Original Article

Doses of Specific Allergens in Early Introduction Foods for Prevention of Food Allergy

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What is already known about this topic? Consumption of food allergens, such as peanut and egg, in infancy can prevent the development of food allergy.

What does this article add to our knowledge? The article reports the concentrations and dose of 18 major food allergens in commercial early introduction foods that are promoted as aids to prevent food allergy.

How does this study impact current management guidelines? Variability in allergen concentration and dose in early introduction foods highlights the need for improved standardization and quality control of these foods.

BACKGROUND: Consumption of common allergenic foods, such as peanut, in early life can reduce the risk of food allergy among high-risk children and is recommended in revised clinical guidelines. Commercial early allergen introduction foods (EIF) containing single or multiple allergenic foods for feeding infants are promoted to consumers and health care providers as aids to prevent food allergy.
OBJECTIVE: To determine the concentration and doses of major food allergens in EIF.
METHODS: Extracts from 32 EIF and 4 control foods were analyzed for 17 allergens: Ara h 1, Ara h 3, Ara h 6, Bos d 5, Bos d 11, Gal d 1, Gal d 2, Ana o 3, Cor a 9, Jug r 1, Gly m 5, Ses i 1, Api g 1, Sin a 1, Cyp c 1, shrimp tropomyosin, and Tri a 19 using a validated fluorescent multiplex array. Ara h 2 was measured by enzyme-linked immunosorbent assay.
RESULTS: The EIF comprised 1-8 samples of 32 foods (n = 86). Combined peanut allergen levels of up to 26,000 μg/g were measured in peanut puffs (doses of 65-182 mg per 7 g serving). Peanut allergens were not detected in mixed food blend puffs. Major allergen levels of >10,000 μg/g were found in several milk, egg, and peanut powders, or combinations thereof, with cumulative allergen doses of 159-2946 mg in the EIF. Mixed food blend powders, puffs crackers, and fruit sauces contained much lower allergen levels, often <10 μg/g, and some had undetectable allergens. The allergen concentration in these EIF varied over a >3 log range and provided lower cumulative doses of allergen.
CONCLUSIONS: Significant variability in allergen composition, concentration, and dose per serving were observed in EIF containing the same foods. The doses of allergens consumed by potentially at-risk infants in early life were EIF dependent. Guidelines should be established to enable consumers and health care providers to make informed decisions about EIF and to improve the formulation and standardization of EIF for prevention of food allergy. **(J Allergy Clin Immunol Pract 2021;■:■-■)**

Key words: *Early introduction foods; Food allergens; Major allergens; Dose; Food allergy; Prevention; LEAP*

The seminal early introduction studies by Du Toit et al[1] showed that peanut consumption in infants aged 4 to 11 months effectively prevented the development of peanut allergy in those at high risk (Learning Early About Peanut [LEAP] study).[1] A follow-up study (LEAP-ON) confirmed that the efficacy of the intervention was maintained after a year of allergen avoidance.[2] The underlying assumption has been that the benefits of the intervention extend to all infants. The clinical outcomes of the LEAP study were so compelling that they triggered a paradigm shift in the treatment of peanut allergy and in the approach to treatment of food allergies in general. Allergen avoidance has been superseded by early introduction of allergenic foods as a primary prevention option to induce tolerance to food allergens. This approach was endorsed by 10 leading medical professional and health care organizations, including the American Academy of Allergy, Asthma and Immunology, American Academy of Pediatrics, US National Institute of Allergy and Infectious Diseases (NIAID), and the European Academy of Allergy and Clinical Immunology, which revised their clinical guidelines and recommended early introduction of peanut to prevent peanut allergy.[3-6] Recent surveys found that these guidelines have been widely implemented by allergists, immunologists, and pediatricians.[7,8]

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Abbreviations used
EAT- Enquiring About Tolerance
EIF- Early introduction foods
ELISA- Enzyme-linked immunosorbent assay
ERD- ErdnußLocken peanut puff
FDA- Food and Drug Administration
HP- Hello, Peanut!
INS- Inspired START
LEAP- Learning Early About Peanut
LMX- Lil Mixins
mAb- Monoclonal antibody
MARIA- Multiplex array for indoor allergens
MARIA for Foods- Multiplex array for food allergens
MDN- Meridian peanut butter
MME- Mission MightyMe
MS- Mass spectrometry
MWP- MeWe Peanut
MYP- MyPeanut
NIAID- US National Institute of Allergy and Infectious Diseases
pAb- Polyclonal antibody
RSF- Ready, Set, Food!
SFO- SpoonfulOne
WOW- Wow Soy Butter

The LEAP studies were inspired by earlier observations that Israeli children had a low prevalence of peanut allergy and regularly consumed peanut foods early in life. Notably, many Israeli infants were weaned on Bamba, a corn puff containing 50% peanut protein.[9] Bamba was the preferred peanut snack used in the LEAP study, and 70% of infants who were enrolled in the treatment group consumed Bamba in the first year of the study. Our previous studies showed that Bamba is a well-formulated peanut food that contains consistent amounts of major peanut allergens.[10] To date, Bamba is the only peanut food brand that has proven efficacy and safety, together with known allergen content. In contrast, peanut butter, peanut flour, and other peanut foods show marked differences in specific allergen content, caused in part by the effect of roasting or other forms of food processing.[11-13] These effects are incompletely understood and can be further complicated by variability in extraction of certain allergens from processed peanut products.[14,15]

Changes in the clinical guidelines in 2016/2017 stimulated innovation in the allergy health care industry. Over a dozen companies were started to manufacture early introduction foods (EIF) or dietary supplements for a variety of foods, including peanut, tree nuts, milk, egg, fish, and shellfish amongst others. The Enquiring About Tolerance (EAT) study, a large multiple food introduction study in the general population, reported significant reductions in peanut and egg allergy, but not for other foods (milk, sesame, fish, or wheat).[16] Unlike Bamba, there are no published data on the allergen content of EIF that are being promoted to consumers and health care professionals for infant consumption, including those from at-risk allergic families.

The aim of this study was to compare specific food allergen levels in EIF using multiplex array technology. Major food allergens were measured using a modification of a multiplex array for indoor allergens (MARIA) that has previously been used to assess environmental allergen exposure.[17,18] The MARIA for Foods measured up to 17 major food allergens and was calibrated using purified allergen standards. Cumulative allergen doses for each EIF were estimated using serving sizes and consumption recommendations provided by the manufacturers.

METHODS

Early introduction foods

Food companies that manufactured EIF were identified by web searches, social media posts, and the promotional activities of the companies. These manufacturers promoted their products for allergy prevention, and most of them referenced recent guidelines provided by medical and professional organizations. The EIF that were compared in this survey were sourced from the following manufacturers (further details and websites are provided in Table E1, available in this article's Online Repository at www.jaci-inpractice.org): Lil Mixins (LMX) peanut powder, LMX baked egg powder; MeWe (MWP) Nut Butter Apple Cinnamon, MWP Nut Butter Berry Coconut, MWP Nut Butter Banana Coconut; SpoonfulOne (SFO) Mix-in 16 blend food powder, SFO 16 blend food puffs, and SFO 16 blend oat crackers (vanilla, cocoa, or blueberry); Ready, Set, Food! (RSF) milk, egg, peanut powder early introduction system; Hello, Peanut! (HP) peanut flour and oat flakes; MyPeanut (MYP) peanut+apple and peanut+tree nuts+apple fruit sauce pouches; inspired START (INS) fruit sauce pouches, apple+peanut, banana+tree nut, mango+soy, banana+shrimp, pear+egg, pear+sesame, apple+wheat, mango+cod; Mission MightyMe (MME) peanut puff; and Bamba peanut puff. Bamba is included as an EIF because it was used in the LEAP study. However, Bamba is not promoted as an EIF by the manufacturer (Osem, Shoham, Israel).

