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**Contact allergy to preservatives: ESSCA* results with the
baseline series, 2009-2012**

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- A.G.A: Registration and management of patients and manuscript responsible.
 - G.D: Registration and management of patients and manuscript support.
 - A.B: Registration and management of patients and manuscript revision.
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ABSTRACT

Background. Allergic contact dermatitis caused by biocides is common, and causes significant patient morbidity.

Objective. To describe the current frequency and pattern of patch test reactivity to biocide allergens included in the baselines series of most European countries.

Methods. Data collected by the European Surveillance System on Contact Allergies (ESSCA) network between 2009 and 2012 from 12 European countries were analysed.

Results. Methylisothiazolinone 0.2% aq. produced the highest prevalence of sensitization during the study period, with an overall prevalence of 4.5%. The mixture methylchloroisothiazolinone/methylisothiazolinone tested at 0.02% aq. followed closely, with 4.1% of positive reactions. Other preservatives with lower rates of sensitisation, but still over 1%, include methyldibromo glutaronitrile (MDBGN) 0.5% pet. and iodopropynyl butylcarbamate (IPBC) 0.2% pet. Formaldehyde releasers and parabens yielded less than 1% positive reactions during the study period. Some regional differences in the prevalence of contact allergy to biocides among European countries were observed.

Conclusions. Contact allergy to biocides is common throughout Europe, and regional differences could be explained by differences in exposure or characteristics of the population tested. Timely regulatory action for isothiazolinones is required. Although MDBGN is banned from cosmetics products since 2005, sensitization prevalence has not appeared to plateau. IPBC is an emerging allergen with an increasing prevalence over the last few years, and its inclusion in the European baseline series may be appropriate.

Keywords: biocides, contact allergy, patch testing, preservatives

INTRODUCTION

Preservatives are biocide chemicals added to cosmetics and household, pharmaceutical, and industrial products to prevent the growth of microorganisms that may enter during manufacture or during usage, thereby prolonging the shelf life and the period of use of the products.^{1,2} Although preservatives can be regarded as indispensable agents, many of them have long been recognized as important skin sensitizer and constitute common causes of both occupational and non-occupational contact dermatitis.¹⁻⁴

Some of the biocide allergens have been responsible for contact allergy outbreaks over the last century, such as the one caused by formaldehyde in the 1950s and 1960s as a result of cutaneous exposure to textile finishes and cosmetics.^{5,6} The introduction of methylchloroisothiazolinone (MCI)/ methylisothiazolinone (MI) resulted in another outbreak in the early 1980s, primarily attributed to cosmetic leave-on products, leading to strict regulations regarding its use.⁷ Later, the use of methyldibromo glutaronitrile (MDBGN) in cosmetic products resulted in the third outbreak, which peaked in the late 1990s and early 2000s. The increasing rates of sensitization to MDBGN prompted the European Commission to restrict its use in leave-on and rinse-off cosmetics.^{8,9} More recently, MI as a single agent was introduced for use in industrial products around the year 2000 and in cosmetics in 2005. In the last years, many centres have reported an unprecedented increase in sensitization rates to both MI and MCI/MI.^{10,11}

Therefore, the knowledge of the clinical and epidemiological features of contact allergy to biocide allergens, along with analyses of time trends, is of critical importance. On the basis of data collected over a 4-year period by the European Surveillance System on Contact Allergies (ESSCA) (www.essca-dc.org) network, this study describes the

current frequency and pattern of patch test reactivity to preservatives, some included in the European Baseline Series (EBS) and some others previously recommended by the ESSCA to be patch tested routinely.

MATERIALS AND METHODS

Retrospective analysis based on data collected by the ESSCA network.¹² Clinical and demographic data, along with patch test results, of all patients tested are documented electronically, using diverse data capture software and partly the multilingual software WinAlldat/ESSCA provided by the ESSCA.¹³ Patch testing was performed according to European Society of Contact Dermatitis (ESCD) guidelines.¹⁴ Data were pooled in the ESSCA data centre in Erlangen for further analysis using R (version 3.1.2) software¹⁵ after anonymised transmission.¹⁶ Pertinent statistical guidelines for the analysis of patch test data were considered.^{17,18} For direct sex- and age-standardization, weights of 0.175 were employed for males age >39 and <40, respectively, and of 0.325 for females age >39 and <40, respectively. The maximum patch test reaction between day (D) 3 and D5 (inclusive) was aggregated as patch test outcome. Reactions designated as either +, ++ or +++ were classified as positive (allergic), and all others as non-allergic. The study period was from January 2009 to December 2012, included 12 European countries and, in total, 54 departments.

