

ORIGINAL ARTICLE

Sensitization to, and allergic contact dermatitis caused by, colophonium in north-eastern Italy in 1996 to 2016 with a focus on occupational exposures

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Background: Colophonium is a natural substance derived from Pineaceae trees. It is used in a wide range of consumer and occupational products because of its tackiness, and is a known sensitizer.

Objectives: To analyse the temporal trend of contact allergy to colophonium in north-eastern Italy, and to evaluate the associations with occupations in our geographical area.

Methodology: From 1996 to 2016, 27 381 patients with suspected allergic contact dermatitis were patch tested in north-eastern Italy. Individual characteristics were documented by use of a standardized questionnaire in 6 departments of dermatology or occupational medicine.

Results: The overall prevalence of colophonium sensitization was 1.67%; the prevalence was significantly higher in women (1.75%) than in men (1.49%). No significant differences among departments were observed. We found significant associations between colophonium sensitization and being a student (odds ratio [OR] 4.64; 95% confidence interval [CI]: 1.04-20.6), being a farmer or fishermen (OR 3.63; 95%CI: 1.21-10.84), being a housekeeper (OR 2.02; 95%CI: 1.08-3.77), and being retired (OR 2.06; 95%CI: 1.08-3.92).

Conclusion: Our study showed that contact allergy to colophonium is still relevant in north-eastern Italy. The frequency of sensitization has dropped significantly over a period of 10 years, but students and farmers/fishermen still have sensitization prevalences above 3.5%. Our results are in line with European data.

KEYWORDS

colophonium, sensitization, epidemiology, occupational contact dermatitis, time trend analysis

1 | INTRODUCTION

Colophonium is a natural byproduct derived from the distillation of Pineaceae tree oil. Because of its tackiness, and emulsifying and insulating properties, it is commonly used in a wide range of products, both at home and in occupational scenarios. There are 3 major types of this substance, which is called colophonium (or colophony) in European countries and rosin in North America, namely, gum, wood and tall oil, which are distinguished by different extraction methods.¹ Gum rosin, the most used, is obtained from the sap of living pine trees, wood rosin is extracted from pine stumps, and tall oil is a byproduct

of paper pulp production.¹ There are several variants of colophonium, in which the mixture of components—more than 100 have been identified—may vary according to the type of tree, and the extraction and storage methods used. According to Annex I of the European Commission Directive 67/548/EEC,² colophonium is classified as a skin sensitizer, but not all of its components have allergenic potential. Resin acids, mainly abietic acid and, to a lesser extent, palustric, levopimaric, pimaric, isopimaric and sandarapimaric acids, constitute 90% of colophonium,³ whereas the other 10% consists of a neutral fraction, including dihydroabietic acid and dehydroabietic acid. These acids, if not oxidized, do not have sensitizing potential, which is

acquired when oxidation takes place, yielding hydroperoxides, peroxides, epoxides and ketones of abietic acid and dehydroabietic acid.⁴

Sensitization to colophonium may lead to asthmatic symptoms⁵ and to allergic contact dermatitis (ACD). In occupational scenarios, colophonium allergy has been reported in the electronic industry, where it can be found in soldering flux,^{6,7} in furniture-making industries, where it is present as pine wood and sawdust,⁸ in house painting,⁹ in paper manufacturing,^{10,11} in clothing industries,¹² and in the metal engineering industry, where it is present in water-miscible metal-working fluids (MWFs),^{13–15} with concentrations ranging from 0.41% to 3.8%.¹⁶ Although, as an allergen, it should be declared when it is present at concentrations of $\geq 0.1\%$ in the safety data sheets of the products used at the workplace, it has been shown that this does not always happen,¹⁶ so colophonium may be a hidden allergen.

