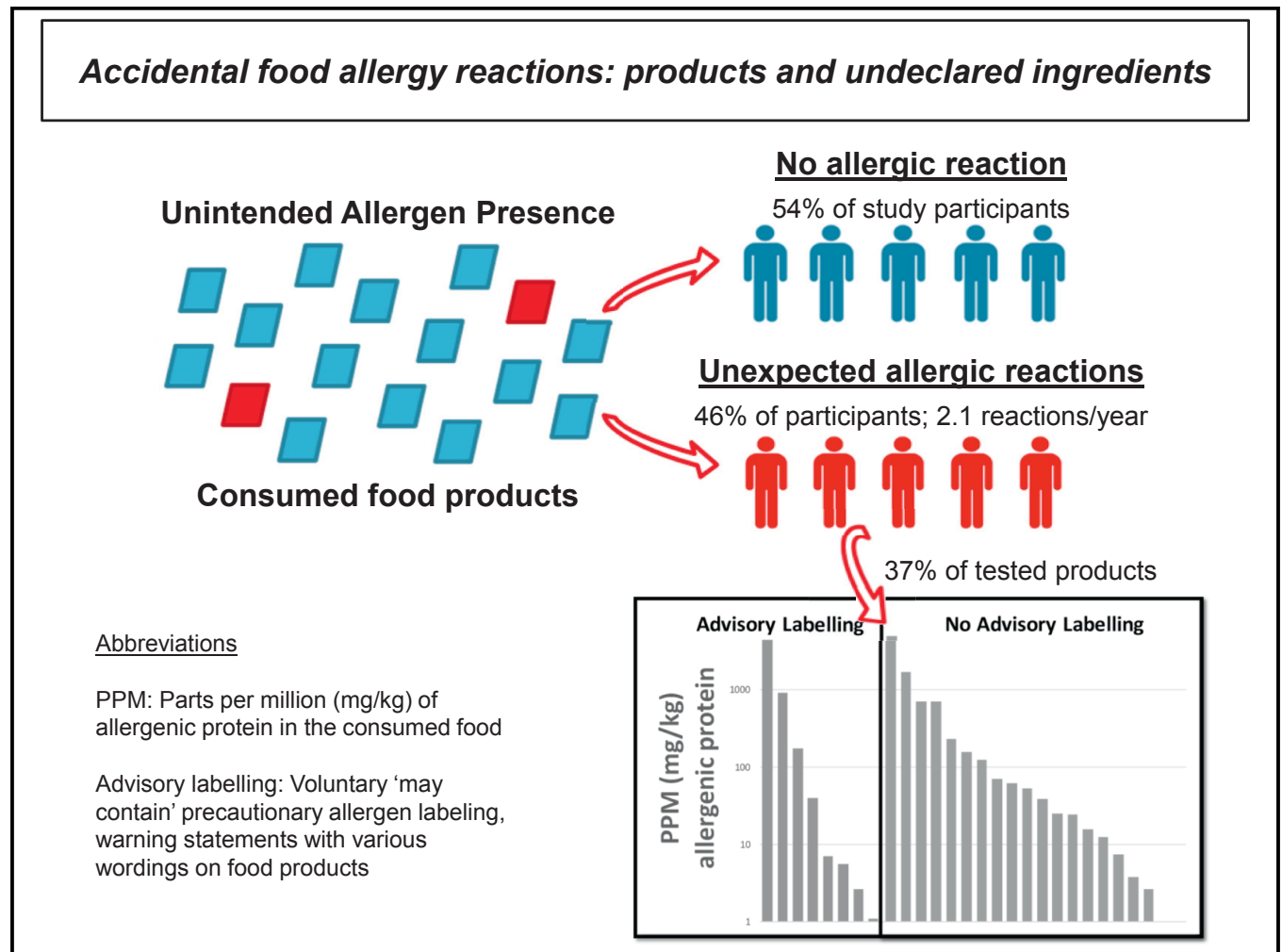


Accidental food allergy reactions: Products and undeclared ingredients

W. Marty Blom, PhD,^{a,b} Anouska D. Michelsen-Huisman, RD,^b Harmieke van Os-Medendorp, PhD,^b Gert van Duijn, PhD,^c Mary-lène de Zeeuw-Brouwer, BSc,^a Astrid Versluis, MSc, MANP,^b Jacqueline J. M. Castenmiller, PhD,^d Hubert P. J. M. Noteborn, PhD,^d Astrid G. Kruizinga, MSc,^a André C. Knulst, PhD, MD,^b and Geert F. Houben, PhD^{a,e} Zeist and Utrecht, The Netherlands

GRAPHICAL ABSTRACT



From ^athe Netherlands Organisation for Applied Scientific Research (TNO), Zeist; ^bthe Department of Dermatology and Allergology and ^cthe Laboratory of Translational Immunology, University Medical Center Utrecht, Utrecht University; ^dTriskelion B.V., Zeist; and ^ethe Netherlands Food and Consumer Product Safety Authority, Utrecht. Supported by the Netherlands Food and Consumer Product Safety Authority. W.M.B. and G.F.H. were also funded in part by the Dutch Governmental TNO Research Cooperation Funds. Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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Corresponding author: W. Marty Blom, PhD, The Netherlands Organisation for Applied Scientific Research (TNO), Department RAPID, Utrechtseweg 48, PO Box 360, 3700 AJ, Zeist, The Netherlands. E-mail: marty.blom@tno.nl. 0091-6749/\$36.00

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Background: Accidental allergic reactions to food are frequent and can be severe and even fatal.

Objective: We sought to analyze the culprit food products and levels of unexpected allergens in accidental reactions.

Methods: A prospective cohort study was conducted in adults ($n = 157$) with a physician-confirmed diagnosis of food allergy. During a 1-year follow-up, 73 patients reported accidental allergic reactions and the culprit food products. Food samples received ($n = 51$) were analyzed for a wide range of suspected noningredient allergens, and risk was quantified.

Results: A very diverse range of food products was responsible for the unexpected allergic reactions. Thirty-seven percent (19/51) of products analyzed had 1 to 4 culprit allergens identified that were not supposed to be present according to the ingredient declaration. Concentrations varied from 1 to 5000 mg of protein of the allergenic food per kilogram of food product and were greatest for peanut, milk, and sesame. Milk proteins posed the highest estimated risk for objective allergic reactions. The intake of culprit allergens by patients varied considerably. For those cases in which culprit allergens were detected, the intake of at least 1 allergen exceeded the reference dose or a culprit allergen with a yet unknown reference dose was present. Both patient neglect of precautionary allergen labeling statements and omission of using a precautionary allergen labeling statement by food manufacturers seem to contribute to accidental reactions.

Conclusion: A wide range of food products are causing accidental reactions in patients with food allergy. Eight different allergens not declared on the ingredient lists were detected in the culprit food products, all of which were representative of allergens regulated in the European Union. (*J Allergy Clin Immunol* 2018;■■■:■■■-■■■.)

Key words: Accidental allergic reaction, allergen management, allergen labeling, allergen intake, food allergy, food product, precautionary allergen labeling, reference dose, undeclared allergen

Food allergy is a serious health problem affecting 1% to 3% of the population.¹ For many patients with food allergy, these allergies are best managed by individualized avoidance of the allergenic food.

Legislation on food labeling is implemented in most regions of the world to help patients with a food allergy. In Europe the presence of 14 ingredients that can cause allergy or intolerance must be listed on food labels of prepackaged foods and be available for unpacked foods according to European Union (EU) Regulation 1169/2011.² However, allergens can accidentally end up in food as a result of contamination during transport, storage, or processing of food. For this, “may contain” precautionary allergen labeling (PAL) is often used in cases in which the absence of allergens cannot be guaranteed. Allergen management guidance lacks thresholds for applying a precautionary allergen statement. This has led to widespread use of PAL on prepackaged foods, even when the chance and expected level of contamination is very low. At the same time, several studies have shown that products without PAL contained undeclared allergens at detectable levels,^{3,4} presenting a potential health risk for the allergic population.⁵⁻⁸ In addition, precautionary statements vary widely in wording, adding to confusion for allergic patients about their meaning.⁸ Consequently, PAL has lost its credibility,

Abbreviations used

EU: European Union

LOD: Limit of detection

PAL: Precautionary allergen labeling

and allergic patients increasingly ignore the precautionary allergen statement.^{9,10}

In the present situation accidental allergic reactions to food occur frequently among allergic patients, including severe and fatal reactions.¹¹⁻¹⁵ The comparability between studies is limited because of different time frames and study populations.¹¹ In those studies, eating outside the home and prepackaged foods are both mentioned frequently as main causes together with patient factors, such as ignoring or missing the (precautionary allergen) label or not informing restaurant staff. However, a systematic study on the relationship between allergic reactions, foods, and the possible unintentional presence of allergens, such as noningredient allergens, is lacking.

