

Patch test results with rubber series in the European Surveillance System on Contact Allergies (ESSCA), 2013/14¹

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Summary

Background. Contact allergy to rubber additives is common, particularly in patients with occupational dermatitis.

Objectives. To descriptively analyse and compare patch test reactions in patients patch tested both with the baseline series and with an additional 'rubber series' as used in the department.

Methods. Patch test data from those members of the European Surveillance System on Contact Allergies (ESSCA) network (www.essca-dc.org) who also contributed data on rubber series patch test results were retrospectively analysed.

Results. Among 29 522 patients patch tested, 2870 were eligible, that is, patch tested both with the baseline series and with a special rubber series. Of these, 8.8% had positive reaction(s) to one of the baseline screening rubber allergens (as compared with 4.2% in all patients), most often to carba mix, 12.3% had positive reaction(s) to allergens from an additional rubber series, and 6.4% had positive reaction(s) to both.

Conclusions. Additional testing with a dedicated rubber series, containing break-down single ingredients of mixes from the baseline series and additional rubber allergens, is warranted in patients with suspected contact allergy to rubber, to avoid false-negative results.

Key words: clinical epidemiology; contact sensitization; patch test; rubber chemicals.

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Conflicts of interest: W. Uter: has accepted travel reimbursement and honoraria for presentations given to cosmetic industry (associations) by these, and has received a lecture fee from Almirall Hermal for educational lectures on contact allergy. J. Geier: has accepted a grant from the German association of the protective glove and skin protection companies (Bundesverband Handschutz), and has received a lecture fee from Almirall Hermal for educational lectures on contact allergy. The IVDK, maintained by the IVDK e.V., of which J. Geier is an employee, is sponsored by the cosmetic and fragrance industry (associations) as well as by public funds. M. Wilkinson: has attended a drug advisory board meeting for GlaxoSmithKline (alitretinoin). The other author does not declare a conflict of interest pertinent to this study.

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Products made of rubber, comprising elastomers of natural rubber (natural rubber latex) or rubber of synthetic origin, have widespread uses in everyday and working life. Examples include protective rubber gloves, balloons, rubber bands, car tyres, machine belts, and rubber tool handles. Residues from the production process (such as vulcanizing agents) or substances deliberately added to enhance product characteristics (such as antioxidants) are well-known contact allergens. Four allergens from this group are included in the 30 allergens comprising the currently recommended European baseline series (1, 2), namely, thiuram mix 1% pet., 2-mercaptobenzothiazole (MBT) 2% pet., mercapto mix (with four constituents, including MBT) 2% pet., and *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine (IPPD) 0.1% pet. Apart from these and other rubber allergens tested in the baseline series (3), many departments patch test with special 'rubber series' when allergy to rubber materials is suspected. Such a series may also be used in patients following a positive reaction to one of the rubber mixes of the baseline series or to a rubber object tested 'as is' or as an extract. The present article provides results from the European Surveillance System on Contact Allergies (ESSCA) network obtained during 2013 to 2014, both with the rubber screening allergens included in the baseline series and with the special rubber series as used in the departments. The focus of analyses was on (i) a description of the frequency of reactions to these allergens, and (ii) an evaluation of concomitant reactivity between the baseline series markers and their single ingredients, to address the question of how far patch testing of single ingredients is necessary in order not to overlook sensitization to the present range of rubber allergens.

Methods

This retrospective analysis is based on routine clinical data collected by the ESSCA (www.essca-dc.org) network, which has been described in detail elsewhere (4). Briefly, clinical and demographic data, along with patch test results, of all patients patch tested in the departments participating in the ESSCA for suspected allergic contact dermatitis resulting from various potential exposures are documented electronically in the local departments. Standardized patch testing follows international (European Society of Contact Dermatitis) recommendations (5). The anonymized data provided by the participants are pooled in the ESSCA data centre in Erlangen for further analysis with R (version 3.1.3) software (6), according to pertinent guidelines (7, 8) and standard operating procedures (9). The maximum patch test reaction between day (D) 3 and

D5 (inclusive) was aggregated as the patch test outcome. Reactions designated as either +, ++ or +++ were classified as positive (allergic), and the remainder were classified as non-allergic. The study period was January 2013 to December 2014.