A total of 63530 consultations were recorded during the study period. Patients not tested with the baseline series were not further considered in this analysis. Altogether, 59922 consultations involved the baseline series including at least one reading at day 3 to 5 after patch test application. Due to multiple consultations of some patients, this number is further slightly reduced to 59728 single consultations (the most recent consultation

was chosen for the analysis). Because of apparent differences in the sensitivity of the TRUE Test® (compared to the investigator-loaded petrolatum-based systems used in the overwhelming majority of departments),¹⁹ TRUE-test® results, which involve 2435 consultations, are reported separately. In all departments using the TRUE Test®, it was always combined with an additional set of allergens using investigator-loaded chambers and petrolatum- or water-based allergens to achieve a better coverage of the desired range of allergens and concordance with the EBS, i.e. fragrance mix II, hydroxisohexyl 3-cyclohexene carboxaldehyde (HICC), MDBGN, sesquiterpene lactone mix, primin, and, more recently, MI.²⁰

The biocides contained in the EBS and further allergens listed in Table 1 were tested in consecutive patients. Please note that in case of biocides, there are partly different test concentrations used because of lack of ESCD recommendations at that time. The corresponding results have been analysed separately.

RESULTS

Prevalence of preservative contact allergy

Table 1 illustrates The overall results with the petrolatum and water-based allergens, all tested in consecutive patients, be it only in certain periods or by a subset of departments only, are presented in table 1. Overall results include stratification by sex and age (dichotomized). MI registered the highest prevalence of sensitization, with 4.9% positive reactions (age-standardized and sex-standardized) of 4755 tested patients at 0.05% aq., and 4.5% positive reactions of 3382 tested patients at 0.2% aq. (MI at 0.1% aq. was patch tested in only 141 patients). The combination MCI/MI, tested at concentration of 0.02% aq., followed closely, with 4.1% of positive reactions. Other

preservatives with lower prevalences, but still over 1%, include MDBGN, the mixture methyldibromo glutaronitrile+ 2-phenoxyethanol, iodopropynyl butylcarbamate (IPBC), and formaldehyde 1%. Other biocides, such as formaldehyde 2% (albeit tested in a still limited number of patients), formaldehyde releasers and parabens, yielded less than 1% of positive reactions during the study period.

~~Table 2 shows~~ The results obtained with the biocide allergens available in the TRUE-test®, including stratification by sex and age, **are shown in table 2**. A wide range of frequencies of positive patch test reactions was found, with the highest prevalence for MCI/MI with 7.5% of positive reactions. At the other end, the paraben mix yielded only 0.4% positive reactions. The distribution of reaction grades as compared with investigator-loaded based allergens varied slightly with a lower proportion of irritant/doubtful reactions (with the exception of formaldehyde and imidazolidinyl urea) and a higher share of weak (+) reactions (except for paraben mix) for the TRUE Test® allergen preparations.

Prevalence by country

A breakdown of test results by country is shown in 'Supplementary table 1'. Some allergens showed marked differences in the frequency of sensitization between European countries, with the highest values seen for MCI/MI tested at 0.02% aq. in Finland and Spain (8.9% and 6.9%, respectively) compared with the United Kingdom (4.2%). The variability of sensitisation to other biocide allergens was on a lower level, for example quaternium-15, which showed a prevalence of sensitization ranging from 0.3% to 1.9% in the 10 countries analysed, and the paraben mix, which ranged from 0.2% to 1.9% in the 11 countries analysed.

Formaldehyde and formaldehyde releasers

Table 3 shows The characteristics of patients positive to a set of 4 formaldehyde releasers and of patients with positive reactions to either 1% or 2% formaldehyde are shown in table 3. The rightmost column gives the proportion of patients with a positive patch test reaction to formaldehyde that also reacted positively to the allergen involved. The column to the left of this is, *vice versa*, the proportion of patients positive to the single allergen also reacting positively to formaldehyde. The formaldehyde releasers with higher co-reactions to formaldehyde were quaternium-15 (39.5%) and diazolidinyl urea (26.4%). On the other hand, just 7.4% of the 2-bromo-2-nitropropane-1,3-diol (Bronopol®) sensitive patients also reacted to formaldehyde.