Recently, we followed a well-defined adult patient cohort with food allergy ($n = 157$) for 1 year to study the frequency, severity, and causes of accidental reactions. The frequency was approximately 1 per person per year. Almost half of the patients reported unexpected reactions. Of those who reacted, food reactions occurred on average 2 times per year (range, 1-11).¹⁶ Here we focus on investigating the type of food products and allergenic protein sources causing these unexpected allergic reactions in the daily lives of this patient group. Products for which the unintended (ie, noningredient) presence of culprit allergens was suspected were analyzed for these allergens. The dose estimated to cause the allergic reaction in individual patients and the potential risk for the allergic population were analyzed.

METHODS

Study design and population

A longitudinal prospective cohort study was conducted by the University Medical Centre Utrecht and TNO, The Netherlands, to collect data on frequency, causes (both product and patient related), severity, and consequences of accidental allergic reactions. Recruitment of participants started in January 2012 and ended in July 2014.

Adult patients (age range, 18-70 years; mean age, 35.3 years; SD, 12.7 years) with a physician-confirmed diagnosed food allergy reported their accidental reactions through a secured Internet portal over 1 year. The physician-diagnosed food allergy was based on the patient's convincing history of allergic complaints to food and a positive skin prick test response and/or IgE level and/or positive food challenge test response. Dietary advice was given per food allergy. This advice was to avoid the allergen present as an ingredient in all cases, and advice for allergens on PAL statements was individualized. Patients who did not have a computer or Internet access and patients without the ability to read or write the Dutch language were excluded.

The outcome for the total study population on frequency, causes, severity, and consequences for the patient, such as medical intervention and sick leave, are described separately.¹⁶ The focus in the present article is on the food products that were assigned to the accidental allergic reactions by the patients and the allergenic protein sources involved.

All patients signed an informed consent form before inclusion. According to the local Medical Ethical Committee, the Medical Research Involving Human Subjects Act did not apply to the study, and therefore official approval by the Medical Ethical Research Committee was not necessary (protocol no. 11-309/c).

Data collection

At the start of the study, an online questionnaire was completed by the participants to determine demographic information (sex, age, education, and living situation [ie, living alone or with others]). Data on history of food allergy and severity of allergy and atopic diseases were collected in a structured interview combined with information from the participant's medical record. After the accidental reaction occurred, patients in the study filled out an online questionnaire and were instructed to provide the causal place, the estimated amount consumed, a sample of the original food product eaten, and/or the label of the product for verification.

Each reaction and submitted label were reviewed by the research team (consisting of a dietician, nurse specialist, allergy specialist, and researcher), determining whether the reaction was compatible with a food-induced allergic reaction and the patient's food allergy. The criteria used to assess this were time interval from ingestion of a food product to reaction (within 2 hours), type of reported symptom to confirm a food-induced allergic reaction and to exclude potential other causes (eg, insect sting, drug reaction, exercise induced, and idiopathic anaphylaxis), and a case-by-case review if the culprit food reported was likely to have caused the reaction in that patient (possible to contain an allergen matching the patient's allergen profile but also which other foods were consumed during the meal, how well the reaction could be assigned to a product, and patient history with the food product). Patients were contacted by telephone or E-mail for further clarification, if necessary.

Product samples were only analyzed if the unexpected reaction was suspected to be caused by noningredient allergens (ie, allergens that were not supposed to be present according to the ingredient declaration). This also included products in which the allergen could be unintentionally present, as indicated by a PAL statement. In cases in which the patient did not read the label or missed the allergen to be declared as an ingredient, the samples were not included in the analyses.

The selection of allergens for analysis was based on the food allergy profile of the individual patient, meaning that a product could be tested for multiple allergens. In principle, a commercial ELISA kit or PCR method was preferred for analysis and quantification. For those allergens for which a commercially available kit was not available, quantitative PCR methods developed by Triskelion BV (Zeist, The Netherlands) were used. Levels of allergen present in the food product were quantified and expressed in milligrams of protein of the allergenic food/kilogram of food product. Detailed information is provided in the [Methods](#) section and [Table E1](#) in this article's Online Repository at www.jacionline.org.

Estimated dose of protein consumed

The estimated dose of protein of the allergenic food (in milligrams) unintentionally present in and eaten through the culprit food was determined. Patients were asked to report consumed amount of food by using prescribed measures, such as portion, tablespoon, and teaspoon. For determination of the amount in grams, this was combined with the sizes reported in the handbook on standardized measures and weights.¹⁷ In cases in which the specific brand name was reported, the brand-specific portion size was used. For a product without a reference measure, the amount of a best representing product was used and checked with the Dutch National Food Consumption Survey.¹⁸ For example, a Seitan roll (vegetarian protein snack) was not indicated in the handbook. However, a sausage roll was indicated, and the reference amount of this product (70 g) was used. The amount consumed at the eating occasion was combined with the analytic results of the allergen concentration to estimate the intake of protein of the allergenic food for the particular reaction.

Quantifying the risk for the population with food allergy

The risk for the population with food allergy posed by the concentrations of culprit allergen or allergens present in each product was calculated by using quantitative probabilistic risk assessment, as described previously.^{19,20} Briefly, the intake distribution of protein of the allergenic food was determined by combining the consumption distribution of the food product and the established concentration of protein of the allergenic food. In a Monte Carlo simulation, allergen intake distribution was compared with the population

threshold dose distribution for the specific proteins of allergenic foods, as described by Taylor et al.²¹ Data sets available for peanut, milk, egg, hazelnut, and sesame are sufficient for population threshold distributions and subsequent risk assessments; for walnut, pecan nut, and celeriac, the data sets were insufficient for risk assessment.^{21,22}

The allergen risk assessment is performed with 3 parametric models (Weibull, log normal, and log logistic) for modeling the threshold doses into population threshold distributions for the respective allergic population. The outcome of all 3 models is included because there is no biological or statistical reason to prefer one over the other,²³ unless model fit disqualified one of these models.²¹ The risk is presented as the range of estimated risks, which means the lowest and highest risk over the models. Risk was expressed as the percentage of responders within the allergic population predicted to react per eating occasion of the described food products.

During curation of data of the Dutch National Food Consumption Survey for food allergen risk assessment, it was chosen to process composed complex dishes (eg, babi panggang and tikka masala) into their individual components. Hence a mixed dish was split into rice, meat, and vegetables. Because of this previous split, a risk assessment in composed complex dishes is not possible with the current food consumption database, and these products were excluded from further risk assessment.

RESULTS

Foods causing accidental allergic reactions

A total of 157 patients completed the study, and 73 reported 151 accidental allergic reactions during the 1-year follow-up (for details on the patient group, see [Table E2](#) in this article's Online Repository at www.jacionline.org).

Overall, a diverse range of food products was reported to cause accidental reactions ([Fig 1](#)). Patients could attribute their reaction to a specific product in 78% (118/151 reactions) of the reported cases. Most of these reactions (62/118 [53%]) were attributed to a small number of foods: bread (rolls), cookies, chocolates, meat and meat products, or fruits (almost equally distributed). The remaining 47% (56/118) was attributed to a wide range of other products, each reported once or twice and occasionally 3 to 5 times.

For 22% (33/151) of the reactions, it was difficult for patients to point to a specific product because of simultaneous consumption of multiple foods (combined meal).

Noningredient allergens present in foods

A total of 53 food samples were received from 32 patients for analysis. On checking the labels, 2 products contained an allergenic ingredient in the ingredient statement for which the patient was allergic, and these were not included for further analysis. Therefore, in total, 51 products from 31 patients fulfilled the criteria for further analysis. The samples received for analysis were the actual leftover product of what the patient had eaten in all but 2 cases. In 2 cases we obtained a sample from a new package (1 negative and 1 positive analytic outcome).