Whereas some departments contributed only baseline series data, seven of the 12 European countries participating, and, in total, 23 departments contributed data on special rubber series as used in the department or national network (see footnote on title page). The present analysis focuses on the above-mentioned departments also using and transmitting to the ESSCA data centre results obtained with their special rubber series (group R). For comparison, selected overall results obtained by the full ESSCA network during this period are presented (group A). Some allergens were tested at more than one concentration; the results for these are presented separately. Moreover, some allergens are apparently included in a special rubber series in some departments, despite being present in the (recommended) baseline series. Conversely, zinc diethyldithiocarbamate (ZDEC), which is present in the German baseline series (<http://dkg.ivdk.org>, 'Testreihen'), is not included in the rubber series of the German Contact Dermatitis Research Group; however, the data have been considered for those patients tested with the special rubber series. The relevant mixes from the baseline series have been described in detail in (1, 3); see also footnote to Table 3.

Results

Rubber allergens from the baseline series

Altogether, 29 522 patients had been patch tested with the baseline series in all 46 departments providing individual data in 2013 to 2014 (group A). The total of patients tested in the subset of departments providing special rubber series data was 15 485 (group R); 2870 of these patients had been patch tested with a rubber series (group RT). The number of patients varied markedly between departments: the largest number of patients ($n = 419$) was seen in Bern, Switzerland, followed by Heidelberg ($n = 379$), Leeds ($n = 363$), and Zürich ($n = 307$). The MOAHLFA index of the patch tested patients is shown in Online Table S1, stratified for the full clinical sample of 2013 to 2014 (group A), for the entire clinical population contributed by those 23 departments providing special rubber series data (group R), for patients tested with such a rubber series (group RT), and finally for those reacting to at least one rubber allergen from the baseline series or the respective rubber series (group RP). The comparison between groups A and R shows a slight

over-representation of occupational and hand dermatitis, and of atopic dermatitis in the centres contributing data on their additional rubber series. In the subset RP of 'rubber-positive' patients, ~50% of all patients suffered from occupational contact dermatitis.

The results obtained with the baseline series rubber allergens and allergen mixes, both with pet.-based allergens and with the TRUE Test[®], are shown in Table 1, stratified for different groups of patients (A, R, and RT). The comparison between all patients tested in the entire network (group A) and the patients tested in subset R showed a slightly higher prevalence of positive reactions in the latter departments, except for MBT; however, the differences were small. Among those patients tested with special rubber series, higher frequencies of positive reactions were seen with all of these rubber allergens, probably because of patient selection. Remarkably, reaction frequencies obtained with the TRUE Test[®] rubber allergens were also higher throughout. This was particularly pronounced among those tested with a special rubber series.

Special rubber series

Patch test results obtained with special rubber series allergens tested in more than one department and in at least 10 patients are shown in Table 2. It is evident that a large number of allergens elicited positive patch test reactions in at least 1% of those tested (in an aimed fashion), but others, such as the group of thiourea derivatives, had a sensitization frequency of <1%.

Concomitant reactivity between the mixes contained in the baseline series and their single constituents was investigated (group RT). The results are shown in Table 3, stratified for the test concentrations of single constituents (see Table 2) where necessary. As only 82 patients had been tested with the TRUE Test[®] baseline series and a special rubber series, no separate analysis for this subgroup was performed. Instead, the TRUE Test[®] results were combined with those from the investigator-loaded, pet.-based system, except for carba mix (see Discussion).

