

Threshold dose distributions for 5 major allergenic foods in children

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Background: For most allergenic foods, insufficient threshold dose information within the population restricts the advice on levels of unintended allergenic foods which should trigger precautionary labeling on prepackaged foods.

Objective: We wanted to derive threshold dose distributions for major allergenic foods and to elaborate the protein doses at which a proportion of the allergic population is likely to respond.

Methods: For 7 allergenic foods double-blind, placebo-controlled food challenges (DBPCFCs) with a positive outcome for allergic reactions were selected from the clinical database of children routinely tested to diagnose food allergy at the University Medical Center Groningen. For each allergen 2 population threshold distributions were determined with the individual minimal eliciting dose and the preceding dose of each DBPCFC for objective symptoms and any symptom (either subjective or objective).

Results: Individual positive DBPCFCs were available for peanut ($n = 135$), cow's milk ($n = 93$), hen's egg ($n = 53$), hazelnut ($n = 28$), and cashew nut ($n = 31$). Fewer children were challenged with soy ($n = 10$) or walnut ($n = 13$). Threshold dose distributions showed a good statistical and visual fit. The protein dose at which 5% of the allergic population is likely to respond with objective reactions was 1.6 mg for peanut, 1.1 mg for cow's milk, 1.5 mg for hen's egg, 7.4 mg for cashew nut, and 0.29 mg for hazelnut. Thresholds for any symptom were on average 2 to 6 times lower than for objective symptoms. The 95% upper and lower confidence intervals of the threshold distributions were overlapping. The peanut threshold distribution on objective

symptoms was similar to the distribution of another European center.

Conclusions: Threshold distribution curves and eliciting doses are a powerful tool to compare different allergenic foods and for informing policy on precautionary labeling. (*J Allergy Clin Immunol* 2012;■■■:■■■-■■■.)

Key words: Allergenic foods, threshold dose distributions, children, eliciting dose

The only option for persons with food allergies to manage their food allergy is the strict avoidance of allergenic food. An increasing number of studies are being published on oral tolerance protocols for peanut, milk, egg, and wheat, although these procedures have not yet become standard practice. Most of the population with food allergy thus still relies on rigorous elimination of culprit allergenic foods from their diet. Legislation in many regions of the world, for instance, the European Union laid down in European Union directives 2003/89/EC and 2006/42/EC, prescribe the labeling of food products for several major allergenic foods or products derived from that allergen when added as ingredients to food. In addition, many food producers have incorporated allergen-auditing programs and voluntarily warn the allergic consumer to the potential presence of allergens by using precautionary labeling of food products, for example, "may contain xxx." However, despite this, recent studies show that a precautionary warning on products is not always valuable to allergic consumers. Surveys of commercially available products show that the presence or absence of a precautionary warning corresponds poorly with the actual presence of the allergen in the product,^{1,2} which can lead to potentially dangerous situations.^{3,4}

A recent study in Canada showed that approximately 17% of persons with food allergies experiencing an accidental exposure attributed this to products with unintentional cross-contamination during manufacturing and no precautionary statement on the label.⁴ Conversely, many products do not contain the allergen to which the precautionary warning on the label refers. As a consequence, consumers increasingly seem to ignore precautionary labels.⁵ To improve this situation, quantitative guidance is needed with advice on levels of unintended allergens (also called action levels) to reduce the number of foods having precautionary labeling. Several initiatives have been created by both food industry and enforcement bodies with the involvement of various stakeholders to improve allergen management and to introduce more uniform and transparent risk information.⁶⁻¹⁰ One of the ultimate goals may be to establish internationally harmonized guidance that includes action levels for labeling unintended allergens.

Previously, a probabilistic risk assessment method for use in population risk assessment has been developed and successfully applied.^{1,3,6,11} The risk assessment method quantifies the number of allergic responders that can be expected when a particular product contains a specified amount of a certain allergen, because it

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge
 ED: Eliciting dose
 LOAEL: Lowest observed adverse effect level
 NOAEL: No observed adverse effect level

combines the consumption patterns of foods in a defined population with the sensitivity of a population for an allergen.¹

The population of young adults may be especially vulnerable, because they represent most of the fatalities in most registries for food-induced anaphylaxis.^{12,13} A large proportion of children allergic to milk, soy, and egg are known to outgrow their allergy.¹⁴⁻¹⁶ In contrast, adults with hazelnut allergy seem to respond with more severe reactions than children.¹⁷ In addition, most adults allergic to cow's milk acquired the allergy at adult age, and reported symptoms which were more severe than symptoms seen in children.^{16,18} Recently, it was shown in a cross-sectional study in a pediatric population that the eliciting dose of peanut (determined by the first symptom occurring during the double-blind, placebo-controlled food challenge [DBPCFC], either subjective or objective) may decrease with increasing age (up to 18 years).¹⁹ However, it is largely unknown whether and to what extent threshold dose distributions are influenced by age. Because several aspects of food allergy seem to vary with age, it can be reasoned that there will be an effect on the eliciting dose (ED) as well. To address these issues and to make more threshold information for the pediatric population available to the community, including the food industry, we performed a structured retrospective study to retrieve data on individual minimal EDs for allergic reactions to several major allergenic foods in the pediatric outpatient population of the University Medical Center Groningen, The Netherlands, which were performed as a routine clinical procedure for the diagnosis of food allergy. These data were used to derive threshold distributions that were used to elaborate ED values.