Four additional products that were not identified as EIF were tested as controls: ErdnußLocken (ERD) peanut puff; PB2 peanut powder; Wow (WOW) Soy Butter; and Meridian (MDN) peanut butter. The ERD peanut puff contains pieces of peanut and is not an EIF. It was used as a positive control for peanut allergen measurements in peanut and other food puffs. PB2 is not promoted as an EIF, but it is used by some allergists to prepare oral food challenge materials. All foods were obtained in 2019/2020 and were analyzed for allergen content before their expiration date.

Extraction of food samples

Food samples (1 g) were extracted in 10 mL of phosphate buffered saline, pH 7.4, containing 2% Tween-20 and 1M NaCl, except for food puffs (100 mg in 2 mL buffer). Samples were vortexed (5 seconds), sonicated for 30 seconds at 35 kHz, heated for 15 minutes in a water bath at 60°C, and then centrifuged at 4°C for 20 minutes at 1250 rcf. The supernatant was pipetted into 2 mL microcentrifuge tubes and stored at −20°C before allergen analysis. Immediately before assay, EIF extracts were centrifuged for 5 minutes at 15,600 rcf. Serial 10-fold dilutions of the supernatant (typically 1/10, 1/100, 1/1000, and 1/10,000) were made and transferred to the MARIA plate. Because of high concentration, several samples were repeated at dilutions up to 1/1,000,000. The food extraction process described above was developed following a literature review and comparison of 3 different buffers and processing procedures, as described in the "Comparison of Extraction Buffers for Foods" section and Figure E1 of this article's Online Repository at www.jaci-inpractice.org.[14,15,19-21]

Multiplex array for food allergens

Food allergens were measured using a modification of a multiplex array for indoor allergens (MARIA) that was previously validated in a multicenter ring trial and used in epidemiologic studies of allergic disease.[17,18,22-24] In most cases, MARIA for Foods used pairs of monoclonal antibodies (mAb) to 1 or more major allergens from each food source. The arrays for Jug r 1, Ses i 1, and tropomyosin incorporated polyclonal antibodies (pAb). Briefly, the MARIA for Foods measured up to 17 specific food allergens in a multiplex array system (xMAP; Luminex Corp, Austin, Tex): peanut, Ara h 1, Ara h 3, Ara h 6; milk, Bos d 5, Bos d 11; egg, Gal d 1, Gal d 2; cashew, Ana o 3; hazelnut, Cor a 9; walnut, Jug r 1; soy, Gly m 5; sesame, Ses i 1; mustard, Sin a 1; celery, Api g 1; fish, Cyp c 1; shrimp, tropomyosin; and wheat, Tri a 19. Capture mAb/pAb that were specific for each allergen were covalently coupled to individual magnetic beads containing unique fluorophores and incubated for 1 hour with dilutions of food extract. After washing, beads were incubated for 1 hour with a cocktail of up to 17 biotin conjugated detector mAb/pAb that had been titrated and optimized for use in the array. Beads were washed and the bound detector mAb/pAb was quantified by the addition of streptavidin-phycoerythrin (30 minutes). The mean fluorescent intensity of food allergen bead sets (50 beads/allergen/sample) was measured in a Bio-plex 200 instrument (Bio-Rad, Hercules, Calif), and the allergen concentration was calculated from control curves of purified allergens. The array was calibrated using purified food allergen standards that showed 95% to 99% purity by mass spectrometry. MARIA for Foods was validated in house for linearity, range, limits of quantification, accuracy, and precision (available at: https://inbio.com/images/pdfs/Food-MARIA-Performance-Characteristics.pdf; accessed November 10, 2020).

In most cases, MARIA for Foods used the same mAb pairs that were previously used in enzyme-linked immunosorbent assay (ELISA). The mAb pair used for Ara h 1 differed from that previously used in ELISA.[25] The Ara h 1 bead set used anti-Ara h 1 mAb 4E2 for allergen capture and biotin conjugated mAb 2F2 for detection. Ara h 2 is not yet available on MARIA for Foods. Ara h 2 was measured in peanut EIF by ELISA, as described previously.[10,11]

All assays were performed in Indoor Biotechnologies laboratory that is ISO/IEC 17025:2017 accredited for biological and environmental testing using MARIA.

RESULTS

The allergen content of EIF from 9 manufacturers (Bamba, HP, INS, LMX, MME, MYP, MWP, SFO, and RSF) and 4 controls (ERD, PB2, WOW, and MDN) was compared for 17 specific allergens using MARIA for Foods. Ara h 2 was measured by ELISA in EIF that contained peanut. In all, between 1 and 8 samples from 32 products were tested (n = 86) providing 1541 data points.

Allergen levels in EIF puffs

Ara h 1, Ara h 2, Ara h 3, and Ara h 6 were measured in peanut puffs (Bamba, ERD, and MME) and in a 16 blend multiallergen puff (SFO). The peanut protein content of Bamba and ERD puffs was listed on the package as 50% and 33%, respectively, but was not specified for the MME or SFO puffs. Bamba contained the highest allergen levels, with Ara h 3 > Ara h 1 > Ara h 2 > Ara h 6, and with Ara h 3 levels that were approximately 10- to 20-fold higher than other peanut allergens (Figure 1). The allergen levels of MME puffs were higher than ERD and comparable to Bamba, except for approximately 3-fold lower levels of Ara h 3. Peanut allergens were undetectable in SFO 16 blend food puffs using MARIA for Foods or ELISA (Figure 1). The combined level of the 4 peanut allergens was calculated and expressed as a dose in a 7 g weekly serving (as per the LEAP study) (Table I).

Comparison of allergen levels in foods containing peanut, milk, and egg

Most EIF were formulated to contain peanut, milk, or egg proteins, and these results were analyzed separately. The foods included milk, egg and peanut powders, peanut butter, peanut flour, and fruit sauces containing peanut and egg. Most foods had a single serving size that was recommended to be consumed either daily or 2 to 3 times per week. Two foods were consumed as increasing daily servings of either peanut flour (HP; 1 serving daily for 7 days plus maintenance) or milk, milk and egg, and milk, egg and peanut powder (RSF; 1 serving daily for 15 days plus maintenance).

The levels of peanut, milk, and egg allergens were compared in selected foods (Table II). The allergen levels in peanut butter products (MWP, MDN) were consistent with those reported previously.[11] Peanut butter samples contained high levels of Ara h 3 (up to 45,000 μg/g), with 10- to 20-fold lower levels of Ara h 1, Ara h 2, and Ara h 6. WOW is a toasted soy butter that is promoted as an alternative to peanut butter for consumption by peanut allergic patients. The WOW butter contained no detectable peanut or allergens other than soy, Gly m 5. The peanut allergen levels in one of the peanut powders (LMX) were very high, approximately 2- to 3-fold higher than in peanut butter and were the highest levels of all the EIF that were tested. In contrast, the peanut allergen levels in fruit sauces (MYP, INS) were very low (Ara h 2, Ara h 6) or at the limits of detection (Ara h 1, Ara h 3).

The HP peanut flour EIF showed a progressive increase in Ara h 3 from day 1 (682 μg/g) to day 7 (7163 μg/g) and maintenance (12,743 μg/g) with similar (approximately 20-fold) dose-related increases of other peanut allergens (Table II and Figure 2). Although the HP product was labeled “dairy free,” trace amounts of milk allergens were detected in 7/8 HP samples at concentrations of up to 10 μg/g. One HP sample also contained trace amounts of egg allergens (Table II).