Stratification for the single ingredients of the thiuram mix showed that the higher test concentration of 1% pet., used in approximately one-fifth of patch tests, was not associated with a (significantly) higher concordance with thiuram mix. Overall, more cases were detected by testing with the mix than by testing with the ingredients separately. For the carba mix and constituents, a comparison was possible in 547 patients, and this showed a pattern that was largely similar to that found in the corresponding analysis of thiurams. In the remaining 2138 patients tested with the three single carba mix ingredients, but not with carba mix, the proportion of positive reactions to any one ingredient was 4.2%. Furthermore,

cross-reactions between carba mix and thiuram mix were investigated, in view of the similarity of these two classes of vulcanizing agent (see Discussion). In all patients tested with both allergens (i.e. group A, not restricted to those tested with a special rubber series), 71 reacted to both, 152 only to thiuram mix, and 265 only to carba mix (significant asymmetry; $p < 0.001$, McNemar test). Interestingly, in the subgroup of patients also tested with a special rubber series and both thiuram and carba mix ($n = 606$, from group RT), 10 reacted to both, 21 only to thiuram mix, and 20 only to carba mix. In other words, the relationship between coupled and isolated reactions remained very similar for thiuram mix, whereas for carba mix the proportion of isolated reactions decreased in this subgroup, achieving almost perfect symmetry ($p > 0.99$, McNemar test).

Two mercapto mixes are in use, one without MBT (1% pet.), and the other with MBT as a fourth constituent (2% pet.); see Discussion. Hence, several comparisons were made, and these are shown in Table 3: the 1% mix versus its three ingredients, the same mix with its single ingredients and also including MBT, and the 2% mix versus all four benzothiazoles (tested in only 541 patients). The pattern across all of the above-mentioned three comparisons involving benzothiazoles was similar, and showed that there were more positive reactions only to single ingredients than only to the mix; thus, the pattern was in contrast to that observed with thiuram and carba mix. It is of note that this asymmetry was significant only in those comparisons that included MBT as the single constituent (Table 3, 'comment').

The overlap between 'black rubber mix' and testing of its single constituents was limited to 80 patients tested with the TRUE Test[®] mix, with 1 patient reacting to the mix and a single constituent, and another to the single constituent but not the mix. For screening for contact allergy to black rubber antioxidants, IPPD is mostly used, in accordance with (1). The two other ingredients of the so-called 'black rubber mix' had been tested in different numbers of patients (Table 3). A comparison between IPPD and *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) was possible in 2569 patients. Of these, 6 reacted only to DPPD, 15 reacted only to IPPD, and 11 reacted to both. In a comparison involving IPPD and *N*-cyclohexyl-*N'*-phenyl-*p*-phenylenediamine (CPPD) ($n = 541$ patients), 4 patients reacted only to CPPD, none reacted only to IPPD, and 4 reacted to both allergens.

Finally, to establish the overall gain when testing is performed with an additional rubber series as compared with when it is performed only with the baseline series and the rubber screening mixes that it contains, results were compared. Among the 2762 patients in

Table 1. Overall results obtained with rubber allergens included in the baseline series with crude prevalence of positive reactions, accompanied by an exact 95% confidence interval (CI)

