dyes or textile dyes), namely, PPD and TDM 6.6% pet. The latter includes Disperse Orange 3, which is chemically very similar to PPD and shows marked cross-reactivity, noted already during the initial study,⁶⁰ and certainly also in the present analysis, with an OR of 68.8 (95% CI: 48.6–97.4). Hence, a Swedish study evaluated a TDM without this dye and concluded that 'results indicate that Disperse Orange 3 might probably be omitted from TDM', but further study is needed to finally decide whether this is sensible or not.⁶¹ Sensitization to PPD itself is more prevalent in central and southern European patch test centres than in Scandinavian,⁶² most likely owing to the common use of dark oxidative hair dye products in the South. A corresponding geographical gradient is also reflected in differences in the share of PPD-containing oxidative hair dye products between countries, which, in addition, has changed over time.⁶³

5 | CONCLUSION

The surveillance results on the prevalence of contact sensitization to EBS allergens, combined with patch test results of possible candidate allergens for inclusion in the EBS,⁵ contributes to maintaining an up-to-date baseline series comprising the most frequent and/or relevant contact sensitizers for routine patch testing in Europe. National adaptations and extensions based on typical geographical allergen exposures are of course possible and useful; these added allergens are often a fruitful starting point for co-operative evaluation on a broader, European scale. The EBS working group offers a forum for this important research aiming at a high level of diagnostic standards and patient care.

AUTHOR CONTRIBUTIONS

Wolfgang Uter: Conceptualization; software; formal analysis; methodology; visualization; project administration; writing – original draft; writing – review and editing; resources. **Mark Wilkinson:** Conceptualization; methodology; data curation; investigation; validation; supervision; resources; project administration; funding acquisition; writing – original draft; writing – review and editing. **Olivier Aerts:** Conceptualization; methodology; data curation; supervision; validation; investigation; project administration; resources; writing – original draft; writing – review and editing. **Andrea Bauer:** Conceptualization; methodology; data curation; supervision; validation; investigation; resources; writing – review and editing. **Leopoldo Borrego:** Conceptualization; methodology; software; data curation; supervision; validation; investigation; funding acquisition; project administration; resources; writing – original draft; writing – review and editing. **Richard Brans:** Conceptualization; methodology; data curation; supervision; validation; investigation; resources; writing – review and editing. **Timo Buhl:** Conceptualization; methodology; data curation; supervision; validation; investigation; writing – review and editing; resources. **Heinrich Dickel:** Conceptualization; investigation; methodology; validation; writing – review and editing; data curation; supervision; resources. **Aleksandra Dugonik:** Conceptualization;

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CONFLICTS OF INTEREST

Wolfgang Uter has accepted travel reimbursement and research funds from the cosmetic industry association IFRA and has received a lecture fee from dermatology-related sponsors for an educational lecture on contact allergy in 2018. Olivier Aerts is investigator, consultant and/or speaker for Leo Pharma and La Roche Posay/L'Oréal. Margarida Gonçalves has received honoraria for advisory boards and lectures from Novartis, Abbvie, Leo, Lilly, Pfizer, Sanofi and Takeda. Cataldo Patruno is an advisor, consultant, speaker and / or investigator for AbbVie, Eli Lilly, Leo Pharma, Novartis, Pfizer, Pierre Fabre, Sanofi. Marie-L.A. Schuttelaar is an advisor, consultant, speaker and/or investigator for Abbvie, Pfizer, LEO Pharma, Regeneron, Sanofi-Genzyme, Eli Lilly and Galderma, and has received research grants from Regeneron, Sanofi-Genzyme, Novartis and Pfizer. The IVDK, maintained by the IVDK e.V., of which Steffen Schubert is an employee, is sponsored by cosmetic and fragrance industry (associations) as well as by public funds. Thomas Werfel is an advisor, consultant, speaker and/or investigator for Abbvie, Pfizer, LEO Pharma, Novartis, Regeneron, Sanofi-Genzyme, Eli Lilly and Galderma. S. Mark Wilkinson has received travel reimbursement to attend meetings with the cosmetic industry. The other authors have no pertinent conflict of interests to declare.

DATA AVAILABILITY STATEMENT

Data sharing not possible owing to privacy restrictions (included patients had not been asked for consent for "open data" sharing)

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SUPPORTING INFORMATION

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

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APPENDIX

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