Regarding possible domestic sources of exposure, colophonium may be present in many products used in leisure and DIY activities, such as in the sawdust produced in carpentry activities^{17,18} and pruning activities,¹⁹ in floor polish,²⁰ and in fabric finishes.²¹ Another scenario of exposure is the health and personal care sector, where colophonium can be found as a tackifier in adhesive tapes and plasters,²² in hydrocolloid dressings,^{23,24} in electrocardiogram electrodes,^{25–27} and as a sealant in dental prostheses,²⁸ potentially causing ACD in patients but also in dental technicians and nurses.^{29,30} Colophonium can be present in topical traditional Chinese medicaments,^{31,32} in nappies,³³ in depilating wax strips,^{34–36} and in cosmetics.^{37–39}

Concerning the clinical relevance of a positive patch test reaction to colophonium, it is often not easy to trace the source of exposure, in view of the wide usage described above. The principal aims of the present study were to investigate the trend of the prevalence of contact allergy to colophonium between 1996 and 2016 in individuals from north-eastern Italy patch tested for suspected ACD, and, particularly, to investigate the role of occupational exposure.

2 | PATIENTS AND METHODS

From 1996 to 2016, 27 381 patients with suspected ACD consulted 1 of the 5 departments of dermatology or occupational medicine in north-eastern Italy: Trieste, Padua, Pordenone, Rovigo, and Trento-Bolzano (the latter being aggregated for analysis). All patients completed a standardized questionnaire⁴⁰ and were patch tested.⁴¹ The questionnaire was aimed at gathering information about individual characteristics, occupational history, and history of personal and familial atopy (asthma and/or allergic rhinoconjunctivitis with at least 1 positive prick test reaction to relevant aeroallergens). Special attention was paid to crafts that, according to the literature and clinical experience, pose a particular risk of exposure to colophonium, while retaining special categories such as welder, beautician, and hairdresser, even though these may have been numerically less well represented. Patients without formal employment were subdivided into the 3 categories of students, retired, and unemployed. Clerks were chosen as a reference group, because, in this occupation, possible sensitization to colophonium is not presumably related to professional exposure. The subjects in the various groups were analysed according to the job held

at the time of consultation, as sensitization was supposed to have occurred during the current occupation. Similarly to job categories, specific body sites (such as fingers, palms, and dorsa of hands) were aggregated into larger categories (eg, "hands"). Sex and age differences were taken into account in order to minimize confounding factors, as described in "Statistical analysis".

2.1 | Patch tests

Finn Chambers on Scanpor tape (Epitest, Tuusula, Finland) and a selection of haptens from FIRMA (Firenze, Italy) were used to perform patch tests on patients with the European baseline series.⁴¹ Patches were applied on the upper back and removed after 2 days (day [D] 2). The sites were examined on removal (D2) and on D3 or D4 according to ICDRG guidelines.⁴² Reactions graded +, ++ and +++ at the second reading were considered to be positive; doubtful reactions (?+) were considered to be negative.

2.2 | Statistical analysis

Data analysis was performed with STATA version 12.0 (Stata, College Station, Texas). Categorical data were cross-tabulated into $k \times k$ contingency tables, and statistically tested by use of the χ^2 square test. Sensitization to colophonium as an outcome was analysed by multivariate logistic regression analysis, with, as independent variables, sex, age (as a continuous variable), and patient occupation (with clerks [white collar workers] as a reference work category). Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated from the coefficients and the standard errors of logistic regression. A colophonium sensitization trend test across ordered groups was performed with Cuzick's test for trend. Patients with missing data for relevant variables were excluded from analysis. A P value of $< .05$ was set as the limit of statistical significance.

3 | RESULTS

The study population included 18 531 women (67.7%) and 8850 men (32.3%). The mean age of the population was similar for male and female patients. There was a higher frequency of occupational dermatitis in males (9.4%) than in females (6.8%), and dermatitis most frequently involved the hands in both sexes (33.9% in males and 29.8% in females), followed by the face, more often in women (16.5% in males and 18.8% in females). The overall prevalence of colophonium sensitization was 1.67%; the prevalence was significantly higher in women (1.75%) than in men (1.49%) ($P < .05$). The characteristics of patients sensitized to colophonium and of non-sensitized patients are shown in Table 1. The former group was older. Occupational and leg dermatitis were common in colophonium-sensitized patients, but this was not statistically significant. Details of patch test results are reported in Figure 1: 63% of patients reacted on D2, mainly with + reactions (38%), whereas the readings on D3 and D4 yielded ++ reactions in 41% and +++ reactions in 22%.

