

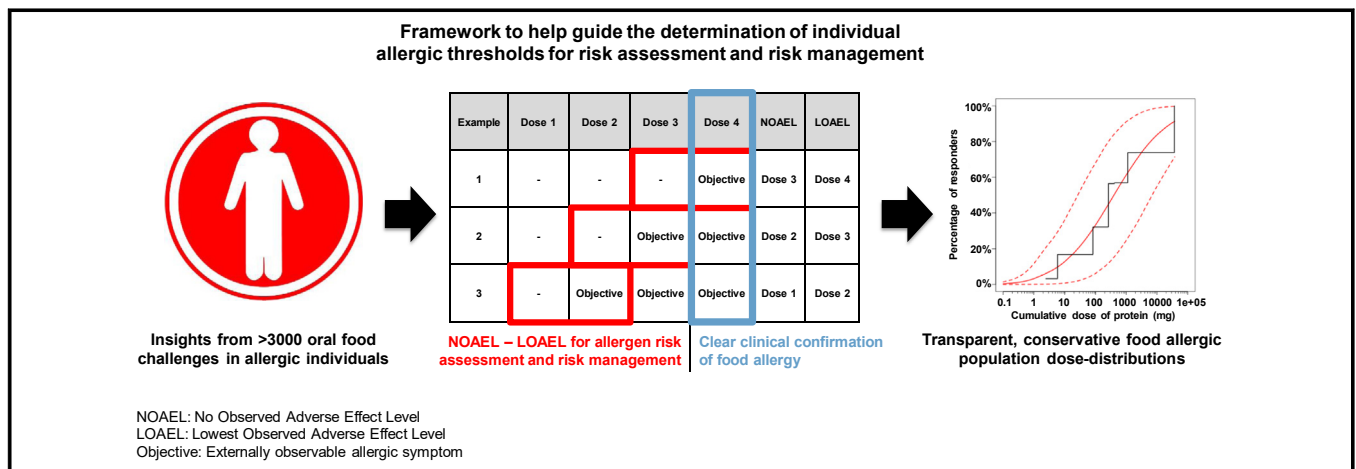
Deriving individual threshold doses from clinical food challenge data for population risk assessment of food allergens



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GRAPHICAL ABSTRACT



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Background: Food allergies are a significant public health issue, and the only effective management option currently available is strict avoidance of all foods containing the allergen. In view of the practical impossibility of limiting risks to zero, quantitative allergen risk assessment and management strategies are needed.

Objective: We sought to develop appropriate methods for informing population-based risk assessments and risk management programs to benefit all stakeholders but particularly patients with food allergy.

Methods: Individual thresholds for food allergens (maximum tolerable doses and minimum eliciting doses) can ideally be established through double-blind, placebo-controlled food challenges. If double-blind, placebo-controlled food challenge data are not available, data from widely used open food challenges using predefined objective criteria can also provide useful data regarding minimum eliciting doses. For more than 20 years, the Netherlands Organisation for Applied Scientific Research and the Food Allergy Research and Resource Program at the University of Nebraska-Lincoln have been collecting individual maximum tolerable doses and minimum eliciting doses that produce objective symptoms from published and unpublished clinical data to better refine knowledge regarding the sensitivity of the population to food allergens.

Results: In this article we provide in-depth insights into the methodology applied by the Netherlands Organisation for Applied Scientific Research and Food Allergy Research and Resource Program to derive individual maximum tolerable doses and minimum eliciting doses for objective symptoms from clinical food challenge data. More than 90 examples for determining individual allergic thresholds are presented.

Conclusion: With the methodology presented in this article, we aim to stimulate harmonization and transparency in quantitative food allergen risk assessment and risk management programs, encouraging their wider adoption. (*J Allergy Clin Immunol* 2019;144:1290-309.)

Key words: Food allergy, food challenge, double-blind, placebo-controlled food challenge, threshold, eliciting dose, risk assessment, risk management, decision-making process, no observed adverse effect level–lowest observed adverse effect level derivation

Food allergies are a significant public health issue, with accumulating evidence that they are increasing in prevalence.¹ Currently, the only effective risk management option available for patients with food allergy is strict avoidance of all foods that contain the allergen. Therefore correct labeling of allergenic foods beyond simple ingredient labeling is critical to the implementation of safe and effective dietary avoidance. In the absence of guidance from public health authorities regarding precautionary allergen labeling thresholds, application of such “may contain” labeling remains inconsistent and sporadic across the industry.^{2–4} If clear thresholds that were acceptable to regulatory authorities were available to guide industry, then this could lead to a clear reduction in the need for precautionary allergen labeling and improve its utility to allergic consumers.^{5,6}

Population-based threshold dose distributions for different food allergens are a prerequisite to perform quantitative population risk assessment and create population risk management

Abbreviations used

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

programs. Population-based threshold dose distributions are developed by using individual maximum tolerable dose and minimum eliciting dose data collected from clinical food challenges, which can be analyzed by using population dose-distribution models.^{7,8} These data can then be used for developing industry-wide risk management programs, such as the quantitative reference dose recommendations, as elaborated by the VITAL Scientific Expert Panel,² and for broader quantitative risk assessment.^{4,9,10}

Individual threshold levels in patients with food allergy (minimum eliciting dose) can be obtained from standardized double-blind, placebo-controlled food challenges (DBPCFCs), which are widely used in research practice and some clinical environments.¹¹ Less optimal unblinded open food challenges are more widely used in clinics to confirm clinical reactivity or tolerance. If DBPCFC data are not available, open food challenges using predefined objective criteria can also provide useful data regarding minimum eliciting doses. Individual thresholds for allergenic foods can be established during a DBPCFC based on the first observation of the development of objective symptoms.¹² A subject is challenged to increasing allergen doses during a DBPCFC. The initial point at which objective symptoms start to occur at a specific dose is designated the lowest observed adverse effect level (LOAEL). The highest dose that does not lead to objective symptoms is designated the no observed adverse effect level (NOAEL).

However, in reality, food challenges and the occurrence or interpretation of clear and clinically unequivocal challenge-stopping symptoms are seldom straightforward. Symptoms can be transient in nature, appearing and disappearing again, or symptoms can be subjective and need to be confirmed over multiple doses. Additionally, the interpretation of the subject's subjective and objective symptoms and their severity depends on the experience of the clinical investigator conducting the food challenges, as well as the prior experience of the patient who might have undergone more than 1 prior food challenge.^{13,14} As such, clinical (nonresearch) challenge protocols can deviate from the standard dosing scheme to include repeated doses to confirm symptoms, whereas others will skip doses based on a patient's history or give partial doses in certain scenarios. Other “real-world” legitimate factors, such as parental concern and desire to stop the challenge, might also affect the outcome of a food challenge.^{15,16}

A DBPCFC is also a typical clinical diagnostic tool to rule in or out the presence of a clinical allergy, which would not aim specifically or only to identify the subject's maximum tolerable dose and minimum eliciting dose in relation to certain symptoms. However, some guidelines suggest that a food challenge can be used to establish a subject's maximum tolerable dose and minimum eliciting dose, and some clinics do this routinely. Therefore, it is crucial to have a clear set of criteria for combining and interpreting individual food challenge data from different

sources in a consistent manner for deriving NOAEL and LOAELs for the purposes of developing population-based threshold dose distributions and applying them to larger risk management programs.

The aim of this article is to provide in-depth insight into the methodology developed and applied by the Netherlands Organisation for Applied Scientific Research and the Food Allergy Research and Resource Program at the University of Nebraska-Lincoln to derive individual threshold doses for objective symptoms from clinical food challenge data in their joint Food Allergen Threshold Database. These individual threshold data points then form the input that is fitted by using parametric models and the resulting model-based prediction of population-based threshold dose distributions for different allergenic foods. The decision-making process and NOAEL-LOAEL derivation for risk management purposes are described in sections focused on study/data inclusion criteria, allergic symptoms taken into consideration during analysis, overarching clinical scenarios observed during food challenges, and insights into NOAEL-LOAEL derivation when symptoms were observed at multiple doses. Each section is further explained below, with detailed examples included.

