CUT-OFF VALUES AND THRESHOLDS OF ALLERGIC REACTION DURING ORAL FOOD CHALLENGE:

A RETROSPECTIVE STUDY

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by

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CONTENTS

PUBLICATION-READY FORMAT

CHAPTER 1:

CHAPTER 2:

APPENDICES

DECLARATION

I, Daria Levina, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor part of it has been, or is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Introduction:

Food allergy (FA) negatively impacts the quality of life of millions of people and represents a substantial physical and economic burden for the patient, his family, and the state. The gold standard for food allergy diagnosis worldwide is the oral food challenge (OFC). However, data on the OFC threshold and the efficiency of tests used for FA diagnosis in the South African population are lacking.

Methods:

A retrospective analysis of all the OFCs carried out at the Red Cross War Memorial Children's Hospital, University of Cape Town, from 2015 to 2023 was performed. Data on the type of food used, OFC outcome, the cumulative amount of protein taken, and data from the previous allergic assessment (skin prick test, specific IgE and components results) were collected.

Results:

Of the 223 oral food challenges conducted, 61% were negative, 36% were positive, and 2% were indeterminate. Diagnostic test performance varied by allergen, with internationally derived cut-offs often demonstrating suboptimal sensitivity. For cow's milk, the fresh skin prick test (SPT) (≥4 mm) showed high sensitivity (100%) and specificity (83.3%), whereas commercial SPT (≥8 mm) had poor sensitivity (0%). Ara h2-specific IgE (sIgE) outperformed peanut sIgE (AUC 0.785 vs. 0.635). ROC analysis identified lower optimal cut-offs for sIgE, improving diagnostic accuracy. Eliciting dose thresholds varied with age, with younger children exhibiting lower thresholds for cooked eggs and peanuts. These findings highlight the need for population-specific diagnostic thresholds and age-adjusted risk assessments.

Conclusion:

This study highlights the limitations of applying international allergy test cut-offs universally and underscores the need for population-specific thresholds. Lower diagnostic cut-offs improved test performance, and younger children exhibited lower eliciting dose thresholds for certain allergens. These findings emphasize the importance of refining allergy testing protocols to balance safety with minimizing unnecessary dietary restrictions.

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LIST OF FIGURES AND TABLES

FIGURES

TABLES

ABBREVIATIONS

AAAAI: the American Academy of Allergy, Asthma and Immunology
ACAAI: the American College of Allergy, Asthma and Immunology
ALLSA: Allergy Society of South Africa
CRD: Component-resolved diagnostics
DRC: Departmental Research Committee
EAACI: the European Academy of Allergy and Clinical Immunology
ED: Eliciting Dose
HREC: Human Research Ethics Committee
LOAEL: Lowest Observed Adverse Effect Level
NOAEL: No Observed Adverse Effect Level
NPV: Negative Predictive Value
OFC: Oral Food Challenge
PPV: Positive Predictive Value
RCWMCH: Red Cross War Memorial Children’s Hospital
SCAH: School of Child and Adolescent Health
SPT: Skin Prick-Test
sIgE: specific Immunoglobulin E
UCT: University of Cape Town

CHAPTER 1:

INTRODUCTION
AND
LITERATURE REVIEW

LITERATURE REVIEW

Food allergy

Food allergy (FA) is an adverse reaction to food mediated by an immunological mechanism [1]. According to the 2024 EAACI classification of hypersensitivity reactions, types I, IVb, and V are involved in the pathophysiology of FA. This indicates the involvement of IgE antibodies, T2 cell immune response, and epithelial barrier dysfunction [2]. Differential diagnosis involves a wide range of non-immune diseases causing similar symptoms, i.e. metabolic (e.g. lactose intolerance), pharmacological (e.g. through consumption of foods containing histamine or serotonin), toxic (e.g. infection related food poisoning) and others. Prevalence of food allergy varies depending on geographic location and tests available: 7.6% in children and 10.8% in adults in the USA by survey-based self-report (no clinical tests) [3], and around 0.8% (0.7% in children and 1.4% in adults) for food challenge-verified FA in Europe [4]. In South Africa, the prevalence of clinically confirmed via open OFC FA in children aged 1 to 3 years is 2.5% in urban children and 0.5% in the rural black African cohort, with the most common culprit foods being egg, cow`s milk, peanut, and fish [5].

Oral food challenge (OFC) is still considered the “gold standard” of food allergy diagnosis worldwide. However, it is a time-consuming and potentially dangerous diagnostic method that requires special training and thorough patient observation. Barriers such as lack of time, staff, space, and experience reduce test availability [6].

Predictive values

Meanwhile, simple *in-vivo* tests such as skin-prick tests (SPT) and *in-vitro* diagnostic tests (e.g., specific IgE) for IgE-mediated FA are widely available, cheap, and rapid to perform [7]. However, neither test has a clear predictive value for an FA diagnosis in every individual [8].

The most commonly used values are the 95% positive predictive value (PPV) and the 95% negative predictive value (NPV). Both determine the probability of any given test being positive or negative in 95% of cases. Commonly used 95% PPVs for different foods were derived from European and North American populations, in subspecialist referral clinics and children with eczema (Table 1). Using a combination of history and 95% PPVs can reduce the need for OFCs by approximately 40–50% [9]. However, both PPV and NPV depend on the prevalence of a condition in the population. The baseline prevalence of allergic diseases varies significantly in different populations and thus PPV and NPV values may differ in each population being studied, requiring local research on predictive values.

Table 1 Diagnostic food-specific IgE values and skin prick tests of greater than 95% positive predictive value in European and North American settings*

Food	IgE (KU/L)	SPT (mm)
Egg	≥7	≥7
≤ 2 yr old	≥2	≥5
Milk	≥15	≥8
≤ 2 yr old	≥5	≥6
Peanut	≥15	≥8
≤ 2 yr old	n/a	≥4
Tree Nuts	≥15	n/a
Fish	≥ 20	n/a

*adapted from Du Toit G, Santos A, Roberts G, Fox AT, Smith P, Lack G. The diagnosis of IgE-mediated food allergy in childhood. Pediatr Allergy Immunol. 2009;20(4):309-319. doi:10.1111/j.1399-3038.2009.00887.x

In 2023 the European Academy of Allergy & Clinical Immunology (EAACI) updated guidelines on diagnosing IgE-mediated food allergy [8]. In the systematic review that informed the new guidelines, the diagnostic performance characteristics (sensitivity and specificity) of various cut-off levels were summarised [10] (Table 2).

Table 2 Stratified analyses of diagnostic performance by predefined cut-offs*

Food allergy	Test	Value	Specificity (95% CI)
Cooked egg allergy	sIgE egg white	≥7 kUA/L	70.0 (61.0; 78.0)
	SPT to egg white	≥7 mm	68.0 (55.0; 79.0)
Cow's milk allergy	SPT to cow's milk	≥8 mm	94.0 (23.0; 100)
Peanut allergy	sIgE to peanut	≥15 kUA/L	98.0 (96.0;99.0)
	SPT	≥8	94.0 (85.0;98.0)

* adapted fromRiggioni C, Ricci C, Moya B, et al. Systematic review and meta-analyses on the accuracy of diagnostic tests for IgE-mediated food allergy. Allergy. 2024;79(2):324-352. doi:10.1111/all.15939

Component-resolved diagnostics (CRD) measures specific IgE to epitopes within a whole protein extract to increase the predictive value. As with specific IgE to whole protein extracts, the levels at which high predictive values are reached, and the performance characteristics achieved, vary widely between allergens. [11] (Table 3, 4).