The RSF food protocol involved consumption of milk powder (days 1-4), milk and egg powder (days 5-8), and milk, egg, and peanut powder (days 9-11 and at days 11-15 on the maintenance dose). The allergen levels in these serving sizes were consistent and contained the highest levels of milk and egg allergens of all the foods tested (Figure 3). The mean Bos d 5 and Bos d 11 levels were 29,627 μg/g and 40,248 μg/g, respectively, for milk powder, whereas mean Gal d 1 and Gal d 2 levels were up to 9143 μg/g and 41,755 μg/g, respectively (Table II). An approximately 3-fold increase in peanut allergen content per serving was measured from the introduction of peanut at day 9 (approximately 3250 μg/g Ara h 3) through maintenance at day 15 (approximately 10,000 μg/g Ara h 3). Similar increases were seen with Ara h 1, Ara h 2, and Ara h 6 (Figure 3).

The levels of milk, egg, and peanut allergens in the SFO 16 blend food powder and cracker were lower than in most EIF, except for MYP and INS. There was also a much lower level of Bos d 5 than Bos d 11 in the SFO blend by comparison with other milk products (Table II).

To enable a direct dose comparison of allergens in foods, the cumulative peanut, milk, and egg allergen doses were calculated based on serving size, the recommended dose provided by the food manufacturer and the combined dose of specific allergens

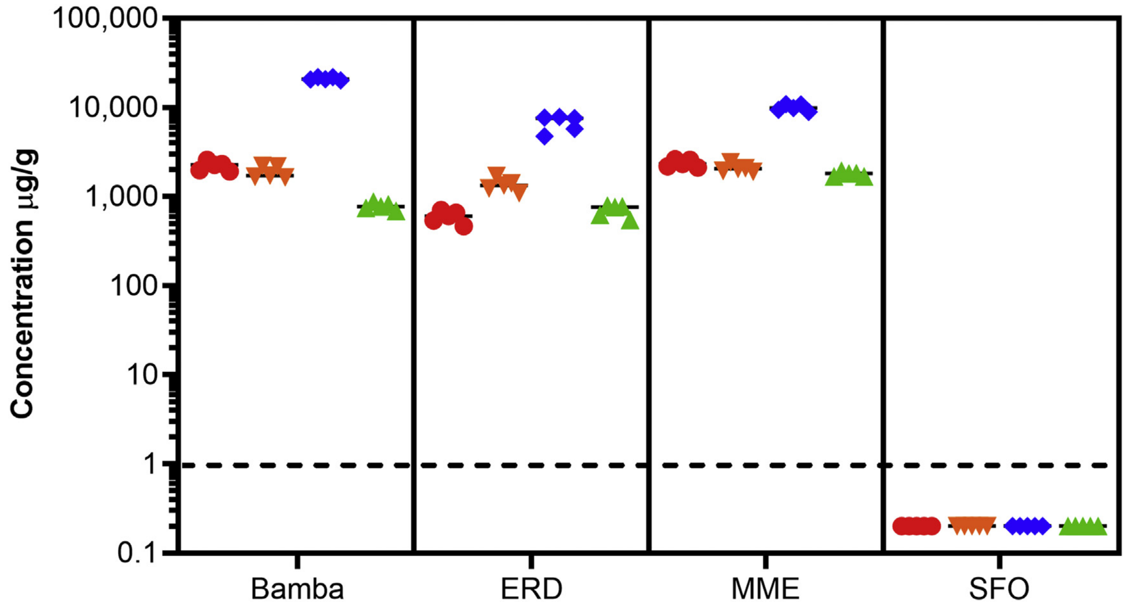

FIGURE 1. Comparison of major peanut allergen levels in food puffs: Ara h 1 (red closed circle), Ara h 2 (orange closed down-pointing triangle), Ara h 3 (blue closed diamond), and Ara h 6 (green closed up-pointing triangle). *ERD*, ErdnußLocken peanut puff; *MME*, Mission MightyMe; *SFO*, SpoonfulOne.

TABLE I. Specific peanut allergen levels in food puff EIF

	Geometric mean (µg/g) ± 95% CI				
Puff EIF	**Ara h 1**	**Ara h 2**	**Ara h 3**	**Ara h 6**	**Total peanut**
Bamba	2211 ± 336	1884 ± 357	21,072 ± 1041	778 ± 87	25,945
ERD	593 ± 118	1355 ± 281	6716 ± 1708	700 ± 136	9364
MME	2368 ± 288	2083 ± 258	9979 ± 1099	1788 ± 136	16,219
SFO	BD	BD	BD	BD	BD

Five samples of each puff EIF were assayed.
BD, Below detection limit of the assay (<0.1 µg/g); *CI*, confidence interval; *EIF*, Early introduction foods; *ERD*, ErdnußLocken peanut puff; *MME*, Mission MightyMe; *SFO*, SpoonfulOne.

(Table III). The results showed significant variation in doses of specific allergens in EIF: peanut (sum of Ara h 1, Ara h 2, Ara h 3, and Ara h 6), 9-2946 mg (n = 12); milk (Bos d 5 and Bos d 11), 120-1245 mg (n = 6); egg, (Gal d 1 and Gal d 2), 2-715 mg (n = 7).

17-plex array analysis of selected foods

The 17-plex MARIA for Foods was designed to measure major allergens from foods that are regulated in the United States (peanut, tree nuts, milk, egg, soy, fish, shellfish, and wheat), together with 3 additional allergens that are regulated in the European Union (sesame, celery, and mustard). None of the EIF contained detectable celery allergen, Api g 1. Three EIF (SFO mixed blend powder, SFO food puffs, and SFO oat crackers) contained up to 16 food allergen groups, except celery and mustard. One MYP fruit sauce EIF contained peanut and 6 tree nuts, including cashew, hazelnut, and walnut. One manufacturer (INS) had 8 fruit sauce EIF that included 1 or more allergens per pouch (Table E1, available in this article's Online Repository at www.jaci-inpractice.org).

The allergen levels of these diverse multi-allergen EIF are shown in Figure 4. Several allergens were present at low levels (<10 µg/g) by comparison with peanut, milk, and egg EIF (Table II). Allergen levels in SFO blend powder ranged from 2 µg/g (shrimp tropomyosin) to 9717 µg/g milk Bos d 11, with doses ranging from <1 to 140 mg (Figure 4 and Table III). Except for Bos d 11, allergen levels in SFO puffs were below the detection limit for 7 allergens and <1 µg/g for Ana o 3, Ara h 3, Ara h 6, Bos d 5, Cor a 9, Jug r 1, and Ses i 1 (Figures 1 and 4). The allergen concentrations in SFO 16 blend oat crackers (vanilla, cocoa, or blueberry flavors) ranged from 0.1 to 60 µg/g, except for shrimp tropomyosin, which was below the detection limit. The INS pear + egg EIF tested negative for Gal d 2, and the INS and MYP tree nut EIF did not contain detectable Cor a 9. Combined allergen doses for tree nut allergens (Ana o 3, Cor a 9, Jug r 1) were 8.5 mg (SFO), 4.2 mg (MYP), and 107 mg (INS).