METHODS

Inclusion criteria and basic principles

Publications and unpublished clinical data were selected for the Food Allergen Threshold Database based on previously described criteria.^{2,8} In short, data from DBPCFCs were included if they started at low doses (<1 mg is ideal, with <10 or <100 mg also used and exceptions depending on the type of allergen and amount of available data) and if the authors/clinics clearly reported the dosing scheme used, indicated if/when repeated doses were used, detailed the challenge material, and reported the symptoms in an individual fashion or reported a grouped symptom classification with a clear separation between objective and subjective symptoms. Additionally, data were included from study protocols by using different time intervals between doses (15-30 minutes, on average, and up to 2 hours between doses). Publications were also used if some, but not all, reported subjects had data available in the desired format.

Because of some extremely high-dose labial challenges, DBPCFCs starting with a positive labial challenge result were excluded, and protocols beginning with labial challenges were discouraged unless the exact labial dose could be verified and it could be safely assumed that the labial dose was smaller than the following ingested doses. Naturally, in contrast to the inclusion criteria, additional general exclusion criteria included results from DBPCFCs with extremely high starting doses if the full dosing scheme was not able to be derived from reported information, if objective and subjective symptoms were unable to be separated from reported grouped symptoms, and if the challenge material or protein content could not be derived from the reported information. Additionally, data from subjects in food challenge data sets who do not react to any dose during challenge and were determined to be tolerant were excluded from further threshold determinations for population risk assessment.

As a subject is challenged with increasing doses during a DBPCFC, objective symptoms occur, and that specific dose is designated as the LOAEL. The highest dose that does not lead to objective symptoms (ie, the immediately preceding step in the progression) is then designated the NOAEL. The exact doses (in milligrams) of total protein for individual subjects' NOAELs and LOAELs were established based on the dosing scheme and challenge material form, as provided in the publication/clinic (eg, scrambled whole egg, scrambled egg white, whole egg powder, and egg white powder). Individual NOAELs and LOAELs were also derived from summarized group data when only objective symptoms were included in the results and the number of subjects was clearly stated. In case of ambiguity regarding reported symptom

data in the publications of interest, the corresponding author of a publication or the responsible clinician was contacted by the researchers for further details.

When the challenge doses were reported in amounts of allergenic food, they were converted to milligrams of total protein of the allergenic food, as previously described by Taylor et al.² All individual NOAELs and LOAELs were expressed in terms of doses (in milligrams) of total protein of the allergenic food.

There are 2 ways of expressing NOAELs and LOAELs, either in discrete or cumulative fashion. Discrete values represent the protein amount for each dose in the challenge scheme, either NOAEL or LOAEL, irrespective of all previous doses ingested during the food challenge. In contrast, cumulative NOAELs and LOAELs take into account the amount of protein of all the preceding doses in the challenge as well. For example, a simple 4-step dosing scheme with discrete doses of 1, 3, 10, and 30 mg of protein would be reported as cumulative doses of 1, 4, 14, and 44 mg of protein. One might assume that a dose-response curve based on discrete doses would be more conservative than when using cumulative doses, but in practice there is little observed difference between the discrete or cumulative population-based eliciting dose (ED) values predicted to cause reactions in 1% or 5% of the allergic population. Additionally, because of differences in the shape and scale of the calculated parametric dose-distribution models fitted to discrete or cumulative data points, curves with steeper slopes can lead to predicted discrete population ED values that are actually slightly greater than the cumulative population ED values in the risk management dosing range of interest (ie, ED01 and ED05 [the milligram protein amount predicted to cause reactions in 1% or 5% of the allergic population] and the lower 95% CI of the ED05 value).² However, differences can occur at greater ED values, such as ED50, and therefore the risk management goal should be considered when choosing discrete or cumulative reporting units. For the purposes of this study, a distinction between discrete and cumulative reporting units is made when necessary, but it is not the main focus of the study.

Additionally, there are 2 different end points that should be considered when interpreting data from food challenges: clear clinical challenge-stopping criteria versus the LOAEL for risk assessment and risk management purposes. Confirming the presence of a clinical food allergy is of extreme importance to a clinician and the potentially allergic patient because of the significant effect of food allergy avoidance on day-to-day life. Thus situations can arise in which challenges are continued after the first appearance of symptoms, either subjective or objective, until clear challenge-stopping criteria (or protocol-defined stopping criteria) have been met and the allergy is confirmed.¹⁶ In these cases the clinical challenge-stopping dose might be different than the determined LOAEL for population risk assessment and risk management purposes. Situations of this nature can include but are not limited to transient objective symptoms, objective symptoms continuing from dose to dose, 1 or multiple doses between objective symptoms, and a lack of objective symptoms. These scenarios and their implications for NOAEL and LOAEL derivation for risk assessment and risk management purposes are described and discussed later in the article.

Symptoms

Objective symptoms include any symptom that is externally observable, whereas subjective symptoms cannot be confirmed by clinical observers (see Table I for a list of possible symptoms).¹⁷⁻²² Although Table I is a rather broad list of symptoms, we acknowledge that it is not an exhaustive list, and other recorded subjective or objective symptoms are also possible. Again, the NOAELs and LOAELs for risk assessment and risk management purposes are derived by using only objective symptoms.

RESULTS

Clinical scenarios during food challenges

More than 3000 individual food challenges (including the data presented in Taylor et al.² and Allen et al.³) could be broadly structured into 5 different clinical scenarios. The 5 scenarios include

TABLE I. Most common signs and symptoms of allergic reactions to food, as reported in publications and unpublished clinical data

Subjective symptoms	Objective symptoms
Oral cavity	
Pruritus (itching) and paresthesia (tingling sensation) of the oral cavity, pharynx and/or lips (so-called oral allergy symptoms)	Lip swelling Redness/swelling of the oral mucosa Blisters of the oral mucosa
Skin	
Pruritus (itching)	Urticaria Angioedema Flush Erythema (redness)
Eyes and nose	
Pruritus (itching)	Red eye/conjunctival hyperemia Tearing Sneezing Rhinorrhea
Gastrointestinal	
Dysphagia	Diarrhea
Abdominal/gastric pain*	Vomiting†
Cramps	
Nausea	
Bloating	
Neurological	
Headache	Seizures
Dizziness	
Anxiety	
Tension/agitation	
Respiratory	
Laryngeal/throat tightness	Laryngeal edema
Thoracic/chest tightness	Dysphonia
Dyspnea/shortness of breath	Wheezing Reduced peak expiratory flow/ FEV ₁ decrease Silence (in lung auscultation) Breathless to speak Rapid breath Chest retractions Cough
Cardiovascular	
Faintness	Change in heart rate/tachycardia
Tiredness	Hypotension/blood pressure decrease Change in consciousness
Other	
Uterine cramps/contractions	

Please note that although the listed symptoms cover the majority of cases, this is not an exhaustive list, and other recorded symptoms are also possible.

*Abdominal pain and gastric pain are considered objective symptoms provided they are observed in children less than 3 years old.

†Vomiting is not considered an objective symptom in children less than 1 year of age unless the clinician stops the challenge because of the vomiting. If vomiting occurs at the final dose of the challenge, it is not considered an objective symptom in children less than 1 year old unless additional objective symptoms are present.²²

the following: A), when an individual completed the dosing protocol; B), when the challenge was stopped before the final dose; C), when a challenge included repeated-skipped-partial doses; D), challenges in infants less than 1 year old who have vomiting/emesis/spit-up during challenge; and E), challenges in children less than 3 years old who have abdominal pain during

challenge. These overarching scenarios formed the basis of the consensus opinion for the methodologies used when deriving individual NOAELs and LOAELs for risk assessment and risk management purposes. Below, we describe these scenarios in more detail.

