

Accepted Manuscript

Dose of Allergens in a Peanut Snack (Bamba[®]) Associated with Prevention of Peanut Allergy

James P. Hindley, PhD, Stephanie Filep, BS, Denise Block, Eva M. King, PhD, Martin D. Chapman, PhD

PII: S0091-6749(17)31092-8

DOI: [10.1016/j.jaci.2017.05.050](https://doi.org/10.1016/j.jaci.2017.05.050)

Reference: YMAI 12903

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 11 January 2017

Revised Date: 8 May 2017

Accepted Date: 30 May 2017

Please cite this article as: Hindley JP, Filep S, Block D, King EM, Chapman MD, Dose of Allergens in a Peanut Snack (Bamba[®]) Associated with Prevention of Peanut Allergy, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2017.05.050.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Dose of Allergens in a Peanut Snack (Bamba®) Associated with Prevention of Peanut Allergy.

James P. Hindley PhD¹, Stephanie Filep BS², Denise Block², Eva M. King PhD², Martin D. Chapman PhD².

INDOOR Biotechnologies, Cardiff, Wales, UK¹ and Charlottesville, VA, USA²

Corresponding Author:

Martin D. Chapman, PhD

INDOOR Biotechnologies Inc.,

700 Harris Street,

Charlottesville, VA 22903

U.S.A.

Tel 434-984-2304; Fax 434-984-2709; e-mail: mdc@inbio.com

Declaration of Funding: This work was funded in part by a grant from the European Union 7th Framework, Project No. FP7-KBBE-2012-6-312147, and by Indoor Biotechnologies.

19 Capsule Summary

20 This letter reports the dose of specific allergens (Ara h 1, Ara h 2 and Ara h 6) in the peanut
21 snack (Bamba®) that was successfully used to prevent peanut allergy in the Learning Early
22 About Peanut (LEAP) study. The results provide weekly doses of specific allergen that are
23 associated with prevention of peanut allergy.

24 Key words: peanut allergens, Bamba®, food allergy, LEAP study, allergy prevention

25

To the Editor,

A randomized trial of peanut consumption in infants at risk of food allergy, the Learning Early About Peanut Study (LEAP), recently showed that peanut consumption in early life reduced the prevalence of peanut allergy among high risk children by 81%.⁽¹⁾ A follow up study (LEAP-On) showed that the reduction in peanut allergy in the consumption group was maintained after 12 months of allergen avoidance.⁽²⁾ The preferred peanut snack used for the LEAP study was a corn puff/stick containing ~50% peanut protein (Bamba®). Sticks of Bamba® were softened with water or milk and mashed with pureed fruit or vegetable.⁽³⁾ Although other peanut-containing foods were used in the LEAP trial (peanut butter, peanuts), 70% of children in the peanut consumption group received Bamba® as the primary peanut food in the first 12 months of the study, from age 4-16 months.^(1,4)

The effective use of Bamba® to reduce the prevalence of peanut allergy raises important questions: what is the allergen composition of Bamba® and what levels of specific peanut allergens are associated with tolerance to peanut? In an accompanying paper, profiles of specific allergens in peanut foods, and in peanut preparations used for diagnostic and therapeutic purposes, were compared.⁽⁵⁾ The results showed qualitative and quantitative variation in three major allergens (Ara h 1, Ara h 2 and Ara h 6). The objectives of this report were i) to measure levels of Ara h 1, Ara h 2 and Ara h 6 in Bamba®; ii) to compare ratios of these allergens in Bamba® with other peanut containing foods; and iii) to estimate the doses of specific peanut allergens associated with oral tolerance in the peanut consumption group.

Samples (100mg) of 16 different lots of Bamba® (Osem Food Industries, Shoham, Israel) from either the UK (n=8) or the US (n=8) were analyzed by ELISA for Ara h 1, Ara h 2 and Ara h 6.⁽⁵⁾ Twenty five individual Bamba® sticks from a single lot were also tested. Allergen consumption in the LEAP study was estimated by calculating the amount of allergen per

Bamba[®] stick and extrapolating from the median peanut consumption reported in the study (7.7g peanut protein/week).⁽¹⁻⁴⁾ For comparison, allergen levels were also measured in another similar peanut snack, ErdnußLocken (Lorenz Bahlsen GmbH, Neu-Isenburg, Germany) (n=10).