Methyldibromo glutaronitrile

Table 4 shows The MOAHLFA index in patients patch tested and in patients positive to MDBGN, as well as the proportion of patients tested with the 3 different concentrations, stratified for 3 age groups, are presented in table 4. Note that the prevalence of positive reactions to MDBGN was higher in the ‘old’ and ‘middle’ age groups than in the youngest age group at the 3 different concentrations tested. Figure 1 shows The time trend regarding MDBGN is shown in figure 1. It should be noted that 3 different patch test concentrations have been used in the study period, namely, 0.2, 0.3 and 0.5% pet. The usage across the different departments differed significantly between the years ($p<0.001$, X^2 test), with 0.2% fairly constantly covering 23 (2009) to 27% (2011/12) of all tests, 0.3% decreasing in usage from 60% in 2009 to 47% in 2012, and 0.5% pet. increasing from 16% in 2009 to 26% in 2012. In view of this, the descriptive results in Fig. 1, stratified for age group, with pooled results from all 3 concentrations, should be

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3 interpreted with some caution, as they are confounded, to some extent, by the above
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5 factor. Statistical analysis, however, does confirm the notion of an upward trend for the
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7 two older groups, and a largely stable prevalence for the youngest age group both in a
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9 bivariate and an adjusted analysis considering potential confounding by test
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11 concentration, sex and country ($p < 0.001$).
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16 **Methylchloroisothiazolinone/methylisothiazolinone (3:1) and methylisothiazolinone**

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18 ~~Figure 2 shows~~ The time trend regarding MCI/MI, stratified for sex and age-group, is
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20 **presented in figure 2.** MCI/MI had been tested in the baseline series throughout the
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22 study period; however, its test concentrations had been increased in some departments,
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24 respectively, pre-empting the ESCD recommendation in the course of the study
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26 period.²⁰ In this case, also taking into account the possibility of detecting a large
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28 proportion of MI contact allergy with MCI/MI tested at 200 ppm, both test
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30 concentrations were analysed separately, with TRUE Test® results allotted to the tests
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32 using 100 ppm. Although MI had been tested separately, in 3 different concentrations
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34 (Table 1), geographic cover age is quite limited. Moreover, the present study period
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36 would only demonstrate the very beginning of the well-recognised epidemic of MI
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38 sensitisation.
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45 **DISCUSSION**

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47 The overall results show that the most important preservatives, in terms of frequency of
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49 contact sensitization, are currently the isothiazolinones (MI and MCI/MI) and MDBGN.
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51 Sensitization frequencies for these biocides were greater than 1% during this period,
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53 justifying their inclusion in the baseline series. Although formaldehyde 2.0% aq. is the
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55 currently recommended patch test concentration,²¹⁻²⁴ the frequency of sensitization
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3 registered was less than 1%. However, this result should not be over-interpreted, as
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5 different patients had been tested with the 2 concentrations, a comparison of the
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7 ‘diagnostic yield’ of (relevant) test reactions cannot be made. The paraben mix and
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9 formaldehyde releasers, such as Bronopol®, imidazolidinyl urea, diazolidinyl urea and
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11 quaternium-15, did not reach the 1% threshold of positive reactions in consecutive
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13 patients; therefore, their inclusion in the EBS should be discussed.
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18 As with previous ESSCA studies,^{12,25} regional variation in the prevalence of
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20 sensitization was observed. Differences were particularly noted and was relevant
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22 specially for formaldehyde, MI/MCI and MDBGN. For example, the Netherlands
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24 shows a particular high prevalence of the following biocides: 2.5% and 3.4% reacted to
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26 formaldehyde 1% and 2% aq., respectively; 8.6% reacted to MCI/MI 0.01% aq.; and
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28 6.9% reacted to MDBGN 0.3% pet. In the other extreme, United Kingdom reported the
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30 lowest prevalence of sensitization to formaldehyde 1% and 2% aq. (1.2% and 1.0%
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32 contact allergy prevalence, respectively), MCI/MI 0.02% aq. (4.2%), and MDBGN
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34 0.3% pet. (0.6%). In spite of a common EU regulation –at least concerning exposure via
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36 cosmetics-, such national or regional differences in the prevalence of sensitivity among
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38 European countries are observed.²⁶ Such These differences could be attributable to
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40 differences in exposure or characteristics of the population tested. Cosmetic-related
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42 contact allergy has been considered to be related to facial dermatitis; indeed facial
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44 dermatitis is about twice as common in patients allergic to formaldehyde releasers,
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46 which are typically cosmetic preservatives, than in the general patch test patients (Table
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48 3).²⁷ Furthermore, despite all efforts for standardization, this regional variation may
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50 partly be explained by residual methodological variation in a network of such a scope.
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MDBGN was launched in the 1980s for use in industrial and cosmetic products. During the early 1990s, the prevalence of allergy to MDBGN was 0.7%, but sensitivity rates rose to 3.5% in 2000, and to 4.5% in 2004-2005. Due to an increase in the prevalence of contact allergy to MDBGN registered in the 2000s,^{3,28} the European Commission restricted its use, and MDBGN was first banned from leave-on products in 2003,²⁹ and subsequently, following a 2005 recommendation of the Scientific Committee on Consumer Products, it was also banned in rinse-off products in 2007.³⁰ Since then Subsequent to these interventions, decreasing trends in MDBGN contact allergy have been reported in Europe. The Danish Contact Dermatitis Group found a decreasing prevalence from 4.6% in 2003 to 2.6% in 2007⁸ and similarly, the IVDK showed a decrease in the prevalence of sensitization from 4.5% in 2004-2005 to 2.4% in 2008-2009.³ Since then, the prevalence of sensitization to MDBGN remains stable, as shown in our data, ranging from 2.5% to 2.8% during the period 2009-2012. Although nowadays most patients sensitised to MDBGN might represent 'historical sensitisation', it should be noted that even in the youngest subgroup of patients, which should reflect changes in exposure, sensitization to MDBGN is still present. This may suggest that other non-regulated sources are still relevant in terms of elicitation or even sensitization. These might include a continued occupational exposure, or topical medicaments where MDBGN is not (necessarily) a disclosed ingredient.³¹ Therefore, MDBGN is still an important allergen to include in the EBS.