A) Completed dosing protocol. When objective symptoms occur only at the final dose of the challenge, the final dose is considered the discrete objective LOAEL, and the sum of all doses is considered the cumulative objective LOAEL. The dose before the final dose is considered the NOAEL. If only subjective symptoms are observed throughout a completed challenge and the subject is considered to be allergic based on the clinician's judgment, it is not possible to derive the LOAEL. In such cases in which only subjective symptoms develop, the final dose given is considered the highest NOAEL and the subject becomes right censored (ie, the LOAEL for objective symptoms is assumed to be greater than the doses received during the challenge). If the challenge is completed without any symptoms (subjective or objective) at the final dose, the subject is considered to be tolerant. Examples of NOAEL and LOAEL derivation for challenges with a completed dosing protocol are presented in Table II.

For each example illustrated in the present article, we assume a hypothetical DBPCFC that has a total of 9 dose steps at the following discrete amounts: 1, 3, 10, 30, 100, 300, 1,000, 3,000, and 10,000 mg of protein. Each example in Table II could be expressed with different or even multiple symptoms from Table I. In the examples that state repeating objective symptoms (Table II, examples 5-6) or reappearing objective symptoms (Table II, from example 11 onward), it should be noted that objective symptoms at the different doses do not need to be the same for derivation of the NOAEL and LOAEL.

B) Challenge stopped before completing the dosing protocol. When the challenge is stopped based on the clinician's judgment before the end of the dosing protocol and the subject is deemed allergic, the objective LOAEL is based on the criteria described below. When the challenge is ended after the first dose because of objective symptoms, it is not possible to derive the NOAEL, the LOAEL is set as the first dose ingested, and the subject is considered to be left censored (ie, the individual threshold is assumed to be less than the first dose received during the challenge). When the challenge is stopped before completing the dosing protocol because of convincing subjective symptoms, it is not possible to establish the objective LOAEL, and the subject is considered to be right censored at the last given dose (ie, the LOAEL for objective symptoms is assumed to be greater than the doses received during the challenge). Examples of NOAEL and LOAEL derivation for challenges that are stopped before the final dose are presented in Table III.

Again, each example in Table III could be expressed with different or even multiple symptoms from Table I. In the examples that state repeating (Table III, examples 7-8) or reappearing (Table III, from example 10 onward) objective symptoms, it should be remembered that the objective symptoms at the different doses do not need to be the same for derivation of the NOAEL and LOAEL.

C) Repeated, skipped, and partial doses. A typical DBPCFC involves a subject ingesting increasing doses during the challenge until objective symptoms manifest. However, sometimes a clinician decides to repeat the previous dose if symptoms

TABLE II. Examples for determining the individual allergic thresholds in case of a completed dosing protocol

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
1	Objective symptoms	—	—	—	—	—	—	—	—	Objective	Dose 8	Dose 9	The LOAEL is conservative because we do not know whether the challenge would have continued or stopped if more doses were available.
2	Subjective symptoms	—	—	—	—	—	—	—	—	Subjective	Dose 9	ND	The subject was considered allergic but without objective symptoms, and therefore the subject was right censored.
3	Repeating subjective symptoms	—	—	—	—	Subjective	Subjective	Subjective	Subjective	Subjective	Dose 9	ND	The subject was considered allergic but without objective symptoms, and therefore the subject was right censored.
4	Objective and subjective symptoms	—	—	—	—	—	Subjective	Subjective	Subjective	Subjective, objective	Dose 8	Dose 9	The LOAEL is conservative because we do not know whether the challenge would have continued or stopped if more doses were available.
5	Repeating objective symptoms	—	—	—	—	—	—	—	Objective	Objective	Dose 7	Dose 8	The LOAEL at first occurrence of objective symptoms was determined.
6	Repeating objective symptoms	—	—	—	—	—	—	Objective	Objective	Objective	Dose 6	Dose 7	The LOAEL at first occurrence of objective symptoms was determined.
7	Transient objective symptoms	—	—	—	Objective	—	—	—	—	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
8	Transient objective symptoms	—	—	—	—	—	—	—	Objective	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
9	Transient objective and subjective symptoms	—	—	Subjective	Subjective	Subjective	Subjective, objective	Subjective	Subjective	Subjective	Dose 9	ND	The subject was considered allergic but without objective symptoms at the highest given dose, and therefore the subject was right censored.
10	Reappearing transient objective symptoms	—	—	—	—	—	—	Objective	—	Objective	Dose 6	Dose 7	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
11	Reappearing transient objective symptoms	—	—	—	—	—	Objective	—	Objective	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
12	Reappearing transient objective symptoms	—	—	—	—	Objective	—	Objective	—	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.

(Continued)

TABLE II. (Continued)

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
13	Reappearing transient objective symptoms	—	—	—	—	—	Objective	—	—	Objective	Dose 8	Dose 9	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between. The LOAEL is conservative because we do not know whether the challenge would have continued or whether the symptom would disappear if more doses were available.
14	Reappearing transient objective symptoms	—	—	—	—	Objective	—	—	Objective	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
15	Reappearing transient objective symptoms	—	—	—	—	—	Objective	—	Objective	Objective	Dose 5	Dose 6	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
16	Reappearing transient objective symptoms	—	—	—	—	Objective	—	Objective	Objective	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
17	Reappearing transient objective symptoms	—	—	—	—	Objective	—	—	Objective	Objective	Dose 7	Dose 8	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
18	Reappearing transient objective symptoms	—	—	—	Objective	—	—	Objective	Objective	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
19	Reappearing transient objective symptoms	—	—	—	—	Objective	—	Objective	Objective	Objective	Dose 4	Dose 5	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
20	Reappearing transient objective symptoms	—	—	—	—	Objective	—	Objective	—	Objective	Dose 4	Dose 5	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
21	Reappearing transient objective symptoms	—	—	—	Objective	—	—	Objective	—	Objective	Dose 6	Dose 7	One dose in between without objective symptoms is allowed for the LOAEL determination, whereas 2 doses or more without objective symptoms are not.
22	Reappearing transient objective and subjective symptoms	—	—	—	—	Subjective	Subjective	Subjective, objective	Subjective	Subjective, objective	Dose 6	Dose 7	The LOAEL at first occurrence of objective symptoms was determined.

(Continued)

TABLE II. (Continued)

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
23	Reappearing transient objective and subjective symptoms	—	—	—	—	Subjective	Subjective, objective	Subjective	Subjective, objective	Subjective	Dose 9	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
24	Reappearing transient objective and subjective symptoms	—	Subjective	Subjective	Subjective	Subjective	Subjective, objective	Subjective	Subjective	Subjective, objective	Dose 8	Dose 9	The LOAEL at reappearance of objective symptoms was determined.
25	Reappearing transient objective and subjective symptoms	—	Subjective	Subjective	Subjective, objective	Subjective	Subjective	Subjective, objective	Subjective	Subjective	Dose 9	ND	The subject was considered allergic but without objective symptoms at the highest given dose, and therefore the subject was right censored.

Indication that a LOAEL is not determined (ND) designates that the data point could be right censored during statistical analysis. If both the NOAEL and LOAEL are not determined, then the subject is considered tolerant and not included during statistical analysis.

develop that are not convincing. It could be that an individual shows convincing objective symptoms after the second administration of the same dose, and in such a case, the discrete NOAEL and the discrete LOAEL would be the same. Therefore in the case of objective reactions to a repeated dose, the previous dosing step is selected as the discrete NOAEL (see Table IV [example 1] for such a case). The cumulative NOAEL and LOAEL already account for all dosing given during a challenge, and thus a repeated dose does not require special adjustments. Other examples of NOAEL and LOAEL derivation for challenges with repeated doses can be found in Table IV (examples 1-6).