METHODS**Study population and database review**

The study population consisted of children in whom DBPCFCs with cow's milk, soy, hen's egg, peanut, hazelnut, walnut, or cashew nut were performed at University Medical Center Groningen between July 2001 and December 2009. Children were referred from primary and secondary care centers because of suspected food allergy. The only exclusion criterion was refusal by parents or the child to undergo the test, which was the case in >2% of patients. Specifically, history of severe reactions was not an exclusion criterion for DBPCFCs. Information on sex, age, the suspected food, baseline symptoms, allergen-specific IgE and/or a positive skin prick test, and allergic symptoms occurring after positive challenge sessions were obtained by retrospective review of the electronic database. This study was exempt from medical ethical approval, because DBPCFCs in children were performed as a routine diagnostic test. All parents consented with the performance of DBPCFCs.

DBPCFCs

DBPCFCs were performed as previously described.²⁰ Clinical symptoms and overall condition had to be stable, and children were instructed to discontinue antihistamines 72 hours before DBPCFC if possible. Before the DBPCFC, the food in question was avoided by the child for at least 6 weeks. Placebo and active challenges were administered in a random order on

separate days with at least 2 weeks' interval in between. Randomization was determined by computer. Recipes for the test foods were prepared for each challenge session individually. For all foods (except walnut) validation of adequate blinding of the test materials was achieved by sensory testing in a dedicated food laboratory.^{20,21}

Incremental scale

The allergenic food was administered in a 4- to 6-step incremental design in which progressively greater quantities of the same allergenic food were administered.²² Pasteurized cow's or ultrapasteurized soy milk, cooked egg, roasted peanuts, roasted cashew nuts, unroasted hazelnuts, or roasted walnuts were used. The incremental scale and total challenge dose used are shown in Table I. The incremental scale was achieved by varying the volume of the test food. Time interval between 2 challenge doses was 30 minutes in almost all cases.

Documentation of symptoms and assessment of challenge outcome

Symptoms were classified as subjective or objective, and immediate (ie, within <2 hours if after the last challenge dose) or late onset (2 to 48 hours after the last challenge dose).²² Challenge sessions in which children consumed <75% of the intended challenge dose in absence of symptoms were considered invalid. The challenge was discontinued when objective allergic symptoms occurred or when subjective allergic symptoms occurred twice on 2 successive administrations of the challenge material.

Subjective symptoms that were noted were itching of the oral cavity, itching of pharynx, abdominal pain, nausea, dizziness, or generalized pruritus. Objective reactions included urticaria, diarrhea, dyspnea, vomiting, lip swelling, rhinoconjunctivitis, and bronchoconstriction. A decrease in peak flow, decrease in heart rate, or anaphylactic shock was not reported. Challenge sessions and total challenge outcome were assessed according to the criteria as previously described.²²

Threshold distributions

DBPCFCs with a positive outcome (allergy confirmed) were analyzed to identify individual lowest observed adverse effect levels (LOAELs) and no observed adverse effect levels (NOAELs), that is, the threshold dose for an allergic reaction (the minimum eliciting dose in an individual) and the preceding dose, respectively, for both *objective* and *any* symptoms. The latter reflects the first reaction to a dose observed, irrespective of the type of reaction. The allergic reactions in the DBPCFC were classified as subjective and/or objective. If a subject reacted while consuming a dose, the LOAEL was set at the dose actually consumed. In patients for whom repeated DBPCFC procedures with the same food were reported in the database, only the results of the first diagnostic session were used for analysis.

For each allergen a population threshold distribution was determined with LOAELs and NOAELs expressed as discrete doses in milligram of total protein of the allergenic food. For each subject, the true threshold lies, by definition, between the NOAEL and LOAEL doses.

Individual thresholds were therefore analyzed with the interval-censoring survival analysis approach as described by Taylor et al.²³ Persons reacting to the first challenge dose were treated as left censored, whereas persons failing to respond to the uppermost challenge dose were treated as right censored. In cases when the challenge was stopped because subjective symptoms occurred on 2 successive administrations, the NOAEL for objective symptoms was set at the last consumed dose and the LOAEL was right censored. Similarly, this approach was done for any symptom; thus, only left censoring takes place here for situations in which the subject immediately responded to the first challenge dose. For each allergen a number of left- and right-censoring cases occurred (see Table II). Data sets were considered of higher quality if more individual data points were interval censored.