Allergen	Concentration (vehicle)	n (test)	% +	% ++/+++	% ? +/IR	% positive	95%CI
All patients (n = 29 522, group A)							
Thiuram mix	1%	27 742	0.98	1.05	1.4	2.03	1.78–2.11
Zinc diethyldithiocarbamate	1%	6 749	0.37	0.15	1.07	0.52	0.36–0.75
Carba mix	3%	12 688	2.37	0.31	1.92	2.68	2.43–3
Mercapto mix (MBT, CBS, MBTS, MOR)	2%	16 858	0.27	0.34	0.29	0.62	0.51–0.76
Mercapto mix (CBS, MBTS, MOR)	1%	10 066	0.45	0.35	2.25	0.79	0.64–1.03
MBT	2%	28 141	0.33	0.32	0.78	0.65	0.56–0.75
<i>N</i> -isopropyl- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine	0.1%	24 081	0.29	0.34	1.16	0.64	0.52–0.72
PPD (black rubber) mix	0.6%	785	0.38	0.38	0.13	0.76	0.14–1.52
PPD (black rubber) mix	1%	121	0	0.83	0	0.83	0–2.35
Consecutive patients from contributing departments (n = 15 485, group R)							
Thiuram mix	1%	14 416	1.5	0.84	1.92	2.34	2.02–2.52
Zinc diethyldithiocarbamate	1%	6 636	0.38	0.15	1.07	0.53	0.37–0.77
Carba mix	3%	7 031	3.1	0.33	1.88	3.43	2.99–3.85
Mercapto mix (MBT, CBS, MBTS, MOR)	2%	5 726	0.68	0.17	0.47	0.86	0.62–1.11
Mercapto mix (CBS, MBTS, MOR)	1%	8 703	0.51	0.37	2.26	0.87	0.68–1.11
MBT	2%	14 441	0.45	0.28	1.07	0.73	0.58–0.87
<i>N</i> -isopropyl- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine	0.1%	13 700	0.44	0.31	1.47	0.74	0.57–0.85
Patients tested with special rubber allergens (n = 2870, group RT)							
Thiuram mix	1%	2 659	3.2	2.14	3.08	5.34	4.42–6.16
Zinc diethyldithiocarbamate	1%	2 046	0.73	0.29	1.5	1.03	0.6–1.55
Carba mix	3%	606	4.46	0.5	2.95	4.95	3.14–6.62
Mercapto mix (MBT, CBS, MBTS, MOR)	2%	541	1.11	0.18	0.29	1.29	0.3–2.03
Mercapto mix (CBS, MBTS, MOR)	1%	2 119	0.71	0.76	2.86	1.46	0.88–1.91
MBT	2%	2 661	0.79	0.68	2.37	1.47	0.99–1.93
<i>N</i> -isopropyl- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine	0.1%	2 650	0.49	0.49	2.85	0.98	0.49–1.15
All patients tested with TRUE Test® (n = 1214)							
Thiuram mix	25 µg/cm ²	1 210	1.4	0.99	0.97	2.4	1.37–3.02
Carba mix	250 µg/cm ²	1 188	2.02	0.76	2	2.78	1.79–3.74
Mercapto mix (CBS, MBTS, MOR)	75 µg/cm ²	1 210	0.5	0.5	0.5	0.99	0.42–1.66
MBT	75 µg/cm ²	1 210	0.74	0.41	0.66	1.16	0.56–1.93
PPD (black rubber) mix	75 µg/cm ²	1 210	0.41	0.66	0.83	1.07	0.44–1.6

The successive parts of the table address (i) results obtained in all European Surveillance System on Contact Allergies departments, (ii) results obtained in those departments contributing data on special rubber series, (iii) results obtained in patients tested with special rubber allergens, (iv) results obtained with the TRUE Test® in all departments.

CBS, *N*-cyclohexylbenzothiazyl sulfenamide; IR, irritant reaction; MBT, 2-mercaptobenzothiazole; MBTS, dibenzothiazyl disulfide; MOR, morpholinylmercaptobenzothiazole; PPD, *p*-phenylenediamine.

whom this comparison was possible, 178 had positive reactions both to baseline rubber allergens and to the additional series, 65 only to baseline series rubber allergens, and, importantly, 161 only to the allergens shown in Table 2 (Fig. S1). In this comparison, the additional series included the single constituents of thiuram, mercapto, and carba mix, respectively. If these single constituents were excluded and only the other additional allergens were considered, there were still 83 of 2870 patients who only had positive reactions to the additional allergens.

Discussion

For the first time, this study reports patch test results of the ESSCA network obtained with a special allergen series,

namely the rubber series. So far, for reasons of better comparability of patch test results between departments and countries, respectively, obtained in consecutively tested patients (4), the ESSCA network has only considered data analyses concerning the baseline series. Thus, the present analysis deliberately did not address comparisons between countries or departments, with their presumably large variations in special series and patient selection. Instead, the aim was to collect patch test results obtained with a special series, notwithstanding the uncertainties mentioned, particularly as such results have mostly been not been published from single departments, countries, or networks. These results will serve as an evidence base for the discussion of the importance of inclusion of 'special' rubber allergens in a unified

Table 2. Overall results obtained with rubber allergens with crude prevalence of positive reactions in group RT, accompanied by an exact 95% confidence interval (CI)