DISCUSSION

The study intent was to provide an objective, third-party analysis of EIF for allergen composition, concentration, and dose. Major allergens elicit IgE antibody responses in >50% of allergic individuals. Measurements of major allergens are used to assess the quality and potency of allergen immunotherapy products and as markers of environmental allergen exposure. The MARIA for Foods measures major food allergen molecules, the

TABLE II. Comparison of specific peanut, milk, and egg allergen levels in selected foods*

		Peanut	Peanut	Peanut	Peanut	Milk	Milk	Egg	Egg
		Ara h 1	Ara h 2	Ara h 3	Ara h 6	Bos d 5	Bos d 11	Gal d 1	Gal d 2
EIF product	Serving size	µg/g (mg)	µg/g (mg)	µg/g (mg)	µg/g (mg)	µg/g (mg)	µg/g (mg)	µg/g (mg)	µg/g (mg)
LMX peanut powder	5 g	9156 (46)	4306 (22)	114,320 (572)	3768 (19)	<0.01	<0.20	0.15	<0.01
LMX baked egg powder	5 g	<0.05	<0.10	<0.02	<0.002	<0.01	<0.20	143 (0.72)	13 (0.07)
MWP peanut butter AC	20 g	3762 (75)	2228 (45)	40,289 (806)	2790 (56)	<0.01	<0.20	<0.10	<0.01
MWP peanut butter BC	20 g	3500 (70)	2679 (54)	37,039 (741)	2548 (51)	0.02	<0.20	<0.10	<0.01
HP peanut flour lowest dose	4 g	31 (0.1)	80 (0.3)	682 (2.7)	107 (0.4)	4.8	1.6	0.39	1.33
HP peanut flour highest dose	4 g	682 (2.7)	1859 (7.4)	12,743 (51)	2109 (8.4)	4.6	0.6	<0.10	<0.01
RSF milk powder	0.02 oz (0.57 g)	<0.05	<0.10	0.48	0.080	28,143 (16)	46,334 (26)	0.23	0.80
RSF milk powder	0.04 oz (1.13 g)	<0.05	<0.10	0.21	0.036	33,324 (38)	47,941 (54)	0.14	0.41
RSF milk and egg powder	0.04 oz (1.13 g)	<0.05	<0.10	0.03	0.006	29,755 (34)	40,683 (46)	1906 (2)	9821 (10)
RSF milk and egg powder	0.05 oz (1.42 g)	<0.05	0.10	<0.02	<0.002	25,481 (36)	44,112 (63)	8817 (13)	39,438 (56)
RSF milk, egg, peanut powder	0.08 oz (2.27 g)	608 (1.4)	969 (2.2)	9828 (22)	1362 (3.1)	14,894 (34)	22,349 (51)	5501 (13)	26,241 (60)
RSF milk, egg, peanut powder	0.08 oz (2.27 g)	586 (1.3)	1064 (2.4)	10,699 (24)	1326 (3.0)	14,642 (33)	23,971 (54)	4837 (11)	26,478 (60)
SFO 16 blend food powder	2 g	80 (0.2)	119 (0.2)	1702 (3.4)	94 (0.2)	403 (0.8)	9717 (19)	294 (0.6)	293 (0.6)
SFO 16 blend food powder	2 g	3.95 (0.01)	118 (0.2)	246 (0.5)	59 (0.12)	31 (0.06)	138 (0.3)	236 (0.5)	446 (0.9)
SFO 16 blend food puff	9 g	<0.10	<0.20	<0.05	<0.004	<0.02	6.95 (0.06)	<0.20	<0.02
SFO 16 blend food cracker	11 g	0.13 (0.001)	17 (0.2)	19 (0.2)	4.8 (0.06)	0.17 (0.002)	4.4 (0.05)	2.53 (0.03)	2.54 (0.03)
MYP peanut	3.5 oz (99 g)	0.13 (<0.1)	140 (14)	0.02 (<0.1)	153 (15)	<0.01	<0.20	<0.10	0.01
MYP peanut with tree nuts	3.5 oz (99 g)	<0.05 (<0.1)	33 (3.3)	<0.02 (<0.1)	56 (5.5)	<0.01	<0.20	<0.10	<0.01
INS apple + peanut	3 oz (85 g)	0.30 (<0.1)	84 (7.1)	0.02 (<0.1)	87 (7.4)	<0.01	<0.20	<0.10	<0.01
INS pear + egg	3 oz (85 g)	<0.05	<0.10	<0.02	<0.002	<0.01	<0.20	191 (16)	<0.01
PB2	12 g	5377 (65)	5748 (69)	52,547 (631)	5890 (71)	<0.02	<0.40	0.23	<0.02
MDN peanut butter	100 g	3983 (398)	4761 (476)	45,373 (4537)	4047 (405)	<0.01	<0.20	<0.10	<0.01
WOW soy butter	0.56 oz (16 g)	<0.05	<0.10	<0.02	<0.002	<0.01	<0.20	<0.10	<0.01

EIF, Early introduction foods; *HP*, Hello, Peanut!; *INS*, inspired START; *LMX*, Lil Mixins; *MDN*, Meridian peanut butter; *MWP*, MeWe Peanut; *MYP*, MyPeanut; *RSF*, Ready. Set. Food.; *SFO*, SpoonfulOne; *WOW*, Wow Soy Butter.
*Results expressed as µg allergen/g food extract and as mg in serving size µg/g (mg). Ara h 2 results were measured by ELISA 2.0.

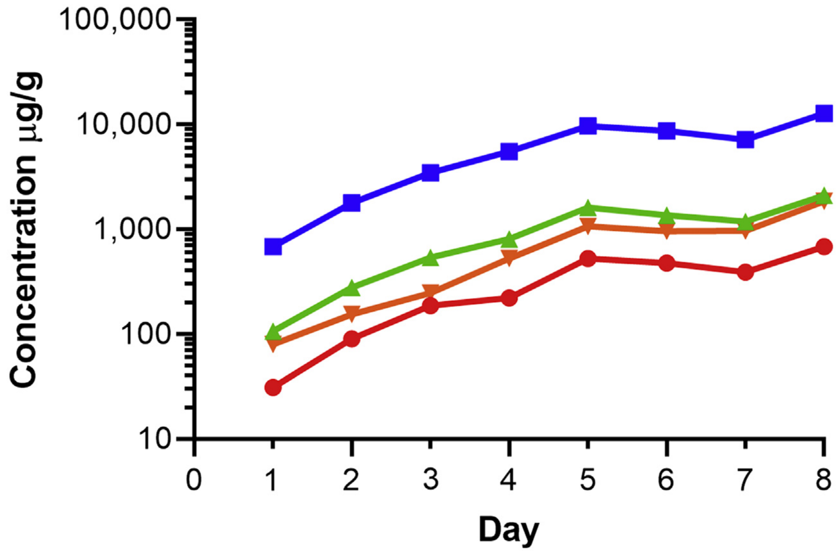

FIGURE 2. Progressive daily increase in allergen concentration in HP peanut powder: Ara h 1 (red closed circle), Ara h 2 (orange closed down-pointing triangle), Ara h 3 (blue closed square), and Ara h 6 (green closed up-pointing triangle). *HP*, Hello, Peanut!.

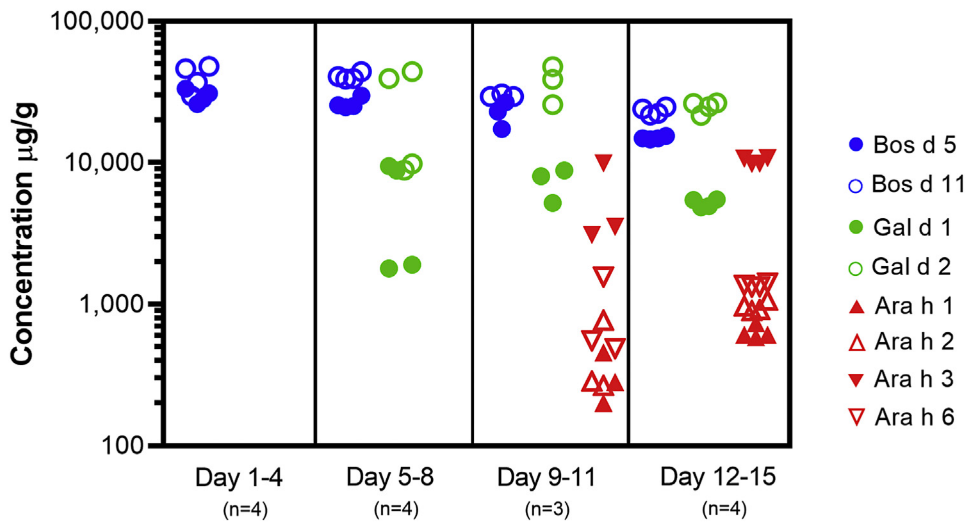

FIGURE 3. Daily concentration of milk, egg, and peanut allergens in the RSF food powder system to be consumed over a 15-day period: Bos d 5 (blue closed circle), Bos d 11 (blue open circle), Gal d 1 (green closed circle), Gal d 2 (green open circle), Ara h 1 (red closed up-pointing triangle), Ara h 2 (red open up-pointing triangle), Ara h 3 (red closed down-pointing triangle), and Ara h 6 (red open down-pointing triangle). *RSF*, Ready, Set, Food!