Colophonium sensitization in different occupational groups, investigated by means of multivariate logistic regression, is shown in

TABLE 1 Characteristics of patients sensitized ($n = 456$) and not sensitized ($n = 26\,925$) to colophonium

	Colophonium-positive		Colophonium-negative			
	%	No.	%	No.	OR	95%CI
Age (years) $\pm$ SD	44.3 $\pm$ 17.9		43.0 $\pm$ 17.5		$P = .055$	
Males	29.9	132	32.4	8707	0.8	0.7-1.0
Occupational dermatitis	9.6	44	7.6	2055	1.3	0.9-1.7
Atopic eczema	7.3	33	7.3	1971	1.0	0.7-1.4
Hand localization	29.6	135	31.1	8373	0.9	0.7-1.1
Leg localization	8.1	37	6.4	1712	1.3	0.9-1.8
Face localization	16.7	76	16.1	4331	1.0	0.8-1.3
Age $\geq$ 40 years	52.8	254	52.7	14\,198	1.2	0.9-1.3

Abbreviations: CI, confidence interval; OR, odds ratio.

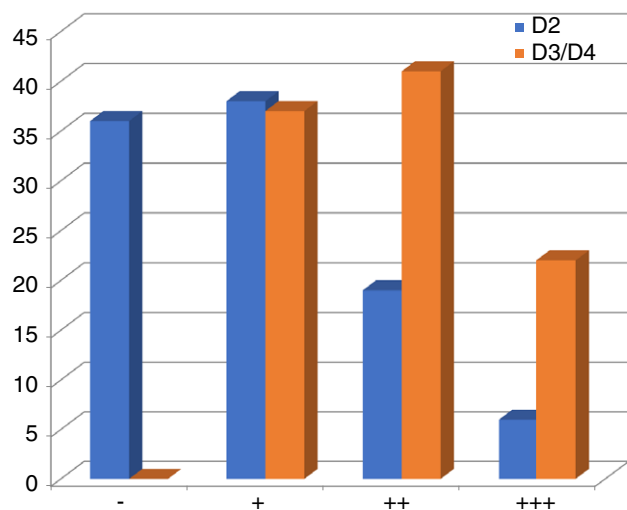
**FIGURE 1** Percentages of patch test reactions on day (D) 2 and D3/D4 in patients sensitized to colophonium ($N = 456$)

Table 2, in which bold type indicates occupations that were significantly associated with colophonium sensitization as compared with clerks. Among the general population of 27 381 patients, 27 were excluded from analysis because of lack of information concerning their jobs. Of the 456 sensitized patients, 414 were distributed among the various occupational categories, and for 42 patients no detailed occupational data were available. We found significant associations between colophonium sensitization and being a student (OR 4.64; 95%CI: 1.04-20.6), being a farmer or fisherman (OR 3.63; 95%CI: 1.21-10.8), being a housekeeper (OR 2.02; 95%CI: 1.08-3.77), and being retired (OR 2.06; 95%CI: 1.08-3.92). The farmer and fisherman group comprised 7 patients, mainly men ($n = 4$); 4 of this group were polysensitized (2 were positive for fragrance mix and *M. pereirae*, 1 was positive for nickel, and 1 was positive for mercaptobenzothiazole and cobalt), 4 had hand dermatitis (1 possibly occupational), 2 had trunk dermatitis, and 1 had shoe dermatitis (also positive for chromium). The student group comprised 4 patients, of whom 2 were male and all were monosensitized, 2 had hand dermatitis, and 2 had trunk dermatitis.