Allergen	Concentration (%)	n (test)	% +	% ++/+++	% ? +/IR	% positive	95%CI
Thiurams							
Tetramethylthiurammonosulfide*	0.25	2119	2.17	0.99	5.69	3.16	2.46–4
Tetramethylthiurammonosulfide*	1.0	558	1.43	0.54	0.29	1.97	0.99–3.5
Tetramethylthiuram disulfide*	0.25	2118	1.32	0.61	2.01	1.94	1.39–2.62
Tetramethylthiuram disulfide*	1.0	558	0.9	0	0	0.9	0.29–2.08
Tetraethylthiuram disulfide (disulfiram)*	0.25	2119	2.27	1.56	2.75	3.82	3.05–4.73
Tetraethylthiuram disulfide (disulfiram)*	1.0	558	4.12	0	0	4.12	2.63–6.12
Dipentamethylenethiuram disulfide*	0.25	2119	0.94	0.38	3.87	1.32	0.88–1.9
Dipentamethylenethiuram disulfide*	1.0	558	0.36	0	0.29	0.36	0.04–1.29
Dithiocarbamates							
Zinc dibenzylidithiocarbamate	1.0	2091	0.1	0	2.44	0.1	0.01–0.35
Zinc dibutylidithiocarbamate*	1.0	2651	0.15	0	2.58	0.15	0.04–0.39
Zinc diethylidithiocarbamate*	1.0	2668	0.67	0.3	1.05	0.97	0.64–1.42
Zinc dimethylidithiocarbamate (ziram)	1.0	625	0.16	0.16	0	0.32	0.04–1.15
Benzothiazoles							
Morpholinylmercaptobenzothiazole*	0.5	1979	0.96	0.56	4.45	1.52	1.03–2.16
Morpholinylmercaptobenzothiazole*	1.0	559	1.61	0.36	1.61	1.97	0.99–3.49
Dibenzothiazyl disulfide*	1.0	2646	0.68	0.34	2.54	1.02	0.67–1.48
N-cyclohexyl-2-benzothiazyl sulfenamide*	1.0	2674	0.79	0.75	5.22	1.53	1.1–2.07
Guanidines							
1,3-Diphenylguanidine*	1.0	2331	2.62	0.64	9.18	3.26	2.58–4.06
Dibutyl thiourea	1.0	2683	0.34	0.11	4.4	0.45	0.23–0.78
Diethyl thiourea	1.0	546	0.55	0.37	0	0.92	0.3–2.12
Diphenyl thiourea	1.0	2677	0.22	0	3.29	0.22	0.08–0.49
PPD derivatives							
Di- β -naphthyl- <i>p</i> -phenylenediamine	1.0	558	0	0	0	0	0–0.54
N,N'-diphenyl- <i>p</i> -phenylenediamine*	0.25	2118	0.66	0.05	3.46	0.71	0.4–1.17
N,N'-diphenyl- <i>p</i> -phenylenediamine*	1.0	558	0.36	0	0	0.36	0.04–1.29
N-cyclohexyl-N'-phenyl- <i>p</i> -phenylenediamine*	1.0	617	1.5	0	3.35	1.5	0.67–2.75
Phenyl- β -naphthylamine	1.0	559	0	0.18	2.82	0.18	0–0.99
Other additives							
4,4'-Diaminodiphenylmethane	0.5	491	1.22	0.2	0	1.43	0.58–2.92
4,4'-Dihydroxydiphenyl	0.1	2061	0.05	0.1	2.52	0.15	0.03–0.42
Cyclohexyl thiophthalimide	0.5	2053	0.44	0.05	1.78	0.49	0.23–0.89
Cyclohexyl thiophthalimide	1	58	1.72	0	5.08	1.72	0.04–9.24
Dodecylmercaptan	0.1	488	0	0	0	0	0–0.61
Ethylenediamine-di-HCl	1.0	2027	0.44	0.25	4.59	0.69	0.38–1.16
Monobenzyl ether of hydroquinone (monobenzene)	1.0	2185	0.82	0	2.93	0.82	0.49–1.3
Methenamine (hexamethylene tetramine)	1.0	2193	0.27	0.14	2.71	0.41	0.19–0.78
Methenamine (hexamethylene tetramine)	2.0	487	0.62	0	0	0.62	0.13–1.79
<i>p</i> -tert-Butylcatechol	0.25	1971	0.71	0.41	1.69	1.12	0.7–1.69
Di-n-butyl phthalate	5	219	0	0	0	0	0–1.36

Results obtained with the rubber series are included if allergens had been tested in more than one department. All allergens are in pet. Mercaptobenzothiazole and N-isopropyl-N'-phenyl-*p*-phenylenediamine as components of a special rubber series are not included, as these single substances are already included in the baseline series.