When a subject has a history of reacting to relatively high doses of allergen, it could be that some of the lower doses or specific intermediate dose steps can be skipped when compared with a clinic's standard DBPCFC protocol. Obviously, cumulative NOAELs and LOAELs are to be established on the basis of the doses actually given during the food challenge. Examples of NOAEL and LOAEL derivation for challenges with skipped doses can be found in Table IV (examples 7-11).

In other instances a subject might react to a partial dose of the challenge material (ie, symptoms appear before the whole portion is consumed). Examples of NOAEL and LOAEL derivation for challenges with partial doses can be found in Table IV (examples 12-17). Ideally, when a subject reacts to a partial dose, the amount of remaining challenge material is weighed or estimated so that the proportion of the intended dose that was ingested can be calculated. The discrete LOAEL is then set at the calculated amount of protein ingested. In the case that the partial dose leads to a calculated discrete LOAEL that is less than the previous dose, then the discrete NOAEL will be set at the highest dose level from the dosing protocol that is lower than the calculated discrete LOAEL. The cumulative LOAEL is set at the total amount of protein ingested at the objective reaction, and the cumulative NOAEL is set at the total amount of protein ingested up to the previous dose, according to the standard protocol. In some cases the amount of remaining challenge material might not have been weighed, and although this is not ideal, the subject could still be included as a right-censored data point because the dose that would elicit objective symptoms is known to be greater than the last measured and quantified cumulative dose but the exact amount given was not quantified.

For previous examples, an assumed DBPCFC with 9 discrete dose steps was used at the following discrete amounts: 1, 3, 10, 30, 100, 300, 1,000, 3,000, and 10,000 mg of protein. In case of repeated and partial doses, as explained above, special attention should thus be paid to the discrete NOAELs and LOAELs because they no longer follow the exact dosing protocol. Again, each example in Table IV could be expressed with different or even multiple symptoms from Table I, and objective symptoms do not need to be identical at each dose for derivation of the NOAEL and LOAEL.

D) Infants more than 1 year old. In general, vomiting in infants less than 1 year of age is not considered an objective symptom.²² Thus transient vomiting occurring in the middle of a continued food challenge is not considered in the LOAEL determination for an infant less than 1 year old. However, if the clinician stopped the challenge because of excessive vomiting, then it is judged as an allergic symptom, and the objective LOAEL will

TABLE III. Examples for determining individual allergic thresholds in case the challenge is stopped by the clinician's judgment before the final dose

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
1	Objective symptoms	—	—	—	—	Objective	Challenge stopped at dose 5	NA	NA	NA	Dose 4	Dose 5	The clinician's judgment stopped the challenge, and we must consider this symptom as the objective LOAEL.
2	Objective symptoms	Objective	Challenge stopped at dose 1	NA	NA	NA	NA	NA	NA	NA	ND	Dose 1	Objective symptoms were present at dose 1, and therefore the subject was left censored.
3	Subjective symptoms	—	—	Subjective	Challenge stopped at dose 3	NA	NA	NA	NA	NA	Dose 3	ND	The clinician's judgment stopped the challenge, and therefore we must consider this subject to be allergic. No objective symptoms were present at the highest given dose, and therefore the subject was right censored.
4	Subjective symptoms	Subjective	Challenge stopped at dose 1	NA	NA	NA	NA	NA	NA	NA	Dose 1	ND	The clinician's judgment stopped the challenge, and therefore we must consider this subject to be allergic. There were no objective symptoms at the highest given dose, and therefore the subject was right censored.
5	Repeating subjective symptoms	—	—	—	—	—	Subjective	Subjective	Subjective	Challenge stopped at dose 8	Dose 8	ND	The clinician's judgment stopped the challenge, and therefore we must consider this subject to be allergic. No objective symptoms were present at the highest given dose, and therefore the subject was right censored.
6	Objective and subjective symptoms	—	Subjective	Subjective	Subjective, objective	Challenge stopped at dose 4	NA	NA	NA	NA	Dose 3	Dose 4	There were objective symptoms at dose 4.
7	Repeating objective symptoms	—	—	Objective	Objective	Challenge stopped at dose 4	NA	NA	NA	NA	Dose 2	Dose 3	The LOAEL at first occurrence of objective symptoms was determined.
8	Repeating objective symptoms	Objective	Objective	Objective	Challenge stopped at dose 3	NA	NA	NA	NA	NA	ND	Dose 1	The LOAEL at first occurrence of objective symptoms was determined. There were objective symptoms at dose 1, and therefore the subject was left censored.
9	Transient objective and subjective symptoms	—	—	—	Subjective	Subjective, objective	Subjective	Subjective	Challenge stopped at dose 7	NA	Dose 7	ND	The subject considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
10	Reappearing transient objective symptoms	Objective	—	Objective	Challenge stopped at dose 3	NA	NA	NA	NA	NA	ND	Dose 1	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
11	Reappearing transient objective symptoms	Objective	—	—	Objective	Challenge stopped at dose 4	NA	NA	NA	NA	Dose 3	Dose 4	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.

(Continued)

TABLE III. (Continued)

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
12	Reappearing transient objective symptoms	Objective	—	Objective	Objective	Challenge stopped at dose 4	NA	NA	NA	NA	ND	Dose 1	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between. There were objective symptoms at dose 1, and therefore the subject was left censored.
13	Reappearing transient objective symptoms	—	Objective	—	Objective	Objective	Objective	Challenge stopped at dose 6	NA	NA	Dose 1	Dose 2	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
14	Reappearing transient objective symptoms	—	—	Objective	—	Objective	—	Objective	Challenge stopped at dose 7	NA	Dose 2	Dose 3	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
15	Reappearing transient objective symptoms	Objective	—	—	Objective	—	Objective	Challenge stopped at dose 6	NA	NA	Dose 3	Dose 4	One dose in between without symptoms is allowed for the LOAEL, whereas 2 doses without symptoms are not.
16	Reappearing transient objective and subjective symptoms	—	—	—	—	Subjective	Subjective, objective	Subjective	Subjective, objective	Challenge stopped at dose 8	Dose 5	Dose 6	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
17	Reappearing transient objective and subjective symptoms	Subjective	Subjective, objective	Subjective	Subjective, objective	Subjective	Challenge stopped at dose 5	NA	NA	NA	Dose 5	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
18	Reappearing transient objective and subjective symptoms	—	Subjective	Subjective, objective	Subjective	Subjective	Subjective, objective	Challenge stopped at dose 6	NA	NA	Dose 5	Dose 6	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
19	Reappearing transient objective and subjective symptoms	—	—	Subjective	Subjective, objective	Subjective	Subjective	Subjective, objective	Subjective	Challenge stopped at dose 8	Dose 8	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.

Indication that a NOAEL or LOAEL is not determined (*ND*) indicates that the data point is left or right censored during statistical analysis.

NA, Not available.

be based on the appearance of challenge-stopping vomiting. Because of the usually large amount of food/liquid given to an infant on the final dose, vomiting that occurs solely at the final dose of the challenge is ignored, and the infant is considered tolerant unless additional (prior or concurrent) symptoms are present.²² For comparative purposes, examples of NOAEL and LOAEL derivation for challenges with vomiting in subjects both less than and greater than 1 year of age are presented in Table V.

E) Children less than 3 years old. In children less than 3 years old, abdominal pain is considered an objective symptom.²² This means that when abdominal pain is reported at a specific dose, the objective LOAEL is based on the criteria described above. Examples of NOAEL and LOAEL derivation for challenges with abdominal pain in both subjects less than and greater than 3 years of age are presented in Table VI.