Table 3 Diagnostic cut-off levels and positive predictive value (PPV) for specific IgE to individual allergen components*

Food	IgE (KU/L)	PPV

Egg ovomucoid		
to diagnose baked egg allergy	50	95%
to diagnose cooked egg allergy	26.6	95%
to diagnose raw egg allergy	5.21	95%
Milk casein		
to diagnose baked milk allergy	20.2	69%
Peanut Ara h 2	0.35-42.2	90%-95%

* adapted from Foong RX, Dantzer JA, Wood RA, Santos AF. Improving Diagnostic Accuracy in Food Allergy. J Allergy Clin Immunol Pract. 2021;9(1):71-80.

Table 4 Stratified analyses of diagnostic performance by predefined cut-offs.*

Food allergy	Test	Value	Specificity (95% CI)
Cooked egg allergy	Ovomucoid-sIgE	≥0.35 kUA/L	90.0 (85.0; 93.0)
Peanut allergy	Ara h 2-sIgE	≥0.35 kUA/L	93.0 (87.0;96.0)

* adapted from Riggioni C, Ricci C, Moya B, et al. Systematic review and meta-analyses on the accuracy of diagnostic tests for IgE-mediated food allergy. Allergy. 2024;79(2):324-352.

Marked geographical differences exist in the diagnostic performance of tests. For example, in Asia, the specificity of Ara h2-sIgE of >0.35kU/L achieves 75%, whereas in Northern Europe and Australia, the specificity reaches 97%. In South Africa, an SPT to egg white resulted in a 70% sensitivity and 81% specificity for challenge-proven food allergy to cooked eggs. In contrast, the Southern European data shows SPTs to result in 83% sensitivity and 78% specificity [10]. This highlights the need to study diagnostic performance in the local population.

A study on peanut allergy diagnosis in South African children with moderate to severe atopic dermatitis calculated sensitivity and specificity for peanut allergy diagnosis in different populations [12] (Table 5). This study also shows a significant difference between Ara h 2 ability to predict peanut allergy (53% in the Xhosa group and 93% in the mixed-race group).

Table 5 Sensitivities and specificities of different tests in peanut-sensitised patients in the overall population

Test	Sensitivity	Specificity	PPV	NPV
SPT ≥3 mm	100	73	55	100
ImmunoCAP ≥0.35 kU/L	100	40	67	100
ISAC Ara h 2 ≥0.3 ISAC units	83	75	80	70
ImmunoCAP Ara h 2 ≥0.35 kU/L	92	60	72	86

Gray CL, Levin ME, Du Toit G. Which test is best for diagnosing peanut allergy in South African children with atopic dermatitis?. S Afr Med J. 2016;106(2):214-220..

The same study determined predictive values for egg allergy [13] (Table 6) and also showed ethnic differences.

Table 6 Sensitivities and specificities of different tests in egg-sensitised patients in the overall population

Test	Sensitivity	Specificity	PPV	NPV
SPT egg white extract ≥7 mm	32	75	53	55

SPT fresh egg white ≥7 mm	96	36	57	91
RAST egg white ≥7 kU/l	68	79	74	73
ImmunoCAP to egg white	96	18	51	-

Gray CL, Levin ME, du Toit G. Egg sensitization, allergy and component patterns in African children with atopic dermatitis. Pediatr Allergy Immunol. 2016;27(7):709-715.

A study conducted at Red Cross Hospital comparing OFC results with the 95% PPV found that a significant proportion of children had negative OFCs despite having IgE levels above this threshold [14]. Among those challenged with egg, 36.1% (17/47) of mixed-race children and 42.9% (3/7) of black African children had negative OFCs. For cow's milk challenges, 40.0% (6/15) of mixed-race children and 80.0% (4/5) of black African children had negative OFCs despite elevated IgE levels (p=0.12). In peanut challenges, 21.7% (5/23) of mixed-race children had negative OFCs with IgE above 95% PPV. Only one black African child with IgE above this threshold underwent a peanut challenge and had a positive result.

Thus, there is a growing need for research on predictive values in the local setting.

Thresholds

For patients` safety, precautionary allergen labelling is used. Labelling legislation varies across the globe. In South Africa, eight major food allergens and sulphites are required to be labelled in clear, common language [15]. Precautionary allergen labelling is less well legislated, with suppliers of prepackaged food being allowed to report the possibility of accidental presence of allergens in products with various statements and without the need for actual testing of allergen content. This creates uncertainty in allergic consumers and limits the usefulness of such labelling. Reform of the use of precautionary labelling is urgently required. A strong case can be made for the standardisation of labelling practices using measurement and risk assessment.

Codex Alimentarius shapes food safety and labelling regulations globally, influencing national laws and regulations, industry practices, and international trade agreements [16]. Codex promotes quantitative risk assessment by measuring the amount of allergen present in the food and comparing it to threshold levels.

Important threshold dose terminology includes the eliciting dose (ED), lowest observed adverse effect level (LOAEL), and no observed adverse effect levels (NOAELs) [17].

Thresholds can be divided into individual and population ones. NOEL and LOAEL can be used to reflect both individual and population thresholds. The highest dose observed not to produce any adverse effect is the NOAEL, and the lowest dose that will produce an adverse effect is the LOAEL. Individual thresholds may be used to counsel patients on their unique risk patterns. Although using population group NOAELs as a threshold by which to determine amounts or concentrations required for food labelling would protect every allergic individual (in that representative population, providing the sample is large enough and reflective of the broader group), it is of no value in a pragmatic approach to finding a level at which most people will be safe, without unnecessarily markedly restricting food choices. This is the rationale for not using NOAELs and LOAELs on a population level.

For the population, eliciting doses can be calculated [18]. ED is a minimum dose causing a reaction to a product in a particular proportion of people who are allergic to it. Eliciting doses are measurements which are used to quantify the potency of an allergen and assess the risk of an allergic reaction. The ED is calculated using the OFC outcomes: the dose of protein received and the percentage of people reacting to it. ED01 is the dose causing a reaction in 1% of individuals allergic to that food, and ED05 is the dose causing a reaction in 5% of individuals allergic to that food. ED50 and ED95 are also often calculated.

Because different allergens have different potencies, the ED05 varies widely between different foods. A lower ED05 value indicates higher allergenic potency, greater risk of reaction and the need for stricter labelling and safety requirements. For example, egg protein has a low ED01 and ED05, while the lowest ED10 and ED50 were for sesame and tree nuts [19]. In another study, those with egg and pistachio allergy had the lowest ED50 [20].

Proposed labelling outcomes include

- Contains [Allergen]: Label required if the amount exceeds the threshold
- May Contain [Allergen]: Voluntary label used when allergen presence is possible but below a threshold
- Free From [Allergen]: Label allowed if rigorous testing and controls ensure allergen absence

Data on the OFC thresholds and the efficiency of tests used for FA diagnosis in the South African population are lacking.

Therefore, we aimed to determine threshold levels and optimal diagnostic cut-offs for cooked egg, whole milk, peanut, and cashew nut allergies.