On average, 1.7 (SD, 1.0) products were sent in by 31 patients, varying from 1 to 5 products per patient. The majority (n = 21) of these patients had more than 1 accidental allergic reaction, although they did not send in a food sample at all incidences. The patient group sending food samples was largely comparable with the group that did not send products (see [Table E2](#) for details on patient data). However, the percentages of patients allergic to peanut (71%) or sesame seed (19%) and the patients with atopic dermatitis (74%) were significantly greater in the group sending food samples compared with the remaining patients (patients

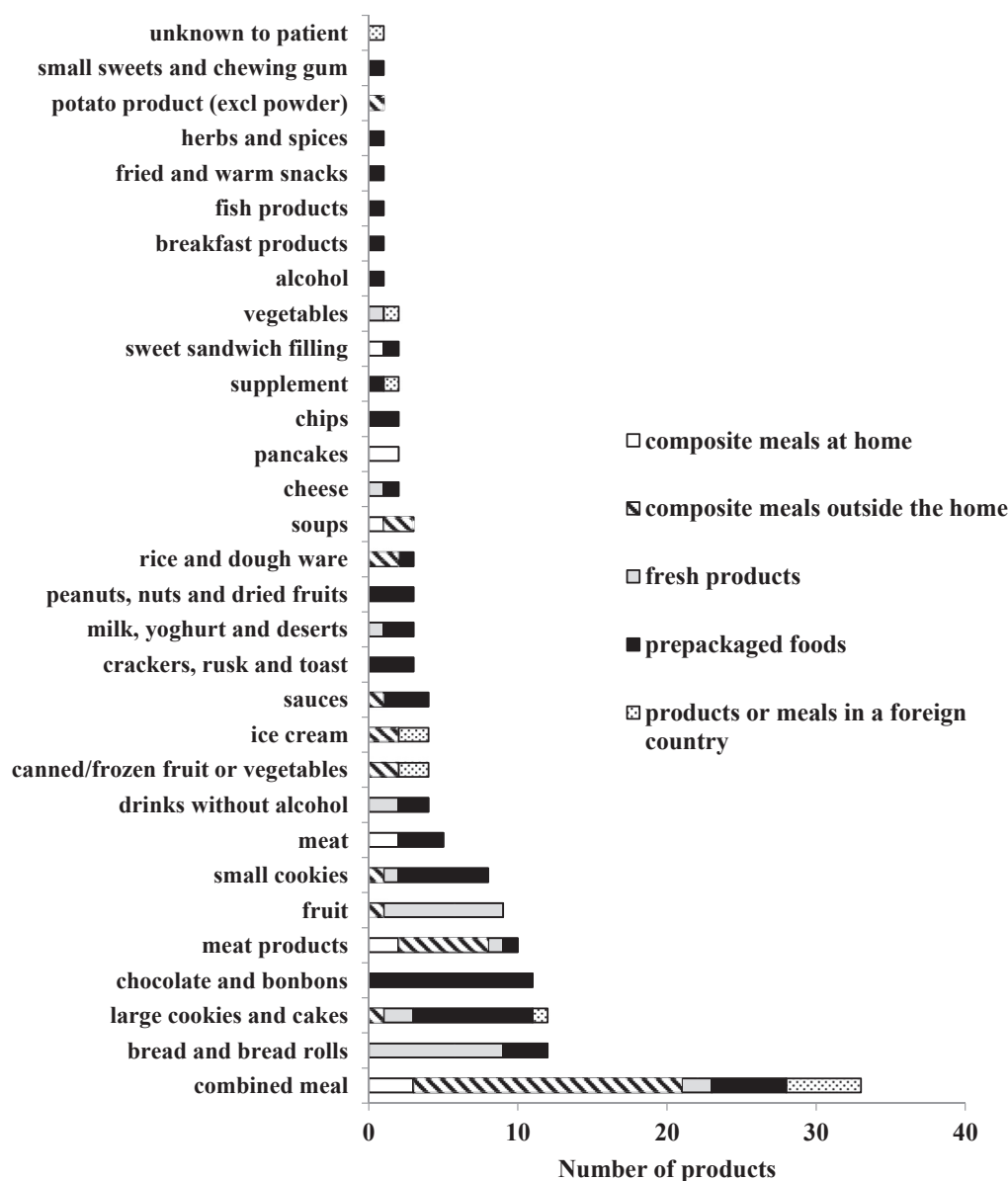


FIG 1. Food products reported by patients to cause an accidental allergic reaction (n = 151). Products reported were classified in 2 ways. First, products were placed into the food group that provided information on the type of food, such as bread and bread rolls, large cookies and cakes, and meat products, as shown. Second, in addition to that, products were designated in groups according to one of the causes for the reaction, such as prepackaged foods, composite meals outside the home, fresh products, products or meals in a foreign country, or composite meals at home. In 1 case the patient reacted in a foreign country and was not able to attribute the reaction to a specific product.

allergic to peanut, 41%; patients allergic to sesame, 5%; and patients with atopic dermatitis, 55%).

Most of the 51 products analyzed were from the category of prepackaged foods (67%), followed by composite meals eaten outside of the home (14%) or in the home (10%) and several fresh products (10%; Table I). Products available for analysis concerned mainly chocolates, bread and bread rolls, cookies (large and small), meat or meat products, and combined dishes (Fig 2). In total, 275 analyses were performed for 28 different allergens, ranging from 1 to 15 allergens per product. On average, 5.4 (SD, 3.4) allergen measurements per product were performed.

TABLE I. Food category origin of products in the total study and of products analyzed for noningredient allergens

	Total study ¹⁶	Products analyzed for noningredient allergens (n = 51)
Prepackaged foods	41%	67% (n = 34)
Composite meals outside the home	24%	14% (n = 7)
Fresh products	19%	10% (n = 5)
Products or meals in a foreign country	9%	0% (n = 0)*
Composite meals at home	7%	10% (n = 5)

*When reactions occurred in foreign countries, products were not sent in for analysis.

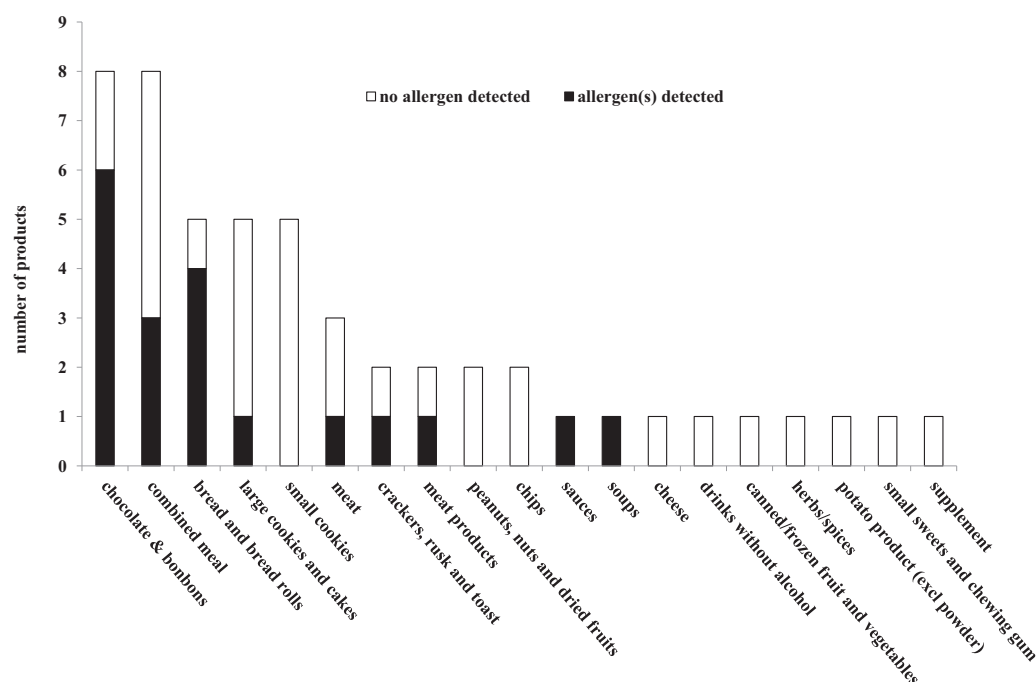


FIG 2. Detection of noningredient allergens in 51 food products causing an accidental allergic reaction. Analysis was performed for multiple allergens that were variable for each product. The selection of allergens for analysis was based on the food allergy profile of the individual patient. For products in which allergens were not detected, this means that multiple allergens could have been analyzed, although the test results were less than the lower LOD, indicating that the allergen concentration could be anything between and including zero and the LOD of the test (see the [Methods](#) section in this article's Online Repository).

Most of the 51 products were analyzed for hazelnut ($n = 38$), peanut ($n = 37$), and other nuts, such as almond ($n = 27$), walnut ($n = 25$), cashew nut ($n = 25$), pistachio ($n = 20$), and pecan nut ($n = 14$). Less frequently, samples were analyzed for milk ($n = 14$), egg ($n = 11$), and sesame ($n = 11$). A report on the allergen analysis results is provided in [Table E1](#).

In 37% (19/51) of the products analyzed, it was possible to detect 1 or more noningredient allergens that could be responsible for the allergic reaction. No culprit allergen was detected in 63% of the products ($n = 32$; [Fig 2](#)). This means that for those products, the test result was less than the limit of detection (LOD), indicating that the allergen concentration could be anything between and including zero and the LOD of the test (eg, the LOD of the ELISA for peanut is 0.25 ppm peanut protein/kg of product).