The NOAEL and LOAEL data for objective symptoms or any symptoms were fitted into the threshold probability distribution models for each allergen separately. Data analyses and modeling were performed in SAS v9.1 (SAS Research Institute, Cary, NC) with the use of the LIFEREG as previously

TABLE I. Challenge doses in milligram of protein used in DBPCFCs

Matrix	Peanut	Hazelnut	Walnut	Cashew nut	Cow's milk/soy*†	Egg*†
	Baked cookies	Baked cookies	Baked cookies	Baked cookies	Milk	Cooked in pancake, custard, or minced meat
Protein content (%)‡	25	14	15	19	3.5	12.6
Mucosal	0.2	0.2	0.2	0.2	0.2	0.2
Dose 1	1.7	1.7	1.8	2.3	1.8	1.8
Dose 2	3.5	3.5	3.5	4.8	3.5	3.5
Dose 3	14	14	14	19	14	14
Dose 4	70	70	71	95	70	70
Dose 5	139	120	161	163	350	350
Dose 6	351	350	350	475	1750	1750
Total	579	559	601	759	2190	2190
Comparable to	~5-7 peanut kernels	~4 small hazelnuts	~1 walnut	~5.5 cashew nut	63 mL milk	~1/3 egg

*A complete description of all matrices can be found in Vlieg-Boerstra et al.^{20,21,38}

†A variety of validated recipes were used for milk, soy, and egg challenges.

‡The protein content of the allergenic food was taken from the Dutch Food Composition Database NEVO (<http://nevo-online.rivm.nl/>) to calculate the protein content of the doses administered.

TABLE II. Number of positive DBPCFCs analyzed for the individual NOAELs and LOAELs for objective and any effects

Allergen	Positive challenges				No. of subjects with symptoms (right censored; left censored)		
	No. of patients	No. per sex (M/F)	Age range (y)	Median age (y)	Any symptoms	Objective symptoms	Subjective symptoms
Cashew nut	31	16/15	3-18	8.3	0;1	16;1	1;3
Hazelnut	28	16/12	3-17	6.5	0;4	11;2	3;4
Hen's egg	53	36/17	0.75-15	4.5	0;3	13;2	18;2
Cow's milk	93	56/37	0.5-18	2.4	0;13	13;7	37;10
Peanut	135	82/53	2-18	6.9	0;10	50;5	12;7
Soy milk	10	8/2	2-15	10.0	0;4	7;1	3;3
Walnut	13	5/8	5-17	8.8	0;0	0;9	0;1

F, Female; M, male.

described.^{1,11,23} The data were fitted as actual, LogLogistic, LogNormal, or Weibull distributions. There is no apparent biological or mathematical basis for choosing one of these distributions; thus, all 3 models are used to fit the data. A visual examination was done to decide on the overall goodness of the fit. If the data set was distributed along the probability curve, it was considered suitable for further analysis.

Elaboration of EDs

A statistical analysis of the parametric models was done to determine the model with the best statistical fit (as determined by the log likelihood) along with the visual examination.²⁴ The model with the best overall statistical fit was selected for deriving EDs (ED₁, ED₅, ED₁₀, and ED₅₀), which are the doses predicted to elicit allergic reactions in 1%, 5%, 10%, and 50% of the allergic pediatric population, respectively. In the present study the Weibull distribution showed the best statistical and visual fits of the data for all data sets.

RESULTS

Study population

The results of 363 individual positive DBPCFCs were included in this study on the basis of completeness of information and a positive response to the tested allergen (Table II). The population was between 2 and 18 years of age, with a trend toward younger children for hen's egg and cow's milk allergies, consistent with the nature of egg and milk allergy. Most DBPCFCs were available for peanut, cow's milk, and hen's egg and a reasonable number of DBPCFCs for hazelnut and cashew nut. Fewer children were challenged with soy or walnut.

TABLE III. Percentage of subjects with subjective, objective, or both type of symptoms at the threshold dose for each allergenic food

First reaction	Allergen				
	Cashew nut	Hazelnut	Peanut	Milk	Egg
Subjective symptoms (A)	52	50	45	13	32
Objective symptoms (B)	10	11	10	39	34
Concurrent subjective and objective symptoms (C)	39	39	46	48	34
Total of subjective symptoms (A + C)	91	89	91	61	66
Total of objective symptoms (B + C)	49	50	56	87	68
Any symptom	100	100	100	100	100

Values are percentage of subjects in the tested population.

In most of the DBPCFCs, subjective symptoms were observed during the challenge (Table III), either preceding or concurrently with objective symptoms. Exclusively objective symptoms were observed in only a small part of the group. In the population with milk and egg allergies a smaller proportion of the population reported subjective symptoms, 61% and 66%, respectively, which is probably because younger children have greater difficulty reporting subjective symptoms. We therefore used the fitted threshold distribution for objective symptoms and any symptom, either subjective or objective, to ascertain the EDs for each allergenic food.