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CHAPTER 2:

PUBLICATION-READY MANUSCRIPT

STRUCTURED ABSTRACT

Introduction:

Food allergy (FA) negatively impacts the quality of life of millions of people and represents a substantial physical and economic burden for the patient, his family, and the state. The gold standard for food allergy diagnosis worldwide is the oral food challenge (OFC). However, data on the OFC threshold and the efficiency of tests used for FA diagnosis in the South African population are lacking.

Methods:

A retrospective analysis of all the OFCs carried out at the Red Cross War Memorial Children's Hospital, University of Cape Town, from 2015 to 2023 was performed. Data on the type of food used, OFC outcome, the cumulative amount of protein taken, and data from the previous allergic assessment (skin prick test, specific IgE and components results) were collected.

Results:

Of the 223 oral food challenges conducted, 61% were negative, 36% were positive, and 2% were indeterminate. Diagnostic test performance varied by allergen, with internationally derived cut-offs often demonstrating suboptimal sensitivity. For cow's milk, the fresh skin prick test (SPT) (≥4 mm) showed high sensitivity (100%) and specificity (83.3%), whereas commercial SPT (≥8 mm) had poor sensitivity (0%). Ara h2-specific IgE (sIgE) outperformed peanut sIgE (AUC 0.785 vs. 0.635). ROC analysis identified lower optimal cut-offs for sIgE, improving diagnostic accuracy. Eliciting dose thresholds varied with age, with younger children exhibiting lower thresholds for cooked eggs and peanuts. These findings highlight the need for population-specific diagnostic thresholds and age-adjusted risk assessments.

Conclusion:

This study highlights the limitations of applying international allergy test cut-offs universally and underscores the need for population-specific thresholds. Lower diagnostic cut-offs improved test performance, and younger children exhibited lower eliciting dose thresholds for certain allergens. These findings emphasize the importance of refining allergy testing protocols to balance safety with minimizing unnecessary dietary restrictions.

MAIN ARTICLE

INTRODUCTION

Food allergy (FA) is an adverse reaction to food mediated by an immunological mechanism [1]. According to the 2024 EAACI classification of hypersensitivity reactions, types I, IVb, and V are involved in the pathophysiology of FA. This indicates the involvement of IgE antibodies, T2 cell immune response, and epithelial barrier dysfunction[2]. Prevalence of food allergy varies depending on geographic location and tests available: 7.6% in children and 10.8% in adults in the USA [3], and around 1% (0.7% in children and 1.4% in adults) in Europe [4]. In South Africa, the prevalence of FA in children aged 1-3 years is 2.5% in urban Cape Town and 0.5% in the rural Eastern Cape, with the most common culprit foods being egg, cow`s milk, peanuts, and fish [5].

Oral food challenge (OFC) is still considered the “gold standard” of food allergy diagnosis worldwide. However, it is a time-consuming and potentially dangerous diagnostic method that requires special training and thorough patient observation. Barriers such as lack of time, staff, space, and experience reduce test availability [6].

Predictive values

Meanwhile, simple in-vivo tests such as skin-prick tests (SPT) and in-vitro diagnostic tests (e.g., specific IgE) for IgE-mediated FA are widely available, cheap, and rapid to perform [7]. However, neither test has a clear predictive value for an FA diagnosis in every individual [8].

The most commonly used values are 95% positive predictive value (PPV) and 95% negative predictive value (NPV). Both determine the probability of any given test being positive or negative in 95% of cases. Using a combination of history and 95% PPVs can reduce the need for OFCs by approximately 40–50% [9]. However, both PPV and NPV depend on the prevalence of a condition in the population. The baseline prevalence of allergic diseases varies significantly in different populations and thus PPV and NPV values may differ in each population being studied, requiring local research on predictive values.

Marked geographical differences exist in the diagnostic performance of tests [10]. For example, in Asia, the specificity of Ara h2-sIgE of >0.35kU/L achieves 75%, whereas in Northern Europe and Australia, the specificity reaches 97%. In South Africa, an SPT to egg white resulted in a 70% sensitivity and 81% specificity for challenge-proven food allergy to cooked eggs. In contrast, the Southern European data shows an SPT to result in 83% sensitivity and 78% specificity. That highlights the need to specify diagnostic performance in the local population.

Thresholds

Thresholds for adverse effects can be categorised as individual or population-based. NOAEL (No Observed Adverse Effect Level) is the highest dose without adverse effects, while LOAEL (Lowest Observed Adverse Effect Level) is the lowest dose that causes adverse effects. Individual thresholds help in advising patients about their specific risk profiles [11].

Population-based thresholds are useful for setting food labelling standards to protect allergic individuals, though they can be overly restrictive. Eliciting doses (EDs) quantify the minimum dose that causes allergic reactions in a percentage of the population. ED01, ED05, ED50, and ED95 represent doses causing reactions in 1%, 5%, 50%, and 95% of allergic individuals, respectively. These are calculated using oral food challenge data to assess allergen potency and risk [12].

Threshold doses quantify allergenic potency and guide safety measures. ED05, indicating the dose causing a reaction in 5% of allergic individuals, varies significantly among allergens. For instance, egg protein has a lower ED05 than tree nuts, necessitating stricter safety requirements [13].

Globally, allergen labelling legislation is inconsistent. While major allergens must be labelled in South Africa, precautionary allergen labelling lacks standardization, creating uncertainty for consumers [13]. Codex Alimentarius advocates quantitative risk assessment to align allergen thresholds with labelling. Proposed reforms include standardized labels such as [14]:

- **Contains [Allergen]:** Mandatory for amounts exceeding thresholds.

- **May Contain [Allergen]:** Voluntary for potential cross-contamination below thresholds.

- **Free From [Allergen]:** Permitted with validated testing protocols.

Advancing FA diagnostics and labelling in South Africa requires population-specific research and regulatory reform. Tailored thresholds, diagnostic performance evaluations, and standardization of labelling will enhance patient safety and quality of life.

We aimed to determine threshold levels and optimal diagnostic cut-offs for cooked egg, whole milk, peanut, and cashew nut allergies.

OBJECTIVES

- To calculate the sensitivity, specificity predictive values of commonly used cut-off levels for egg, milk, peanut, cashew, and hake proteins in the South African food-allergic population
- To calculate optimal cut-off values for SPT and IgE levels for egg, milk, peanut, cashew, and hake proteins in the South African food-allergic population
- To calculate 95% PPV for the SPT and IgE levels for egg, milk, peanut, cashew, and hake proteins in the South African food-allergic population
- To calculate NOAEL, LOAEL, ED01, ED05, ED10, ED50 and ED95 for egg, milk, peanut, cashew, and hake proteins in the South African food-allergic population

METHODS

STUDY DESIGN

A retrospective analysis of the OFCs and sensitisation data (skin prick test and specific IgE results) was performed on children who underwent OFCs at the Red Cross War Memorial Children's Hospital (RCWMCH) Allergy Clinic from 2017 to 2023.

SETTING

RCWMCH is a dedicated paediatric hospital in Cape Town, providing secondary, tertiary and quaternary care to an average of 250,000 patients per year, referred from the Western Cape, the rest of South Africa, and across broader Africa. The Division of Asthma and Allergy provides diagnostic and treatment services on an inpatient, outpatient and outreach basis, providing multi-disciplinary care with established collaborations between associated services, including gastroenterology, dietetics, dermatology, pulmonology, ophthalmology, otolaryngology and infectious diseases.[17] Approval was received from the UCT Human Research Ethics Committee and institutional approval was obtained from RCWMCH.