In total, 8 different noningredient allergens were detected ([Table II](#) and see [Table E2](#)); all were representative of the regulated allergens in the EU. Cow's milk ($n = 8$), peanut ($n = 6$), and hazelnut ($n = 5$) were detected most often. Allergen concentrations in the products varied over several orders of magnitude because peanut protein levels ranged from 4 to 5000 ppm, milk protein levels ranged from 6 to 4388 ppm, and sesame protein levels ranged from 3 to 1690 ppm.

One culprit allergen was detected in 12 of 19 products (bread rolls, chocolates, meat and meat products, soup, combined food dishes, and sauce). Two potentially culprit allergens were identified in 6 of 19 products (bread and bread rolls, chocolate, and bonbons). In 1 product (bread roll) 4 different potential culprit allergens were present.

PAL

Overall, 59% (30/51) of the products analyzed did not have PAL, whereas 41% (21/51) did. Use of PAL was distributed almost equally among products with or without noningredient allergens detected ([Fig 3](#)).

The 21 PAL statements (of 51 products) mentioned single allergens or combinations of particular allergens, including peanut, nuts (occasionally specified into hazelnut and almond), milk, egg, sesame, and/or soy. Most precautionary statements were for nuts (17/21 [81%] products with PAL for nuts), followed by 19% to 29% of the 21 products with PAL for peanut, sesame, milk, and/or soy, and 5% of the products warned against egg. Interestingly, all products in which peanut ($n = 6$) or egg ($n = 2$) was detected did not have PAL statements, whereas of the products with PAL for peanut, egg, and soy, none had any of these allergens detected. For other allergens, various situations were noticed: for example, milk was detected in 8 products, of which 3 with and 5 without a PAL for milk and 1 additional product with a PAL for the unintended presence of milk was negative for milk proteins.

Of 19 products in which 1 or more allergens were detected, 10 (53%) did not have a PAL statement, and in these cases the accidental reaction is caused by omission of a precautionary statement ([Table III](#)). On 9 (47%) of 19 products, a PAL statement was provided, and 1 or more culprit allergens were present, indicating that the accidental reaction is likely caused by patient's disregard for the PAL statement (patients 3, 4, 6, 7, and 8), although a role of an unknown food allergy of the patient (patient 2) or of an omission of a correct PAL statement (product J, patient

TABLE II. Concentrations of allergenic foods detected in patient-provided food products (n = 19)

Allergenic food	No.	Concentration of allergen (minimum–maximum [ppm])*
Cow's milk	8	6-4388
Peanut	6	4-5000
Sesame	2	3-1690
Hazelnut	5	1-126
Pecan nut	1	920
Walnut	2	40-159
Hen's egg	2	3-25
Celery	2	0.2-0.4

*Concentration in milligram of total protein of the allergenic food/kilogram of food product.

9) cannot be excluded. In this latter case product J, a nougat, carried a PAL statement for nuts and sulfites and was consumed by a patient allergic to sesame and fruits. Subsequent analysis demonstrated the presence of sesame.

Risk assessment: Intake of and percentage of allergic reactions to noningredient allergens

The estimated intake of protein of the allergenic food consumed at the eating occasion varied considerably, from 0.01 mg of celeriac protein up to 170 mg of peanut protein (Table III). Comparison of the allergens detected corresponded with the patients' known food allergen sensitization profile (highlighted in boldface in Table III). For peanut, hazelnut, sesame, cow's milk, and hen's egg, estimated amounts of protein consumed at the eating occasion and causing the unexpected allergic reaction were compared with allergen management reference doses expected to protect up to 99% of the allergic population, as proposed by Taylor et al (Table III).²¹ In 16 of 19 positive products, the estimated intake of 1 or more noningredient allergens exceeded the corresponding reference doses. In 2 of these products (products E and H), the intake of hazelnut protein was less than the corresponding reference dose. Yet both products E and H also contained another noningredient allergen for which the patient was allergic, and the intake exceeded the reference dose for those allergens, 0.4 mg of peanut protein (product E) and 0.4 mg of milk protein (product H), respectively. In a number of products, a culprit allergen was present for which a reference dose is not currently available (pecan, walnut, or celeriac). For all cases, either intake of at least 1 allergen exceeded the reference doses or a culprit allergen was present with a not yet established reference dose.

For all allergens for which we analyzed but that were not detected, we estimated a theoretical dose, which is the potential doses consumed by patients using their intake of the product and an allergen concentration at the LOD of the test (Table IV). Average consumption for products with or without allergens detected was comparable (61.7 ± 112 and 67.3 ± 124 g, respectively). The average theoretical dose that patients consumed for different allergens was very low and ranged from 0.1% to 15% of the reference dose. This is less than the lowest eliciting dose of responders in our database.²¹ We consider it unlikely that those low allergen doses were causing

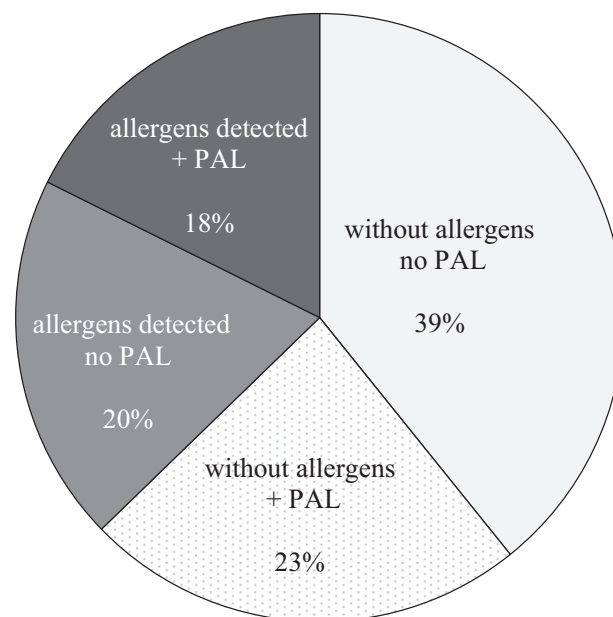


FIG 3. Distribution of PAL statements among products with or without noningredient allergens detected. The presence or absence of a PAL statement on the 51 food products received for analysis and either with or without noningredient allergens detected (also displayed in Fig 2) is shown. Note: A PAL statement could mention the potential presence of 1 or multiple allergens, and in 74% (14/19 products) the allergen warned for does not necessarily correlate with the undeclared ingredients detected (see also Table III).

the unexpected allergic reaction. The exception might be for products consumed at very high quantities, such as a pasta bolognese or spinach lasagna consumed (200-400 g). Here, the intake of a product with an allergen at the LOD could lead to a dose just below or at the reference dose. For example, 1 patient consumed pasta with multiple ingredients at 375 g, but no allergens were detected. The theoretical hazelnut dose in this case is 0.053 mg of hazelnut protein, which is less than the reference dose of 0.1 mg, but the minimal eliciting dose of the most sensitive subject in the database is 0.02 mg of hazelnut protein.

The patients reported multiple (up to 10 different) symptoms for accidental reactions to the 19 products in which allergens were detected. Reactions varied from mild subjective symptoms related to oral allergy syndrome (ie, itchy mouth, throat, or eyes) to several clear objective symptoms (ie, vomiting and blisters) in half of the group (Table V). Reported symptoms were scored according to the Müller classification and in most cases were mild or moderate, although in 3 cases the accidental reaction was classified as severe. In most of the patients, the nature of the accidental reactions was of lower severity than indicated by the historical Müller score. The amount of data is too limited for conclusion on a relationship between the dose and severity of reactions. Most patients used antihistamines, although none used an adrenaline injection, whereas most of them had this treatment prescribed.