The numbers of patients collected by the 5 departments that participated in the survey were: 8960 (32.8%) for Padova, 8701 (31.8%) for Trieste, 6060 (22.2%) for Pordenone, 2706 (9.9%) for Trento-Bolzano, and 927 (3.4%) for Rovigo. Among the 456 sensitization cases,

165 were diagnosed in Padua, 115 in Pordenone, 107 in Trieste, 60 in Trento-Bolzano, and 9 in Rovigo. The prevalence of sensitization in Trieste was lower than that in Padua (OR 0.66; 95% CI: 0.52-0.85), whereas the prevalence in Trento-Bolzano was higher. The temporal analysis for colophonium sensitization showed a fluctuating trend (Table 3), with a significant reduction in prevalence from the first 3 years to the last 3 years (1996-1998 vs 2014-2016, $P = .011$), and three single periods for which results from multivariate logistic regression showed a significantly lower prevalence of positive patch test reactions (2005-2007, 2011-2013, and 2014-2016, respectively). No age-related pattern of sensitization was found (Table 4), and nor was an age-related temporal trend (data not shown). Concurrent sensitization in colophonium-sensitized subjects is shown in Table 5: the strongest association was found for *p*-tert-butylphenol formaldehyde resin (OR 5.1; 95%CI: 3.4-7.9), followed by *M. pereirae* (balsam of Peru) (OR 4.9; 95%CI: 3.9-6.2), mercaptobenzothiazole, and fragrance mix.

4 | DISCUSSION

Colophonium is widely used in occupational, non-occupational and healthcare settings. No exhaustive data on sensitization prevalence and the role of occupational exposure are available in the literature, specifically regarding the Italian population. This study investigated the prevalence of colophonium sensitization in a database of 27 381 subjects with suspected ACD who were patch tested during 1996 to 2016 in north-eastern Italy. The overall average prevalence of colophonium sensitization in this time frame was 1.67%. On comparison of our results with those of other European studies, such as the European Surveillance System on Contact Allergies (ESSCA) results presented by Frosch et al,⁴³ we confirm a low prevalence of sensitization to this allergen in Italy. In the ESSCA study, colophonium sensitization prevalence showed marked differences between the 12 European countries that participated in the study. Lithuania, Austria, The Netherlands, Germany and Finland were the countries with the highest prevalences, ranging from 4.03% to 5.61%; Denmark, Sweden and the United Kingdom showed lower prevalences, ranging from 2.67% to 3.31%; and Poland, Spain, Slovenia and Italy had the lowest prevalences, ranging from 1.19% (Italy) and 1.68%.

In our study, colophonium sensitization prevalence significantly decreased, from 2.18% in 1996 to 1998 to 1.11% in 2014 to 2016,

TABLE 2 Prevalence of colophonium sensitization in occupational groups

Profession	Total (N)	Colophonium-positive (n)	%	OR	95%CI
Clerks	6933	107	1.54	1.00	
Retired	3984	76	1.90	2.06	1.08-3.92
Housekeepers	3192	73	2.28	2.02	1.08-3.77
Healthcare workers	2747	54	1.96	1.74	0.94-3.24
Workers in building and extraction	1202	24	1.99	1.92	0.89-4.14
Mechanics	1245	18	1.44	1.42	0.64-3.15
Domestic workers and restaurant workers	1426	12	0.84	0.71	0.26-1.90
Woodcraftsmen and similar	369	8	2.16	0.48	0.06-3.63
Textile workers	568	8	1.40	1.24	0.36-4.23
Farmers and fishermen	233	7	3.00	3.63	1.21-10.8
Unemployed	563	6	1.06	0.99	0.22-4.27
Delivery workers	441	5	1.13	1.00	0.40-3.72
Students	148	4	2.70	4.64	1.04-20.6
Hairdressers, barbers, and beauticians	343	3	0.87	0.47	0.06-3.55
Shop assistants	253	3	1.18	0.76	0.15-2.3
Chemical machine workers	223	2	0.89	1.71	0.39-7.42
Drivers	252	2	0.79	1.76	0.40-7.65
Technicians	92	1	1.08	2.94	0.38-22.6
Service workers	86	1	1.16	0.75	0.1-5.4
Others (including paper machine and machine workers, teachers, and chefs)	3054	42	1.37	0.9	0.6-1.3
Total	27 354	456			

Abbreviations: CI, confidence interval; OR, odds ratio. The ORs and 95%CI were evaluated with multivariate logistic analysis adjusted for age, with clerks as the reference category. Statistically significant results are in bold.