PARTICIPANTS

Population

All children who underwent OFCs to cooked egg, whole milk, peanut butter, cashew butter, and hake at RCWMCH between January 2017 and December 2023 were included. Cooked egg, whole milk, peanut butter, cashew butter, and hake OFC were selected due to the highest proportion of positive outcomes, which is needed to provide statistical value. This was established based on a recent retrospective review of food challenge outcomes [15].

Recruitment

Clinical notes of the medical records of all patients undergoing an OFC to cooked egg, whole milk, peanut, cashew nut, and hake were reviewed retrospectively.

MEASUREMENTS

Amount of protein in OFC

The amount of allergen protein in each product used for food challenges was estimated. The amounts of protein per 1 gram of foods used in the OFC were estimated from the package information and are presented in the Appendix A. Patient records were examined to extract data on the cumulative dose of food ingested and the occurrence of symptoms. Protein doses ingested were calculated, and in the case of a positive OFC, the cumulative dose at which the reaction appeared (the sum of all doses ingested including the dose at which the reaction occurred) was considered the LOAEL, and the NOEL was defined as the cumulative dose of protein ingested without the dose at which the reaction accrued. Where deviations from the standard protocol occurred (due to partial ingestion of the food dose or other factors), the actual doses consumed were estimated.

Variables

- Demographics: age at OFC
- Diagnostic investigations (SPT, sIgE)
- Doses of protein ingested during the challenge
- Positive/negative outcome of the OFC

Diagnostic investigations (SPT, sIgE) were chosen individually by the doctor according to the patient`s clinical history, test availability, and parent`s preference. SPTs to egg white, cow`s milk and peanut were provided by the hospital and some patients had additional skin tests with fresh egg, cow`s milk, and peanut butter are also used. sIgE was measured with the ImmunoCAP 300 instrument (Thermo Fisher Scientific) to allergen extracts (egg white, cow`s milk, peanut, cashew, hake) and components (ovomucoid, casein, Ara h2). OFCs were open-label, with incremental dosing, based on standard clinical protocols at RCWMCH. Food allergy diagnosis was confirmed by an OFC. Therefore participants with positive OFC were considered allergic, when those with negative outcome were considered non-allergic.

DATA MANAGEMENT

Patient details were anonymised, and variables were entered into an Excel spreadsheet stored and analysed on a secure computer.

STATISTICAL ANALYSIS

1. The median, the interquartile range, and the mode were calculated to describe the patient's age at the time of challenge.

2. Statistical analysis of cut-off values for the following allergens was performed: cooked egg, whole milk, peanut butter, cashew butter, and hake. Including the following:

    - The proportion of patients passing the oral food challenge with diagnostic test results above internationally derived and commonly used 95% PPV levels [9].

    - The specificity, sensitivity, PPVs, and NPVs were calculated using the internationally derived and commonly used cut-off levels to assess the utility of using these values in this patient cohort [10]

International cut-offs according to the literature combined [9,10]

Food	IgE (KU/L)	SPT (mm)	CRD (KU/L)
Egg	≥7	≥7	OVM ≥0.35
≤ 2 yr old	≥2	≥5	n/a
Milk	≥15	≥8	Casein ≥0.35
≤ 2 yr old	≥5	≥6	n/a
Peanut	≥15	≥8	Ara h2 ≥0.35
≤ 2 yr old	n/a	≥4	n/a
Tree Nuts (Cashew)	≥15	n/a	
Fish	≥ 20	n/a	

Peanut:	Cooked egg:	Cow`s milk:	Cashew:
SPT to peanut ≥4 mm sIgE to peanut ≥4.3 kUA/L	SPT to egg ≥5 mm SPT to fresh egg ≥6 mm	SPT to cow`s milk ≥4 mm SPT to fresh cow`s milk ≥4 mm	sIgE to cashew ≥1.1 kUA/L

Ara h 2-sIgE ≥0.44 kUA/L	sIgE to egg white ≥3.5 kUA/L OVM-sIgE ≥0.8 kUA/L	sIgE to cow`s milk ≥3.5 kUA/L Casein-sIgE ≥2.6 kUA/L	

- A receiver operating characteristic (ROC) curve analysis was used to identify the population's optional cut-offs.

3. Interval-censoring survival analysis was used to demonstrate eliciting dose curves for each food and to obtain population-specific NOAEL, LOAEL, ED01 ED05, ED50, and ED95 values.

RESULTS

Population

Data on 223 OFC were analysed. 137 OFC were negative, 81 were positive, and 5 were indeterminate. Indeterminate OFCs were excluded from the final analysis.

Children’s age varied from 6 months to 17 years. There were no statistical differences in patient age in the negative and positive OFC groups (Wilcoxon rank sum test; p-value = 0.2). The median age was 59 months in the negative OFC group and 75 months in the positive OFC group. The minimum and maximum age in groups was nine months and 209 months in patients with negative OFCs and seven months and 180 months with positive OFCs. Of all the OFCs 43 were performed in children two years old or younger, and 175 were older than two.

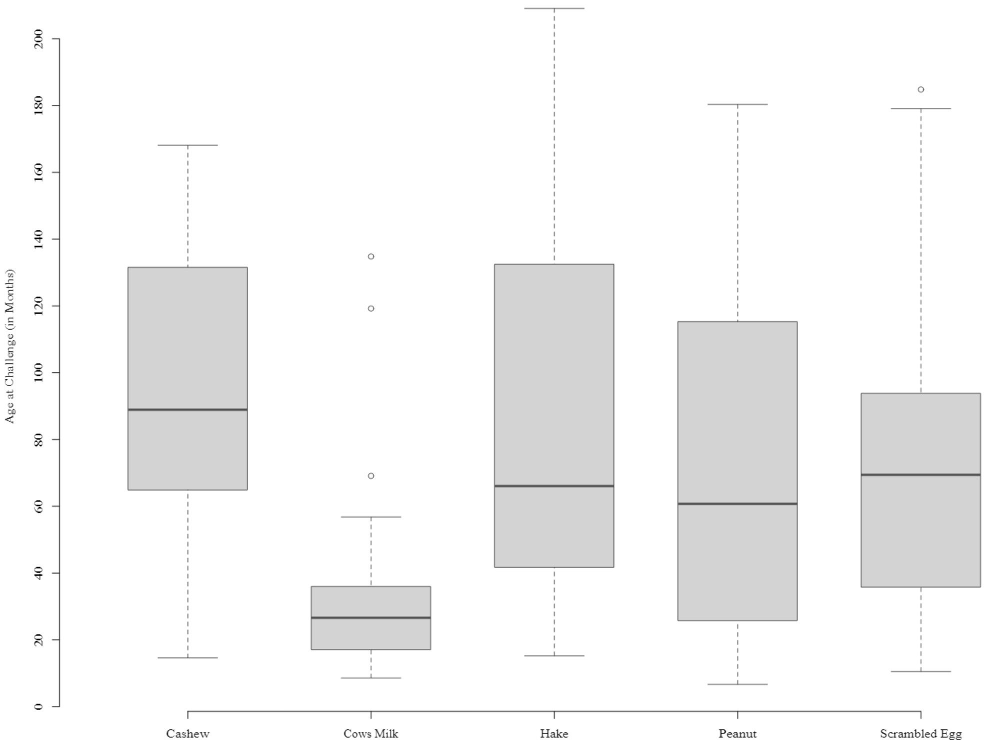
Most of the OFCs were performed to cooked egg - 79 (36%), followed by peanut - 66 (30%), cashew - 27 (12%), cow`s milk - 24 (11%), and hake - 22 (10%). The most commonly challenged foods at the age of 2 years and below were peanut - 15 (35%) and cooked egg - 13 (30%), cow`s milk was challenged 9 times (21%), hake 5 times (12%), and cashew just once (2.3%). In children older than 2 cooked egg was challenged 66 times (38%), peanut 51 (29%), cashew 26 (15%), hake 17 (9.7%), and cow`s milk 15 (8.6%) (Table 1).