The absolute amounts of peanut allergens in Bamba[®] were reproducible between lots obtained in the US and the UK (Figure 1). The mean ratio of Ara h 1 to Ara h 2 was 0.8, and for Ara h 1 to Ara h 6 was 1.0. Mean allergen levels were: Ara h 1, 2,388µg/g Bamba[®] (12% CV); Ara h 2, 1,988µg/g (15% CV); and Ara h 6, 2,341µg/g (16% CV). (Table 1A). Previous ELISA data showed that peanut butter typically contained 2-4 fold more Ara h 1 than Ara h 2 or Ara h 6, while peanut flour showed up to 20 fold higher levels of Ara h 2 and Ara h 6 compared to Ara h 1.⁽⁵⁾ Moreover, peanut flour used in clinical trials of oral immunotherapy had 2-10 fold more Ara h 2 than Ara h 1.⁽⁵⁾ Thus, unlike other peanut food products, Bamba[®] contained similar levels of each major allergen. Coincidentally, these data are concordant with semi-quantitative slot-blot analysis of major allergen content of Bamba[®] that were reported in the original studies showing a lower prevalence of peanut allergy in Israeli infants, as compared to those living in the UK.⁽⁶⁾

ErdnußLocken peanut snack had 3-4 fold lower amounts of peanut allergens compared to Bamba[®], but showed similar tight ranges of allergen levels, especially for Ara h 2 and Ara h 6 (Figure 1, Table 1A). The ErdnußLocken snack is commonly eaten by older children and adults in Germany and has a more crunchy texture. ErdnußLocken sticks contain pieces of peanut which makes it unsuitable as a weaning food for infants. The allergen profiles of ErdnußLocken were included as an objective control for Bamba[®] without prejudice to the food itself. The data confirms that the methods used herein provide quantitative comparisons of specific allergen levels in these peanut foods.

One serving of Bamba[®] used in the LEAP study was two thirds of a 1oz/25g bag or 21 sticks of Bamba[®].^(3, 4) To estimate the doses of specific peanut allergens used in the LEAP

study, the amount of each allergen per Bamba[®] stick was first determined. Median weekly doses of allergens were then calculated based on consumption of 80 Bamba sticks (equivalent to 7.7g peanut protein).⁽¹⁻⁴⁾ Individual Bamba[®] sticks contained similar levels of peanut allergens, from 1.03 – 1.57mg allergen/stick (Table 1B). The calculated median weekly doses of allergens amounted to: 83mg Ara h 1, 120mg Ara h 2 and 127mg Ara h 6; (total 330mg/week, Table 1B). Bamba[®] was the preferred food that was consumed by ~70% of children in the consumption group of the LEAP study over the first two years of life.⁽⁴⁾ In subsequent years, peanut butter and peanuts were the major sources of dietary peanut. Thus, the data appear to represent allergen doses for the first two years of the LEAP study.

Bamba[®] is a well formulated peanut product, with each of the three major allergens present in uniform amounts. The estimates obtained here suggest that cumulative weekly doses of ~330mg specific peanut allergens are associated with prevention of peanut allergy and with the induction of clinical tolerance to peanut. This is the first time that doses of specific food allergens have been calculated for a clinical trial of food allergy prevention. The results highlight that clinical trials using foods as interventions should measure allergen content, record food intake, define, where possible, the effects of food processing, and investigate the importance of the relevant allergen through clinical and immune responses. Allergen measurements will allow the dose of allergen administered to be compared with clinical and immune outcomes of food immunotherapy trials, including symptom scores, specific antibody responses, T cell responses and changes in effector cell function, as well as environmental exposure assessments. The caveats are that this is a preliminary study and the amounts of allergens measured in this study, may overestimate the amount of allergen necessary to explain the clinical outcomes of the LEAP study. The preventative effect of Bamba[®] (and other peanut foods) may be obtained with lower cumulative allergen doses (which could be followed as part of future clinical trials).

99 The results provide a roadmap for investigating whether similar doses apply to the
100 induction of tolerance to other food allergens, such as milk and egg, and for investigating
101 mechanisms of tolerance. Specific allergen dosing allows food formulations with defined
102 allergen content to be developed for clinical trials of oral immunotherapy products and for
103 prophylactic interventions. This will allow different food formulations to be directly compared,
104 provide improved standardization for regulatory authorities and strengthen the evidence base
105 for prophylactic approaches to prevent food allergy.