TABLE 3 Sensitization to colophonium during the years considered

Years	Sensitized	%	OR	95%CI
1996-1998	83	2.18		
1999-2001	130	1.68	0.77	0.58-1.01
2002-2004	67	1.77	0.81	0.58-1.12
2005-2007	47	1.35	0.62	0.43-0.88
2008-2010	67	1.92	0.88	0.64-1.22
2011-2013	35	1.30	0.59	0.40-0.88
2014-2016	27	1.11	0.50	0.32-0.78
Total	456			

Abbreviations: CI, confidence interval; OR, odds ratio. Statistically significant results are in bold.

TABLE 4 Sensitization to colophonium in different age groups

Age (years)	Sensitized	%	Total	OR	95%CI
<26	78	1.61	4841	1.00	
26-35	84	1.43	5888	0.88	0.65-1.20
36-45	87	1.70	5132	1.05	0.77-1.43
46-55	78	1.77	4395	1.10	0.80-1.51
56-65	59	1.62	3643	1.00	0.71-1.41
>65	70	2.03	3455	1.26	0.91-1.75
Total	456	1.67	27 354		

with a fluctuating trend in between. This decrease is in line with other European countries, such as Sweden, where colophonium sensitization prevalence decreased from 5.4% (1992) to 3.4% (2000) and then to 2.4% (2009).⁴⁴ One hypothesis to explain this decrease could be

the replacement of colophonium-containing products with other, synthetic-based products, which do not release abiatic acid, and possibly the efficiency of local exhaust systems in activities in which abiatic acid fumes could be produced. This happens, for example, during welding processes, and, in fact, we did not find any increased risk in welders and mechanics. Moreover, the different prevalences across Europe could be related to different habits during leisure time, for instance, exposure to wood or adhesives.

Our results showed that colophonium sensitization affected women more often (1.75%) than men (1.49%), which is in line with other European studies,^{43,45,46} whereas it seems that no sex-related differences are present in paediatric populations.⁴⁷ The trend concerning patient age showed a slight increase in sensitization, albeit not significant, in the older group (>65 years) (OR 1.26 95%CI 0.91-1.75). This confirms the data from German studies on allergies in the elderly,^{48,49} in which colophonium is listed in the top 5 sensitizing agents. We think that there could be different reasons for this increased prevalence. It may be partially explained by the time spent in hobby activities (gardening, DIY, etc.) during this period of life, and partially by the presence of comorbidities, one of the most common of which is venous insufficiency, the treatment of which may involve the use of topically applied hydrocolloid dressings, which may contain colophonium.

Regarding the association between colophonium sensitization and occupation, we found an increased prevalence in farmers and fishermen (OR 3.63; 95%CI: 1.21-10.8), housekeepers (OR 2.02; 95% CI: 1.08-3.77), and retired people (OR 2.06; 95%CI: 1.08-3.92). The first-mentioned result can be explained by the well-documented

TABLE 5 Concurrent sensitization to colophonium and other haptens (all in pet., except where indicated otherwise)