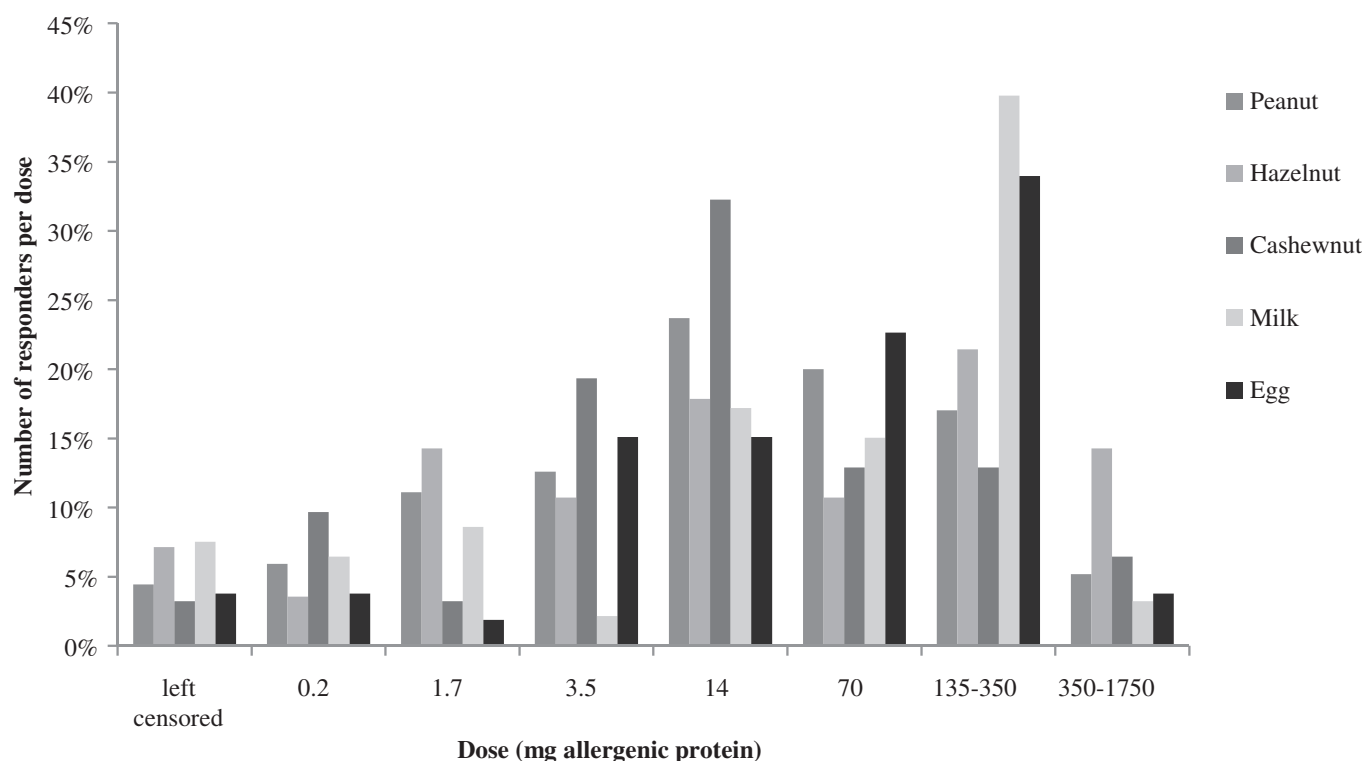


FIG 1. Distribution of individual thresholds on the range of doses for each allergenic food. The percentage of responders within the population with peanut, hazelnut, cashew nut, cow's milk, and hen's egg allergies is shown. Details on the number of persons and the exact dose per allergenic food are presented in [Tables I and II](#).

Threshold distributions

For cashew nut, hazelnut, hen's egg, cow's milk, and peanut the data sets were of good quality, and the 3 parametric models showed a good statistical fit. Visual examination showed that the data sets of these 5 allergenic foods were distributed along the curve and that for each dose a number of responders was observed ([Fig 1](#)). The log likelihood of the LogLogistic, LogNormal, and Weibull distributions was not very different, and the Weibull distribution, which gave the best fit for these allergenic foods, is shown ([Fig 2](#)).

Because the data sets for persons with walnut allergy or soy allergy appeared too small to fit into distribution models and visual examination showed that the data retrieved covered only a part of the threshold distribution curve, these NOAEL and LOAEL data are presented in [Table IV](#). In 4 of the 13 subjects with walnut allergy and 3 of 10 subjects with soy allergy objective symptoms were recorded, which is insufficient for statistical dose-distribution modeling for objective symptoms.