IR, irritant reaction; PPD, *p*-phenylenediamine.

*Allergens that are components of the respective mix.

European rubber series (K Warburton et al. 2016, in preparation).

The previous analysis of ESSCA data on rubber screening allergens in the baseline series (3) obtained similar results, in terms of the prevalence of positive reactions, on comparison of the TRUE Test[®] and investigator-loaded,

pet.-based systems, except for carba mix, for which the proportion of positive reactions was significantly lower with the TRUE Test[®] allergen preparation. Note that different patients had been tested with the respective systems, so no formal comparison of diagnostic performance can be made. Otherwise, the similarity between results

Table 3. Concomitant reactivity between rubber chemical mixes included in the baseline series and their single constituents included in the special rubber series, in terms of reactions to at least one of the mix ingredients

Single constituents		Mix		Comment
		Positive	Negative	
Thiurams 0.25%	Positive	Thiuram mix 1% 65	10	(a)
	Negative	55	1964	
Thiurams 1.0%	Positive	Thiuram mix 1% 13	0	(b)
	Negative	15	530	(c)
		Carba mix 3% 11	2	(d)
ZDBC, ZDEC, 1,3-DPG	Positive	19	515	(e)
	Negative	Carba mix 3% 10	21	
Thiuram mix	Positive	20	555	(f)
	Negative	Mercapto mix 1% 26	19	
CBS, MBTS, MOR	Positive	12	2131	(g)
	Negative	Mercapto mix 1% 30	25	
CBS, MBTS, MOR, MBT	Positive	8	2136	(h)
	Negative	Mercapto mix 2% 6	11	
CBS, MBTS, MOR, MBT	Positive	1	523	
	Negative			

'Mix': respective mix pertinent to the single constituents listed, that is, thiuram mix, carba mix, mercapto mix without MBT, and mercapto mix with MBT (see 'Comment' for further explanation). All allergens are in pet.

CBS, *N*-cyclohexylbenzothiazyl sulfenamide; 1,3-DPG, 1,3-diphenylguanidine; MBT, 2-mercaptobenzothiazole; MBTS, dibenzothiazyl disulfide; MOR, morpholinylmercaptobenzothiazole; ZDBC, zinc dibutylthiocarbamate; ZDEC, zinc diethylthiocarbamate.

(a, b) Tetramethylthiuram disulfide, tetramethylthiuram monosulfide, tetraethylthiuram disulfide (disulfiram) and dipentamethylenethiuram disulfide versus thiuram mix 1% pet.

(c) The proportion of concordantly positive reactions did not differ between the two concentrations ($p < 0.001$, χ^2 test).

(d) ZDBC, ZDEC and 1,3-DPG versus carba mix 3% pet.

(e) $p > 0.99$ for asymmetry (exact McNemar test).

(f) CBS, MBTS, and MOR ($p = 0.28$, exact McNemar test). For MOR, results obtained with 0.5% and 1.0% pet., in 2121 and 559 patients, respectively, were pooled.

(g) CBS, MBTS, MOR, and MBT ($p = 0.0046$, exact McNemar test).

(h) CBS, MBTS, MOR, and MBT ($p = 0.0063$, exact McNemar test).

serves as a justification for pooling both results for the sake of the comparison with single constituents in the present analysis, except for carba mix.