Table 1: Population characteristics of children undergoing oral food challenges (OFCs)

Food challenged	Overall n=218	<= 2 n=43	> 2 n=175
Cow`s milk	24 (11%)	9 (21%)	15 (8.6%)
Scrambled egg	79 (36%)	13 (30%)	66 (38%)
Peanut	66 (30%)	15 (35%)	51 (29%)
Cashew	27 (12%)	1 (2.3%)	26 (15%)
Hake	22 (10%)	5 (12%)	17 (9.7%)

Cow’s milk challenges were performed at a median age of 28 months, whereas peanut, egg, fish and cashew challenges were performed in older children (p=0.0001) (Figure 1)

Figure 1: Age distribution of children undergoing OFCs by allergen



Predictive values

The proportion of patients passing the OFC with test results above commonly used 95% PPV levels was calculated for all of the foods. For cooked eggs, 8% were negative with IgE >7 kUA/L, and 1% of OFC to peanut were negative with IgE >14 kUA/L. For hake and cow`s milk, none of the tests were negative above the 95% PPV, for cashew, there were no patients challenged with PPV above 95%.

Performance characteristics of internationally derived and commonly used cut-off levels

Table 2 presents the sensitivity and specificity of cut-off values reported in the literature and commonly used as 95% PPV levels to identify candidates for the OFC. Table 3 presents predictive values of the optimal cut-offs proposed by the latest EAACI systematic review [10] in the study population.

Table 5 shows a comparison between the optimal cut-offs in the systematic review [10] and the ones calculated in our studied population (presented in the Table 4).

Table 2: Performance of diagnostic tests for food allergy prediction

Test	Cut-off	AUC	Youden-test results
Cow`s milk sIgE	15 kUA/L	0.75	Sensitivity: 0.5; Specificity:1
Casein sIgE	0.35 kUA/L	0.75	Sensitivity: 1; Specificity:0.5
Cow`s milk commercial SPT	8 mm	0.5	Sensitivity: 0; Specificity:1
Cow`s milk fresh SPT	8 mm	0.8333	Sensitivity: 0.667; Specificity:1
Egg white sIgE	7 kUA/L	0.5693	Sensitivity: 0.318; Specificity:0.821
Ovomucoid sIgE	0.35 kUA/L	0.5393	Sensitivity: 0.727; Specificity:0.351
Egg white commercial SPT	7 mm	0.5192	Sensitivity: 1; Specificity:0.038
Egg white fresh SPT	7 mm	0.6125	Sensitivity: 0.6; Specificity:0.625
Peanut sIgE	15 kUA/L	0.5296	Sensitivity: 0.0938; Specificity:0.9655

Ara h2 sIgE	0.35 kUA/L	0.7425	Sensitivity: 0.781; Specificity:0.704
Peanut commercial SPT	8 mm	0.6071	Sensitivity: 0.214; Specificity:1
Peanut fresh SPT	8 mm	0.6250	Sensitivity: 1; Specificity:0.25
Cashew sIgE	15 kUA/L	0.5455	Sensitivity: 0.0909; Specificity:1
Hake sIgE	20 kUA/L	0.5	Sensitivity: 0; Specificity:1

Table 3: Sensitivity, specificity, and predictive values of international cut-offs according to the latest EAACI systematic review [10]

Test	**Cut-off (median)**	**AUC**	**Youden-test results**	**According to EAACI 2023**
Cow`s milk sIgE	3.5 kUA/L	0.7917	Sensitivity: 0.75; Specificity:0.833	Sensitivity: 0.82; Specificity:0.92
Casein sIgE	2.6 kUA/L	0.75	Sensitivity: 0.5; Specificity:1	Sensitivity: 0.67; Specificity:0.93
Cow`s milk commercial SPT	4 mm	0.6667	Sensitivity: 0.333; Specificity:1	Sensitivity: 0.52; Specificity:0.8
Cow`s milk fresh SPT	4 mm	0.9167	Sensitivity: 1; Specificity:0.833	Sensitivity: 0.9; Specificity:0.8

Egg white sIgE	3.5 kUA/L	0.5478	Sensitivity: 0.455; Specificity:0.641	Sensitivity: 0.85; Specificity:0.73
Ovomucoid sIgE	0.8 kUA/L	0.6020	Sensitivity: 0.636; Specificity:0.568	Sensitivity: 0.74; Specificity:0.91
Egg white commercial SPT	5 mm	0.5256	Sensitivity: 0.167; Specificity:0.885	Sensitivity: 0.68; Specificity:0.77
Egg white fresh SPT	6 mm	0.7708	Sensitivity: 1; Specificity:0.542	Sensitivity: 0.94; Specificity:0.66
Peanut sIgE	4.3 kUA/L	0.5857	Sensitivity: 0.344; Specificity:0.828	Sensitivity: 0.81; Specificity:0.83
Ara h2 sIgE	0.44 kUA/L	0.7795	Sensitivity: 0.781; Specificity:0.778	Sensitivity: 0.82; Specificity:0.92
Peanut commercial SPT	4 mm	0.5	Sensitivity: 0; Specificity:1	Sensitivity: 0.84; Specificity:0.86
Peanut fresh SPT	4 mm	0.6071	Sensitivity: 0.714; Specificity:0.5	NA
Cashew sIgE	1.1 kUA/L	0.6648	Sensitivity: 0.455; Specificity:0.875	Sensitivity: 0.94; Specificity:0.64

Optimal cut-offs

The optimal cut-offs for the population were identified by the largest area under the curve (AUC).