"active ingredients" of EIF. For milk, egg, and peanut allergens, 2 to 4 major allergens were measured. Quantitative results were obtained over a 4-log range, allowing the relationship between different allergens to be compared. An important caveat is that for other foods only a single major allergen was measured. This is a limitation of the present array, especially in foods that contain multiple allergens (eg, tree nuts and soy). It is assumed that single allergen measurements correlate with other allergen(s) that may be present in the food, but this may not always be the case. Nonetheless, failure to detect one of the major allergens may be a cause for concern. Another potential limitation of MARIA for Foods is that the array only measures allergens that are recognized by the mAb/pAb used for capture and detection. The molecular structure of these epitopes has yet to be determined.

Overall, the survey revealed that several EIF contained significant levels of major food allergens that were consistent and reproducible. Dose escalation was evident in EIF with protocols that increased over a period of up to 15 days and broadly correlated with serving size. The weekly cumulative allergen doses in EIF for peanut, milk, and egg allergens ranged from 100 to 3000 mg. For peanut, the doses were in keeping with previous estimates of the weekly allergen dose in Bamba (330 mg, Ara h 1, Ara h 2, Ara h 6) and in peanut butter/peanut powder.[10,11] The NIAID guidelines recommend Bamba or peanut butter/powder for prevention of peanut allergy as

TABLE III. Combined doses of specific allergens in peanut, milk, and egg intervention products

EIF product	Serving size	Dosing schedule	Cumulative allergen dose* (mg specific allergens combined)		
			Peanut	Milk	Egg
LMX peanut powder	5 g	One serving, 3× per week	1977	NA	NA
LMX baked egg powder	5 g	One serving, 3× per week	NA	NA	2.4
MWP nut butter AC	20 g	One serving, 3× per week	2946	NA	NA
MWP nut butter BC	20 g	One serving, 3× per week	2748	NA	NA
HP peanut flour	4 g	Days 1-7	197	NA	NA
HP peanut flour highest dose	4 g	Maintenance serving 3× per week	208	NA	NA
RSF milk powder	0.02-0.4 oz (0.57-1.13 g)	Days 1-4: milk	NA	235	NA
RSF milk and egg powder	0.04-0.5 oz (1.13-1.42 g)	Days 5-8: milk, egg	NA	341	170
RSF milk, egg, peanut powder	0.06-0.08 oz (1.7-2.27 g)	Days 9-11: milk, egg, peanut	46	323	272
RSF milk, egg, peanut powder	0.08 oz (2.27 g)	Days 12-15: milk, egg, peanut	119	346	273
RSF milk, egg, peanut powder	Combined	Days 1-15 milk, egg, peanut total	165	1245	715
SFO 16 blend food powder	2 g	One serving, 7× per week	28	140	8.4
SFO 16 blend food cracker	11 g	One serving	<1	<1	<1
MYP peanut	3.5 oz (99 g)	One serving	29	NA	NA
MYP peanut with tree nuts	3.5 oz (99 g)	One serving	9	NA	NA
INS apple + peanut	3 oz (85 g)	One serving	14	NA	NA
INS pear + egg	3 oz (85 g)	One serving	NA	NA	16
WOW soy butter	0.56 oz (16 g)	One serving	<0.1	<0.1	<0.1

EIF, Early introduction foods; *HP*, Hello, Peanut!; *INS*, inspired START; *LMX*, Lil Mixins; *MDN*, Meridian peanut butter; *MYP*, MyPeanut; *MWP*, MeWe Peanut; *NA*, not applicable; *RSF*, Ready, Set, Food!; *SFO*, SpoonfulOne; *WOW*, Wow Soy Butter.
*Cumulative doses of specific allergens were calculated based on the dosing recommendations provided by the manufacturer on the product insert or website.

having proven safety and efficacy based on the LEAP study. Using Bamba as a performance standard, similar high cumulative allergen doses of milk and egg allergens (170-1245 mg) were found in 1 powdered food mix. The maintenance doses of milk and egg allergens in that EIF, 1245 mg and 715 mg, respectively, are comparable to those reported in successful controlled trials of early introduction of egg for prevention of egg allergy.[26-28]

Most EIF conformed to what might be considered as the Bamba standard for allergen content (Tables I-III). This was not the case for other EIF, including fruit sauces, multiallergen blend powder, puffs, and crackers. As a group, these foods had low allergen levels compared with other foods in the survey and by comparison with Bamba (for peanut). Three milk/egg powders or fruit sauces contained lower cumulative doses of these allergens (Table III). They also showed evidence of loss of, or perhaps selective denaturation, of certain allergens, for example, fruit sauces containing peanut with undetectable Ara h 1 and Ara h 3, and low levels of Ara h 2 and Ara h 6, or low levels of Bos d 5 (compared with Bos d 11) in mixed food powder. The cause of these idiosyncrasies is unknown. Variability in the food source material used to prepare the EIF or the effects of food processing are possible explanations. Acid precipitation, leading to the formation of insoluble allergen aggregates in fruit sauces, could also explain the low allergen levels in those EIF. Allergen levels in multiallergen blended EIF were inconsistent and varied over a 3-log range, with low or undetectable levels of several allergens. This is perhaps not surprising given the complexities of sourcing a diverse array of foods, validating the source ingredients and formulating the final food blend. The trace levels of some allergens found in this group of EIF (<10 μg/g) raises questions about whether they are sufficient to induce tolerance in a similar manner to Bamba and peanut butter, or to milk and egg powders with high levels of major allergens.

Previous early intervention trials have used different empirical approaches to the diet, amount, and dose of the various foods used in the intervention arms. The EAT trial provided guidelines for the amounts of specific foods (eg, fish fingers, yoghurt, egg, cereal) to be eaten per week.[16,29] Egg intervention trials have used whole egg or egg, egg white, or heated egg powders in amounts ranging from 875 mg to 7.5 g per week.[26-28] The NIAID guidelines recommended amounts of peanut protein (ie, 2 g peanut protein in Bamba or peanut butter per serving) based on LEAP.[5] Although these are all reasonable approaches, the challenge lies in ensuring the consistency and dose of foods used in clinical trials to ensure comparability between studies. The variability in allergen levels in EIF seen in this study suggests that total protein may not correlate with allergen levels and may be affected by food processing. The present study suggests that specific allergen measurements provide a useful adjunct to total protein. Multiplex technology is one of the tools that can be used to assess allergen content of foods. This approach, coupled with ELISA for specific allergens, measures natural allergen molecules that are present in the food (or its ingredients) and is quantitative. Another approach is to use mass spectrometry (MS) to analyze allergen sequences or peptides in the food. The advantages of MS are that it can determine the profile of multiple allergens in foods, provides estimates of relative abundance, and has the potential to detect allergen peptides/sequences in processed foods.[30-33] In recent studies, we have observed a good correlation between multiplex technology, ELISA, and MS for comparing Ara h 1, Ara h 2, Ara h 3, and Ara h 6 in peanut flour extracts and have been able to formulate peanut allergen preparations with defined allergen content (manuscript in preparation). The combination of immunoassay and MS allows precise determination of food allergens, which is applicable to EIF, as well as to oral immunotherapy products and food reference