The probability of objective allergic reactions among the allergic population could be estimated for 14 products (Table VI). For other products, insufficient threshold dose information was available for the allergenic food (pecan, celeriac, and walnut) or a food consumption distribution was lacking

TABLE III. Patient-reported intake and dose of noningredient allergens expressed in milligrams of protein of the allergenic food for each food product

Patient no. (sensitization profile)	Product description	PAL*	Intake (gram of product)†	Pea	Haz	Pec	Wal	Ses	Egg	Mi	Cel	Other allergens analyzed (ND)	
				Reference dose (mg of protein)									
				0.2	0.1			0.2	0.03	0.1			
Patient 1: Egg, Pea , Haz, Alm, Wal, Fru, Veg	A Bread	—	Whole portion (35 g)	0.6	ND		ND		0.09			Alm, Sf, Bp	
Patient 2: Egg, Pea , Haz, Alm, Wal, Crus, Cel, Fru, Veg	B Prebaked wheat baguette	Milk, nuts, sesame	Half portion (22.5 g)	ND	ND	ND	ND		ND	0.13	ND	Cas, Alm, Pis, B, C, M, Fi, Shf	
Patient 3: Pea , Haz, Alm, Wal, Ses, Cel , Fru, Veg	C Kaiser bread rolls‡	Sesame, milk, soya	Whole portion (40 g)	ND	ND	ND	ND	0.1§			0.01	Cas, Alm, Pis, Soy	
Patient 4: Haz, Wal , Veg	D Filled vegetarian pastry (Seitan roll)	—	Half portion (35 g)		0.8	ND	5.6		0.9	2.5			
	O Diary butter apple cakes (small)	Nuts	Whole portion (30 g)		0.2	ND	12						
	I Dark chocolate (peppermint)	Nuts	Pinch (1 g)		ND	0.9	ND						
Patient 5: Pea, Haz , Cas, Veg	E Christmas chocolate	—	Whole portion (6 g)	0.4	0.07§	ND						Cas, Pis, Lu	
Patient 6: Mi , Egg , Pea , Haz, Wal, Fru	F Dark chocolate (extra dark)	Nuts	Two thirds of a cube of chocolate bar (4.7 g)	ND	ND		ND		ND	3.3			
Patient 7: Mi	G Dark chocolate with orange pieces	Milk, hazelnut, almond, soya	Four cubes of chocolate bar (28 g)	ND	3.5		ND			123		Cas, Alm, Pis, Soy	
Patient 8: Mi, Haz , Wal	H Rice milk chocolate	Nuts	Small piece of chocolate (7 g)		0.01§		ND			0.4			
	P Steak tartar	—	Tablespoon (17 g)		ND		ND			0.7			
Patient 9: Ses , Fru	J Prepackaged nougat	Other nuts, sulfite	Approximately 25 g (25 g)					42					
Patient 10: Pea , Haz, Ses	K Grilled pork meat in sauce (babi panggang)	—	Half portion (175 g)	13	ND			ND				Pis, Soy	
Patient 11: Pea , Cas, Fru	L Pad Thai Kai Fried noodles	—	Two tablespoons (34 g)	170								Cas	
Patient 12: Pea , Haz, Alm, Ses, Cel, Fru, Veg	M Spinach lasagna	—	Half portion (200 g)	0.8	ND		ND	ND				Cas, Alm	
Patient 13: Mi , Egg , Pea , Veg	N Rice wafer with dark chocolate	Milk, nuts	Half portion (8.4 g)	ND					ND	1.5		Gl	
Patient 15: Pea , Soy, Lup, Veg	R Hamburger	—	Whole portion (70 g)	16	ND							Alm	
Patient 17: Pea , Haz, Alm, M, Soy, Cel , Fru, Veg	T Canned mushroom ragout	—	Table spoon (17 g)	ND	ND						0.01	Cas, Alm, Pis, Soy, M, Shf, Sh	
Patient 18: Mi , Egg , Pea , Haz, Wal, Cas, Fru	U Tomato soup	—	Two tablespoons (36 g)	ND	ND	ND	ND		ND	25		Cas, Alm, M	

ND indicates that intake was not determined because allergens were not detected with the test used, and allergen concentrations could be 0 or between 0 and the LOD of each test. Cel, Celeriac; Egg, hen's egg; Haz, hazelnut; Mi, cow's milk; Pea, peanut; Pec, pecan nut; Ses, sesame; Wal, walnut. Other allergens analyzed: Alm, almond; B, Brazil nut; Bp, bell pepper; C, candle nut; Cas, cashew nut; Crus, crustacean; Fi, fish; Fru, fruits; gl, gluten; Lu, lupin; M, macadamia nut; Pis, pistachio; Sf, stonefruit; Sh, shrimp; Shf, shellfish; Soy, soya; Veg, vegetables. Boldface indication of the allergen highlights if an allergen was detected and matched the patient allergy profile.

*In this case the PAL statement was the precautionary statement on the product or asking the food business selling a fresh product or composite meal outdoors for the possible unintended presence of allergens for specific product.

†Samples received for analysis were the actual leftover product of what the patient had eaten. For example, the patient had consumed part of a loaf of bread, a slice of a brownie cake, a portion of a whole meal, a chocolate, or a cookie from a package.

‡The product analyzed was from a new package of the same brand and the same store.

§The dose estimated is less than the reference dose.²¹

(complex meals with various individual components). The simulation estimated risk to be greater than 1% of the respective allergic user population for almost all allergen concentrations (maximum estimated risk, up to 64%). The risk for products with or without PAL statements was comparable.

Only 1 product (product H, a rice milk chocolate with hazelnut protein at 1.1 ppm) had a predicted risk to less than 1% of the population with hazelnut allergy for experiencing an objective allergic reaction (0% to 0.9%). However, product H also contained 53 ppm cow's milk protein, which posed an

TABLE IV. Estimated theoretical doses, such as potential doses consumed by patients for allergens present at the LOD of each test (n = 51 products)

Allergen	Taylor et al ²¹		Theoretical dose			
	Reference dose (mg)	Lowest eliciting dose* (mg)	No. of samples	Average theoretical doses (mg)	Percentage of reference dose	Range (minimum-maximum [mg])
Peanut	0.2	0.1	30	0.008	4.0%	0.0005-0.06
Milk	0.1	0.2 mg	14	0.007	6.7%	0.0026-0.01
Egg	0.03	0.014 mg	8	0.003	8.7%	0.0006-0.005
Hazelnut	0.1	0.019 mg	32	0.007	7.4%	0.0001-0.05
Soy Flour	1	11.1 mg	12	0.03	3.2%	0.0028-0.15
Wheat	1	2.6 mg	1	0.0008	0.1%	0.0008
Lupin	4	36.2 mg	3	0.01	0.2%	0.0022-0.02
Sesame	0.2	1.0 mg	9	0.03	15.0%	0.0023-0.13

*Lowest eliciting dose of responders with objective symptoms (in milligrams) in the database of Taylor et al.²¹

TABLE V. Patient-reported symptoms after consuming a product causing an accidental allergic reaction

Patient no.	Müller history	Product causing unexpected reaction	All symptoms reported*	Müller grade†	Medication
1	3	A Bread	Oral itch, itching eyes, swelling eyes, flush , pruritis, swelling of skin , abdominal pain	2	Antihistamines
2	4	B Prebaked wheat baguette	Feel of oral swelling, blisters , nausea, dyspnea	3	Antihistamines + inhaler
3	3	C Kaiser bread rolls	Feel of oral swelling, oral swelling, vomiting, diarrhea	2	Antihistamines
4	4	D Filled vegetarian pastry (Seitan roll)	Oral itch, feel of oral swelling, swelling oral, flush , pruritis, abdominal pain	2	Antihistamines
4	4	O Diary butter apple cakes (small)	Oral itch, feel of oral swelling, oral swelling, itching eyes, flush , pruritis	1	Antihistamines
4	4	I Dark chocolate (peppermint)	Oral itch, feel of oral swelling, oral swelling, abdominal pain	2	Antihistamines
5	3	E Christmas chocolate	Blisters, vomiting	2	Antihistamines
6	4	F Dark chocolate (extra dark)	Oral itch, feel of oral swelling, dysphagia, pruritis, cough	3	None
7	3	G Dark chocolate with orange pieces	Oral itch, feel of oral swelling, oral swelling	1	None
8	3	H Rice milk chocolate	Feel of oral swelling	0	None
8	3	P Steak tartar	Oral itch, feel of oral swelling, blisters	1	Antihistamines + prednisone
9	4	J Prepackaged nougat	Flush , pruritis, urticaria	1	Antihistamines
10	3	K Grilled pork meat in sauce (babi panggang)	Feel of oral swelling, abdominal pain	2	Antihistamines
11	3	L Pad Thai Kai Fried noodles	Feel of oral swelling	0	Antihistamines
12	3	M Spinach lasagna	Feel of oral swelling, dysphagia, abdominal pain, vomiting, diarrhea	2	Antihistamines
13	2	N Rice wafer with dark chocolate	Oral itch, feel of oral swelling	0	None
15	4	R Hamburger	Feel of oral swelling, dysphagia, blisters	1	Antihistamines + visit GP
17	2	T Canned mushroom ragout	Feel of oral swelling, hoarseness, cough	3	Antihistamines
18	4	U Tomato soup	Feel of oral swelling, dysphagia, abdominal pain	2	None

*Subjective and objective symptoms were reported. Objective symptoms are in boldface.