For cashew nut, hazelnut, hen's egg, cow's milk, and peanut the statistical Weibull model was used to estimate EDs (ED_1 , ED_5 , ED_{10} and ED_{50}) of the allergic pediatric population ([Table V](#)). The ED_5 for objective symptoms was lowest for hazelnut (0.29 mg hazelnut protein) and highest for cashew (7.4 mg cashew nut protein). The ED_5 s for objective symptoms in the population with milk allergy, egg allergy, and peanut allergy were 1.1, 1.5, and 1.6 mg of protein, respectively.

Comparing the threshold dose distribution curves for objective symptoms showed that the dose response within the allergic population for the 5 allergenic foods was not all equally

distributed ([Fig 2, A](#)). The dose distributions for hazelnut, egg, and milk showed a similar pattern, but the population with cashew allergy and peanut allergy reacted within a smaller dose range. Overall, the threshold distributions of the 5 allergenic foods were not statistically different from each other, because the upper and lower confidence limits were overlapping ([Table V](#)). The thresholds for any symptoms were on average 2 to 6 times lower than that for objective symptoms ([Table V, Fig 2, B](#)). Analysis of the threshold dose distribution curves for any symptom showed that the population with hazelnut allergy responded to the lowest doses, with ED_5 of 0.05 mg protein, followed by peanut (ED_5 , 0.14 mg protein) and cashew nut (ED_5 , 0.32 mg protein). In addition, the population with milk allergy and egg allergy were the least sensitive, responding with an ED_5 at 0.27 and 0.75 mg protein, respectively.

The threshold levels for objective and any symptoms in children with egg allergy and milk allergy tended toward a higher dose range compared with the 3 other allergenic foods, which might be related to the different age distribution. In the populations with egg allergy and milk allergy 14 of 53 persons with egg allergy and 50 of 93 the persons with milk allergy were between 7 and 30 months old when they underwent the diagnostic DBPCFC. In the population with peanut allergy, hazelnut allergy, and cashew nut allergy children were older than 30 months before they were tested. Therefore, subpopulations were selected from the populations with egg allergy and milk allergy to obtain an age distribution similar to the children with peanut allergy. A small shift in the threshold distribution to the right was observed for objective symptoms in the population with milk allergy and egg