The single ingredients of baseline screening mixes are straightforward components of any special 'rubber series', to allow for break-down testing and identification of the actual allergen(s). Consistently, both thiuram mix and carba mix caused more reactions than their respective single ingredients combined (considering at least one positive reaction to a single ingredient). Regarding thiuram mix, it has been claimed that patch testing with the single thiurams in the same concentrations in which they are used in the mix, that is, 0.25%, will result in an underdiagnosis of contact allergy. However, the present results obtained with single thiurams tested at 1% pet.,

albeit in a limited number of patients, do not show greater concordance with the mix. This is in line with Information Network of Departments of Dermatology (IVDK) results published in 2002 (10). Considering the very good negative predictive value but the relatively poor positive predictive value of thiuram mix in our data, one can conclude that (i) thiuram mix should be kept in the baseline series and its single constituents should be kept in the rubber series, and (ii) the rubber series should be tested right from the start in cases of suspected thiuram allergy. Moreover, it seems warranted to further study the most adequate patch test concentrations of single thiurams, and the clinical implications of thiuram mix reactions.

Carba mix is a difficult allergen to test, because it can be irritating, and thus may cause false-positive

reactions (11, 12). In fact, the proportion of putatively false-positive reactions (19/30; Table 3) is very similar to the proportion of 60% previously found in a large analysis (12). It has been suggested this may be attributable to 1,3-diphenylguanidine (1,3-DPG) being present at too high a concentration (13). It is interesting to note that the significant excess of positive reactions only to carba mix, as compared with the number of reactions only to the thiuram mix, on comparison of both reactions, was observed only in an unselected group A of consecutive patients, whereas, in the more selected group RT, discordance was symmetrical (Table 3). Although firm conclusions can probably not be drawn from this observation, this phenomenon may indicate that, in unselected patients, spurious, false-positive reactions are indeed observed, whereas, with a higher *a priori* likelihood in the RT group, such spurious reactions do not play a similar role. Moreover, the recent increase in the frequency of positive reactions to carba mix observed in earlier ESSCA results (3) and in Odense, Denmark (14) most likely indicates a true increase in sensitization, as an increase only in the frequency of irritant reactions to carba mix is not conceivable. Recently, an increasing frequency of sensitization to 1,3-DPG (part of the carba mix) from medical gloves has been observed (15, 16), which also might explain the increasing frequency of carba mix reactions. In fact, the high proportion of positive reactions to 1,3-DPG observed in our analysis may provide an explanation for the increase. Further studies could examine whether exposure to 1,3-DPG has indeed increased indeed in the last few years, after rather infrequent use in the past, which would offer an explanation for the trend. Besides 1,3-DPG, carba mix contains two dithiocarbamates [ZDEC and zinc dibutyldithiocarbamate (ZDBC)], which have very different chemical structures from that of 1,3-DPG. Positive reactions to ZDBC are very rare (Table 1). Thus, the truly relevant components of carba mix are ZDEC and 1,3-DPG. Unfortunately, in cases of positive patch test reactions to carba mix, it is impossible to determine to which compound the patient is sensitized, although it is important for the patient to know this for future allergen avoidance. Hence, patch testing with ZDEC and 1,3-DPG instead of carba mix should be considered, possibly even as part of the baseline series. In a previous setting of low 1,3-DPG use and sensitization prevalence, carba mix had been found to be 'redundant', in view of cross-reactivity with thiuram mix (17). However, with apparently increased 1,3-DPG exposure, testing with at least 1,3-DPG, if not ZDEC, in the baseline series may be advisable. Moreover, the test concentration of 1,3-DPG could be evaluated in this context, for example by additionally testing with 1,3-DPG 0.1% pet. (13).

There has been much discussion about the best way to screen for contact allergy to MBT and/or its derivatives. Hansson and Agrup showed rapid conversion of *N*-cyclohexylbenzothiazyl sulfenamide (CBS), dibenzothiazyl disulfide (MBTS) and morpholinylmercaptobenzothiazole (MOR) to MBT in the presence of glutathione, which is abundant in the epidermis (18). They assumed that MBT is probably the true hapten among all of the benzothiazoles included in the mercapto mix. The patch test manufacturer Hermal showed, some 20 years ago, by chemical analyses, that, owing to chemical interactions, the concentration of MBT in the four-component mercapto mix (MBT, CBS, MBTS and MOR at 2% pet. in total) is unstable. Therefore, they withdrew this mix in 1995, and replaced it with the three-component mercapto mix (CBS, MBTS, and MOR, but not MBT, 1% pet. in total) (19). There is consistent evidence from the IVDK (20) and EECDRG (21) studies that the combined use of MBT and mercapto mix in the baseline series is the best option for screening for contact sensitization to mercaptobenzothiazoles (22). The same conclusion can be drawn from the data presented in this article. However, considering the above-mentioned stability aspects, the three-component mercapto mix (without MBT) should be preferred.