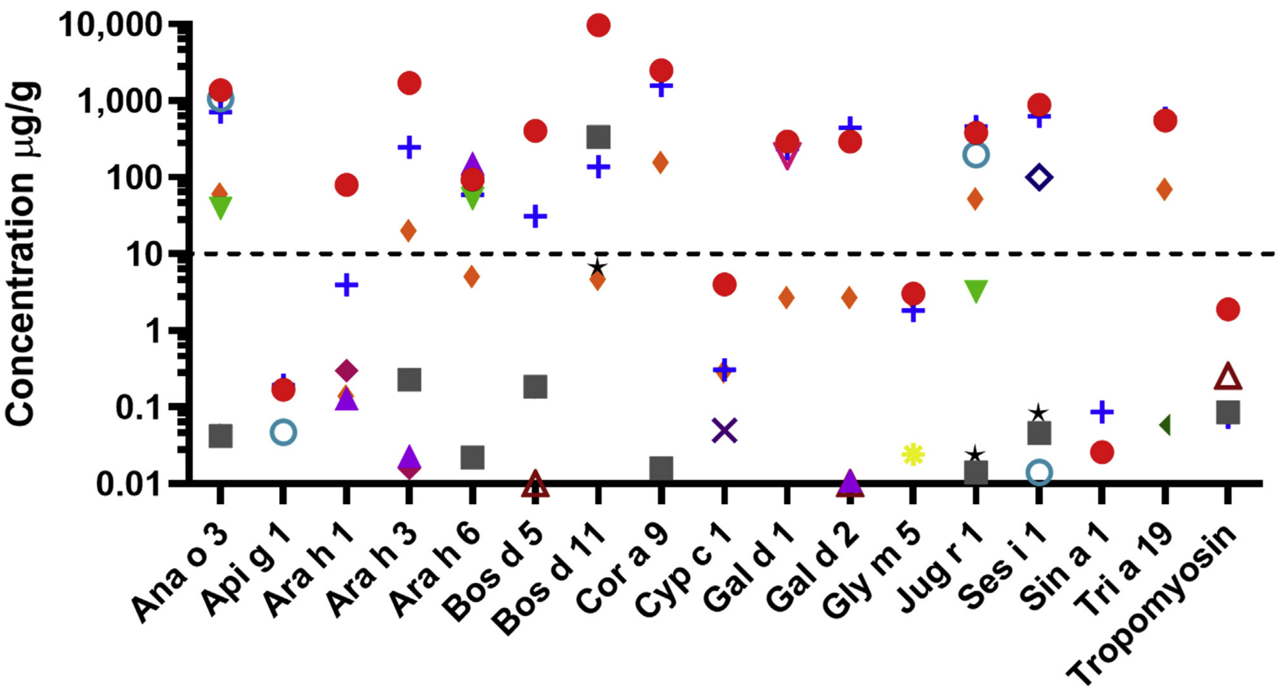

FIGURE 4. Concentration of 17 major allergens in multifood products: SFO Powder #1 (red closed circle), SFO Powder #2 (blue plus symbol), SFO Puff #1 (gray closed square), SFO Puff #2 (black star symbol), SFO Cracker (orange closed diamond), MYP (purple closed up-pointing triangle), MYP Tree Nuts (green closed down-pointing triangle), INS Peanut (pink closed diamond), INS Tree Nut (blue open circle), INS Soy (yellow asterisk symbol), INS Shrimp (brown open up-pointing triangle), INS Egg (pink open down-pointing triangle), INS Sesame (blue open diamond), INS Wheat (green closed left-pointing triangle), and INS Cod (purple cross symbol). The dashed line at 10 µg/g represents a low level of allergen. This was arbitrarily defined based on the allergen levels of EIF in Table II, which typically had allergen levels that were >>10-fold higher than 10 µg/g. *EIF*, Early introduction foods; *INS*, inspired START; *MYP*, MyPeanut; *SFO*, SpoonfulOne.

materials. This synergistic molecular approach will improve performance testing and standardization of food allergen measurements and promote greater harmonization of methods in the food industry.[34]

Since the publication of the LEAP study, there has been intense interest in consumption of foods in early life to prevent peanut and other food allergies. Although the evidence that peanut consumption can prevent peanut allergy is strong, the evidence for egg and milk allergens is mixed, with some studies reporting a positive benefit and others not.[6,26-28] The rapid commercialization of EIF has provided consumers and allergic families with a choice of EIF as aids for allergy prevention, which has several consequences. The foods may be perceived as drugs or over-the-counter treatments and to have similar efficacy. Consumers may also consider them to be equally effective for different allergens. Food manufacturers can petition the Food and Drug Administration (FDA) for a "qualified health claim" that has specific language describing the claim. One manufacturer has been successful in petitioning the FDA for a qualified health claim for a peanut product based on the evidence of the LEAP study (available at: https://www.fda.gov/food/food-labeling-nutrition/qualified-health-claims; accessed on November 10, 2020). Qualified health claims have not yet been applied to other allergens.

The information on doses of major allergens provided in this survey allows consumers and their health care providers to make informed decisions about EIF. Measurements of allergen content also allow food manufacturers to modify and improve EIF (eg, by monitoring food ingredients, altering the dose, reducing trace contamination or aberrant processing or formulation effects on allergens). This will be especially useful for boosting allergen levels in tree nuts, soy, fish, shellfish, and sesame. More rigorous standardization should improve the quality of food allergens used in intervention studies, as well as in oral food challenges. Further clinical trials are needed to establish the efficacy of EIF. Ultimately, guidelines should be established to define therapeutic doses of allergens in food.

NOTE

Since this study went to press, one manufacturer (LMX) asked the authors to clarify that their egg powder was made from baked egg. This may explain the low level of Gal d 2, which is heat sensitive, in LMX baked egg powder.

REFERENCES

1. Du Toit G, Roberts G, Sayre PH, Bahnson HT, Radulovic S, Santos AF, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med 2015;372:803-13.
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;374:1435-43.
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;136:258-61.
4. Sicherer SH, Bock SA, Zeiger RS. Implications of the "Consensus Communication on Early Peanut Introduction in the Prevention of Peanut Allergy in High-

Risk Infants" for allergists, primary care physicians, patients, and society. J Allergy Clin Immunol Pract 2015;3:649-51.