NOAEL-LOAEL derivation when objective symptoms were observed over multiple doses

Objective symptoms continue from dose to dose. If a clinician continues dosing and observes objective symptoms after 2 (or more) consecutive doses before ending the challenge, then the discrete and cumulative objective LOAEL will be based on the first occurrence of objective symptoms. The same principle also applies to cases in which multiple consecutive objective symptoms are observed but the challenge ends because of the end of the dosing protocol. Examples of food challenges with reappearing symptoms, as well as with their NOAEL and LOAEL derivation, can be found in Tables II to VI.

Transient objective symptoms. In principle, discrete and cumulative objective LOAELs will be based on the first appearance of objective symptoms. However, transient objective symptoms can appear and disappear within a single dosing interval of a food challenge. In the case of transient symptoms, many clinicians will choose to continue dosing until more stable or more significant symptoms appear for a clear clinical end point. Alternatively, clinicians might elect to wait an additional period of time before administering the next dose for determination of the degree of transience of the symptoms. When determining NOAEL and LOAEL values for population risk assessment and risk management purposes, a single occurrence of a transient objective symptom before the final dose of the challenge scheme is ignored and the subject is considered tolerant, provided that no other objective symptoms occur during the remainder of the challenge. When a single occurrence of a transient objective symptom is considered a reason to stop the challenge, the dose at which the symptom occurs is deemed the discrete objective LOAEL, and the sum of all doses given is deemed the cumulative objective LOAEL. Examples of food challenges with transient symptoms and their NOAEL and LOAEL derivation can be found in Tables II to VI.

Only 1 dose between objective symptoms. When transient objective symptoms are present and only a single dose occurs without objective symptoms before the reappearance of symptoms at the next dose, after which the challenge is ended, discrete and cumulative objective LOAELs will be based on the first occurrence of objective symptoms. This principle is applicable to cases in which the challenge ends because of the clinician's judgment or because of the end of the

dosing protocol. Examples of food challenges with transient symptoms with a single dose between symptoms, along with their NOAEL and LOAEL derivation, can be found in Tables II, III, V, and VI.

Multiple doses between objective symptoms. When transient objective symptoms are present and multiple doses without objective symptoms occur before the reappearance of objective symptoms and the challenge being stopped, the discrete objective LOAELs will be based on the dose at which the objective symptoms reappear. The sum of all doses given is then designated the cumulative objective LOAEL. This principle is applicable to cases in which the challenge ends because of the clinician's judgment or because of the end of the dosing protocol. Examples of food challenges with transient symptoms with multiple doses between objective symptoms, along with their NOAEL and LOAEL derivation, can be found in Tables II, III, V, and VI.

Continuous presence of a physical finding/objective symptom. Sometimes, a visible finding, such as flush or eczema, is consistently present in a subject's day-to-day life and in the patient's constant medical state. Thus the finding is already present before administration of the first dose of a food challenge, is reported as a background observation throughout the challenge, and thus is not attributable to any allergic reaction. In such cases the background physical finding is present and not discriminative for deriving a LOAEL. Thus the background physical finding is ignored, and other objective symptoms are required for derivation of discrete and cumulative objective LOAELs.

Left- or right-censored data. It is normal for subjects undergoing a food challenge to react with objective symptoms and signs to an intermediate dose of the challenge scheme (LOAEL) after ingesting multiple doses with no symptoms. The highest dose that does not lead to objective symptoms is designated the NOAEL, and the data point would be interval censored because it has derived both a NOAEL and LOAEL. However, subjects can have objective symptoms already at the first dose of a DBPCFC or subjects can complete the challenge with multiple subjective symptoms but without objective symptoms. When a subject shows challenge-stopping objective symptoms at the first dose (ie, no NOAEL can be established), the first dose is considered the LOAEL, and the data are considered to be left censored because the subject's true threshold is at or less than the first dose (Fig 1). When a challenge is completed or stopped without observation of objective symptoms (ie, no objective LOAEL can be established) but the subject is considered to be allergic based on the clinician's judgment, the final discrete or cumulative dose given is then designated the NOAEL. In such a case the data are considered to be right censored because the dose that would elicit objective symptoms is assumed to be greater than the last given dose (Fig 1). Because both scenarios include confirmation of allergy by a clinician, left-censored and right-censored data provide additional useful information when determining the population-based threshold dose distribution.

Placebo reactions. Symptoms occurring during the placebo arm of the food challenge are an indication that a more in-depth study of that particular food challenge result is needed. If similar objective symptoms appear on both the active and placebo challenge days, additional different objective symptoms or stronger reported symptoms on the challenge day would be

TABLE IV. Examples for determining individual allergic thresholds in case of repeated, skipped, or partial doses

Example	Description	Consumed doses of protein (mg) with symptoms indicated at each dose	Final dose of protein (mg)	Discrete NOAEL (mg)	Discrete LOAEL (mg)	Cumulative NOAEL (mg)	Cumulative LOAEL (mg)	Remarks
1	Repeated dose with objective symptoms at repeated dose	1 (—), 3 (—), 10 (—), 30 (subjective), 30 (subjective, objective)	30	10	30	44	74	There were objective symptoms only at the repeated dose, after which the challenge was ended. A discrete NOAEL was set at the previous dose level. Cumulative NOAELs and LOAELs include the repeated dose.
2	Repeated dose with objective symptoms before repeated dose	1 (—), 3 (—), 10 (objective), 10 (objective)	10	3	10	4	14	The LOAEL at first occurrence of objective symptoms was determined. The cumulative LOAEL does not include the repeated dose.
3	Repeated dose with only subjective symptoms	1 (—), 3 (—), 10 (—), 30 (—), 100 (subjective), 300 (subjective), 300 (subjective)	300	300	ND	744	ND	The subject was considered allergic, but there were no objective symptoms at the highest (repeated) given dose, and therefore the subject was right censored. The cumulative NOAEL includes the repeated dose.
4	Repeated dose, thereafter challenge is continued	1 (—), 3 (—), 10 (—), 30 (subjective), 30 (subjective), 100 (subjective), 300 (subjective), 1000 (subjective, objective)	1000	300	1000	474	1474	There were objective symptoms after the repeated dose. Cumulative NOAELs and LOAELs include the repeated dose.
5	Repeated dose, thereafter challenge is continued	1 (—), 3 (objective), 3 (objective), 10 (objective)	10	1	3	1	4	The LOAEL at first occurrence of objective symptoms was determined. The cumulative LOAEL does not include the repeated dose.
6	Repeated dose, thereafter challenge is continued	1 (—), 3 (—), 10 (—), 30 (—), 100 (—), 300 (subjective), 1000 (subjective), 1000 (subjective), 3000 (subjective)	3000	3000	ND	5444	ND	The subject is considered allergic, but there are no objective symptoms at the highest given dose, and therefore the subject was right censored. The cumulative NOAEL includes the repeated dose.
7	Skipped single starting dose	3 (—), 10 (—), 30 (objective)	30	10	30	13	43	The first dose was skipped, and therefore cumulative NOAELs and LOAELs were adjusted accordingly.
8	Skipped multiple starting doses	100 (—), 300 (—), 1000 (objective)	1000	300	1000	400	1400	First doses are skipped, and therefore cumulative NOAELs and LOAELs are adjusted accordingly.
9	Skipped multiple starting doses	10 (objective)	10	ND	10	ND	10	There were objective symptoms at the lowest given dose, and therefore the subject was left censored.