	Colophonium-positive (N = 456)		Colophonium-negative (N = 26 925)		OR	95%CI
	%	n	%	n		
4-tert-butylphenolformaldehyde resins 1%	5.5	25	1.1	297	5.1	3.4-7.9
Myroxylon pereirae 25%	23	104	5.7	1527	4.9	3.9-6.2
Mercaptobenzothiazole 2%	2.8	13	0.6	162	4.8	2.7-8.6
Mercapto mix 2%	2.6	12	0.7	174	4.1	2.2-7.4
Lanolin alcohol 30%	6.1	28	1.6	432	4.0	2.7-5.9
Fragrance mix I 8%	22.7	103	7.0	1888	3.9	3.1-4.9
Benzocaine 5%	2.9	13	0.8	201	3.9	2.2-6.8
Paraben mix 16%	3.1	14	1.0	273	3.1	1.8-5.3
Epoxy resin 1%	2.2	10	0.8	205	2.9	1.5-5.5
Thiuram mix 1%	4.4	20	1.6	429	2.8	1.8-4.5
Formaldehyde 1% (aq.)	5.9	26	2.9	784	2.0	1.3-3.0
Diaminodiphenylmethane 0.5%	4.4	20	2.4	646	1.9	1.9-2.9
p-Phenylenediamine 1%	5.5	25	3.6	972	1.5	1.0-2.3

Abbreviations: CI, confidence interval; OR, odds ratio.

presence of colophonium in the bark of some trees and in the working material of fishermen (hooks, lances, and fishing rods), whereas, in the case of housekeepers, sources of sensitization could be wood care and cleaning products. In our study, healthcare workers (HCWs) had a prevalence of 1.74% (95%CI: 0.94%-3.24%), which is lower than in other European studies. Molin et al⁵⁰ conducted a study on 4386 German nurses, who were patch tested in 2002 to 2012. The prevalence of positive reactions to colophonium in that study was 3.4% (95%CI: 2.5%-4.3%) in the group that received a diagnosis of occupational contact dermatitis (OCD), whereas it was 1.9% (95%CI: 1.4%-2.4%) in nurses without OCD. Moreover, a study on 120 Danish HCWs showed a higher prevalence in this occupational group, in which colophonium was in the list of the top 5 sensitizing agents, with a prevalence of 4.1%.⁵¹ This could possibly be explained by the greater use of colophonium-containing products, such as medical devices, wound dressings, and adhesives.

Regarding mechanics, chemical machine workers, and building and extraction workers, we found colophonium sensitization prevalences of, respectively, 1.42% (95%CI: 0.64%-3.15%), 1.71% (95%CI: 0.39%-7.42%), and 1.92% (95%CI: 0.89%-4.14%), which are lower than in other studies investigating similar occupational groups. Geier et al⁵² patch tested 144 metalworkers exposed to MWF and with suspected OCD (44 in Dortmund, 37 in Malmö, 30 in Osnabrück and the remainder in Homburg/Saar, Mainz, Heidelberg, and Göttingen), and found a sensitization prevalence of 8% for colophonium, confirming results obtained in a previous study by the same group.⁵³

Surprisingly, in the group of students (18-24 years), we found a high prevalence of sensitization to colophonium: 4.64% (95%CI: 1.04%-20.6%). This prevalence is higher than in other European studies. Czarnobilska et al⁵⁴ found a prevalence of sensitization to colophonium of 1.0% among Polish children with eczema (aged 7-16 years). Fortina et al,⁴⁷ who investigated 608 children and adolescents with ACD aged 1 to 16 years between 2002 and 2010, found colophonium sensitization in 2.04% (95%CI: 1.5%-2.7%) in the 13-year to 16-year subgroup, and Mortz et al⁴⁶ patch tested 442 young Danish adults from a cohort of schoolchildren previously

enrolled to follow up the course of contact dermatitis and ACD, and found colophonium sensitization in 2.0%. We think that a possible reason for the prevalence that we found could be the use of materials such as rubber, glue and adhesives during school or university activities, while other sources of sensitization are currently unknown.

We found differences in the prevalence of colophonium sensitization in different areas. The prevalence of sensitization in Trieste was lower than in Padua (OR 0.66; 95%CI: 0.52-0.85), whereas the prevalence in Trento-Bolzano was higher. There could be different reasons for this. In Trieste, much of the population is employed in the tertiary sector; moreover, the city is by the sea, so much free time is devoted to outdoor activities. In contrast, the cities of Trento-Bolzano are more Nordic, and leisure-time activities involving carpentry, restoration and the creation of artefacts are popular, which could in part explain the greater prevalence of sensitization in these cities. Another reason could involve different referral patterns following the presence of dermatological symptoms.