The 'black rubber' mix (with IPPD, CPPD and DPPD) is barely used, except in the TRUE Test® (3); following a recommendation in 1995 (23), the European baseline series includes IPPD as a screening allergen (1). However, our results seem to indicate that this strategy will result in underdiagnosis of sensitization to this class of allergen. Hence, it is recommended to patch test with an adequate additional rubber series from the start in cases of suspected (occupational) contact dermatitis caused by this class of allergen. Sometimes, patch testing with pieces of rubber 'as is' may be necessary to identify contact allergy to the rubber material that the patient is actually exposed to; it may not always be possible to obtain the antioxidant used in this material for patch testing (24).

The chemical 'families' represented by the above-mentioned mixes (or single substances) used for screening in the baseline series presumably cover the vast majority of cases of contact sensitization caused by rubber chemicals. However, the results of the present study indicate that a substantial proportion of contact allergies to these and other rubber chemicals are missed when only the baseline screening allergens are used: (i) in terms of single ingredients of thiuram mix, mercapto mix or carba mix when the respective mix gives a negative result; and (ii) in terms of other chemicals not covered by the mixes at all. However, it should be noted this statement is based on a selected group of patients, in whom the

additional series had been applied because rubber allergy was already suspected from the start, or in whom initial screening with the baseline series had given positive reactions. In strictly consecutive patients, the 'gain' obtained by testing with an additional series is presumably considerably smaller. As a further limitation, carba mix had been tested only in a subset of patients, so the proportion of contact allergies undetected by the screening mixes may be underestimated. Moreover, some of the 'additional chemicals' – and some of the ingredients of the mixes – are used not only in rubber manufacturing or handling, but also in other types of product that may have caused sensitization. Thereby, the benefit of testing with such an additional series may be overestimated to some extent. Notwithstanding this, particularly in

patients with occupation-related contact dermatitis, patch testing with an additional rubber series is certainly warranted.

Supporting Information

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

Table S1. MOAHLFA index in group A, R, RT and RP (rubber positive).

Figure S1. Proportionate Venn (Euler) diagram of the contribution of positive patch test reactions from baseline vs. special rubber series.

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Table 1. MOAHLFA index of (i) the full 2013–2014 European Surveillance System on Contact Allergies (ESSCA) sample (group A), (ii) all patients patch tested in the ESSCA departments contributing rubber series data ($n = 15485$, group R), (iii) all patients tested with a rubber series ($n = 2870$, group RT), and (iv) patients with at least one positive reaction to a rubber allergen either from the baseline series or from the respective rubber series ($n = 1394$, group RP)

Factor	Full sample A		Group R		Group RT		Group RP	
	n	%	n	%	n	%	n	%
Male	9547	32.3	5325	34.4	1272	44.3	189	46.8
Occupational	3594	12.2	2586	16.7	1099	38.3	204	50.5
Atopic eczema	7372	25.0	4821	31.1	1040	36.2	136	33.7
Site: hand	4692	15.9	4515	29.2	922	32.1	124	30.7
Site: leg	886	3.0	782	5.1	53	1.9	2	0.5
Site: face	3728	12.6	3000	19.3	74	2.6	13	3.2
Age ≥ 40 years	17 532	59.4	9644	62.3	1675	58.4	246	60.9

Fig S1. Proportionate Venn (Euler) diagram of the number of patients with positive patch test reactions, among 2762 patients tested with the baseline series and the rubber allergy screening allergens that it includes (thiuram, mercapto, 'black rubber', and carba mix, as well as 2-mercaptobenzothiazole and *N*-isopropyl-*N*'-phenyl-p-phenylenediamine) and additionally a special patch test series with rubber allergens, comprising (some of) the above-mentioned mixes and further rubber allergens, see Table 3.

