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# DER P 1, DER P 2, AND DER P 23 CONTENT IN HOUSE DUST MITE ALLERGEN EXTRACTS IN RELATION TO THEIR BIOLOGICAL POTENCY MEASURED THROUGH STANDARDIZED SKIN PRICK TESTING

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**Abstract**

House dust mites (HDM) allergy possesses great clinical and social significance. It is extremely heterogeneous on a molecular level, which poses various difficulties for allergen extract manufacturers, in terms of their composition. Great attention is paid to the concentration of the so-called major allergens, to which most patients are sensitized and due to which, in large, the biological activity of these extracts is expressed.

The aim of our study was to conduct a pilot measurement of concentration of three of the most clinically significant major allergens – Der p 1, Der p 2, Der p 23, within 8 series of HDM extracts, produced by the Laboratory of Allergy at Bul Bio – NCIPD using monoclonal antibody ELISA. Results showed the presence of all three major allergens within the extracts, but no statistically proven correlation was found between their concentration, and the reported biological activity of the same extracts from skin prick testing ($\rho < 0.20$ and $p \geq 0.05$).

**Key words:** allergen extract, major allergen, biological potency, house dust mite, HDM allergy

**Introduction.** House dust mite (HDM) allergy is one of the most common inhalant allergies worldwide provoking bronchial asthma and allergic rhinitis. Sensitivity to HDM allergens can develop relatively early [1], and often progressively

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worsens over time, which has serious health and socio-economic consequences for affected patients [2].

HDM allergens are protein-based and show hierarchical allergenicity, i.e. some proteins dominate (major) over others (minor), in terms of the level of IgE antibody response they generate upon entry into the body, which is primarily through the respiratory tract [3]. This is where most of the immune response against them manifests, alongside the clinical symptoms of allergy [1].

The most potent allergens are possessed by members of the genus *Dermatophagoides*, with main representatives *pteronysinnus* and *farinae*, for which numerous allergenic molecules have been described [3, 4]. Most patients show sensitivity to the so-called group 1 and group 2 allergens, with the main serodominant representatives being Der p 1 and Der f 1, together with Der p 2 and Der f 2, respectively [3], but immune response is far from limited to them only [3, 4]. Recently, the influence of Der p 23, a recently described serodominant allergen, has also been discussed in literature, especially in the context of the pediatric asthmatic population, where it is associated with greater severity of the course of asthma, and harder to establish control over the condition [5, 6].

The manufacturing of allergen extracts for diagnosis and treatment of allergic diseases in the form of allergen immunotherapy has to meet a set of internationally established strict legal requirements for safety and efficacy, as part of their quality control. Since they are extracted directly from their natural sources, it is difficult to specify and fully standardize their composition. Therefore, one of the main regulatory documents for pharmaceutical manufacturing in the EU – The European Pharmacopoeia, allows for concentration variability of an individual allergenic component between 50–200%, and 50–150% for biological activity, or potency, both in comparison to those measured within the in-house reference extract (IHRP) [7,8]. This variability is confirmed by comparative studies investigating the content of different commercial allergen extracts, and can be significant, potentially affecting the diagnosis and subsequent treatment of allergic diseases in the form of allergen immunotherap [9, 10].

Since allergy to house dust mites is quite common and of great clinical importance [2], we analyzed the composition of eight randomly selected house dust mite extracts produced by the Laboratory of allergy at Bul Bio – NCIPD, and investigated the presence of a correlation between the composition and the reported biological reactivity of these extracts, as measured by standardized skin prick testing for the purpose of extract quality control.

**Materials and methods.** ***Extracts.*** Eight museum extracts from house dust mites, at a diagnostic concentration of 1000 BU, produced in different years were randomly selected. The raw material for the extracts was sourced from house dust mites cultured in the Laboratory of Allergy for the respective year, according to the laboratory's manufacturing protocols.

***Quantitative measurement of major allergens.*** Quantitative measurement of major allergens Der p 1, Der p 2, and Der p 23 was performed with quantitative ELISA kits (Indoor Biotechnologies, Cardiff, UK), for each major allergen, in each of the 8 extracts, according to the manufacturer's protocol. Each extract was tested at its native dilution of 1000 BU. Each test of each extract was repeated 3 times, and was analyzed against the standard curve for each ELISA kit. Concentrations were calculated, with mean values and standard deviation.

***Skin prick testing and further analysis.*** Data, in the form of papule diameters in mm, was taken from previous skin prick testing campaigns of each manufactured HDM series in the Laboratory of Allergy, for the purpose of quality control of biological potency. Each campaign testing of a series was performed on 11 selected allergic patients with sensitivity to house dust mites. Each previous series is used as an in-house reference for the next.