5. Togias A, Cooper SF, Acebal ML, Assa'ad A, Baker JR Jr, Beck LA, et al. Addendum guidelines for the prevention of peanut allergy in the United States: Report of the National Institute of Allergy and Infectious Diseases-sponsored expert panel. J Allergy Clin Immunol 2017;139:29-44.
6. de Silva D, Halken S, Singh C, Muraro A, Angier E, Arasi S, et al. Preventing food allergy in infancy and childhood: Systematic review of randomised controlled trials. Pediatr Allergy Immunol 2020;31:813-26.
7. Johnson JL, Gupta RS, Bilaver LA, Hu JW, Martin J, Jiang J, et al. Implementation of the Addendum Guidelines for Peanut Allergy Prevention by US allergists, a survey conducted by the NIAID, in collaboration with the AAAAI. J Allergy Clin Immunol 2020;146:875-83.
8. Gupta RS, Bilaver LA, Johnson JL, Hu JW, Jiang J, Bozen A, et al. Assessment of pediatrician awareness and implementation of the addendum guidelines for the prevention of peanut allergy in the United States. JAMA Netw Open 2020;3:e2010511.
9. Du Toit G, Katz Y, Sasieni P, Mesher D, Maleki SJ, Fisher HR, et al. Early consumption of peanuts in infancy is associated with a low prevalence of peanut allergy. J Allergy Clin Immunol 2008;122:984-91.
10. Hindley JP, Filep S, Block DS, King EM, Chapman MD. Dose of allergens in a peanut snack (Bamba) associated with prevention of peanut allergy. J Allergy Clin Immunol 2018;141:780-2.
11. Filep S, Block DS, Smith BRE, King EM, Commins S, Kulis M, et al. Specific allergen profiles of peanut foods and diagnostic or therapeutic allergenic products. J Allergy Clin Immunol 2018;141:626-31.
12. Pomés A, Butts CL, Chapman MD. Quantification of Ara h 1 in peanuts: why roasting makes a difference. Clin Exp Allergy 2006;36:824-30.
13. Maleki SJ, Chung SY, Champagne ET, Raufman JP. The effects of roasting on the allergenic properties of peanut proteins. J Allergy Clin Immunol 2000;106:763-8.
14. Sharma GM, Chatim A, Ferguson M, Williams KM. Extraction conditions affect the immunoreactivity of peanut allergens. J Food Sci 2019;84:2357-63.
15. Jayasena S, Wijeratne SSK, Taylor SL, Baumert JL. Improved extraction of peanut residues from a wheat flour matrix for immunochemical detection. Food Chem 2019;278:832-40.
16. Perkin MR, Logan K, Marrs T, Radulovic S, Craven J, Flohr C, et al. Enquiring About Tolerance (EAT) study: feasibility of an early allergenic food introduction regimen. J Allergy Clin Immunol 2016;137:1477-86.
17. Earle CD, King EM, Tsay A, Pittman K, Saric B, Vailes L, et al. High-throughput fluorescent multiplex array for indoor allergen exposure assessment. J Allergy Clin Immunol 2007;119:428-33.
18. King EM, Filep S, Smith B, Platts-Mills T, Hamilton RG, Schmechel D, et al. A multi-center ring trial of allergen analysis using fluorescent multiplex array technology. J Immunol Methods 2013;387:89-95.
19. Westphal CD, Pereira MR, Raybourne RB, Williams KM. Evaluation of extraction buffers using the current approach of detecting multiple allergenic and nonallergenic proteins in food. J AOAC Int 2004;87:1458-65.
20. Ito K, Yamamoto T, Oyama Y, Tsuruma R, Saito E, Saito Y, et al. Food allergen analysis for processed food using a novel extraction method to eliminate harmful reagents for both ELISA and lateral-flow tests. Anal Bioanal Chem 2016;408:5973-84.
21. Pilolli R, De Angelis E, Monaci L. Streamlining the analytical workflow for multiplex MS/MS allergen detection in processed foods. Food Chem 2017;221:1747-53.
22. Gold DR, Adamkiewicz G, Arshad SH, Celedon JC, Chapman MD, Chew GL, et al. NIAID, NIEHS, NHLBI, and MCAN Workshop Report: the indoor environment and childhood asthma-implications for home environmental intervention in asthma prevention and management. J Allergy Clin Immunol 2017;140:933-49.
23. Salo PM, Wilkerson J, Rose KM, Cohn RD, Calatroni A, Mitchell HE, et al. Bedroom allergen exposures in US households. J Allergy Clin Immunol 2018;141:1870-9.
24. Gergen PJ, Mitchell HE, Calatroni A, Sever ML, Cohn RD, Salo PM, et al. Sensitization and exposure to pets: the effect on asthma morbidity in the US population. J Allergy Clin Immunol Pract 2018;6:101-7.
25. Pomés A, Helm RM, Bannon GA, Burks AW, Tsay A, Chapman MD. Monitoring peanut allergen in food products by measuring Ara h 1. J Allergy Clin Immunol 2003;111:640-5.
26. Wei-Liang TJ, Valerio C, Barnes EH, Turner PJ, Van Asperen PA, Kakakios AM, et al. A randomized trial of egg introduction from 4 months of age in infants at risk for egg allergy. J Allergy Clin Immunol 2017;139:1621-8.
27. Natsume O, Kabashima S, Nakazato J, Yamamoto-Hanada K, Narita M, Kondo M, et al. Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial. Lancet 2017;389:276-86.
28. Bellach J, Schwarz V, Ahrens B, Trendelenburg V, Aksunger O, Kalb B, et al. Randomized placebo-controlled trial of hen's egg consumption for primary prevention in infants. J Allergy Clin Immunol 2017;139:1591-9.
29. Perkin MR, Logan K, Tseng A, Raji B, Ayis S, Peacock J, et al. Randomized trial of introduction of allergenic foods in breast-fed infants. N Engl J Med 2016;374:1733-43.
30. Chapman MD, Briza P. Molecular approaches to allergen standardization. Curr. Allergy Asthma Rep 2012;12:478-84.
31. Parker CH, Khuda SE, Pereira M, Ross MM, Fu TJ, Fan X, et al. Multi-allergen quantitation and the impact of thermal treatment in industry-processed baked goods by ELISA and liquid chromatography-tandem mass spectrometry. J Agric. Food Chem 2015;63:10669-80.
32. Nitride C, Lee V, Baricevic-Jones I, Adel-Patient K, Baumgartner S, Mills ENC. Integrating allergen analysis within a risk assessment framework: approaches to development of targeted mass spectrometry methods for allergen detection and quantification in the iFAAM Project. J AOAC Int 2018;101:83-90.
33. Pilolli R, Nitride C, Gillard N, Huet AC, van PC, de LM, et al. Critical review on proteotypic peptide marker tracing for six allergenic ingredients in incurred foods by mass spectrometry. Food Res Int 2020;128:108747.
34. Holzhauser T, Johnson P, Hindley JP, O'Connor G, Chan CH, Costa J, et al. Are current analytical methods suitable to verify VITAL 2.0/3.0 allergen reference doses for EU allergens in foods? Food Chem Toxicol 2020;145:111709.

ONLINE REPOSITORY

COMPARISON OF EXTRACTION BUFFERS FOR FOODS

A food blend was formulated by mixing 400-2400 mg of the following food reference materials or food samples: NIST SRM 1549a (whole milk), NIST SRM 8445 (spray-dried whole egg), NIST SRM 3234 (soy flour), peanut flour (12% light roast, Byrd Mill), cashew flour (Wild Tree Farms Premium), hazelnut flour (Sincerely Nuts), and shrimp powder (Bolner's Fiesta Extra Fancy ground shrimp). The food blend (6650 mg) was vortexed at 3200 rpm to homogenize the sample. Thirty aliquots of 100 mg of the food product blend were extracted in 1 mL of different extraction buffers.

Three extraction buffers were prepared based on a review of published literature on food allergen extraction procedures.[E1-E5] Buffer #1 was phosphate buffered saline (PBS), pH 7.4, containing 1M NaCl and 2% Tween 20. Buffer #2 was 50 mM carbonate-bicarbonate, pH 9.6, containing 2% Tween 20. Buffer #3 was 0.1M Tris, pH 8.5, containing 1% sodium lauryl sulfate and 0.1M sodium sulfite. Control buffer was PBS, pH 7.4, containing 0.05% Tween 20.

In addition to multiple buffers, different extraction processes were compared: Extraction process A: vortexed (5 seconds), sonicated 30 seconds at 35 kHz, heated for 15 minutes in a water bath at 60°C; Extraction process B: vortexed (5 seconds), sonicated 30 seconds at 35 kHz, incubated for 15 minutes at room temperature; Extraction process C: vortexed (5 seconds), sonicated 30 seconds at 35 kHz, incubated for 1 hour at room temperature on a laboratory rocker; and Extraction process control: vortexed (5 seconds), incubated for 2 hours at room temperature on a laboratory rocker. A total of 10 different buffer/extraction process scenarios were tested in triplicate. Samples were centrifuged at 4°C for 20 minutes at 1250 rcf. The supernatant was pipetted into 2 mL microcentrifuge tubes and stored at −20°C before allergen analysis. Immediately before assay, extracts were centrifuged for 5 minutes at 15,600 rcf. Serial 10-fold dilutions of the supernatant (typically 1/1000, 1/10,000, 1/100,000, and 1/1,000,000) were made and transferred to the MARIA for Foods microplate.