106

107 James P. Hindley PhD¹,
108 Stephanie Filep BS²,
109 Denise Block²
110 Eva M. King PhD²,
111 Martin D. Chapman PhD².
112
113 INDOOR Biotechnologies Ltd¹
114 Vision Court, Cardiff, CF23 8HA, Wales, UK¹
115 INDOOR Biotechnologies Inc²
116 700 Harris Street, Charlottesville, VA 22903, USA²

117 Acknowledgements

118 This work was funded in part by Indoor Biotechnologies and by a grant from the European
119 Union 7th Framework, Project No. FP7-KBBE-2012-6-312147, Integrated Approaches to Food
120 Allergen and Allergy Management (iFAAM). The authors are grateful to Mary Feeney, Dr.
121 George Du Toit and Dr. Gideon Lack for helpful discussions on the doses of Bamba[®] and other
122 peanut foods that were administered during the LEAP study.

123

References

1. Lack G, Plaut M, Sayre PH. Peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; 372(22):2165-6.
2. Du Toit G, Sayre PH, Roberts G, Sever ML, Lawson K, Bahnson HT et al. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. *N Engl J Med* 2016; 374(15):1435-43.
3. Fleischer DM, Sicherer S, Greenhawt M, Campbell D, Chan E, Muraro A et al. Consensus communication on early peanut introduction and the prevention of peanut allergy in high-risk infants. *J Allergy Clin Immunol* 2015; 136(2):258-61.
4. Feeney M, Du Toit G, Roberts G, Sayre PH, Lawson K, Bahnson HT, Sever ML, Radulovic S, Plaut M, Lack G. Impact of peanut consumption in the LEAP study: feasibility, growth and nutrition. *J Allergy Clin Immunol* 2016; 138:1108-1118.
5. Filep S, Block D, King EM, Commins S, Kulis M, Vickery BP, Burks W, Chapman MD. Specific allergen profiles of peanut foods and of diagnostic or therapeutic allergenic products. *J Allergy Clin Immunol*, 2017; In press.
6. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, Fox AT, Turcanu V, Amit T, Zadik-Mnuhin G, Cohen A, Livne I, Lack G. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. *J Allergy Clin Immunol* 2008; 122:984-91.

Legend to Figure 1

Specific allergen levels in peanut snacks. Comparison of Ara h 1, Ara h 2 and Ara h 6 concentrations in Bamba® (blue symbols) and in ErdnußLocken (green), showing individual data points, mean $\pm$ 2 standard deviations. Bamba® lots from the UK and the US are indicated by open and closed blue symbols, respectively.

Table 1A

Specific allergen content of peanut snacks.

Snack	Country	Ara h 1 µg/g (CV)	Ara h 2 µg/g (CV)	Ara h 6 µg/g (CV)
Bamba	U.S. (n=8)	2,195 (11%)	1,953 (13%)	2,288 (13%)
Bamba	U.K. (n=8)	2,582 (8%)	2,023 (18%)	2,393 (19%)
ErdnußLocken	Germany (n=10)	626 (77%)	687 (29%)	557 (18%)
	Total (n=26)	1,711 (55%)	1,488 (46%)	1,655 (56%)

Table 1B

Estimated Doses of Peanut Allergens in Bamba[®] Administered During the LEAP Study

Dose		Ara h 1 (mg)	Ara h 2 (mg)	Ara h 6 (mg)
Mean allergen/stick*		1.03	1.48	1.57
Range		(0.68-1.61)	(1.10-1.73)	(1.04-2.33)
21 sticks**	One serving	21.6	31.1	33.0
80 sticks***	Median weekly dose	83	120	127
80 sticks x 52 weeks***	12 months consumption	4,326	6,224	6,604

* Mean of 25 individual sticks analyzed for each allergen. Each stick was weighed (mean stick weight = 0.68g) and then extracted in PBS-Tween 20 and assayed for allergen content. ⁽⁴⁾ The range of allergen content per stick is indicated in parentheses.

**One serving as described in an international consensus document; 21 sticks = ~17g Bamba = 2g peanut protein. ⁽³⁾

***Median weekly dose, equivalent to 7.7g peanut protein