Table 4: Population-specific optimal diagnostic cut-offs based on ROC analysis

Test	**Cut-off**	**AUC**	**Youden-test results**
Cow`s milk sIgE	1.72 kUA/L	0.9167	Sensitivity: 1; Specificity:0.833
Casein sIgE	1.447 kUA/L	0.8125	Sensitivity: 0.75; Specificity:0.875
Cow`s milk commercial SPT	1 mm	0.8125	Sensitivity: 1; Specificity:0.625
Cow`s milk fresh SPT	4 mm	0.9167	Sensitivity: 1; Specificity:0.833
Egg white sIgE	4.59 kUA/L	0.6247	Sensitivity: 0.455; Specificity:0.795
Ovomucoid sIgE	0.686 kUA/L	0.6474	Sensitivity: 0.727; Specificity:0.568
Egg white commercial SPT	1 mm	0.6731	Sensitivity: 1; Specificity:0.346
Egg white fresh SPT	6 mm	0.7708	Sensitivity: 1; Specificity:0.542
Peanut sIgE	1.42 kUA/L	0.6352	Sensitivity: 0.719; Specificity:0.552
Ara h2 sIgE	0.599 kUA/L	0.7853	Sensitivity: 0.719; Specificity:0.852

Peanut commercial SPT	7 mm	0.6071	Sensitivity: 0.214; Specificity:1
Peanut fresh SPT	1 mm	0.7500	Sensitivity: 1; Specificity:0.5
Cashew sIgE	1.838 kUA/L	0.6960	Sensitivity:0.455; Specificity:0.9375
Hake sIgE	0.532 kUA/L	0.7929	Sensitivity: 0.8; Specificity:0.786
Hake commercial SPT	0 mm	0.8750	Sensitivity: 1; Specificity:0.75

Table 5: Comparison of population-specific optimal diagnostic cut-offs with those proposed in the latest EAACI systematic review [10]

Test	**Cut-off (South African population)**	**Cut-off (EAACI 2023)**
Cow`s milk sIgE	1.72 kUA/L	3.5 kUA/L
Casein sIgE	1.447 kUA/L	2.6 kUA/L
Cow`s milk commercial SPT	1 mm	4 mm
Cow`s milk fresh SPT	4 mm	4 mm
Egg white sIgE	4.59 kUA/L	3.5 kUA/L

Ovomucoid sIgE	0.686 kUA/L	0.8 kUA/L
Egg white commercial SPT	1 mm	5 mm
Egg white fresh SPT	6 mm	6 mm
Peanut sIgE	1.42 kUA/L	4.3 kUA/L
Ara h2 sIgE	0.599 kUA/L	0.44 kUA/L
Peanut commercial SPT	7 mm	4 mm
Peanut fresh SPT	1 mm	4 mm
Cashew sIgE	1.838 kUA/L	1.1 kUA/L

Figure 2: ROC curves for cow`s milk allergy tests (Cow`s milk and casein ImmunoCAP, cow`s milk commercial and fresh milk SPT)

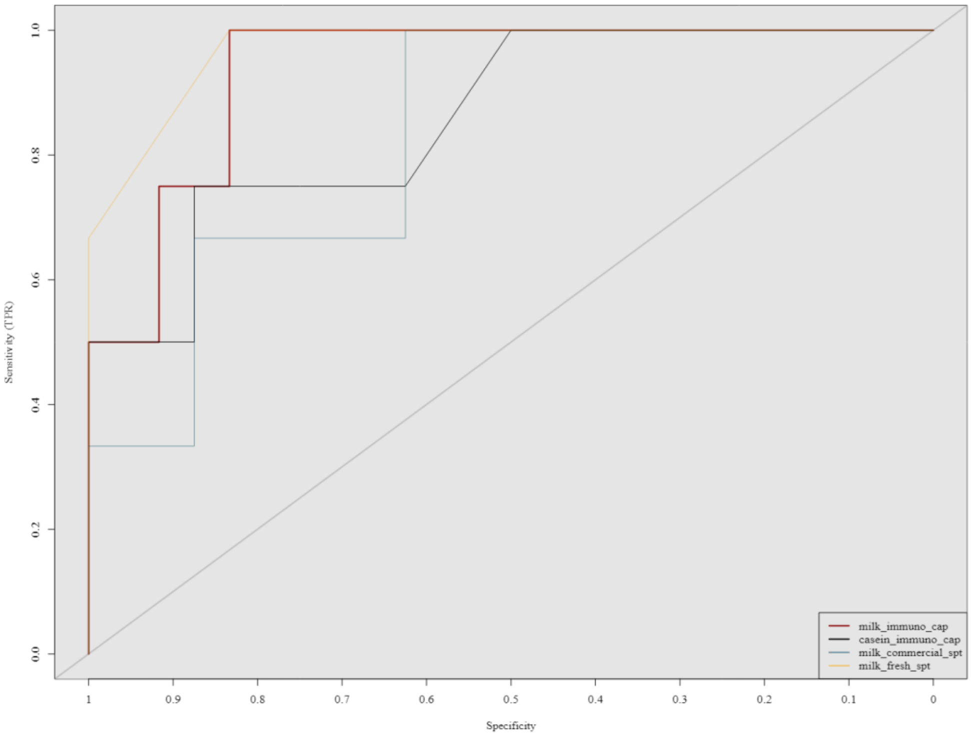


Figure 3: ROC curves for cooked egg allergy tests (Egg white and ovomucoid ImmunoCAP, egg`s white commercial and fresh egg SPT)

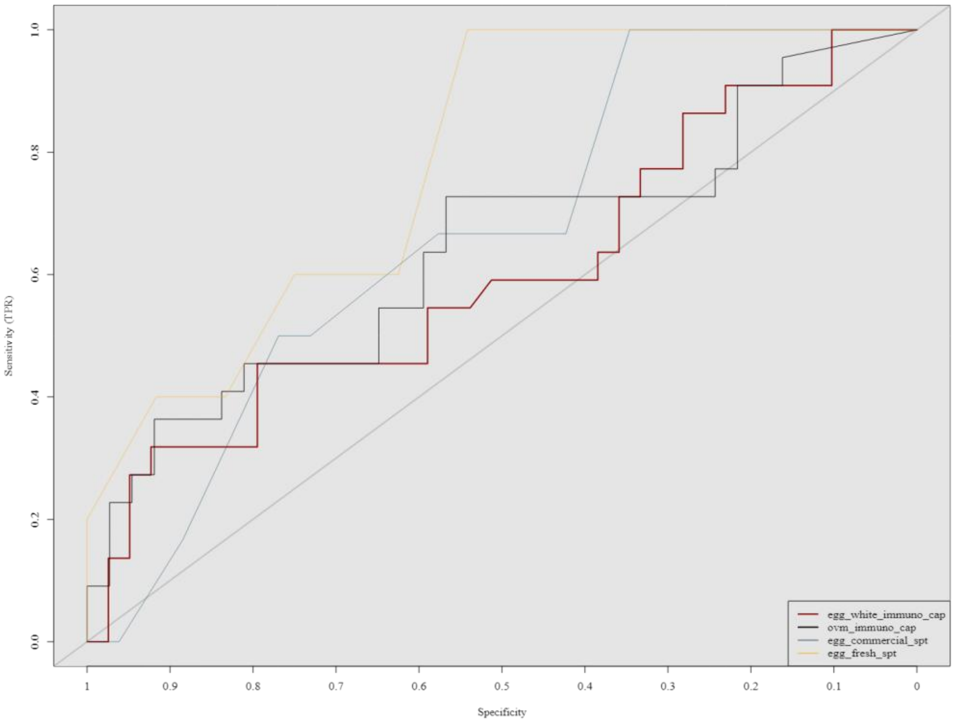


Figure 4: ROC curves for peanut allergy tests (Peanut and Ara h2 ImmunoCAP, peanut commercial and fresh peanut SPT)