(Continued)

TABLE IV. (Continued)

Example	Description	Consumed doses of protein (mg) with symptoms indicated at each dose	Final dose of protein (mg)	Discrete NOAEL (mg)	Discrete LOAEL (mg)	Cumulative NOAEL (mg)	Cumulative LOAEL (mg)	Remarks
10	Skipped single intermediate dose	1 (—), 10 (—), 30 (—), 100 (objective)	100	30	100	41	141	Single intermediate doses are skipped, and therefore cumulative NOAELs and LOAELs are adjusted accordingly.
11	Skipped multiple intermediate doses	1 (—), 10 (—), 100 (—), 300 (objective)	300	100	300	111	411	Multiple intermediate doses are skipped, and therefore cumulative NOAELs and LOAELs are adjusted accordingly.
12	Partial dose with objective symptoms	1 (—), 3 (—), 10 (—), 30 (—), 50 (objective)	50	30	50	44	94	The partial dose is greater than the previous dose, and therefore discrete NOAELs and LOAELs are as usual.
13	Partial dose with objective symptoms	1 (—), 3 (—), 10 (—), 30 (—), 100 (—), 50 (objective)	50	30	50	144	194	The partial dose is lower than the previous dose, and therefore the discrete NOAEL is set lower than the discrete LOAEL. The cumulative NOAELs and LOAELs include the partial dose.
14	Partial dose with objective symptoms before partial dose	1 (—), 3 (—), 10 (—), 30 (—), 100 (—), 300 (objective), 250 (objective)	250	100	300	144	444	The LOAEL at first occurrence of objective symptoms was determined. The cumulative LOAEL does not include the partial dose.
15	Partial dose with subjective symptoms	1 (—), 3 (—), 10 (—), 20 (subjective)	20	20	ND	33	ND	The partial dose was greater than the previous dose. The subject was considered allergic, but there were no objective symptoms at the greatest (partial) given dose, and therefore the subject was right censored. The cumulative NOAEL includes the partial dose.
16	Partial dose with subjective symptoms before the partial dose	1 (—), 3 (—), 10 (—), 30 (—), 100 (—), 300 (subjective), 1000 (subjective), 250 (subjective)	250	1000	ND	1694	ND	The partial dose was lower than the previous dose. The subject was considered allergic, but there were no objective symptoms at any given dose, and therefore the subject was right censored with a discrete NOAEL at the highest dose given. The cumulative NOAEL includes all doses given.
17	Partial dose with subjective symptoms before the partial dose	1 (—), 3 (—), 10 (—), 30 (—), 100 (—), 300 (subjective), 1000 (subjective), 250 (objective)	250	100	250	1444	1694	The subject was considered allergic, with objective symptoms at the highest (partial) given dose. The partial dose was lower than the previous dose, and therefore the discrete NOAEL was set lower than the discrete LOAEL. The cumulative NOAEL and LOAEL include the partial dose.

Indication that a NOAEL or LOAEL is not determined (ND) indicates that the data point is left or right censored during statistical analysis.

TABLE V. Examples for determining individual allergic thresholds in children less than a specific age with regard to vomiting

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
1	Vomiting, age <1 y, challenge completed	—	—	Vomiting (nonobjective)	—	—	—	—	—	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
2	Vomiting, age >1 y, challenge completed	—	—	Vomiting (objective)	—	—	—	—	—	—	ND	ND	The subject was considered tolerant because there were no symptoms at the final dose.
3	Vomiting, age <1 y, challenge completed	—	—	—	—	—	—	—	—	Vomiting (nonobjective)	ND	ND	The subject was considered tolerant. Vomiting at the final dose was not considered a symptom (objective or subjective) in an infant of <1 y.
4	Vomiting, age >1 y, challenge completed	—	—	—	—	—	—	—	—	Vomiting (objective)	Dose 8	Dose 9	LOAEL is conservative because we do not know whether the challenge would have continued or stopped if more doses were available.
5	Vomiting, age <1 y, challenge completed	—	—	—	—	—	—	Vomiting (nonobjective)	—	Vomiting (nonobjective)	Dose 9	ND	Vomiting before the final dose was considered a subjective symptom in an infant of <1 y. The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
6	Vomiting, age >1 y, challenge completed	—	—	—	—	—	—	Vomiting (objective)	—	Vomiting (objective)	Dose 6	Dose 7	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
7	Vomiting, age <1 y, challenge completed	—	—	—	—	—	Vomiting (nonobjective)	—	—	Vomiting (nonobjective)	Dose 9	ND	Vomiting before the final dose was considered a subjective symptom in an infant of <1 y. The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
8	Vomiting, age >1 y, challenge completed	—	—	—	—	—	Vomiting (objective)	—	—	Vomiting (objective)	Dose 8	Dose 9	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
9	Vomiting, age <1 y, challenge stopped	—	—	—	—	Vomiting (objective)	Challenge stopped at dose 5	NA	NA	NA	Dose 4	Dose 5	The clinician's judgment stopped the challenge, and we must consider this symptom as the objective LOAEL.
10	Vomiting, age >1 y, challenge stopped	—	—	—	—	Vomiting (objective)	Challenge stopped at dose 5	NA	NA	NA	Dose 4	Dose 5	The LOAEL at first occurrence of objective symptoms was determined.

(Continued)

TABLE V. (Continued)

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
11	Vomiting, age <1 y, challenge stopped	—	—	—	—	—	Vomiting (objective)	—	Vomiting (objective)	Challenge stopped at dose 8	Dose 5	Dose 6	The clinician's judgment stopped the challenge, and we must consider this symptom as objective. Normally, vomiting before the final dose was considered a subjective symptom in an infant of <1 y, but because it was challenge stopping. The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
12	Vomiting, age >1 y, challenge stopped	—	—	—	—	—	Vomiting (objective)	—	Vomiting (objective)	Challenge stopped at dose 8	Dose 5	Dose 6	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
13	Vomiting, age <1 y, challenge stopped	—	—	—	—	—	Vomiting (nonobjective)	—	Objective	Challenge stopped at dose 8	Dose 7	Dose 8	The LOAEL at first occurrence of objective symptoms was determined because vomiting before the final dose was considered a subjective symptom in an infant of <1 y.
14	Vomiting, age >1 y, challenge stopped	—	—	—	—	—	Vomiting (objective)	—	Objective	Challenge stopped at dose 8	Dose 5	Dose 6	The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
15	Vomiting, age <1 y, challenge stopped	—	—	—	—	Vomiting (nonobjective)	—	—	Vomiting (objective)	Challenge stopped at dose 8	Dose 7	Dose 8	The clinician's judgment stopped the challenge, and we must consider this symptom as the objective LOAEL. Vomiting before the final dose was considered a subjective symptom in an infant of <1 y.
16	Vomiting, age >1 y, challenge stopped	—	—	—	—	Vomiting (objective)	—	—	Vomiting (objective)	Challenge stopped at dose 8	Dose 7	Dose 8	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
17	Vomiting, age <1 y, challenge stopped	—	—	—	—	Vomiting (nonobjective)	—	—	Objective	Challenge stopped at dose 8	Dose 7	Dose 8	The LOAEL at first occurrence of objective symptoms was determined because vomiting before the final dose was considered a subjective symptom in an infant of <1 y.
18	Vomiting, age >1 y, challenge stopped	—	—	—	—	Vomiting (objective)	—	—	Objective	Challenge stopped at dose 8	Dose 7	Dose 8	The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.

Indication that a NOAEL or LOAEL is not determined (ND) indicates that the data point is left or right censored during statistical analysis. If both the NOAEL and LOAEL are not determined, then the subject is considered tolerant and not included during statistical analysis.

NA, Not available.