Isothiazolinones are well-known contact sensitizers and effective biocides. The mixture of MCI and MI in the ratio of 3:1 (MCI/MI) was responsible of an increasing number of allergic patients in the 1980s. The European Union restricted the MCI/MI concentration to an upper limit of 15 ppm in cosmetics in 1989. Even lower limit of 7.5 ppm in leave-

~~on products was recommended by the US Cosmetic Ingredient Review. Nowadays,~~
MCI/MI is completely banned from use in leave-on products in Europe.³² **However,**
several years ago, MI was introduced as a ~~single~~ **stand-alone** preservative in skin care
products and cosmetics (at a maximum use concentration of 100 ppm).³³ Since then, an
alarming and unprecedented increase in the prevalence of sensitization to both MCI/MI
and MI has been reported.^{11,34} ~~According to the IVDK, the prevalence of contact allergy~~
~~to MCI/MI was constant between 2.1 and 2.3% from 1998 to 2009. After that, it rose to~~
~~3.9% in 2011, paralleled by an increase in the frequency of sensitization to MI 0.05%~~
~~aq. from 1.9% in 2009 to 4.4% in 2011. Similarly, an increasing frequency of~~
~~sensitization to MCI/MI from 0.9% in 2008 to 4.9% in 2011-2012, paralleled by an~~
~~increase in the frequency of allergic reactions to MI 0.02% aq. from 0.6% in 2009 to~~
~~2.5% in 2012 was reported in the United Kingdom. Our analysis shows similar results,~~
and a prevalence of contact allergy to MCI/MI of 3.3-4.1% and to MI of 4.5-4.9% was
found during the study period. The presented ESSCA data on MI alone are somewhat
limited in terms of geographical coverage, and only show the very beginning of the
increased frequency of contact allergy due to MI reported elsewhere. ~~Along with timely~~
Following regulatory action for these two preservatives, the impact on sensitization
frequencies in the future should be ~~checked~~ **monitored**.

Formaldehyde is a difficult allergen to test, since most of the allergic reactions are only
weakly positive, although they often have a clinical relevance.³⁵ When patients are
tested with formaldehyde and one or more formaldehyde-releasers, concomitant
positive reactions may be observed.^{3,36-38} However, there is currently insufficient
evidence regarding whether formaldehyde releasers present a risk for patients who are
allergic to formaldehyde. One reason is that it is impossible to determine the levels of

free formaldehyde in products containing formaldehyde-releasers, as a wide range of factors, such as nature and concentration of the releaser, pH, temperature, storage time, level of microbial contamination, and presence of other components, can influence the amount of free formaldehyde released.³⁸ Previous studies showed that just 15% of the Bronopol®-positive patients also reacted to formaldehyde, while a higher percentage (40-60%) of the patients reacting to the other releasers also reacted to formaldehyde.³⁸ In our study, these rates of cross-reactivity between formaldehyde and formaldehyde-releasers were considerably lower: only 7% of all reactions to Bronopol® and 21-40% of the reactions to the other releasers are associated with a positive patch test reaction to formaldehyde in the test material. Similar results were found in a recent study from the IVDK,³ with 5% of cross-reactivity for Bronopol® and 16-47% for the other releasers. Notwithstanding, the recommendation of advising patients who are allergic to formaldehyde to avoid leave-on cosmetics preserved with formaldehyde releasers, especially when are used regularly and/or on “sensitive” or damaged skin, seems very reasonable.³⁸ Although most patients would tolerate these products, If there would be no alternative, Bronopol®-containing products would probably be the safest option, since most patch test reactions to Bronopol® are caused by a sensitivity to this preservative *per se* and are not related to formaldehyde allergy.³⁹