A non-parametric Spearman analysis was performed on the obtained mean concentrations of major allergens, with the median values of the papule in mm for each series.

**Results.** We conducted a pilot quantitative measurement of major HDM allergens in manufactured HDM extracts by the Laboratory of allergy at Bul Bio – NCIPD, and analyzed the data in accordance with the results obtained from skin prick testing with the same extracts by year, to investigate whether a correlation is present between the amount of the major allergen contained in an extract, and the diameter size of the papule, obtained through skin prick testing.

The mean concentration of the major allergens Der p 1, Der p 2, and Der p 23 in all 8 extracts, together with the average values of the papule obtained through skin prick testing for the purpose of standardization for each series, expressed in mm median diameter, are presented in Table 1.

Table 1

Results of quantitative measurement of major allergens and sizes of the obtained papule from prick testing of each extract by year

| Extracts by year | Der p 1 Mean ± SD [ng/ml] | Der p 2 Mean ± SD [ng/ml] | Der p 23 Mean ± SD [ng/ml] | Wheal Median (IQR) [mm] |
|---|---|---|---|---|
| 2009 | 19.4 ± 0.17 | 13.8 ± 0.31 | 12.26 ± 0.38 | 5 (1) |
| 2013 | 15.56 ± 0.28 | 30.51 ± 1.93 | 15.33 ± 0.41 | 4 (2) |
| 2015 | 16.91 ± 0.13 | 5.7 ± 0.15 | 10.4 ± 0.25 | 6 (2) |
| 2018 | 58.0 ± 1.09 | 31.15 ± 0.22 | 22.78 ± 0.40 | 5 (2) |
| 2020 | 22.16 ± 0.63 | 14.57 ± 9.24 | 14.51 ± 0.52 | 7 (2) |
| 2022 | 24.38 ± 1.40 | 33.38 ± 0.91 | 16.33 ± 0.20 | 8 (4) |
| 2024 | 14.33 ± 0.32 | 15.51 ± 0.52 | 8.91 ± 1.07 | 7 (3) |
| 2025 | 30.58 ± 0.81 | 55.01 ± 1.15 | 17.88 ± 0.61 | 5 (3) |

After Spearman analysis of the data, no statistically significant correlation was found between major allergen concentrations obtained in all extracts, and the results of skin testing – $\rho < 0.20$ and $p \geq 0.05$ (Der p 1 – $\rho = -0.02$ and $p > 0.9$, Der p 2 – $\rho = -0.06$ and $p = 0.8$, and Der p 23 – $\rho = -0.3$ and $p = 0.05$).

**Discussion.** Our study on the composition of eight HDM extracts produced by the Laboratory of Allergy (Bul Bio – NCIPD) in different years found major allergens Der p 1, Der p 2, and Der p 23 presented in all 8 extracts, but quantitative analysis shows significant variation between different years for all three allergenic molecules.

This variation has been confirmed by other studies investigating the composition of commercial house dust mite extracts, using different methods, confirming the great heterogenicity of raw natural material, which despite the streamlined manufacturing process still results in noticeable variations in content [9, 10].

Attempts have been made to produce recombinant allergenic molecules from house dust mites in order to counteract this obstacle, but the problems encountered in these studies mainly relate to the structural features of the resulting protein molecules, although some of them express good IgE binding activity [4]. However, data compiled from the CREATE project between 2001 and 2008, show that these molecules, at least as far as house dust mite is concerned, are not a good substitute for their naturally derived equivalents [7]. This brings the discussion back in the field of allergenic extracts and the need for their composition to elicit an appropriate reaction in sensitive patients.

Our study aimed to investigate whether the composition of major allergens in the HDM extracts of the Laboratory of Allergy (Bul Bio – NCIPD) shows a correlation with skin prick testing on sensitive patients. The initial statistical and residual analysis showed heteroscedasticity, so non-parametric Spearman analysis was performed, as it does not assume equal variance or linearity. As a result, no statistically significant correlation was demonstrated between major allergen concentrations in all extracts, and the average diameter in mm, of the papule obtained during skin prick testing – $\rho < 0.20$ and $p \geq 0.05$.

The protein content of an extract is not entirely representative of its biological activity [10], and indeed Brunetto et al. [9] did not find a relationship between the levels of Der p 1 and Der p 2 and the intensity of the skin prick reaction that extracts produced in patients. Although the concentration of major allergens is a mandatory traceable parameter in allergen extract manufacturing, with acceptable percentages of deviation set in place [8], so far no straightforward relationship has been established between the levels of these molecules in an extract, and its biological activity, which is probably a multifaceted phenomenon [11].