†Severity of symptoms was classified by using the Müller classification. Reactions with local oral allergy symptoms (Müller grade 0) were classified as being mild, reactions with symptoms from skin and mucous membranes (Müller grade 1) and/or the gastrointestinal tract (Müller grade 2) were classified as moderate, and reactions with respiratory symptoms (Müller grade 3), and/or cardiovascular symptoms (Müller grade 4) were classified as being severe. Müller history is the historically highest Müller reaction of patients.

estimated risk to 3.3% to 5.0% of the user population with milk allergy.

Seven of the 14 products posed a serious concern for public health, with a predicted risk to greater than 10% for the respective allergic user populations (maximum, 64%), and this was caused by the presence of milk, egg, peanut, or sesame. For 4 of these products (a filled vegetarian pastry, 2 dark chocolate products, and a soup), the presence of milk caused this high risk. In 1 of the products of serious concern, a second allergen of serious concern, was present in addition to the risk posed by milk. Product D also contained egg protein, which was predicted to cause objective allergic reactions

per eating occasion in 10% to 15% of the user population with egg allergy. Overall, products containing the unintentional presence of milk protein were most frequently detected (8/14 positively identified) and posed the highest percentage risk (up to 64%) to the respective allergic population.

DISCUSSION

To our knowledge, this is the first prospective study systematically investigating food products and levels of allergens causing accidental allergic reactions in the daily life of patients

TABLE VI. Estimated risk of the concentration of culprit allergens in food products for the population allergic to the allergenic food

Product description	Causal category	Information on PAL*	Culprit allergen (milligrams of protein of the allergenic food/kilogram of food product) and the predicted probability of an objective reaction in the allergic user population (range of estimated risk [%])†					
A Bread	Fresh	No PAL	Peanut, 16 ppm	4.9% to 6.9%	Hen's egg, 2.6 ppm	2.8% to 5.4%		
B Prebaked wheat baguette	Prepack	PAL on milk, nuts, and sesame	Cow's milk, 5.6 ppm	1.7% to 2.9%				
C Kaiser bread roll	Prepack	PAL on sesame, milk, soya	Sesame, 2.6 ppm	0.3% to 1.6%				
D Filled vegetarian pastry	Fresh	No PAL	Hazelnut, 25 ppm	4.0% to 9.1%	Hen's egg, 25 ppm	10.3% to 15.5%	Cow's milk, 70 ppm	12.6% to 18.3%
E Christmas chocolate	Prepack	No PAL	Peanut, 63 ppm	3.8% to 5.3%	Hazelnut, 12 ppm	0.5% to 2.8%		
F Dark chocolate (extra)	Prepack	PAL on nuts	Cow's milk, 702 ppm	18.6% to 22.4%				
G Dark chocolate with orange pieces	Prepack	PAL on milk, hazelnut, almond, soya	Hazelnut, 126 ppm	4.3% to 8.2%	Cow's milk, 4388 ppm	41.9% to 46.4%		
H Rice milk chocolate	Prepack	PAL on nuts	Hazelnut, 1.1 ppm	0.0% to 0.9%	Cow's milk, 53 ppm	3.3% to 5.0%		
J Prepacked nougat	Prepack	PAL on nuts, sulfite	Sesame, 1690 ppm	20.8% to 28.2%				
N Rice wafer with dark chocolate	Prepack	PAL on milk, nuts	Cow's milk, 176 ppm	9.0%				
O Dairy butter apple cakes (small)	Prepack	PAL on nuts	Hazelnut, 7 ppm	1.0% to 3.8%				
P Steak tartar	Prepack	No PAL	Cow's milk, 39 ppm	5.3% to 6.8%				
R Hamburger	Prepack	No PAL	Peanut, 233 ppm	25.6% to 29.5%				
U Tomato soup	Composite outdoors	No PAL	Cow's milk, 702 ppm	62.1% to 63.8%				

*In this case PAL statement was the precautionary statement on the product or asking the food business selling a fresh product or composite meal outdoors for the possible unintended presence of allergens in the specific product.

†Percentage risk estimated for total allergic user population with the minimum and maximum percentage risk estimated for the Weibull, log normal, and log logistic models and of female and male consumer populations. Among the risks estimated for 3 parametric models (Weibull, log normal, and log logistic), there is only a small difference. For peanut and milk, the WB threshold distributions did not fit and were excluded from the risk assessment.²¹

with food allergy. A wide range of different food products were reported to cause allergic reactions. Patients indicated a single product as being the cause of their reactions in 78% of reactions. Products received and analyzed showed up to 4 allergens not declared on the ingredients list being detected. Levels ranged from 1 to 5000 ppm (total protein of the allergenic food in consumed food product), with the highest levels detected for peanut, milk, and sesame. The unintended allergen intake by individual patients varied from 0.01 mg of celeriac protein up to 170 mg of peanut protein.

Milk was the most frequently detected allergen in the products causing an accidental reaction. Concentrations detected in this study posed a serious health concern for the population with milk allergy because 4 of 8 products estimated a high (10% to 25%) to extremely high (>40%) risk of objective allergic reactions. These results are important because milk is one of

the allergenic foods with widespread use in food production²⁴ and the prevalence of milk allergy is considerable, particularly in childhood.¹

A wide variety of food products was reported by patients as the cause of the accidental reaction, with some food categories appearing to stand out. Bread and bread rolls, cookies and cakes, chocolates and bonbons, and meat products were reported relatively often and in equal fashion (62/151 products). These food products were also typically identified in previous market surveys analyzing for the presence of noningredient allergens, such as milk in dark chocolate products,^{4,6} chocolates or cookies cross-contaminated with peanut and/or hazelnut,³ or milk or egg in bakery products²⁴⁻²⁶ and meat products.²⁷ However, these surveys contain a selection bias in products analyzed.²⁸ Also, a conclusion that these products are more dangerous or that other products are less dangerous cannot be made because the

observation in our study might well be due to a difference in frequency of consumption.

In the food consumption survey of The Netherlands, these products are typically consumed by Dutch adults during 40% and 93% of the reported consumption days (cookies/bonbons and bread, respectively). In contrast, soups, crisps, and snacks are less frequently consumed (range, 11% to 25%).¹⁸ It is possible that those products are therefore less frequently reported in our study. Because we are dependent on what products have been sent to us by patients, we have a selection of products for analysis. Therefore it is not possible to indicate whether a specific food category and allergen combination is more often involved in accidental reactions.

In addition to this, the present study setup cannot provide insight into the products and possible allergen levels that might have been consumed by patients without causing any accidental reaction. In that case a thorough food survey on all foods consumed by the patients and analyses of all foods would have been necessary.

The unintentional presence of milk, peanut, and other allergens detected in various product categories indicated that the risk for unintended presence and unexpected reactions is not a problem of particular food categories but should be regarded as a general problem. Food production is very complex, and food manufacturers apply allergen management to reduce the risk. However, the people preparing the food (patients, family/friends, and restaurants) contribute to the risk of food contamination. It would have provided valuable insight if it had been possible to trace back the origin of the unintentional presence of an allergen in each of the cases. This can be very difficult and a lengthy operation, such as in the case reported in which almond was substituted by peanut (reviewed by Walker et al²⁹).

A total of 51 products were analyzed for the presence of noningredient allergens, and detectable levels were found in 37% (19/51) of the food products. In 63% of samples, culprit allergenic substances were not detected, which could have various reasons that are discussed in no particular order because the relative contribution of each is unknown. Part of the reported accidental reactions took place during an eating occasion with multiple food products. Thus in some cases a patient might have wrongly attributed a reaction to a particular food product and provided the wrong food for analysis. Furthermore, most of our patients had multiple food allergies, and it could be that in some cases, the patients had a yet unknown allergy. The research team carefully checked the patient's allergy history and the reported information and contacted patients to limit receiving the wrong product or missing allergy information.

Also, products were analyzed for a large number of allergens. In some cases the culprit allergen might not have been equally distributed in the product or might have been present as a particle. Sesame, peanut, hazelnut, and other nuts are typically used as particulates in food processing, and accidental cross-contamination with particles can happen during processing. In one case a patient provided a picture of a dark chocolate product in which a big piece of walnut was clearly visible, although we expect that to be an extreme situation, and particles will normally be present in smaller pieces not easily visible for the allergic patient. However, in principle one small piece of peanut or nut can present a dose sufficiently high for inducing an allergic reaction.³⁰ Thus a sample provided to us might not have had the allergen particle present or it might have been present at a concentration

less than the LOD of the test. In many products with allergens absent or present at less than the LOD, normal consumption is too low, and we consider that it is unlikely that such a low dose would cause an allergic reaction. However, occasionally, products in which the allergen is close to the LOD of the test can be consumed in very high quantities (250–400 g), which might lead to a dose that is close to the lowest eliciting dose in allergic subjects published.²¹ A further problem is that current analytic approaches are not optimal for all scenarios; for example, protein recovery might have been affected by processing of the food.^{29,31,32} Despite the many possible causes for not detecting the targeted allergens in food products, we still found the presence of noningredient allergens in nearly 40% of the products analyzed.