Other biocide allergens include parabens and IPBC. Parabens are not only used in cosmetics, but are also widely used in food and industrial products. Since the 1990s, the prevalence of sensitization to parabens has been relatively stable in Europe around 0.5 to 1%.^{4,12,28} The European Environmental and Contact Dermatitis Research Group (EECDRG) reported a prevalence of contact allergy of 0.5-1% between 1991 and 2000; a Danish study found a overall prevalence of 0.5% between 1985 and 2008; and studies

from Italy and the United Kingdom reported a prevalence below 1% (0.7% and 0.6% respectively) in the years 2007-2008. Although the IVDK found a sensitization rate of 1.3% between the years 1996 and 2009, a decreasing trend was noted.³ In the current analysis, the prevalence of contact allergy to parabens has apparently remained stable in the last few years, with an overall sensitization frequency of 0.7% between 2009 and 2012. Conversely, IPBC, a small lipophilic molecule that may readily penetrate the skin, is an emerging allergen with an increasing prevalence over the last few years. This preservative is now being used with increasing frequency, especially in cosmetic products.⁴⁰ The rate of sensitization frequency to IPBC in Europe varied from 0.2- 0.3% in the late 1990s to 0.6% from 2000 to 2011.⁴¹⁻⁴³ The IVDK showed a prevalence of contact allergy of 0.9% between 1996 and 2009, with an relevant increase in the last years.³ With a sensitization rate to IPBC 0.2% pet. (the recommended patch test concentration since 2005) of 1.2% during 2009-2012, our data suggests that this allergen could be included in the EBS.

In conclusion, preservatives are nowadays one of the major causes of contact allergy. MI, with an overall prevalence of sensitization during 2009-2012 of 4.5%, is a current problem across Europe. Although MDBGN is banned from leave-on and rinse-off products since 2005, sensitized patients are still diagnosed, suggesting other non-regulated sources of exposure. Prevalence of contact allergy to parabens has remained stable in the last years, whereas IPBC is an emerging allergen with an increasing prevalence. Regional differences in the prevalence of sensitivity among European countries could be explained by differences in exposure and test populations. Continued surveillance is needed to monitor the impact of biocides allergens across Europe.

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For Peer Review

Table 1: Overall results with the biocide allergens. ‘%(pos.std.)’, proportion of positives, directly age- and sex-standardised, 95% CI, 95% confidence interval to the standardised prevalence. Allergens marked with a # are part of the ESCD-recommended European baseline series

Allergen	Conc. %	nr (test)	% (+)	% (++/+++)	% (? +/IR)	% (pos.)	% (pos.std.)	95% CI
BRONOPOL®	0.25	11000	0.84	0.08	0.38	0.92	0.94	(0.76–1.13)
BRONOPOL®	0.5	22086	0.62	0.17	1.59	0.78	0.72	(0.61–0.84)
Diazolidinyl urea	2	16673	0.53	0.2	0.35	0.74	0.72	(0.59–0.85)
Methyldibromoglutaronitrile + 2-Phenoxyethanol	1.5	4590	1.55	0.41	0.23	1.96	2.04	(1.62–2.46)
Formaldehyde	1	48676	0.94	0.63	1.22	1.57	1.52	(1.41–1.63)
Formaldehyde #	2	12195	0.80	0.23	0.21	1.03	0.99	(0.82–1.17)
Imidazolidinyl Urea (Germall 115)	2	23336	0.45	0.17	0.28	0.62	0.61	(0.51–0.71)
Iodopropynylbutyl carbamate (IPBC)	0.1	7956	0.11	0.03	NaN	0.14	0.14	(0.05–0.22)
Iodopropynylbutyl carbamate (IPBC)	0.2	12360	1.21	0.1	2.83	1.31	1.23	(1.04–1.42)
Methylchloroisothiazolinone Methylisothiazolinone (MCI/MI)	0.01	48720	2.14	1.74	1.31	3.88	3.31	(3.15–3.47)
Methylchloroisothiazolinone Methylisothiazolinone (MCI/MI) #	0.02	17085	2.71	1.69	0.54	4.40	4.12	(3.83–4.41)
Methyldibromo glutaronitrile	0.2	13581	2.41	0.68	3.79	3.09	2.77	(2.49–3.05)
Methyldibromo glutaronitrile	0.3	30984	1.36	0.39	0.68	1.97	2.73	(2.47–3)
Methyldibromo glutaronitrile #	0.5	11873	0.8	1.72	0.87	2.52	2.46	(2.18–2.74)
Methylisothiazolinone	0.05	4755	3.3	2.19	1.56	5.49	4.91	(4.31–5.51)
Methylisothiazolinone	0.1	141	0.71	0	4.12	0.71	0.21	(0–0.61)
Methylisothiazolinone #	0.2	3382	3.13	1.57	0.15	4.7	4.48	(3.78–5.17)
Paraben mix #	16	52586	0.47	0.26	1.78	0.7	0.7	(0.63–0.77)
Quaternium 15 #	1	39342	0.55	0.38	0.34	0.91	0.87	(0.78–0.96)

Table 2: Overall results with the biocide allergens available in the TRUE-Test®. ‘%(pos.std.)’, proportion of positives, directly age- and sex-standardised, 95% CI, 95% confidence interval to the standardised prevalence.