TABLE VI. Examples for determining individual allergic thresholds in children less than a specific age with regard to abdominal pain

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
1	Abdominal pain, age <3 y, challenge completed	—	—	Abdominal pain (objective)	—	—	—	—	—	—	ND	ND	The subject was considered tolerant.
2	Abdominal pain, age >3 y, challenge completed	—	—	Abdominal pain (subjective)	—	—	—	—	—	—	ND	ND	The subject was considered tolerant.
3	Abdominal pain, age <3 y, challenge completed	—	—	—	—	—	—	—	—	Abdominal pain (objective)	Dose 8	Dose 9	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL was conservative because we do not know whether the challenge would have continued or stopped if more doses were available.
4	Abdominal pain, age >3 y, challenge completed	—	—	—	—	—	—	—	—	Abdominal pain (subjective)	Dose 9	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
5	Abdominal pain, age <3 y, challenge completed	—	—	—	—	—	—	Abdominal pain (objective)	—	Abdominal pain (objective)	Dose 6	Dose 7	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL at the first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
6	Abdominal pain, age >3 y, challenge completed	—	—	—	—	—	—	Abdominal pain (subjective)	—	Abdominal pain (subjective)	Dose 9	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose.
7	Abdominal pain, age <3 y, challenge completed	—	—	—	—	—	Abdominal pain (objective)	—	—	Abdominal pain (objective)	Dose 8	Dose 9	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
8	Abdominal pain, age >3 y, challenge completed	—	—	—	—	—	Abdominal pain (subjective)	—	—	Abdominal pain (subjective)	Dose 9	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.

(Continued)

TABLE VI. (Continued)

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
9	Abdominal pain, age <3 y, challenge stopped	—	—	—	—	Abdominal pain (objective)	Challenge stopped at dose 5	NA	NA	NA	Dose 4	Dose 5	Abdominal pain was considered an objective symptom in a child <3 y of age, and therefore LOAEL at first occurrence of objective symptoms was determined.
10	Abdominal pain, age >3 y, challenge stopped	—	—	—	—	Abdominal pain (subjective)	Challenge stopped at dose 5	NA	NA	NA	Dose 5	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
11	Abdominal pain, age <3 y, challenge stopped	—	—	—	—	—	Abdominal pain (objective)	—	Abdominal pain (objective)	Challenge stopped at dose 8	Dose 5	Dose 6	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
12	Abdominal pain, age >3 y, challenge stopped	—	—	—	—	—	Abdominal pain (subjective)	—	Abdominal pain (subjective)	Challenge stopped at dose 8	Dose 8	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.
13	Abdominal pain, age <3 y, challenge stopped	—	—	—	—	—	Abdominal pain (objective)	—	Objective	Challenge stopped at dose 8	Dose 5	Dose 6	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL at first occurrence of objective symptoms was determined because there was only 1 dose without objective symptoms in between.
14	Abdominal pain, age >3 y, challenge stopped	—	—	—	—	—	Abdominal pain (subjective)	—	Objective	Challenge stopped at dose 8	Dose 7	Dose 8	LOAEL at first occurrence of objective symptoms.
15	Abdominal pain, age <3 y, challenge stopped	—	—	—	—	Abdominal pain (objective)	—	—	Abdominal pain (objective)	Challenge stopped at dose 8	Dose 7	Dose 8	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
16	Abdominal pain, age >3 y, challenge stopped	—	—	—	—	Abdominal pain (subjective)	—	—	Abdominal pain (subjective)	Challenge stopped at dose 8	Dose 8	ND	The subject was considered allergic, but there were no objective symptoms at the highest given dose, and therefore the subject was right censored.

(Continued)

TABLE VI. (Continued)

Example	Description	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Dose 9	NOAEL	LOAEL	Remarks
17	Abdominal pain, age <3 y, challenge stopped	—	—	—	—	Abdominal pain (objective)	—	—	Objective	Challenge stopped at dose 8	Dose 7	Dose 8	Abdominal pain was considered an objective symptom in a child <3 y of age. The LOAEL at reappearance of objective symptoms was determined because there were 2 doses without objective symptoms in between.
18	Abdominal pain, age >3 y, challenge stopped	—	—	—	—	Abdominal pain (subjective)	—	—	Objective	Challenge stopped at dose 8	Dose 7	Dose 8	The LOAEL at first occurrence of objective symptoms was determined.

Indication that a NOAEL or LOAEL is not determined (ND) indicates that the data point is left or right censored during statistical analysis. If both the NOAEL and LOAEL are not determined, then the subject is considered tolerant and not included during statistical analysis.

NA, Not available.

needed to continue with a LOAEL derivation for that subject. Weaker symptoms during the active challenge or identical symptoms for both the active and placebo challenges would lead to the subject being deemed a placebo reactor or tolerant.

DISCUSSION

In this article we describe the methodology that is applied to derive the objective individual allergic thresholds from clinical food challenge data in the Food Allergy Research and Resource Program–Netherlands Organisation for Applied Scientific Research Food Allergen Threshold Database (including the data presented in Taylor et al² and Allen et al³) for population food allergen risk assessment and risk management purposes. Additionally, select data derived from food challenges conducted during the European Union's 7th Framework Project Integrated Approaches to Food Allergen and Allergy Management were analyzed in this fashion, as were other published works by a number of the authors.

The methodology presented in this article can be considered as a conservative, pragmatic, and practical approach to balance data analysis from individual diagnostic food challenges for population risk assessment and risk management purposes without overcorrecting in the presence of transient or repeated symptoms. In principle, the threshold dose is based on the first appearance of objective symptoms. However, as previously demonstrated, a challenge is often continued by the clinician after the observation of objective symptoms, possibly because the investigator requires stronger evidence to confirm the diagnosis of food allergy. Thus, if the derivation of the threshold dose would be in each case based on the first objective symptom, this might lead to overconservative estimations. For example, transient urticaria in patients with atopic dermatitis on dose 1 and no other symptoms until multiple objective symptoms on dose 9 would be too conservative if dose 1 was chosen as the LOAEL. Conversely, if only the challenge-stopping dose would be used for derivation of the LOAEL, then the population-based threshold dose-distribution values could be apparently greater than they are in reality and therefore insufficiently protective.^{20,23-25} For example, a patient who presents with transient hives to doses 1 and 2 and with hives and angioedema to dose 3 would not represent the objective LOAEL if dose 3 was chosen.

Still, it should be noted that a number of assumptions have been made regarding different clinical scenarios and their interpretations described earlier in this article. To the best of the author group's knowledge, there are no data about the relative specificities of subjective versus objective symptoms (ie, appearance of symptoms on challenge vs placebo day), but objective symptoms were chosen to minimize potential patient reporting biases. Similarly, there are no data on the specificity of symptoms occurring at a subsequent dose with only 1 or multiple intervening symptoms. Additionally, the specificity of abdominal pain in children less than 3 years old and the nonspecificity of vomiting in those less than 1 years of age are assumptions based on clinical consensus from prior research or NOAEL-LOAEL derivation interactions between the authors. Again, the assumptions made in this article were for the purposes of balancing clinical consensus for allergy diagnosis with a conservative, pragmatic, and practical approach to derive data for risk assessment purposes.

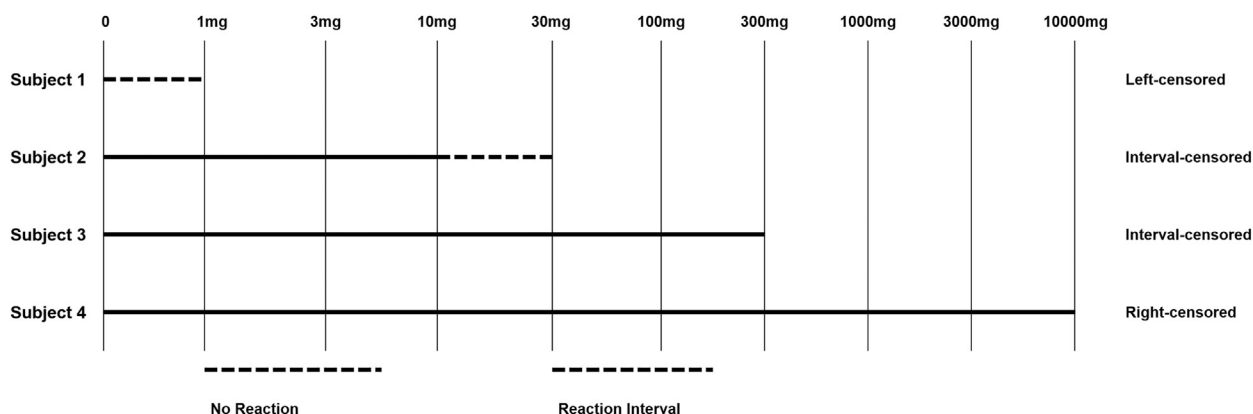


FIG 1. Graphic representation of left-censored, interval-censored, and right-censored data.