The detected concentrations of noningredient allergens in products (just greater than the LOD up to 5000 ppm) led to an estimated intake of protein of the allergenic food that exceeded reference doses, as proposed by Taylor et al,²¹ in the majority of these accidental reactions and often at considerable excess. In some cases the intake of a culprit allergen was less than the reference dose, but another patient-specific culprit allergen was present and exceeded the reference dose or another culprit allergen was present for which a reference dose is not currently available. This study did not show the occurrence of accidental allergic reactions triggered by allergen intakes at or less than the reference dose. Unfortunately, a difficulty of our study is that it is not possible to confirm (or exclude) whether particular unintended allergens present are responsible for the reaction because our patients generally have multiple allergies, multiple allergens can be present in a product eaten, and we do not have the threshold dose for all allergies of the patients. For 5 accidental reactions, we had an individual threshold dose for one of the allergens in the products. In all cases the intake exceeded the minimal eliciting dose of the patient (results not shown). Using the comparison to the reference doses, we have insight into how doses causing an accidental reaction relate to what we know for the population allergic to the respective food.

As known from the literature, both patient negligence of a PAL statement on products and food producer negligence by not providing a PAL statement on products contribute to accidental reactions.^{6,8,11,33} In the present study accidental reactions were reported at an approximately similar rate to products with or without a PAL statement. Previously, several surveys investigated the risk of unintended allergens present and showed that this did not correlate to the presence (or absence) of a PAL statement.^{6,7,34} The products analyzed here show similar results. More importantly, sometimes the allergens detected did not match the allergens for which the precautionary statements were provided and could have contributed to the accidental reaction.

In conclusion, the food products reported by patients to cause accidental allergic reactions varied widely, with particular food products reported more often, likely because those products are in general frequently consumed. Patients send in products in which 8 different allergens not declared on the ingredient lists were detected. All were representatives of allergens regulated in the EU. Concentrations detected varied by several orders of magnitude, and in particular, the unintentional presence of milk was significant (in number of products, estimated intake, and risk). Both patient neglect of PAL statements and omission of using a PAL statement by food manufacturers contribute to accidental reactions. Overall, the

study shows that in the current situation choosing a safe food product is problematic for allergic patients, especially for the majority of multiple allergies.

Key messages

- A wide range of food products and different noningredient allergens were involved in accidental allergic reactions.
- In particular, the unintentional presence of milk in food products would warrant more attention both in allergen and allergy management.
- For the allergens detected, the estimated intake by patients was in almost all cases at doses of greater than the proposed reference dose for allergens.

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E1 Analysis of allergens in food products

Methods

Food samples received were analyzed for possible culprit allergens. The allergens for which each product was analyzed were selected based on the food allergy profile of the individual patient, which meant that a product could be tested for multiple allergens. In principle a commercial ELISA kit or PCR method was preferred for analysis and quantification. For those allergens without commercially available kits Q-PCR methods used in this study were developed within Triskelion BV. The levels of allergen present in the food product were quantified, and expressed in mg allergen protein/kg food product.

ELISA Procedures

All ELISA type of experiments were performed using commercially available test kits. For the quantitative analysis of the presence of sesame proteins in food samples, the BioKits Sesame Assay Kit (Cat. Nr. 902070X) was used. For the quantitative detection of wheat a kit measuring Gluten was used, the Gliadin R5 specific Neogen Veratox® assay (Cat. Nr. 8510). In order to detect mustard allergens, also a Neogen Veratox® assay kit was used (Cat. Nr. 8400). Hazelnut allergen and peanut allergen were detected using Neogen Veratox® assay kits (Cat. Nr. 8420 and 8430, respectively). For the quantitative detection of egg allergen, also a Neogen Veratox® assay kit was used (Cat.Nr. 8450). Finally, the Neogen Veratox® assay kit (cat.Nr. 8470) was used for the detection of total milk allergen.

Prior to perform any of these ELISA type of assays, the complete food sample available for analysis was homogenized using a Retsch Grindo-mixer (GM200).

Following homogenization, the preparation and protein extraction of samples was performed essentially according to the respective manual available for each individual ELISA assay kit. Also the complete ELISA test procedure itself was performed using the detailed description in the manuals of each individual ELISA assay kit.

Calibration curves for all ELISA assays were obtained using the reference samples as delivered with all respective ELISA kits.

In order to possibly allow quantification of any of the allergens present in a food sample within the dynamic range of the ELISA assay of interest, various dilutions of the sample extracts were

measured. These sample extract dilutions will also gain insight into the parallelism between the calibration curve and the data obtained from the sample, thereby allowing quantitative interpretation of the results. In addition, inhibitory components might be present in food matrices leading to underestimation of the quantity of allergen detected in the sample extract. This inhibitory effect can be overcome by further dilution of the sample extract as used in the ELISA assay. Finally, in order to elucidate possible false-negative results, spiking experiments were included for all samples in each type of ELISA. For this, reference samples included in the ELISA test kits, were added to the sample extracts prior to analysis.

For all ELISA type of tests used in this study, the dynamic range for quantification is between 2.5 and 25 ppm of the allergenic food component. As a consequence, the limit of quantification for the individual allergens is 2.5 ppm. Using extrapolation of the Optical Density values obtained for all calibration curves between 2.5 ppm and 0 ppm, a limit of detection of 1 ppm can be reached. It should be noted that users should not extrapolate below the lowest standard in the standard curve. As a consequence n.d. means allergens could not be detected and the actual concentration of the allergen could be 0 or between 0 and the LOD of each test.

Quantitative PCR (Q-PCR) Procedures

With the exception of Celery, all Q-PCR methods used in this study were developed within TNO Triskelion BV. For this, specific DNA sequences for several nuts were identified using NCBI databank in combination with the BLAST programs (Basic Local Alignment Search Tools). Primers and probes were designed using Primer Express software (Applied Biosystems). PCR primers and fluorogenic probes were purchased from Eurogentech. The TaqMan probes are labelled on the 5' end with the fluorescent FAM reporter dye and with the quencher dye 6 - carboxytetramethylrhodamine (TAMRA) attached to its 3' end. As a result of this procedure specific and unique primer-probe combinations were obtained for almond, pecan, walnut, cashew and macadamia. All individual nut-specific primer-probe combinations have been cross-validated with respect to specificity for the 5 relevant nut species. Using a comparable strategy also specific primer-probe combinations have been designed for soy and a group of crustaceans. For celery, a commercially available Q-PCR kit was purchased from Congen (Cat. Nr. S3105).

The specific primer-probe DNA sequences are listed below:

63

Almond	Sequence (5' to 3')
primer F	TGA TTG AAG GTG CCC CAA CAT
primer R	TCC GAA TGA TAG AGA CCA ACC AAA AT
Probe	6-FAM- GTA GCA CAC CAA ATA GTA CAA TA -TAMRA

64

Pecan	Sequence (5' to 3')
primer F	CCG CGA AGA GAA AGC AGA G
primer R	TCA TGT CTC GAC CTG AGT CC
Probe	6-FAM- ATT GTG CCT CTG TTG CTC CTC TTC CCG -TAMRA

65

Walnut	Sequence (5' to 3')
primer F	CCG GTT GAC TCC GTA ATT ATT TAT C
primer R	AAA GAG TCA AAT CAG TGA GAA ACA TAA CA
Probe	6-FAM- TCT GCT CTT TTC TTT TCG TTA GCG GTT TCA A TAMRA

66

Cashew	Sequence (5' to 3')
primer F	GGT GGA AGC CTG GGA TCC TA
primer R	GGT ATG CCT AAC CAA GGC AAC A
Probe	6-FAM- CCA TGA GCA ATT CCG ATG CGC TG -TAMRA

67

Macadamia	Sequence (5' to 3')
primer F	GGT TAA AGC ACG TTT GTC GAA GA
primer R	AAG CAG CAG GTT CTC GTT TCC
Probe	6-FAM- CAT CCC GTC GTC TTC GTT TCA TCC G -TAMRA

68

Crustaceans	Sequence (5' to 3')
primer F	TAA AGT CTG GCC TGC CCA
primer R	GCT TTA TAG GGT CTT ATC GT
Probe	6-FAM- CTA ATG ATT ATG CTA CCT TCG CA-TAMRA

69

70

Soy	Sequence (5' to 3')
primer F	TGG AGC AAG TGA AGG CTT CTA TG
primer R	CTT AAC ATC AGG CTT CTT AAC CTC TTC T
Probe	6-FAM- AAA ATG GTG TTC TCA CAG TTA CCG TTC CCA TAMRA

71

72 Prior to performing any of these Q-PCR type of assays, the complete food sample available for
73 analysis was homogenized using a Retsch Grindo-mixer (GM200).