Allergen	Conc. <i>mg/cm²</i>	nr (test)	% (+)	% (++/+++)	% (? +/IR)	% (pos.)	% (pos.std.)	95% CI
Methylchloroisothiazolinone Methylisothiazolinone (MCI/MI)	0.004	2362	4.23	3.77	0.51	8	7.51	(6.46–8.56)
Quaternium 15	0.1	2362	0.93	0.55	0.34	1.48	1.32	(0.88–1.75)
Diazolidinyl urea	0.55	2316	0.69	0.22	0.41	0.91	0.91	(0.51–1.31)
Formaldehyde	0.18	2362	1.06	0.55	0.19	1.61	1.42	(0.97–1.88)
Imidazolidinyl Urea (Germall 115)	0.6	2316	0.73	0.22	0.54	0.95	0.95	(0.55–1.36)
Paraben mix	1	2362	0.21	0.17	0.27	0.38	0.35	(0.12–0.59)

Table 3: MOAHLFA index (Male, Occupational, Atopic dermatitis, Hand eczema, Leg dermatitis, Facial dermatitis, and Age ≥ 40 years) in all patients patch tested (first row) and in patients positive to formaldehyde and formaldehyde releasers, respectively. For this analysis, different concentrations of an allergen (see Table 1) and TRUE-test® allergens were regarded equivalent and pooled. ‘pos.(F’ald.)’; proportion of patients positive to the allergen in the row, who also had positive reactions to formaldehyde. ‘% of F’ald. pos’; proportion of patients positive to formaldehyde (tested with the allergen in the row) who are also positive to the allergen in the row.

Allergen	n(test)	% Male	% Occupational	% Atopic	% Hand	% Leg	% Face	Age 40+	pos.(F’ald.)	‘% of F’ald. pos’
All patients	57146	33.6	13.6	22.5	21.8	4.9	14.3	59.9		
Formaldehyde	54896	33.5	22.0	22.9	33.0	4.0	11.5	63.5	100	
Bronopol	30820	32.6	16.9	31.0	21.5	2.9	17.8	66.5	7.4	4.4
Diazolidinyl urea	19109	31.9	17.4	39.6	29.9	6.2	26.4	63.2	26.4	12.2
Imidazolidinyl urea	23640	36.3	14.6	39.5	28.7	7.0	24.8	59.9	21.0	9.4
Quaternium 15	37745	24.9	16.7	25.7	32.2	3.1	22.6	66.1	39.5	23.9

Table 4: MOAHLF(A) index in patients patch tested (left column) and in patients positive to methyldibromo glutaronitrile (right column), respectively, as well as proportions of patients tested with the 3 different concentrations, stratified for 3 age groups. ‘pos. %’ indicates the percentage of positive reactions in the subgroup defined by row and column variables. *p*-value: adaptation of Fisher’s exact test for heterogeneity of reactivity to MDBGN across the 3 age groups in the strata of the MOAHLF(A) factors (A for age 40+ evidently omitted), e.g., in male patients, in patients with occupational dermatitis, etc..

	Age < 16		Age 16 to 64		Age > 64		<i>p</i> -value
	tested	pos. %	tested	pos. %	tested	pos. %	
Number	1931	0.7	42958	2.2	8774	3	< 0.0001
M	772	0.6	14136	2.7	3043	3.2	< 0.0001
O	22	4.5	7213	3.2	122	4.9	0.30
A	915	0.3	10381	1.7	685	3.8	< 0.0001
H	224	0.4	10650	2.1	917	2.1	0.23
L	64	0	1418	3.5	1062	2.2	0.086
F	379	0.3	6369	1.5	1006	2.3	0.020
Conc. 0.2%	228	1.8	10512	2.9	2711	3.7	–
Conc. 0.3%	1280	0.5	22446	1.6	4629	2.6	–
Conc. 0.5%	423	0.7	10000	2.5	1434	3	–

Supplementary Table 1: Results with the biocide allergens patch tested in petrolatum or water, stratified for country. ‘%(pos.std.)’, proportion of positives, directly age- and sex-standardised, 95% CI, 95% confidence interval to the standardised prevalence. Results from 2 German departments using a patch test exposure of 1 day (‘DE (1 day)’) are presented separately.