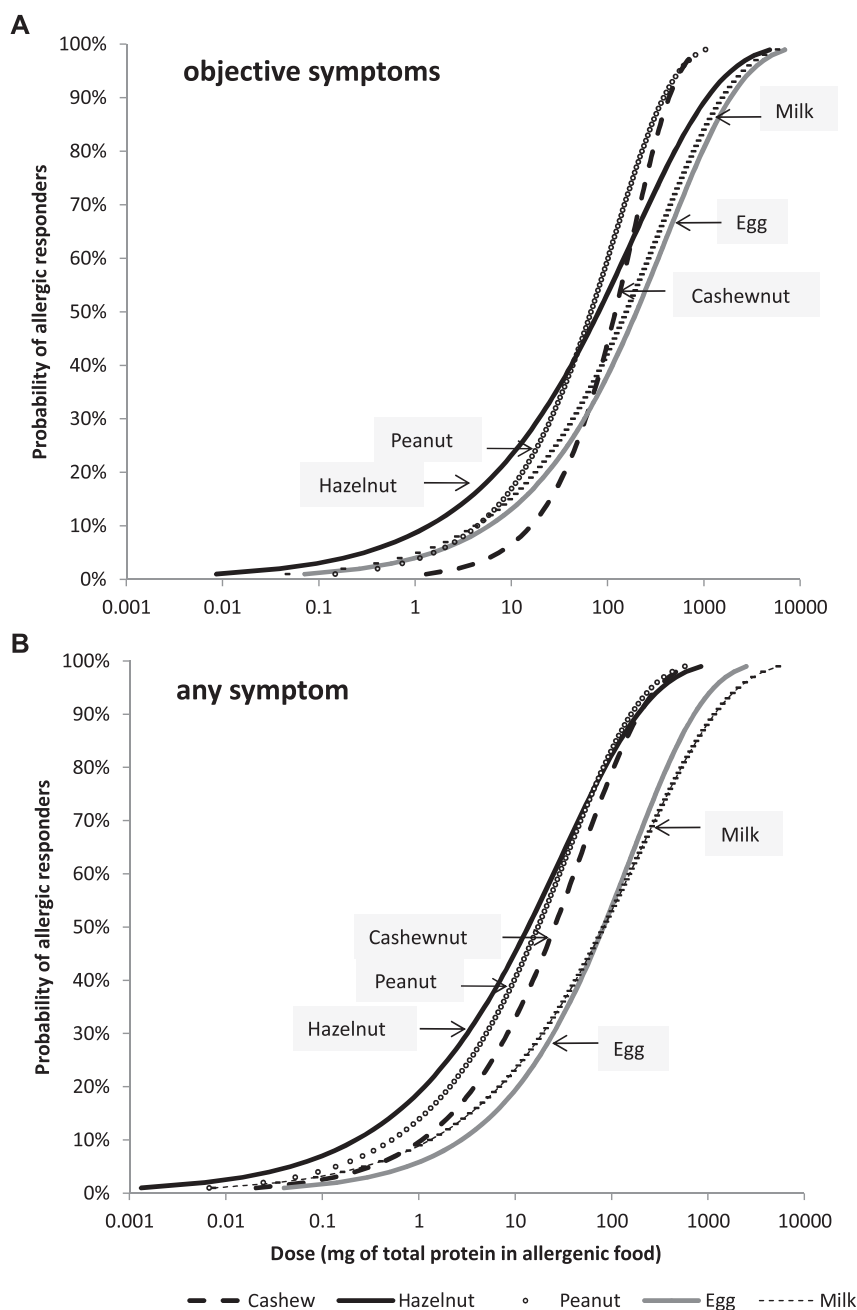


FIG 2. Probability distribution curves of thresholds for peanut, hazelnut, cashew nut, cow's milk and hen's egg (as discrete doses in milligram of total protein of the allergenic food) in the allergic pediatric population (Table II). Distributions based on LOAEL and NOAELs for objective symptoms (A) and any symptoms (B). Data were fitted with the use of different statistical models; the Weibull distribution is shown.

allergy older than 30 months compared with the population younger than 30 months (not shown). For any symptoms the threshold distribution for the different age groups remained similar. Infants and young children often outgrow their milk and egg allergies. This resolution of milk and egg allergies is likely a continuous process of moving closer to oral tolerance for individual patients, although that is not well established yet in terms of threshold doses. Thus, as age increases, a shift toward higher thresholds is expected. This would be less likely to occur for peanut, cashew, and hazelnut because these allergies are less

likely to be outgrown. Overall, a clear age effect was not observed for the dose response difference between the allergenic foods, and for risk assessment the complete threshold data sets could be applied.

DISCUSSION

The present study is the first to present a large number of NOAELs and LOAELs for the pediatric population of one clinical center for 7 major allergenic foods relevant for precautionary

TABLE IV. NOAELs and LOAELs (mg protein) for children with soy and walnut allergies

Patient no.	Age (mo)	Sex	Objective		Any	
			NOAEL	LOAEL	NOAEL	LOAEL
Soy allergy						
1	180	M	1.8		0.2	1.8
2	88	M	1.8			1.8
3	30	M	350	1750	350	1750
4	28	M	70	350	70	350
5	184	F	1750		350	1750
6	149	M	1750		350	1750
7	173	F	1.8			1.8
8	109	M		0.2		0.2
9	164	M	1750		350	1750
10	91	M	14			14
Walnut allergy						
1	177	F	1.8		0.2	1.8
2	140	M	71		14	71
3	157	F	1.8		0.2	1.8
4	97	F	1.8		0.2	1.8
5	91	F	350		161	350
6	104	M	0.2	0.9	0.2	0.9
7	122	F	14		3.5	14
8	105	F	3.5	14	3.5	14
9	198	M	71		14	71
10	99	F	71	161	71	161
11	65	M	161	350	161	350
12	79	M	14		3.5	14
13	206	F	3.5		1.8	3.5

NOAEL and LOAEL are expressed as discrete doses in milligram of total protein in the allergenic food. See Table II for characteristics of each population. F, Female; M, male.

labeling: peanut, cow's milk, hen's egg, hazelnut, cashew nut, walnut, and soy. Especially, the NOAELs and LOAELs for walnut and cashew nut are unique because the potency of these nuts in sensitive persons has not been described before. Assessing the public health effect of cross-contamination represents a considerable challenge to risk managers of public authorities and the food industry. An approach aimed at zero risk can pose serious practical, technical, and financial problems to all stakeholders. Therefore, a structured risk-based approach that is based on threshold doses with tolerable level of risk for the allergic population was proposed by Crevel et al.²⁵ Insight into the actual risk associated with the presence of allergenic constituents in food is possible with the probabilistic risk assessment method that quantifies the risk.¹ However, except for peanut,^{23,26} only limited or summarized threshold information is currently publically available,²⁷ which hinders establishing the threshold dose distributions needed for risk assessment. These data are present in multiple publications and often with only a limited number of data available per allergen. In addition, only limited data have been published on threshold doses for children with hazelnut allergy²⁸ and soy allergy.²⁹⁻³¹ Overall, the present study presents sufficient clinical data to allow statistical dose distribution modeling into threshold distribution curves for peanut, milk, hen's egg, hazelnut, and cashew nut and thereby forms a unique opportunity to compare these 5 allergenic foods at the population level.

Responders were observed at all doses, and the peak number of responders was within the dose range tested for each allergenic food. This shows that the responders covered the entire dose range likely to be encountered in the allergic population. The statistical

parametric models allow for low-dose extrapolation such as for the elaboration of the ED₅ and ED₁.²⁵ Aside from a good statistical fit, a visual examination of the overall goodness of the fit is needed, both at the low-dose range and the distribution of the data set along the probability curve. This approach is used in the present study and in the studies of Taylor et al.^{23,26} The number of data points that are needed to make the estimates with adequate confidence has not yet been determined.²⁵ The 95% upper and lower confidence intervals of the ED values that are presented in Table IV show that those associated with the ED₁ are particularly wide. Thus, determination of the ED₁ would be strengthened by larger numbers of individual threshold data from the allergic population.