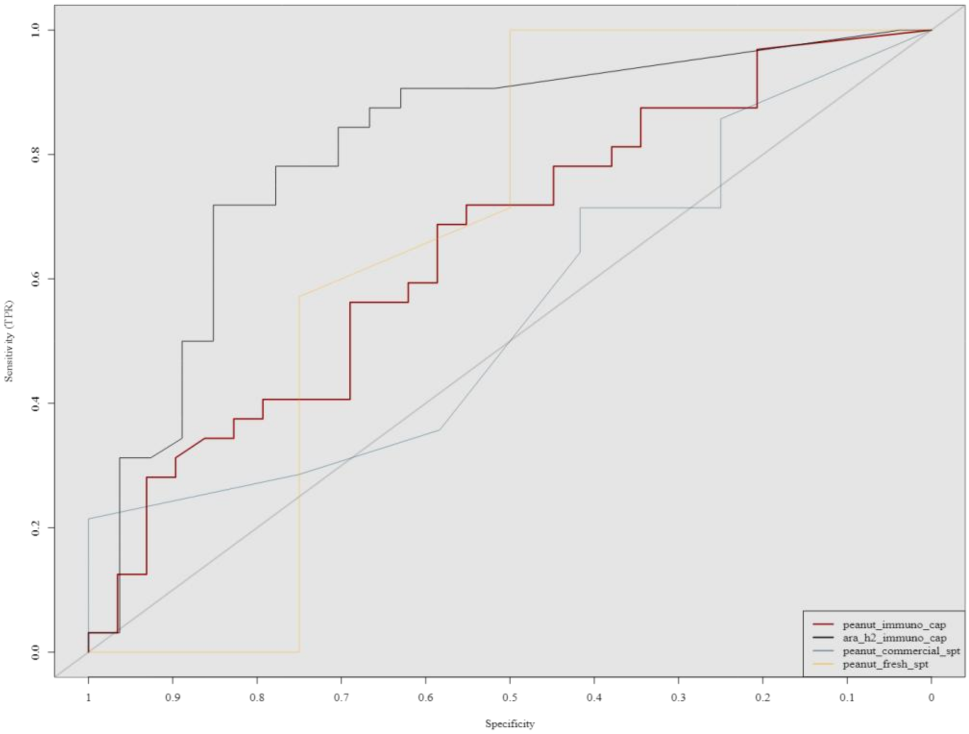


Figure 5: ROC curves for cashew allergy test (Cashew ImmunoCAP)

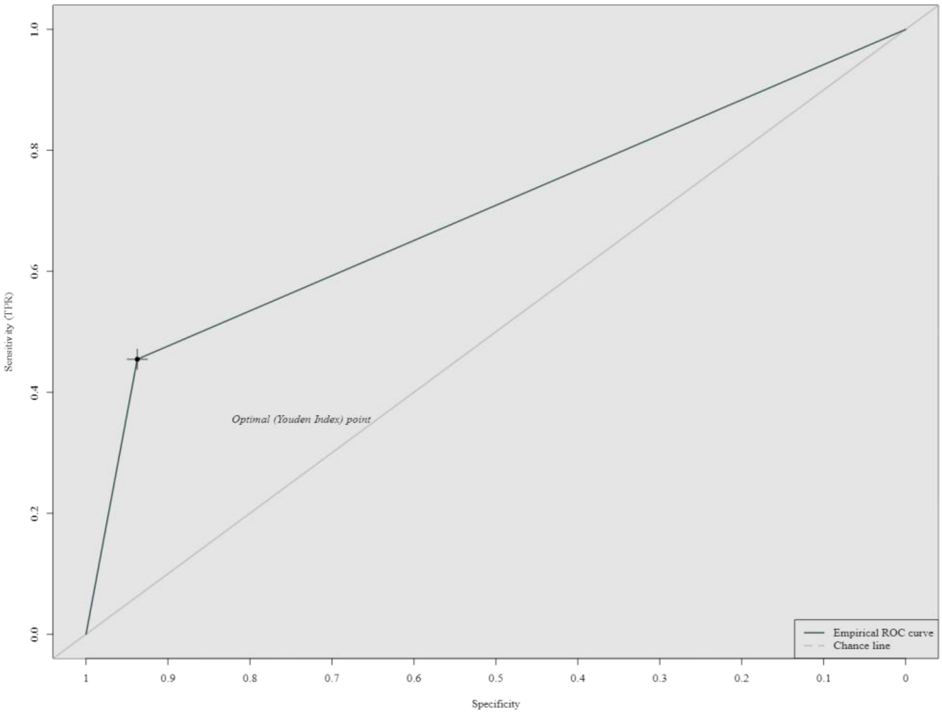
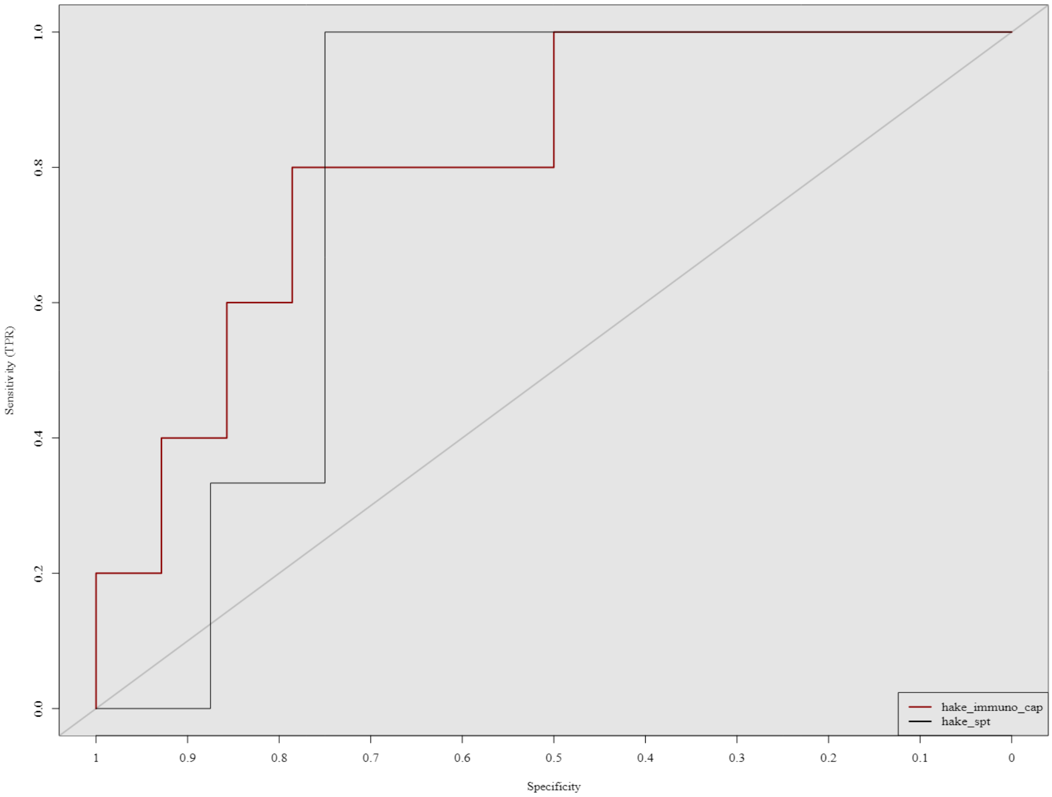


Figure 6: ROC curves for hake allergy tests (Hake ImmunoCAP and hake SPT)



Thresholds

Table 5 presents calculated thresholds for each allergen. For hake and peanuts, no NOAEL was calculated, as some participants reacted at the lowest dose on the challenge schedule. Appendix B presents ED curves by allergen and by age.