a) Formaldehyde, 1% aqu.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
AT	1109	15	1.35	1.34	(0.65-2.03)
CH	4966	64	1.29	1.28	(0.95-1.62)
DE	5535	76	1.37	1.31	(0.99-1.63)
DE (1 day)	1904	27	1.42	1.28	(0.73-1.83)
DK	2573	58	2.25	2.06	(1.13-3)
ES	3272	64	1.96	1.93	(1.45-2.42)
FI	1055	53	5.02	5.03	(3.69-6.37)
IT	9211	125	1.36	1.37	(1.13-1.6)
LT	865	19	2.2	1.88	(1-2.76)
NL	2823	70	2.48	2.49	(1.9-3.07)
PL	2828	64	2.26	2.19	(1.65-2.73)
SI	5224	61	1.17	1.16	(0.87-1.46)
UK	9418	115	1.22	1.21	(0.99-1.44)

b) Formaldehyde, 2% aqu.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
IT	690	9	1.3	1.44	(0.47-2.41)
NL	498	18	3.61	3.41	(1.83-4.98)
UK	9177	98	1.07	1.03	(0.83-1.24)

c) MCI/MI, 0.01% aqu.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
AT	1113	72	6.47	6.89	(5.26-8.53)
CH	4999	217	4.34	4.27	(3.68-4.86)
DE	5556	223	4.01	3.77	(3.24-4.31)
DE (1 day)	1924	59	3.07	2.86	(2.04-3.68)
DK	2561	41	1.6	1.46	(0.66-2.25)
ES	2645	106	4.01	4.04	(3.26-4.81)
FI	597	22	3.69	3.81	(2.23-5.39)
IT	9241	321	3.47	3.49	(3.12-3.87)
LT	865	16	1.85	1.74	(0.87-2.61)
NL	2791	255	9.14	8.61	(7.59-9.63)
PL	2828	62	2.19	2.21	(1.65-2.76)
SI	5224	140	2.68	2.7	(2.25-3.15)
UK	4101	149	3.63	3.57	(2.99-4.15)

d) MCI/MI, 0.02% aqu.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
ES	896	58	6.47	6.89	(5.09-8.69)
FI	459	41	8.93	8.92	(6.27-11.56)
UK	14839	647	4.36	4.15	(3.83-4.47)

e) Methylisothiazolinone, 0.05 % (aqu.)

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
CH	424	28	6.6	6.46	(3.95-8.97)
DE	658	25	3.8	3.3	(1.93-4.66)
DE (1 day)	237	12	5.06	4.28	(1.58-6.97)
ES	622	19	3.05	3.06	(1.65-4.48)
NL	2814	177	6.29	5.89	(5.03-6.74)

f) Methylisothiazolinone, 0.1 % (aqu.)

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
FI	141	1	0.71	0.56	(0-1.67)

g) Methylisothiazolinone, 0.2 % (aqu.)

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
IT	690	15	2.17	2.39	(1.14-3.63)
UK	2683	143	5.33	5.09	(4.27-5.91)

h) Paraben mix, 16% pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
AT	1109	15	1.35	1.34	(0.65-2.03)
CH	4980	54	1.08	1.14	(0.82-1.46)
DE	5530	40	0.72	0.73	(0.48-0.98)
DE (1 day)	1903	36	1.89	1.87	(1.18-2.57)
DK	2572	5	0.19	0.21	(0-0.45)
ES	3361	13	0.39	0.37	(0.16-0.58)
IT	5766	20	0.35	0.35	(0.2-0.51)
LT	865	11	1.27	1.32	(0.46-2.19)
NL	2823	22	0.78	0.77	(0.44-1.09)
PL	2825	30	1.06	1.17	(0.74-1.59)
SI	5224	52	1	1.02	(0.73-1.3)
UK	15592	71	0.46	0.45	(0.34-0.55)

i) Methylidibromo glutaronitrile, 0.2% pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
AT	1113	39	3.5	3.38	(2.26-4.5)
CH	5000	181	3.62	3.27	(2.78-3.77)
DE	5551	125	2.25	2.03	(1.64-2.41)
DE (1 day)	1917	75	3.91	3.55	(2.65-4.44)

j) Methylidibromo glutaronitrile, 0.3% pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
ES	3721	56	1.5	1.35	(0.98-1.72)
FI	1015	16	1.58	1.54	(0.78-2.29)
IT	4388	66	1.5	1.47	(1.12-1.82)
LT	865	49	5.66	5.01	(3.49-6.53)
NL	2823	211	7.47	6.87	(5.97-7.77)
UK	15588	98	0.63	0.59	(0.47-0.71)

