Distribution of peanut protein in the home environment

Helen A. Brough, MSc, MRCPCH,[a,b] Kerry Makinson, MSc,[a] Martin Penagos, MSc, MD,[a] Soheila J. Maleki, PhD,[c] Hsiaopo Cheng, MS,[c] Abdel Douiri, PhD,[d] Alick C. Stephens, PhD,[a] Victor Turcanu, PhD,[a]* and Gideon Lack, MD, FRCPCH[a]* *London and Southampton, United Kingdom, and New Orleans, La*

Background: To halt the increase in peanut allergy, we must determine how children become sensitized to peanut. High household peanut consumption used as an indirect marker of environmental peanut exposure is associated with the development of peanut allergy.
Objective: We sought to validate a method to quantify environmental peanut exposure, to determine how peanut is transferred into the environment after peanut consumption, and to determine whether environmental peanut persists despite cleaning.
Methods: After initial comparative studies among 3 ELISA kits, we validated and used the Veratox polyclonal peanut ELISA to assess peanut protein concentrations in dust and air and on household surfaces, bedding, furnishings, hand wipes, and saliva.
Results: The Veratox polyclonal peanut ELISA had the best rate of recovery of an independent peanut standard. We demonstrated 100% sensitivity and specificity and a less than 15% coefficient of variation for intra-assay, interassay, and interoperator variability. There was high within-home correlation for peanut protein levels in dust and household surface wipes. Airborne peanut levels were lower than the limit of quantitation for the Veratox polyclonal peanut ELISA in a number of simulated scenarios, except for a brief period directly above peanuts being deshelled. Peanut protein persisted on hands and in saliva 3 hours after peanut consumption. Peanut protein was completely removed from granite tables after cleaning with detergent, and levels were reduced but still present after detergent cleaning of laminate and wooden table surfaces, pillows, and sofa covers.
Conclusions: Peanut spread easily around the home and might be resistant to usual cleaning methods. Peanut protein can be transferred into the environment by means of hand transfer and saliva but is unlikely to be aerosolized. (J Allergy Clin Immunol 2013;132:623-9.)

Key words: *Peanut, sensitization, allergy, environment, dust, aerosolized, airborne, saliva, hand, ELISA, validation*

Peanut allergy is an important public health concern.[1] Ongoing studies on oral tolerance induction to peanut aim to address these issues (www.leapstudy.co.uk).[2] To halt the increase in peanut allergy,[3,4] we must first understand the mechanism of peanut sensitization. Household peanut consumption is 10 times higher in infants with peanut allergy versus high-risk (with egg allergy) control subjects.[5] In this study household peanut consumption was considered an indirect marker of environmental peanut exposure; however, peanut protein levels in the home were not directly quantified.

Few studies have assessed the distribution of peanut in the environment. Surface wipes from desks, cafeteria tables, and water fountains of 6 schools found little evidence of peanut using a monoclonal ELISA against Ara h 1 (INDOOR Biotechnologies, Warminster, United Kingdom).[6] Most cleaning agents (plain water, dishwashing liquid, sanitizing wipes, and bleach cleaner) were able to remove Ara h 1 from tables and hands spiked with 5 mL of peanut butter. Dish soap left residual Ara h 1 on 33% of tables (40-140 ng/mL), and Ara h 1 remained on 25% and 50% of hands after use of water and hand sanitizer, respectively.[6] Previous studies have quantified egg (ovomucoid), milk (β-lactoglobulin), and fish levels in household settled dust.[7,8] More recently, Ara h 2 has been quantified in bedroom dust of 18 (23.4%) of 77 children with asthma.[9] We have shown that peanut levels increase on bed sheets (on which participants have slept) the day after a single peanut-containing meal.[10]

As well as quantifying environmental peanut exposure, it is important to determine how peanut can be transferred into the environment from persons eating peanut. Aircraft often impose restrictions on peanut consumption because of concerns that persons with peanut allergy might inhale airborne peanut from other passengers eating peanuts on board.[11] There are anecdotal reports of allergic reactions after inhalation of peanut; however, when children with severe or reported inhalational reactions to peanut

From [a]the Department of Paediatric Allergy, MRC & Asthma UK Centre in Allergic Mechanisms of Asthma, Division of Asthma, Allergy and Lung Biology, King's College London and Guy's and St Thomas' NHS Foundation Trust, London; [b]Faculty of Medicine, University of Southampton; [c]United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, New Orleans; and [d]the Department of Public Health Science, School of Medicine, King's College London.