Our study comprises a small patient pool, with a small pool of studied allergen extracts, which raises the need for more extensive data and studies regarding the effect of concentration of major and other allergenic molecules on the biological potency of an extract.

Patient variation in skin reactivity is also well documented, which should be taken into account in interpretation [9, 10]. The individual level of sensitivity determines a different threshold for skin reaction to the extract. Therefore, the quantity of allergenic proteins within the extract could have an impact on the sensitivity and specificity of skin prick testing performed with the respective extract, based in part, on the patient's individual sensitization [12].

Lastly, composition of the raw source material is also important. There is evidence that, depending on whether more fecal particles or bodies of house dust mites are concentrated within the source material, the content and ratio of major allergens Der p 1 and Der p 2 are also affected [9, 10]. In a previous study we demonstrated how the way in which raw source material is processed during the manufacturing process also leads to noticeable differences in the concentration of Der p 1 [13]. This shows that further studies are necessary to elucidate the factors that lead to the accumulation of major allergenic proteins in the source material itself, and what potentially influences this process.

**Conclusion.** All the studied allergenic extracts manufactured by the Laboratory of Allergy (Bul Bio – NCIPD) contained different levels of the three major allergens of house dust mite. Quantification of major allergens is an important step in the quality control process of allergen extract manufacturing, but further studies are needed to establish clear relationships between their quantitative and qualitative presence in an extract's composition and biological activity. Biological potency of naturally derived allergen extracts is a complex and multifactorial phenomenon, which is possibly reliant on many other molecular aspects, in addition to major allergenic protein composition.

## REFERENCES


[1] Jurkiewicz K., M. Jutel, S. Smolinska (2025) Update on HDM allergy: principal changes over the years, Int. J. Mol. Sci., **26**(12), 5660.

[2] Biagtan M., R. Viswanathan, R. L. Bush (2014) Immunotherapy for house dust mite sensitivity: where are the knowledge gaps?, Curr. Allergy Asthma Rep., **14**(12), 482.

[3] Abu Khweek A., E. Kim, M. R. Joldrichsen et al. (2020) Insights into mucosal innate immune responses in house dust mite-mediated allergic asthma, Front. Immunol., **11**, 534501.

[4] Thomas W. R., B. J. Hales, W. A. Smith (2010) House dust mite allergens in asthma and allergy, Trends Mol. Med., **16**(7), 321–328.

[5] Weghofer M., M. Grote, Y. Resch et al. (2013) Identification of Der p 23, a peritrophin-like protein, as a new major *Dermatophagoides pteronyssinus* allergen associated with the peritrophic matrix of mite fecal pellets, J. Immunol., **190**(7), 3059–3067.

[6] Jiménez-Feijoo R., M. Pascal, R. Moya et al. (2020) Molecular diagnosis in house dust mite-allergic patients suggests that Der p 23 is clinically relevant in asthmatic children, J. Investig. Allergol. Clin. Immunol., **30**(2), 127–132.
[7] Zimmer J., J. Bridgewater, F. Ferreira et al. (2021) The history, present and future of allergen standardization in the United States and Europe, Front. Immunol., **12**, 725831.
[8] European Pharmacopoeia, Monograph on allergen products 01/2025:1063.
[9] Brunetto B., R. Tinghino, M. C. Braschi et al. (2010) Characterization and comparison of commercially available mite extracts for in vivo diagnosis, Allergy, **65**(2), 184–190, https://doi.org/10.1111/j.1398-9995.2009.02150.x.
[10] Casset A., A. Mari, A. Purohit et al. (2012) Varying allergen composition and content affects the in vivo allergenic activity of commercial *Dermatophagoides pteronyssinus* extracts, Int. Arch. Allergy Immunol., **159**(3), 253–262.
[11] Becker S., F. Fassio, R. Muñoz-Cano et al. (2022) Major allergen content in allergen immunotherapy products: the limited value of numbers, J. Investig. Allergol. Clin. Immunol., **32**(5), 345–356.
[12] Larenas-Linnemann D., J. J. Matta, K. Shah-Hosseini et al. (2010) Skin prick test evaluation of *Dermatophagoides pteronyssinus* diagnostic extracts from Europe, Mexico, and the United States, Ann. Allergy Asthma Immunol., **104**(5), 420–425.
[13] Hristova M., G. Nikolov, A. Michova et al. (2008) Quantification of Der p 1 – a major allergen from *D. pteronyssinus* in different mite cultures, [Poster Abstract], 3rd National Congress of Immunology, Varna, Bulgaria.

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