k) Methyldibromo glutaronitrile, 0.5% pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
DK	2564	114	4.45	3.76	(2.56-4.95)
IT	2362	23	0.97	0.93	(0.54-1.32)
NL	1451	42	2.89	2.81	(1.97-3.66)
PL	2827	93	3.29	3.28	(2.62-3.95)
SI	5224	141	2.7	2.66	(2.22-3.1)

l) Methyldibromo glutaronitrile + 2-Phenoxyethanol (Euxyl ®K 400), 1.5% pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
IT	4575	90	1.97	2.04	(1.62-2.46)

m) Quaternium 15, 1 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
DE	1004	3	0.3	0.48	(0-1.07)
DK	2565	21	0.82	0.75	(0.17-1.33)
ES	3361	27	0.8	0.75	(0.46-1.05)
FI	1055	18	1.71	1.86	(1-2.72)
IT	4008	11	0.27	0.27	(0.11-0.44)
LT	865	3	0.35	0.28	(0-0.6)
NL	2823	34	1.2	1.18	(0.78-1.58)
PL	2828	33	1.17	1.14	(0.74-1.53)
SI	5224	29	0.56	0.57	(0.36-0.79)
UK	15583	181	1.16	1.09	(0.93-1.25)

n) Diazolidinyl urea, 2 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
ES	1706	8	0.47	0.47	(0.13-0.81)
FI	544	4	0.74	0.67	(0-1.36)
IT	690	1	0.14	0.1	(0-0.31)
LT	130	0	0	0	(0-2.28)
NL	4268	34	0.8	0.78	(0.52-1.05)
PL	303	1	0.33	0.28	(0-0.83)
UK	9025	75	0.83	0.82	(0.63-1)

o) Imidazolidinyl urea, 2 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
ES	1795	10	0.56	0.52	(0.19-0.85)
FI	544	5	0.92	0.86	(0.07-1.65)
IT	690	0	0	0	(0-0.43)
LT	130	0	0	0	(0-2.28)
NL	4267	43	1.01	1.02	(0.71-1.32)
PL	303	1	0.33	0.28	(0-0.83)
UK	15587	86	0.55	0.54	(0.43-0.66)

p) 2-Bromo-2-nitropropane-1,3-diol (Bronopol ®), 0.25 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
FI	544	4	0.74	0.69	(0-1.38)
IT	690	2	0.29	0.42	(0-1.01)
UK	9758	95	0.97	0.99	(0.79-1.2)

q) 2-Bromo-2-nitropropane-1,3-diol (Bronopol ®), 0.5 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
AT	1108	10	0.9	0.72	(0.26-1.17)
CH	4979	50	1	0.97	(0.68-1.25)
DE	5537	40	0.72	0.6	(0.4-0.8)
DE (1 day)	1905	25	1.31	1.07	(0.61-1.53)
ES	720	6	0.83	0.9	(0.16-1.65)
LT	130	1	0.77	0.53	(0-1.58)
NL	1453	5	0.34	0.34	(0.04-0.64)
UK	6242	36	0.58	0.55	(0.37-0.73)

r) Iodopropynyl butylcarbamate, 0.1 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
UK	7952	11	0.14	0.14	(0.05-0.22)

s) Iodopropynyl butylcarbamate, 0.2 % pet.

Country	nr(test)	nr(pos.)	%(pos.)	%(pos.std.)	95% CI
CH	424	13	3.07	2.43	(1.06-3.79)
DE	659	7	1.06	1.16	(0.22-2.1)
DE (1 day)	236	3	1.27	0.93	(0-2)
ES	1015	5	0.49	0.4	(0.05-0.75)
FI	544	2	0.37	0.26	(0-0.63)
NL	2814	109	3.87	3.8	(3.08-4.51)
PL	303	1	0.33	0.28	(0-0.83)
UK	6361	22	0.35	0.3	(0.18-0.43)

FIGURE LEGENDS

Figure 1: Time trend, in 3 age groups, of contact allergy to methyldibromoglutaronitrile (MDBGN), all test concentrations pooled. For explanations see text.

Figure 2: Time trend, in 4 age groups according to sex and age (<40 *versus* ≥40), of contact allergy to methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), patch tested at 0.01% (100 ppm; left four prevalences of the eight centered around the year of patch test) and at 0.02% (200 ppm) aq., right four prevalences in the figure; see also figure legend.

Time trend, in 3 age groups, of contact allergy to methyldibromo glutaronitrile (MDBGN), all test concentrations pooled. For explanations see text.

177x177mm (300 x 300 DPI)

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8. Manuscript title

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9. Full name

Ana M Giménez-Arnau

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Andrea Bauer

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9. Full name

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WU has received travel reimbursement and partly honoraria from cosmetic industry accociations for presentations given to these, and a lecture fee from Almirall/Hermal.

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