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Results of patch testing propolis in the European baseline series: A 4-year retrospective study

Gizem Kocabas[1] | Norbertus A. Ipenburg[2] | Anton de Groot[2] | Thomas Rustemeyer[2]

[1]Amsterdam University Medical Centers, Amsterdam, The Netherlands

[2]Dermato-Allergology and Occupational Dermatology, Amsterdam University Medical Centers, Amsterdam, The Netherlands

Correspondence
Anton de Groot, Dermato-Allergology and Occupational Dermatology, Amsterdam University Medical Centers, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
Email: antondegroot@planet.nl

Abstract

Background: Propolis was added to the European baseline series (EBS) in 2019.

Objectives: To investigate the frequency and relevance of positive patch tests to propolis in the EBS and to study co-reactivities.

Patients and Methods: Retrospective study in patients patch tested between June 2019 and November 2023 in a university hospital in Amsterdam, The Netherlands.

Results: Of 3134 consecutive patients, 299 (9.5%) had a positive reaction to propolis 10% pet. Only nine reactions (3%) were judged to be clinically relevant. There were significant co-reactivities to *Myroxylon pereirae* resin (balsam of Peru), colophonium, fragrance mixes 1 and 2, and to limonene and linalool hydroperoxides. A steep increase in rates of positive reactions to propolis was observed from 2020 to 2023. This was highly likely the result of the replacement of Chinese propolis with Brazilian propolis by the manufacturer.

Conclusions: Positive patch tests for propolis are very frequent in Amsterdam, but only a few of these reactions are relevant. Most are probably (pseudo-)cross-reactions in patients with fragrance allergies. Propolis in the EBS has very limited value for dermatologists and patients in The Netherlands. Changes in patch test materials should be provided to all users to avoid misinterpretation of patch test results.

KEYWORDS

allergic contact dermatitis, balsam of Peru, bee glue, Brazilian propolis, Chinese propolis, colophonium, contact allergy, cosmetics, cross-reactions, fragrance mix 1, fragrance mix 2, *Myroxylon pereirae* resin, propolis, pseudo-cross-reactions

1 | INTRODUCTION

In January 2019, the European Society of Contact Dermatitis included propolis 10% pet. in the European baseline series (EBS) for routine testing.[1] The decision was largely based on the results of ESSCA 2013–2014 patch test studies in seven countries, of which five (Switzerland, Germany, Austria, Poland and Italy) showed a high prevalence of positive patch test reactions (2.1%–6.3%) and two had low rates: United Kingdom (0.3%) and Lithuania (0.7%).[2] We have investigated the usefulness of the addition of propolis to the EBS for The Netherlands, focussing on the frequency of positive patch tests, relevance, propolis-containing exposures and co-reactivities.

2 | METHODS

Information of all patients who had positive patch tests to propolis 10% pet. in the EBS at Amsterdam UMC between 11 June 2019 and 27 November 2023 was retrieved from the patch test database and electronic patient files. Data included sex, age, patch test results, clinical relevance of the reactions (current, past, unknown), current and past professions, and products responsible for allergic contact dermatitis. The commercial test haptens used were obtained from Allergeaze (SmartPracticeEurope, Greven, Germany). Patch testing was performed with Van der Bend patch test chambers® (Van der Bend, Brielle, The Netherlands), fixation with Omnifix® elastic (Paul Hartmann BV, Nijmegen, The Netherlands). The occlusion time was 48 h, and the results were read on Day (D)2 with a second reading on D3 or D4 according to ESCD criteria.[3] Patients were instructed to contact the department when new reactions were observed after the final reading.

3 | RESULTS

In the study period of 4 years and 5.5 months, 3134 consecutive patients (69.1% women, 30.1% men) were patch tested with propolis 10% pet. A total of 299 positive reactions to propolis (9.5%) were observed, 205 in women (68.6%) and 94 (31.4%) in men (age range 9–84 years, median 42, mean 42.9). The percentages of positive reactions by year were 3.4% (2019, 5.5 months), 2.8% (2020), 6.1% (2021), 16.1% (2022) and 16.4% (2023, 11 months), showing a significant rise from 2020 to 2023 ($p < 0.0001$, chi^2 test).