The data on soy and walnut are less robust, and more threshold data are needed before a threshold dose distribution may be accurately established. Combining the soy threshold data with the limited threshold data available within literature might be a valuable next step; however, this is not the purpose of the present study.

The ED₁₀ and ED₅ for objective symptoms induced by peanut in our pediatric population with peanut allergy (age range, 2 to 18 years; median, 6.9 years) is 4.4 mg and 1.6 mg allergenic peanut protein, respectively, which is within the same range as reported for peanut by Taylor et al.²⁶ Assuming a level of protein of 25% (NEVO, the Dutch Food Composition Database; <http://nevo-online.rivm.nl/>) in peanut, the ED₁₀ and ED₅ for the 256 subjects presented in the study reported by Taylor et al.²⁶ were 3.6 mg (equal to 14.4 mg whole peanut) and 1.8 mg (equal to 7.3 mg whole peanut), respectively. The population characteristics in that study (age range, 1 to 48 years; median, 7 years, with an unknown number of the subjects being adults) differed from our population, and there were also differences in study protocol and geographic region of patients recruitment. For instance, there is a difference in the preparation and matrix between the peanut challenges used by us and those reported by Taylor et al.,²⁶ namely, peanut in a baked cookie and roasted peanut in apple sauce, respectively. In addition, the EDs derived from the threshold distribution curves generated in our study are based on discrete doses, whereas the study of Taylor et al.²⁶ reported cumulative doses. The use of discrete doses for modeling the threshold distribution curves represents a conservative approach in risk assessment, because in theory the threshold distribution curve is shifted left to lower doses compared with using cumulative doses and would lead to lower EDs. However, currently no data exist on this notion.²⁵

On the whole, the peanut EDs found in our study and the study of Taylor et al.²⁶ were in the same range, suggesting that the total effect of the reporting differences mentioned was small.

The threshold distribution curves for each allergenic food in the present study appeared in proximity although with different steepness. At the lower dose end the populations with peanut allergy, milk allergy, and egg allergy reacted with similar probability, with ED₅ for objective symptoms of 1.6 mg, 1.1 mg, and 1.5 mg, respectively. However, the dose range to which the population with peanut allergy was reacting was smaller, and at higher doses the population with peanut allergy was more likely to respond. Fifty percent of the population with peanut allergy had reacted at 67 mg peanut protein, whereas at that dose approximately 35% of the persons with milk allergy and egg allergy had responded with objective symptoms.

TABLE V. The ED₁, ED₅, ED₁₀, and ED₅₀ (mg protein) and the 95% confidence interval for each allergenic food as determined in the Weibull distribution curve

	ED ₁ (95% CI)	ED ₅ (95% CI)	ED ₁₀ (95% CI)	ED ₅₀ (95% CI)
Objective symptoms				
Cashew nut	1.30 (0.18 ± 9.57)	7.41 (1.9 ± 28.7)	16.0 (5.4 ± 47.4)	120 (67.3 ± 213)
Egg	0.07 (0.01 ± 0.79)	1.51 (0.3 ± 7.7)	5.82 (1.6 ± 21.4)	199 (106 ± 374)
Peanut	0.15 (0.04 ± 0.51)	1.56 (0.7 ± 3.6)	4.42 (2.3 ± 8.5)	67.3 (48.7 ± 93)
Milk	0.05 (0.01 ± 0.30)	1.07 (0.3 ± 3.8)	4.24 (1.6 ± 11.6)	156 (96.4 ± 253)
Hazelnut	0.01 (0.00 ± 0.56)	0.29 (0.0 ± 4.6)	1.38 (0.2 ± 12.0)	80.6 (28.3 ± 230)
Any type of symptom				
Cashew nut	0.02 (0.002 ± 0.25)	0.32 (0.1 ± 1.8)	1.07 (0.3 ± 4.5)	25.4 (12.4 ± 52)
Egg	0.04 (0.005 ± 0.35)	0.75 (0.2 ± 3.3)	2.75 (0.8 ± 9.2)	82.0 (45.6 ± 147)
Peanut	0.007 (0.002 ± 0.03)	0.14 (0.1 ± 0.4)	0.52 (0.2 ± 1.2)	17.2 (11.8 ± 25.1)
Milk	0.007 (0.001 ± 0.06)	0.27 (0.1 ± 1.1)	1.31 (0.4 ± 4.2)	82.6 (48 ± 142)
Hazelnut	0.001 (0 ± 0.05)	0.05 (0.0 ± 0.6)	0.22 (0.0 ± 1.8)	13.5 (5.1 ± 36.3)

In our study we show that for the 5 allergenic foods the threshold distribution curves for any symptom, which are predominantly subjective and relatively mild reactions, were left shifted relative to the curves for objective, generally more severe symptoms. For each allergenic food the ED for any symptom was lower than the ED for objective symptoms. This implies that threshold distribution curves for individual symptoms or reaction types may be different. It is of interest to ascertain EDs for individual symptoms, although this would require data sets large enough for each type of effect to generate reliable fitting distribution curves that can be used to estimate EDs.