The results (Figure E1) show that for 8/10 allergens that were tested, buffer/extraction process 1-A was most effective. This procedure was used for extraction of the EIF that were compared in the study. This buffer was less effective for Ana o 3 and most significantly for Bos d 11. However, the buffer containing SDS and sodium sulfite that was most effective for Bos d 11 (3A-C) was not suitable for most of the other allergens. Nonetheless, Bos d 11 was readily detected in most milk-containing EIF, although the amounts may be an underestimate of total Bos d 11.

TABLE E1. Early introduction foods: products, manufacturers and websites

Product name	Abbreviation	Manufacturer	Product description	Serving size
Lil Mixins Early Allergy Introduction	LMX (n = 2)	Lil Mixins, LLC	Peanut powder	5 g
			Baked egg powder	5 g
MeWe	MWP (n = 4)	Edesia	Apple cinnamon nut butter (jar)	20 g
			Apple cinnamon nut butter (pack)	20 g
			Banana coconut nut butter (pack)	20 g
			Berry coconut nut butter (pack)	20 g
SpoonfulOne	SFO (n = 8)	BEFORE Brands, Inc	Daily mix-in food blend (n = 2)	2 g
			Strawberry puffs (n = 2)	9 g
			Banana puffs	9 g
			Vanilla oat crackers	11 g
			Cocoa oat crackers	11 g
			Blueberry oat crackers	11 g
Ready, Set, Food!	RSF (n = 15)	Prollergy Corporation	Milk powder (n = 2)	0.02 oz
			Milk powder (n = 2)	0.04 oz
			Milk and egg powder (n = 2)	0.04 oz
			Milk and egg powder (n = 2)	0.05 oz
			Milk, egg, and peanut powder (n = 1)	0.06 oz
			Milk, egg, and peanut powder (n = 6)	0.08 oz
Hello, Peanut!	HP (n = 8)	Assured Bites, Inc	Peanut powder (n = 8)	4 g
MyPeanut	MYP (n = 2)	Everidis Health Sciences	Peanut and apples baby food blend	3.5 oz (99 g)
			Peanuts, tree nuts, and apples baby food blend	3.5 oz (99 g)
inspired START	INS (n = 8)	Little Big Brands	Apple and peanut baby food	3 oz (85 g)
			Banana and tree nut baby food	3 oz (85 g)
			Mango and soy baby food	3 oz (85 g)
			Banana and shrimp baby food	3 oz (85 g)
			Pear and egg baby food	3 oz (85 g)
			Pear and sesame baby food	3 oz (85 g)
			Apple and wheat baby food	3 oz (85 g)
			Mango and cod baby food	3 oz (85 g)
WOWBUTTER	WOW (n = 1)	WOWBUTTER Foods	Soy butter—nut free	0.56 oz (16 g)
Bamba	Bamba (n = 1)	Osem Corporation	Peanut puff	1 oz (28 g)
ErdnußLocken	ERD (n = 1)	Lorenz Snack-World	Peanut puff	100 g
Mission MightyMe	MME (n = 1)	Mission MightyMe	Peanut puff	7 g
Original PB2	PB2 (n = 1)	PB2 Foods, Inc	Powdered peanut butter	13 g
Meridian	MDN (n = 1)	Meridian Foods Limited	Peanut butter	100 g

Product name	Website
Lil Mixins Early Allergy Introduction	https://www.lilmixins.com/http://www.lilmixins.com/
MeWe	https://www.edesianutrition.org/mewe/www.edesianutrition.org/mewe/
SpoonfulOne	https://www.spoonfulone.com/http://www.spoonfulone.com/
Ready Set Food	https://readysetfood.com/
Hello, Peanut!	https://www.hello-peanut.com/www.hello-peanut.com/
MyPeanut	https://my-peanut.com/
inspired START	https://www.littlebigbrands.com/portfolio/inspired-start/www.littlebigbrands.com/portfolio/inspired-start/
WOWBUTTER	https://wowbutter.com/
Bamba	https://www.osem.co.il/product/classic-bamba-he/http://www.osem.co.il/product/classic-bamba-he/
ErdnußLocken	https://www.lorenz-snackworld.at/en/brands-and-products/erdnusslocken
MightyMe	https://missionmightyme.com/
Original PB2	https://pb2foods.com/
Meridian	https://shop.meridianfoods.co.uk/

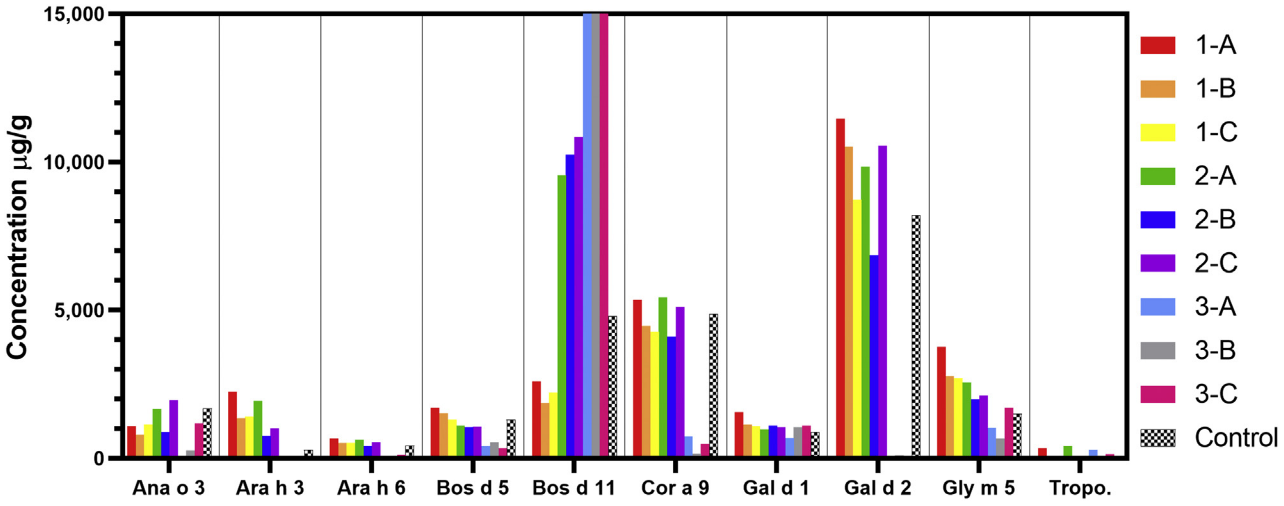

FIGURE E1. Comparison of extraction methods for food allergens.

REFERENCES

E1. Westphal CD, Pereira MR, Raybourne RB, Williams KM. Evaluation of extraction buffers using the current approach of detecting multiple allergenic and nonallergenic proteins in food. J AOAC Int 2004;87:1458-65.

E2. Ito K, Yamamoto T, Oyama Y, Tsuruma R, Saito E, Saito Y, et al. Food allergen analysis for processed food using a novel extraction method to eliminate harmful reagents for both ELISA and lateral-flow tests. Anal Bioanal Chem 2016;408:5973-84.

E3. Pilolli R, De Angelis E, Monaci L. Streamlining the analytical workflow for multiplex MS/MS allergen detection in processed foods. Food Chem 2017;221:1747-53.

E4. Sharma GM, Chatim A, Ferguson M, Williams KM. Extraction conditions affect the immunoreactivity of peanut allergens. J Food Sci 2019;84:2357-63.

E5. Jayasena S, Wijeratne SSK, Taylor SL, Baumert JL. Improved extraction of peanut residues from a wheat flour matrix for immunochemical detection. Food Chem 2019;278:832-40.