Of the 299 reactions to propolis, 8 (2.7%) were considered to be currently relevant and one (0.3%) had past relevance; causative products were 6 cosmetics, 2 food supplements and one topical biopharmaceutical product. The relevance of the other 290 patients (97%) remained unknown. Not a single patient was a beekeeper.

Of the 299 patients with positive reactions to propolis, 25 (8.4%) had a single reaction to propolis. 179 (59.9%) individuals co-reacted to one or more fragrances in an extension of the EBS (linalool hydroperoxides, limonene hydroperoxides) or to one or more fragrance indicators in the EBS (*Myroxylon pereirae* resin, colophonium, fragrances mixes 1 and 2). In patients with positive patch tests to propolis, co-reactivity to individual haptens and mixes was as follows: *M. pereirae* resin 20.7% (6.5% in propolis-negative individuals), colophonium 11.4% (2.1% in propolis-neg.), fragrance mix 1 23.4% (vs. 8.3%), fragrance mix 2 14.7% (vs. 4.9%), linalool hydroperoxides 32.4% (vs. 14.9%) and limonene hydroperoxides 30.1% (vs. 10.4%). All differences were statistically significant (chi^2 test, $p < 0.0001$).

4 | DISCUSSION

Contact allergy to propolis has been known for a long time and appears to be frequent. High prevalences (up to 7.6%) of positive patch test reactions to propolis have been observed in many studies performing routine testing, especially in mid- and eastern European countries such as Germany, Austria, Switzerland, Czech Republic, Poland and Lithuania[4] and recently also in the USA (8.6%).[5] Co-reactions to other plant-based materials such as *M. pereirae* resin (balsam of Peru) and colophonium, fragrances and essential oils are frequent.[4,6–8]

There are few studies on the frequency of positive patch tests to propolis by its presence in the EBS. A multinational and multicentre study in 10 European countries performed in 2019–2020 found a mean rate of 3.5% positive reactions to propolis in 11 952 patients. The rates ranged from 0% in Portugal to 10.4% in Austria, with a median of 4.3%. No data on clinical relevance was provided.[7] Our study shows a very high 9.5% positive reaction to propolis in Amsterdam over the past 4.5 years. Yet, as only a few reactions were found to be relevant, adding propolis to the EBS appears not to be very useful for The Netherlands, even when assuming that we have missed identifying culprit propolis-containing products in a number of patients. In other studies, higher rates of relevancy have been observed.[4–6] However, in a study from the USA, the top source of exposure for positive patch tests to propolis judged to have current clinical relevance was coded as 'unspecified'.[5] In an IVDK study, suspected allergen sources included 'plants (not food)' and 'perfume'.[6] However, plants do not contain propolis and perfumes are very unlikely to contain propolis and are not labelled, which means that the presence of propolis cannot be ascertained and relevance established. These data show the difficulties in accurately assessing relevance, possibly resulting in overreporting of relevant reactions.

In The Netherlands, the use of propolis in cosmetics, pharmaceuticals and food supplements does not appear to be widespread. Why, then, did we find nearly 10% positive reactions? We assume that most are cross-reactions or, more frequently, pseudo-cross-reactions (the same allergens present in 2 or more materials) in patients sensitised to fragrances. Indeed, as in other studies[4,6–8] we have found significant co-reactivities with *M. pereirae* resin (at least 26 chemicals may be present in both propolis and *M. pereirae* resin[4]), colophonium, fragrance mixes 1 and 2, and also to linalool and limonene hydroperoxides. Nearly 60% of propolis-positives were associated with one, but far more often, several of the fragrances and fragrance indicators (details will be provided in another publication).