*These authors contributed equally to this work.

Supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's & St Thomas' NHS Foundation Trust and King's College London and the Medical Research Council.

Disclosure of potential conflict of interest: H. A. Brough, K. Makinson, and V. Turcanu have received research support from the Department of Health through the NIHR comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust. S. J. Maleki is on the Scientific Advisory Council to the National Peanut Board. G. Lack has received research support from the Department of Health through the NIHR comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust; is on the DBV Technologies Scientific Advisory Board; has received consultancy fees from the Anaphylaxis Campaign and National Peanut Board; has received lecture fees from Sodilac, Novartis, Nestlé Nutrition, GlaxoSmithKline, and Serono Symposia International Foundation; and has stock/options in DBV Technologies. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 29, 2012; revised February 20, 2013; accepted for publication February 28, 2013.

Available online April 19, 2013.

Corresponding author: Gideon Lack, MD, FRCPCH, Children's Allergy Unit, 2nd Fl, Stairwell B, South Wing, Guy's and St Thomas' NHS Foundation Trust, Westminster Bridge Rd, London SE1 7EH, United Kingdom. E-mail: Gideon.lack@kcl.ac.uk.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.02.035

Abbreviations used
IOM: Inhalable occupational medicine
LLQ: Lower limit of quantitation
VPPE: Veratox polyclonal peanut ELISA

underwent blind inhalational peanut challenges (peanut butter held 12 inches from the face for 10 minutes), these children had no allergic symptoms or signs.[12] Peanut protein has been detected in the ventilation system filters of commercial airliners after 5000 flight hours by using an inhibition assay with peanut extract[13]; however, the results of this abstract have not been replicated. Peanut protein might be transferred into the environment after peanut consumption through hand transmission[5] or saliva.[14] Ara h 1 has been measured in saliva in levels up to 40 μg/mL (enough to cause an allergic reaction) immediately after peanut consumption; however, it was undetectable in 87% of participants after 1 hour using a monoclonal ELISA against Ara h 1.[14]

This study was designed to validate a method to quantify environmental peanut protein levels in household dust, surfaces, bedding, furnishings, and air to quantify environmental peanut exposure and its potential role in peanut sensitization and allergy. We also wished to assess potential routes of peanut transfer into the environment and the effect of usual detergent cleaning on reducing environmental peanut levels.

METHODS

The study was approved by the Brent Medical Research Ethics Committee. Informed consent was obtained before environmental sampling and from participants who provided saliva and hand-wipe samples before and after peanut consumption.

Validation of method to quantify peanut protein in dust and wipes

The Veratox polyclonal peanut ELISA (VPPE) used in this study was validated according to the International Conference on Harmonization guidelines for validation of analytic procedures.[15] We also assessed aspects of dust processing related to peanut protein. Details of the methods used are included in the Methods section in this article's Online Repository at www.jacionline.org, including the following:

1. Details of samples used
2. Rate of recovery of an independent peanut standard comparing 3 validated commercial ELISA kits:
   A. VPPE (Neogen, Lansing, Mich)
   B. Biokits polyclonal Ara h 1 ELISA (Tepnel Research Products and Services, Flintshire, United Kingdom)
   C. Monoclonal ELISA against Ara h 1 (INDOOR Biotechnologies)
3. Performance characteristics of VPPE:
   A. Sensitivity and specificity
   B. Lower limit of quantitation (LLQ)
   C. Assay precision
4. Dust processing:
   A. Peanut protein in sieved fine dust versus residual fluff
   B. Extraction assays
   C. Effect of freezing and thawing extracted dust samples.