Finally, changes in NOAELs and LOAELs are increasingly being used as outcome measures in clinical intervention studies for food allergy (ie, food allergen immunotherapy). In this context the exclusion of significant subjective symptoms (ie, persistent abdominal cramps in subjects >3 years of age) in derivation of NOAEL/LOAEL values for risk assessment purposes might need to be reviewed in the future. This article provides a transparent and flexible template for interpretation of food challenge results that can be updated in the future based on potential changes in stakeholder opinions/requirements. For example, if the symptoms listed in Table I were no longer separated into objective and subjective symptoms but rather “relevant/not relevant symptom criteria for risk assessment & risk management NOAEL/LOAEL derivation,” then Tables II to VI could be updated to reflect these potential future changes, and the general framework for decision making would still apply.

In a recent article by Hourihane et al,²⁰ it was shown that the dose expected to elicit an allergic reaction in 5% of the population with peanut allergy (ED05), which was previously established by using the methodology described in this article, was moderately overconservative when unselected patients with peanut allergy were exposed in an open challenge to a single dose equal to the ED05. Of the 387 patients who took part in the study, only 8 (2.1%) had objective symptoms according to predefined criteria agreed upon by all participating clinical partners on a “single-dose” ingestion of 1.5 mg of peanut protein. These results indicate that the methodology used by the authors and presented in detail in the present article is sufficiently conservative for population risk assessment and risk management purposes. This “single-dose” approach was recently shown to be quick, safe, and highly cost-effective compared with recommending strict avoidance of precautionary labeling. Adaptation of a single-dose approach to establish tolerance to this ED05 value in combination with an industry-wide application of precautionary labeling according to the risk management programs by using the same ED05 value could have significant clinical implications.^{20,26}

The standardized DBPCFC is used in clinics for the analysis of individual threshold levels of an allergic food in patients with food allergy.¹¹ However, there are some characteristics or practices of the DBPCFC that can limit its usefulness for derivation of individual NOAELs and LOAELs. Sometimes, the first exposure during a DBPCFC is through a labial challenge,

in which a small amount of challenge material is smeared on either the outside or inside of the lip for several seconds. As a result, it is difficult to determine how much of the labial dose will actually be absorbed versus ingested. By default, such DBPCFCs are excluded unless the labial challenge was performed with a matrix and the labial dose was estimated to be around 1 mg or less. In addition, the labial dose should always be lower than the first oral dose of the dosing scheme. If this is the case, we assume that the full labial dose is ingested and the data for the DBPCFC can be used for derivation of threshold doses.

Another point of attention is that most DBPCFCs have a dosing protocol with increasing doses given every 15 to 30 minutes. This way, even a challenge with 9 dose steps can be performed during a single-day visit to the hospital. However, when there is a relatively short time between doses, it usually cannot be unequivocally established whether the observed reaction was due to the previous dose, or the cumulative dose ingested. Blumchen et al²⁷ used a protocol with 120 minutes between doses and showed that the majority of patients (45/63) had their first objective symptoms more than 30 minutes after dosing. Using a protocol with a short dosing interval could therefore result in a potential erroneous derivation of the actual discrete or cumulative threshold dose. To limit the burden for individual patients, a reduced protocol with fewer but longer time intervals might be an option, although at the expense of accuracy.²⁸

With regard to the expression of NOAELs and LOAELs by either discrete or cumulative amounts of protein, there are different arguments that can be used to favor one expression over the other. On the one hand, discrete NOAELs and LOAELs should be more representative of acute exposure during a single accidental consumption. On the other hand, cumulative NOAELs and LOAELs might be more representative of the situation in which each dose step contributes to the provocation of an allergic reaction. One major limitation of the discrete representation is apparent when dealing with partial doses. Based on the methodology presented in this article, the discrete NOAEL and LOAEL based on the partial dose might be lower than the highest consumed dose that did not result in objective symptoms, thereby resulting in overconservative estimations of the threshold dose. Currently, no evidence exists to prefer one expression over the other from a biological point of view,⁷ and in practice, there is

little actual difference between the discrete or cumulative ED values (with 95% CIs) in the population risk management dosing range of interest (ie, ED01 and ED05) for food allergen population-based thresholds for precautionary labeling.² However, differences can occur at higher ED values, such as the ED50. Thus it is suggested to express and analyze the data both as discrete and cumulative values²⁹ and to consider the risk management goal³⁰ when choosing to focus on results with discrete or cumulative reporting units.

For appropriate statistical power, it is essential that a representative sample of sufficient size (≥ 20 -30 but preferably ≥ 60 patients) is obtained to allow accurate estimation of population-based threshold dose distributions for each allergen.²⁸ In addition, risk assessment for specific subpopulations (ie, children vs adults, races/ethnicities, and geographic regions) might also be of interest, although prior research has found no differences in subpopulations or the analysis has been limited because of dosing protocol variations known to influence results when comparing data from single studies or single protocol-specific data sets.^{3,28} Consequently, the demand for additional data for some (ie, many tree nut species and lupin), but not all, allergenic foods still remains. Such data can be obtained from DBPCFCs and open food challenges, as performed in clinics worldwide. Open food challenge data are currently typically only included for specific food allergens for which limited or no data from DBPCFCs are available. If stakeholders agree to use open food challenge data for other allergenic foods as well, perhaps when these are conducted by using predefined objective stopping criteria, then these could be analyzed in a similar fashion.

Because the main objective of a clinically indicated food challenge is often to merely confirm whether a subject is allergic to a specific food, what happens at the individual dose steps is often not of primary interest. This leads to loss of information because documentation is not structured to capture valuable details that would permit more nuanced risk assessment. Therefore it is important that awareness is increased among clinicians that the data obtained in such studies is also valuable for other purposes, such as risk assessment and larger risk management programs. To have the data available to extract the threshold doses needed for risk assessment, it is essential that every observation is noted and reported for each dose during a DBPCFC and not just the challenge-stopping symptoms. In addition, every deviation (repeated, skipped, or partial doses) from the dosing protocol should be noted, and all symptoms (both objective and subjective) should be reported instead of only the severity scores (eg, Sampson, Muller) because in many cases the severity score alone does not allow identification of the objective LOAELs. Additionally, the relationship (or lack of relationship) between dose and type of symptoms experienced during an allergic reaction is complex and not clear from currently available data.³¹⁻³³ Reporting of individual food challenge data points in the detailed manner suggested above would help enable future research in this area.

With the methodology presented in this article, we aim to stimulate standardization and transparency in deriving population-based threshold dose distributions used within quantitative food allergen risk assessment and risk management programs. Harmonization and transparency will help form better risk assessments to benefit all stakeholders, although with the particular aim of improving the quality of life and quality of care of patients with food allergy. We would also hope that

harmonization fosters a more widespread adoption of risk assessment approaches based on quantitative criteria, such as reference doses.

Key messages

- This study presents an in-depth insight into the methodology to derive individual threshold doses for objective symptoms from clinical food challenge data.
- More than 90 examples for determining individual allergic thresholds are presented.
- Harmonized transparent individual threshold derivation methods will lead to better food allergen risk assessment and risk management programs.

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