We found a striking increase in the prevalence of positive reactions to propolis from 2020 to 2023: 2.8% in 2020, 6.1% in 2021, 16.1% in 2022 and 16.4% in 2023, which could not be explained by (apparent) increased exposure to propolis-containing products. Therefore, we looked at possible changes in the patch test material. Throughout this period, test materials from Allergeaze had been used. The website of SmartPractice Canada, beginning of March 2024, listed two propolis test materials, propolis and propolis [B]. The propolis patch test material used by us at that moment had the label propolis [B]. Information on the website revealed that this was propolis from Brazil and not from the usual source, China, which is the main supplier of the European poplar-type propolis.[4] Email correspondence with SmartPractice (19 March 2024) learned that Chinese propolis (coded NA71) had been discontinued in October 2019 because of

supplier problems and no suitable alternative Chinese propolis source was found. Therefore, a new propolis patch test material was created, Propolis [B] (company code NH400), originating from Brazil, which was used from that moment on in the EBS and (most likely) other series, including the American Core baseline series (NACDG recommended) and the Paediatric series. SmartPractice stated to have advised its customers, including worldwide distributors, that NA71 Propolis was being discontinued and would be substituted with NH400 Propolis [B]. However, this information had not reached us. We contacted several European colleagues working in academic centres and none was aware of the switch from Chinese to Brazilian propolis.

In Amsterdam, three technicians perform patch testing, each with their own materials. From checking ordering data with our distributor of Allergeaze test materials, we could reconstruct that up to the end of 2020, >90% of patients have been tested with the Chinese propolis, and that from the end of 2021 on, probably all patch tests have been performed with the Brazilian propolis. Therefore, we assume that the high percentages in 2022 and 2023 (both >16%) may be related to the switch (of which we were not aware) from Chinese to Brazilian propolis. Apparently, the rise in positive reactions from Brazilian propolis was known to the manufacturer, who wrote, 'We have seen higher rates in our own clinics and reports of higher prevalence for propolis in the literature' (Email 19 March 2024). The latter possibly refers to the 2019–2020 study of the NACDG.[5] Propolis became a top 10 allergen in 2019–2020, with a prevalence of 8.6%. There was a statistically significant increase both for 2019–2020 versus 2017–2018 (4.7%) and versus 2009–2018.[5]

Why Brazilian propolis gives (or appears to give) more positive reactions than Chinese poplar-type propolis is unknown. Brazilian propolis is significantly different from propolis found in the temperate zones.[4] The chemical composition of any propolis type is highly variable and only detailed knowledge of the ingredients in the Chinese propolis and the Brazilian propolis used for preparing the Allergeaze test materials could possibly explain the observed increase in positive reactions. Unfortunately, the manufacturer could not provide this information. However, assuming that most propolis reactions are related to fragrance sensitisation, a possible explanation is that the Brazilian propolis used for preparing propolis [B] contains a larger number of sensitising fragrance chemicals or in higher concentrations than the Chinese propolis used for the patch test material. For Chinese propolis, bud exudate of poplars, mainly *Populus nigra* L. (black poplar) is the main source; black poplars are not known for their fragrance.[4] For Brazilian green propolis, *Baccharis* species, predominantly *Baccharis dracunculifolia* DC, is the main source. This is indeed an aromatic plant species, the leaf essential oil of which is highly appreciated in the fragrance/perfumery industry by its woody, floral, and green notes, which is mainly attributed to (*E*)-nerolidol and other sesquiterpene alcohols.[9] High concentrations of β-caryophyllene (up to 9.8%), β-pinene (up to 27%), limonene (up to 26%) and α-pinene (up to 11%) have also been observed in samples of the essential oil.[10] Whether this will also reflect in Brazilian propolis is unknown, but in general, propolis composition is directly related to that of the leaf bud exudates.[4]

In the NACDG study, not only the prevalence of positive reactions to propolis rose significantly, but also that of the fragrance mix 1,[5] which may be in line with our propolis-fragrance hypothesis.

5 | LIMITATIONS

The limitations of this study include its retrospective design, selection of patients investigated in a tertiary referral centre and inexact knowledge of when patients were first tested with Brazilian propolis and how many.

AUTHOR CONTRIBUTIONS

Gizem Kocabas: Conceptualization; methodology; data curation; investigation; writing – original draft; writing – review and editing. **Norbertus A. Ipenburg:** Formal analysis; methodology; visualization; resources; project administration; writing – review and editing. **Anton de Groot:** Conceptualization; methodology; project administration; writing – original draft; writing – review and editing. **Thomas Rustemeyer:** Supervision; writing – review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ORCID

Norbertus A. Ipenburg https://orcid.org/0000-0003-0679-4131
Anton de Groot https://orcid.org/0000-0002-6666-7292
Thomas Rustemeyer https://orcid.org/0000-0001-7580-0684

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