Peanut protein in household dust and surfaces

Forty-five families with infants were recruited from pediatric allergy clinics. Dust samples were obtained from the bed sheets of all household members and from the infant's play area; participants were asked not to wash or vacuum these for 5 days before the home visit. Dust samples were taken from each side of the parent's bed. The infant's play area was the place where the infant spent most of his or her day (eg, play mat/quilt and living room carpet).

A Philips cylinder vacuum FC8262 (1600 W) was connected to a Dustream adaptor and collector with a disposable nylon collection filter (pore size, 40 μm; INDOOR Biotechnologies). Bed sheets and the infant's play area were vacuumed for 2 minutes within a 1-m^2 surface area; the infant's bed sheet was vacuumed for 1 minute within a 0.5-m^2 area. Dust samples were sieved with a 300-μm copper sieve (Endecotts, London, United Kingdom), and fine dust was weighed to express results in micrograms of peanut protein per gram of dust. Dust was extracted in proportional volumes of the VPPE extraction solution and heated for 15 minutes at 60°C (see the Methods section in this article's Online Repository for further details). Dust samples of less than 5 mg were excluded.

Wipe samples made from Benchkote filter paper (Whatman, Maidstone, United Kingdom) cut to 4 × 4 cm and moistened with 0.5 mL of PBS were obtained from the parent's table, infant's highchair table, tap, dishwasher handle, refrigerator handle, and infant's crib rail. Table-surface wipes were collected within A4 paper–sized templates. Wipes were weighed before and after sampling to calculate results in micrograms of peanut protein per gram. Wipe samples were extracted in 2 mL of VPPE extraction solution in a sealed syringe. We used the VPPE to quantify peanut protein levels in dust and wipes. All samples collected were blinded from the researcher performing the ELISAs.

Airborne peanut

Airborne peanut was captured with glass-fiber filters (pore size, 0.7 μm) inserted into the inhalable occupational medicine (IOM) sampling head of a personal air-sampling monitor (TUFF; Casella Measurement, Bedford, United Kingdom). The pump was run at 2 L/min, as recommended by the manufacturer, which is equivalent to an infant's minute volume (tidal volume [5 mL/kg] × respiratory rate [40 breaths/min]), using an estimated weight of 10 kg. Glass-fiber filters were processed in the same way as wipes and analyzed with the VPPE. The VPPE LLQ was 100 ng/mL (equivalent to 2.5 μg/m^3). The following experiments were performed to detect airborne peanut:

1. The sampling head was held 1 cm (n = 3) and 1 m (n = 3) above a peanut butter jar/dry-roasted peanut bag for 22 hours and above a simmering pan of satay sauce (10.8 g of peanut; Amoy, Hayes, United Kingdom) for 10 minutes.
2. While eating peanut butter or dry-roasted peanuts, the sampling head was pinned to the researcher's clothes, placed on the dining room table, breathed on for 10 minutes, or placed overnight on the bedside table (n = 3).
3. The IOM was run for 22 hours in homes with high peanut protein levels in dust (n = 5; median peanut protein, 163.8 μg/g; range, 51.2-365.2 μg/g).
4. The sampling head was held 1 cm and 1 m above peanuts being deshelled. New glass-fiber filters were run in the IOM for 10 minutes before, during, immediately after, and 30 minutes and 1 hour after deshelling peanuts (n = 6).

Peanut protein on hands and saliva after peanut consumption

Hand-wipe and saliva samples were taken before and 3 hours after consuming 50 g of salted peanuts (n = 6; KP Nuts, Hayes, United Kingdom). Participants were asked not to eat peanut for 24 hours before and 3 hours after this peanut meal. Hand samples were taken with Benchkote wipes of the right palm (all subjects were right handed) and processed as described above. Saliva samples were collected into Eppendorf tubes and analyzed directly for peanut protein by using the VPPE without extraction.

Persistence of peanut despite cleaning

Table surfaces. Three table surfaces (wood [unpainted], granite, and laminate) were cleaned with water and allowed to air dry. A5 paper templates were sellotaped to the tables (n = 3). Smooth peanut butter (0.5 mL; Sun-Pat; Premier Foods Group, Manchester, United Kingdom) was spread evenly onto