Compared with other studies both some consistency and variation in reported thresholds for allergenic foods are observed. The study of Morisset et al³² reported that at 65 mg food, corresponding to 6.5 mg egg protein or 16 mg peanut protein, 16% of the patients with egg allergy and 18% of the patients with peanut allergy had reacted.³² In the present study we found similar results for the populations with egg allergy and peanut allergy: an estimated 11% of the population with egg allergy and 23% of the population with peanut allergy would have reacted at this dose. The response of the population with milk allergy was 24% of the persons with milk allergy in our study and was much higher than 5% of the population with milk allergy in the study of Morisset et al³² (at a dose of 0.8 mL milk, approximately 32 mg milk protein). However, a recent study presented clinical thresholds expressed as the ED₁₀ to egg (5.4 mg protein), hazelnut (18.7 mg protein), milk (3.5 mg protein), and peanut (26.6 mg protein).³³ The reported ED₁₀s for milk and egg were in the same range as found in the present study. However, the ED₁₀s of the populations with peanut allergy and hazelnut allergy were much higher. Many reasons are possible why some of the EDs differ between studies, including issues of patient selection, protocols, and challenge materials. However, an extensive and thorough analysis of factors operative in the data from different centers and their relation to the outcome thresholds is beyond the scope of the present study, which focuses on differences between different allergenic foods in a single center. Reasons for differences between centers should be a focus of future studies and will help to standardize food challenge procedures.

The threshold distribution of cashew nut showed a different shape. The ED₅ of the population with cashew nut allergy was 7.4 mg, and at this low-dose end of the probability curve the population with cashew allergy seems less sensitive than the population

with hazelnut allergy, peanut allergy, milk allergy, and egg allergy. At higher doses, however, the population with cashew nut allergy was more sensitive than the population with milk allergy and egg allergy. Several publications report that cashew nut allergy causes relatively severe reactions, and cashew nut allergy it is more likely to cause anaphylaxis than peanut allergy.^{34,35} However, information on the dose causing the anaphylactic reaction was lacking³⁴ and was only present for 4 patients consuming from 1 to 2 cashew nuts to as much as 20 cashew nuts.³⁵ In the present study we did not observe anaphylactic symptoms. In addition, Rance et al³⁶ reported 8 patients who received cumulative doses (13 to 1753 mg protein) in an open food challenge without cardiovascular collapse.³⁶ It is not clear what causes these differences in the reported severities of the reactions because the challenge dose of cashew nut in the present study was approximately 5 cashew nuts and thus was in the same range as in the studies that reported severe reactions. A potential explanation could be that cashew nuts are typically consumed without food matrix as particulates,³⁷ so that the doses of exposure are higher. As yet unknown differences between the subjects in these studies could also be a contributing factor.

The population with hazelnut allergy was the most sensitive group, with 5% of the population with hazelnut allergy likely to respond with objective symptoms to 0.3 mg hazelnut protein (ED₅), whereas 10% of the population was likely to respond to 1.4 mg (ED₁₀). This population with hazelnut allergy was also the most sensitive group of the 5 populations when comparing the threshold distributions for any symptom.

The probabilistic risk assessment approach by now has been accepted as the step forward for risk management purposes,⁶ but it was lacking sufficient underlying information on the sensitivity of the allergic pediatric population for most priority allergenic foods. The similarity of our threshold distribution curve that was based on objective symptoms for peanut with the distribution curve from another center in Europe supports the legitimacy of combining threshold data from multiple centers together to obtain sufficient numbers for statistically modeling a threshold distribution curve. However, distribution curves generated in different centers and populations must be compared to assess the limits of generalizability of the data.

To conclude, in the present situation in which only limited threshold information is available on most allergenic foods within the public literature, analysis of the results from food challenges

is a valuable resource. The threshold distribution of several major relevant allergenic foods presented here for the pediatric population gives insight into the sensitivity of the allergic pediatric population. This data can be used to extract the protein dose at which part of the allergic population is likely to respond with objective or any reactions (ED values) as a basis for elaboration of precautionary labeling action levels and for incorporation in the probabilistic risk assessment for allergic reactions in the pediatric population after consumption of products that are contaminated with allergens.

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Clinical implications: The distribution of threshold doses for 5 major allergenic foods is crucial to assess the risk of allergen exposure for allergic consumers and to elaborate action levels for precautionary labeling.

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