The evaluation of concurrent sensitization showed a significant association between colophonium positivity and positivity for many other haptens, mainly resins, such as *p*-tert-butylphenol formaldehyde resin, *M. pereirae* (balsam of Peru), and epoxy resin, but also mercaptobenzothiazoles, lanolin alcohol, and fragrance mix. Coupled reactivity between colophonium, fragrance mix and *M. pereirae* has already been reported by other authors,⁵⁵⁻⁵⁸ probably because all 3 allergens contain phenols. Moreover, colophonium is present in many cosmetics that often contain the other haptens mentioned. Concurrent sensitization between *p*-tert-butylphenol formaldehyde resin and colophonium was reported by Özkaya et al⁵⁹; both haptens are probably used as glues or additives in neoprene shorts. In the farmer/fisherman group, concurrent sensitization to some of these haptens may also depend on the use of rubber protective equipment (gloves and shoes) and possibly exposure to MWFs in machinists. Byers⁶⁰ reported that colophonium can be present in MWFs; moreover, mercaptobenzothiazoles could be used as biocides and corrosion inhibitors, *M. pereirae* and some fragrances such as cinnamal may be used as deodorizers and fresheners, and nickel and other metals such as cobalt can be released

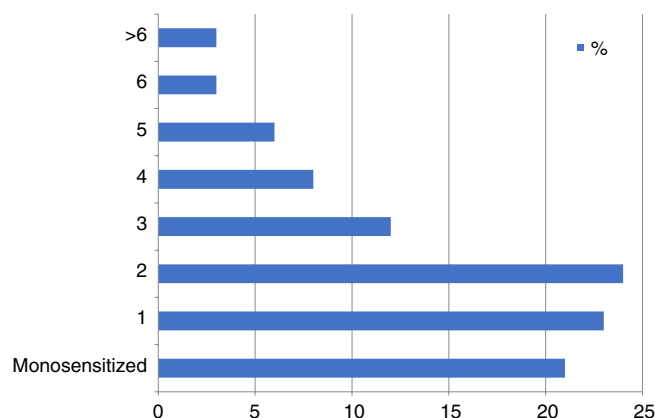


FIGURE 2 Percentages of colophonium-sensitized patients reacting to a growing cumulative number of haptens

from the recirculation of the fluids inside the work machinery. Furthermore, colophonium can be used as a tackifier in shoes or in rubber products.⁵⁵

Multiple reactions among colophonium-sensitized patients are summarized in Figure 2. Most of the co-sensitized subjects reacted to 2 other haptens (24%), followed by those who reacted to only 1 other hapten (23%). Twenty-one per cent were monosensitized to colophonium, whereas only a few were sensitized to ≥ 3 other haptens.

In conclusion, our study adds some important data regarding colophonium sensitization prevalence in the north-eastern Italian population, and points to a possible occupational role in sensitization. The downward time trend probably reflects the results of comprehensive training and vocational education supplied by the occupational health-care providers, the use of personal and collective protective equipment by workers, and the replacement of sensitizing agents such as colophonium with others with a safer profile. In contrast, hobby activities of the young adults and of the retired people may explain the high sensitization prevalence observed. However, some limitations of this study have to be discussed. Although the study included a large sample, the study population included patients who attended health services for suspected allergic dermatitis. Hence, our results may be affected by selection bias. The merging of smaller groups into larger ones—owing to the statistical limitations of small-group analysis—may also have led to some loss of information, even though we retained occupational categories previously reported in the literature to have a higher prevalence of sensitization than the average. Another possible limitation of this study is related to its multicentre design, which may have affected the data recording in different centres, although all participants accepted the use of a standardized protocol.

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Conflict of interest

The authors declare no potential conflict of interests.

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