74 Following homogenization, DNA extraction was performed using a TNE buffer including Guanidine
75 HCl and Proteinase K. Next to every set of samples also a water blank DNA isolation was performed.
76 Following DNA extraction, further purification was performed using a chloroform extraction step,
77 followed by specific binding of DNA to Wizard™ resin. In a final step the DNA was washed with 2-
78 Propanol and eluted from the Promega Wizard™ minicolumn with sterilized H₂O. All DNA extracts
79 are stored below -18°C.

80 All Q-PCR experiments were performed using an ABI Prism 7500 or a Roche Light Cycler 480.

81 Each Q-PCR experiment was started with a cleaning and denaturation step, followed by 40 repetitive
82 sequences of amplification according to the next scheme.

Step	Stage	T°C	Time (sec)	Data collection	Cycles
1	UNG	50°C	120"	no	1x
2	Initial denaturation	95°C	600"	no	1x
3	Amplification Denaturation	95°C	15"	no	40x
4	Annealing & Extension	60°C	60"	yes	

83

84 In all Q-PCR experiments, positive and negative control samples were included. Besides water blank,
85 the negative extraction control sample was included. Further, for all different allergens, positive
86 control DNA was obtained from the raw materials of interest. These positive control samples were
87 also used as calibrators for the semi-quantification of the respective allergens in the samples. For

88 this, the so-called cycle-threshold (Ct) value obtained in the Q-PCR for the allergen DNA of interest is
89 compared with the value obtained for the sample.

90 For all allergens, a limit of detection of around 1 ppm of food allergen was found.

91 In order to allow quantification of any of the allergens present in a food sample, various dilutions of
92 the sample extracts were measured. Also, as mentioned above for ELISA type of assays, sample
93 extract dilution might reveal the possible presence of inhibitory components in de the food matrix.
94 Finally, in order to elucidate possible false-negative results, spiking experiments were included for all
95 samples in each type of Q-PCR.

96

97 Results of the analysis are presented in Table E1.

1 **Table E1 All non-ingredient allergens analysed in the food products with culprit allergens present (mg total protein of the allergenic food/kg food**
2 **product); n=19.**

	Product description	PAL	peanut	hazelnut	almond	pecan nut	walnut	cashew nut	Brasil nut	lupine	soya	sesame	hen's egg	cow's milk	macadamia	fish	shell fish	celeriace	gluten	mustard
A	Bread	-	15.8	LD	LD		LD						2.65							
B	Prebaked wheat baguette	milk, nuts sesame	LD	LD	LD	LD	LD	LD	LD				LD	5.6	LD	LD	LD	LD		
C	Kaiser bread rolls	sesame, milk, soya	LD	LD	LD	LD	LD	LD				2.6						0.2		
D	Filled vegetarian pastry (Seitan roll)	-		24.5		LD	159						25.2	70.2						
E	Christmas chocolate	-	62.5	12.5		LD		LD		LD										
F	Dark chocolate (extra dark)	nuts	LD	LD			LD						LD	702						
G	Dark chocolate with orange pieces	milk, hazelnut, almond, soya	LD	126	LD		LD	LD			LD			4388						
H	Rice milk chocolate	nuts		1.1			LD							52.7						
I	Dark chocolate (peppermint)	nuts		LD		920	LD													
J	Prepackaged nougat	other nuts, sulfite										1690								
K	Grilled pork meat in sauce (babi pangang)	-	7.5	LD							LD									
L	Phad Thai Kai Fried noodles	-	5000					LD												
M	Spinach lasagne	-	3.8	LD	LD		LD	LD												
N	Rice wafer with dark chocolate	milk, nuts	LD										LD	176					LD	
O	Diary butter apple cakes (small)	nuts		7		LD	39.8													
P	Steak tartar	-		LD			LD							38.6						
R	Hamburger	-	233		LD															
T	Canned mushroom ragout	-	LD	LD	LD			LD			LD				LD		LD	0.4		
U	Tomato soup	-	LD	LD	LD	LD	LD	LD					LD	702	LD					

additional allergens analyzed in other products received : candle nut or kemirint; split pea; stonefruit; bell pepper; poppy seed; shr: shrimp.
LD = below limit of Detection

1 **Online Repository to JACI-D-17-00699 BlomWM et al Accidental allergic reactions caused**
2 **by multiple products and non-ingredient allergens present**

3 **Patient characteristics**

4 **Table E2 Demographic and illness-related data for the patient group**

Demographics and illness related data	Total patient group n=157	Patient group not sending in products for analysis n=125	Patient group sending in products for analysis n=32
Gender Female	116 (74%)	89 (71%)	27 (87%)
Age Mean age, in years (SD, min-max)	35.3 (sd 12.7; range 18-70)	36.0 (sd 13.2; range 18-70)	32.7 (sd 10.3, range 18-52)
Food allergy for Hazelnut Other nuts Peanut Milk Egg Soybean Sesame Celery Crustaceans Fish Lupin Mollusc Mustard Other allergens, not belonging to the major allergens*: - Fruit - Vegetables - Other food allergies	87 (55%) 75 (48%) 73 (47%) 29 (19%) 25 (16%) 17(11%) 12 (8%) 12 (8%) 6 (4%) 5 (3%) 4 (3%) 2 (1%) 0 96 (61%) 50 (32%) 11 (7%)	69 (55%) 55 (44%) 51 (41%) 20 (16%) 17 (14%) 14 (11%) 6 (5%) 8 (6%) 4 (3%) 5 (3%) 3 (2%) 2 (2%) 0 79 (63%) 37 (30%) 11 (9%)	18 (58%) 19 (61%) 22 (71%)# 9 (29%) 8 (26%) 3 (10%) 6 (19%)# 4 (13%) 2 (7%) 0 1 (3%) 0 0 17 (55%) 13 (42%) 0
Number of allergens Mean number of allergies*	4.9 (sd 3.1; range 1-17)	4.6 (sd 2.9; range 1-14)	6.5 (sd 3.3, range 2-17)\$
Years of having food allergy Years of having food allergy (SD, min-max) Missing n=3	n=154 15.1 (12.7, 0-54)	N=122 (3 missing) 14.2 (sd 13.2; range 0-54)	18.5 (sd 10.2; range 0-46)\$
Severity Mild (Müller 0) Moderate (Müller 1-2) Severe (Müller 3-4)	7 (5%) 36 (23%) 114 (73%)	7 (6%) 31 (15%) 87 (70%)	0 5 (16%) 26 (84%)
Prescribed medication (more answers possible) in case of unexpected reactions Oral antihistamines - Daily antihistamines - Antihistamines on	128 (82%) - 49 (31%) - 78 (50%)	109 (72%) - 46 (31%) - 71 (47%)	26 (84%) - 12 (39%) - 17 (55%)

demand			
Adrenaline auto injector	110 (70%)	108 (72%)	23 (74%)
Oral corticosteroids	89 (57%)	83 (55%)	18 (58%)
Inhaler	33 (21%)	26 (17%)	13 (42%)#
No emergency medication	4 (3%)	18 (12%)	2 (6%)
Atopic comorbidities			
Atopic dermatitis	92 (59%)	69 (55%)	23 (74%)#
Asthma	84 (54%)	64 (51%)	19 (61%)
Pollinosis	120 (76%)	95 (76%)	25 (81%)

* The number of food allergies for fruit, vegetables or other allergens than those of the major groups was each taken as 1

Significant difference with the chi-square tests between the patient group sending in food samples and not sending in food samples $p < 0.05$

\$# Significant difference with the t- tests between the patient group sending in food samples and not sending in food samples $p < 0.05$

Data on the patients were analyzed with IBM SPSS statistics 22 as described in Michelsen et al (submitted for publication). Briefly, baseline characteristics were achieved by calculating the frequencies or means and ranges of variables. Disease characteristics of food allergic patients providing a food sample were compared with the remaining group of patients using the chi-square tests (#) or t-test (\$). All outcome parameters were reported by descriptive statistics.

E1. Michelsen-Huisman, A.D.; Os-Medendorp, H.; Blom, W.M.; Versluis, A.; Castenmiller, J.J.M.; Noteborn, H.P.J.M.; Kruizinga, A.G.; Houben GF; Knuls. AC. Prepackaged foods are the most frequent cause of accidental allergic reactions which are generally moderate in severity. *Manuscript submitted for Publication.*