Table 6: Eliciting dose thresholds by allergen (g)

Food protein	NOAEL	LOAEL	ED01	ED05	ED10	ED50	ED95
Cow`s milk	0.021	0.525	0.1383	0.4021	0.6441	2.2103	5.7612
Egg	0.00121	0.0013	0.0092	0.0639	0.1501	1.4038	7.9722
Peanut	NA	0.0023	0.0010	0.0112	0.0329	0.5499	4.9097
Cashew	0.00203	0.0203	0.0012	0.0125	0.0353	0.5340	NA
Hake	0.00178	0.0623	0.0014	0.0148	0.0417	0.6231	NA

Allergen thresholds varied by age (Table 6). No data was available on positive reactions to cashews below 2 years.

Table 7: Eliciting dose thresholds for different allergens in younger vs. older children (g)

Food protein	NOAEL	LOAEL	ED01	ED05	ED10	ED50	ED95
Cow`s milk <=2 years	0.581	1.281	0.1815	0.4459	0.6633	1.8745	4.2021
Cow`s milk >2 years	0.231	0.525	0.2970	0.7297	1.0854	3.0675	6.8766
Egg <=2 years	0.334	0.0907	0.0091	0.0629	0.1479	1.3823	7.8494
Egg >2 years	NA	0.0013	0.0093	0.0641	0.1505	1.4068	7.9886
Peanut <=2 years	0.0023	0.0603	0.0008	0.0094	0.0276	0.4556	4.0278
Peanut >2 years	NA	0.0023	0.0011	0.0120	0.0350	0.5790	5.1184
Hake <=2 years	0.49128	1.3813	0.2180	0.6113	0.9639	3.1733	8.0090
Hake >2 years	0.00178	0.0623	0.0170	0.0477	0.0751	0.2474	0.6244

Table 8: Eliciting dose thresholds compared to the data from Purington et al [16] (g)

Food protein	ED05 (study data)	Purington et al	ED10 (study data)	Purington et al	ED50 (study data)	Purington et al
Cow`s milk	0.4021	0.00021	0.6441	0.00074	2.2103	0.02042
Egg	0.0639	0.00004	0.1501	0.00018	1.4038	0.00707
Peanut	0.0112	0.00049	0.0329	0.00152	0.5499	0.0299
Cashew	0.0125	0.00007	0.0353	0.00025	0.5340	0.00878

Table 9: Eliciting dose thresholds compared to the data from Mortz et al [17] (g)

Food protein	ED01 (study data)	Mortz et al	ED05 (study data)	Mortz et al	ED10 (study data)	Mortz et al	ED50 (study data)	Mortz et al
Cow`s milk	0.1383	0.0082	0.4021	0.0279	0.6441	0.0535	2.2103	0.5327
Egg	0.0092	0.0014	0.0639	0.0063	0.1501	0.0139	1.4038	0.2236
Peanut	0.0010	0.0042	0.0112	0.0121	0.0329	0.0211	0.5499	0.1507
Cashew	0.0012	0.004	0.0125	0.0104	0.0353	0.0174	0.5340	0.1046

DISCUSSION

This study aimed to evaluate the diagnostic performance of commonly used cut-off values for food allergy testing and assess eliciting dose thresholds for children undergoing oral food challenges to cooked egg, whole milk, peanut butter, cashew butter, and hake. The findings provide valuable insights into

the applicability of international predictive values in this population and highlight differences in allergen thresholds by age.
The study included 223 OFCs, with 137 negative, 81 positive, and five indeterminate challenges. OFCs were most frequently conducted for cooked egg (36%) and peanut (30%), with the least challenges performed for hake (10%). The distribution of challenges by age indicates that peanuts and cooked eggs were the most commonly challenged allergens in children aged two years or younger, whereas cashew was the least frequently challenged allergen in this age group. No significant difference in age was observed between children who had positive and negative OFCs (p=0.2), suggesting that age alone may not be a primary determinant of challenge outcome.

The predictive performance of internationally derived cut-off values varied across allergens. While some tests showed high specificity, sensitivity was often suboptimal. Notably, for cow's milk, the fresh skin prick test (SPT) (≥4 mm) demonstrated high specificity (83.3%) and sensitivity (100%), whereas commercial SPT at ≥8 mm exhibited poor sensitivity (0%). Similarly, Ara h2-specific IgE (sIgE) performed better than peanut sIgE in distinguishing allergic from non-allergic individuals (AUC 0.785 vs. 0.635). For cooked eggs, ovomucoid sIgE demonstrated moderate sensitivity (72.7%) but low specificity (35.1%), suggesting limitations in using ovomucoid alone to predict challenge outcomes.

Despite the high specificity of most tests, the proportion of patients who passed an OFC with diagnostic results above commonly used 95% positive predictive value thresholds highlights the need for context-specific interpretation. Notably, 8% of patients passed the cooked egg challenge despite having a sIgE >7 kUA/L, and 1% of patients passed the peanut OFC with a sIgE >14 kUA/L. These findings indicate that strict reliance on these thresholds may lead to unnecessary dietary restrictions in some patients.

Receiver operating characteristic curve analysis identified population-specific cut-offs with improved diagnostic accuracy. For cow's milk, a lower sIgE cut-off (1.72 kUA/L) provided a higher AUC (0.917) than the commonly used 3.5

kUA/L threshold. Similarly, a peanut sIgE cut-off of 1.42 kUA/L outperformed the standard 4.3 kUA/L cut-off (AUC 0.635 vs. standard sensitivity of 81%). These findings suggest that lower cut-offs may improve sensitivity without significantly compromising specificity, allowing for better patient selection for OFCs.

Threshold data demonstrated variation in eliciting doses by age. For peanut and hake, no NOAEL was established, as some children reacted to the lowest challenge dose. Younger children had lower eliciting doses for cooked eggs and peanuts than older children, emphasising the need for age-specific risk assessment when interpreting threshold values. For cow's milk, the NOAEL was higher in younger children (0.581 g vs. 0.231 g in older children), possibly reflecting immunological differences or early tolerance development. Cow's milk LOAEL, ED01, ED05, ED10 and ED50 were markedly higher than for other allergens. Our data shows that various EDs in out population are much higher then the ones in the previous research [17,18]. However, authors of those papers do not differentiate between children and adult populaton. Differences in thesholds may be also caused by a smaller sample size due to the insufficient number of positive OFCs in our setting.

These findings underscore the limitations of applying international predictive cut-offs universally. The study highlights the need for population-specific reference values to optimise patient selection for OFCs. The identification of lower diagnostic thresholds with improved performance may help refine allergy testing protocols, reducing unnecessary food avoidance while ensuring safety.

The observed age-related differences in eliciting doses suggest that younger children may have lower reaction thresholds, reinforcing the importance of cautious OFC interpretation in this age group. However, it may be connected to the fact that more diagnostic OFCs are performed in younger children, while in older children OFCs are used to reintroduce food to those who possibly gained tolerance. Further studies are needed to validate these findings in broader populations and explore additional factors influencing OFC outcomes, such as atopic comorbidities and environmental exposures.

Our main study limitations are that data was collected retrospectively. Also, in some occasions, the protocols were not adhered to strictly, most often because the child refused to ingest a full portion of a dose or the final dose was reduced to take into account the typical daily dose of a child of that size or to exceed the target dose for a particular child.

CONCLUSION

This study provides valuable data on the diagnostic performance of common allergy tests and allergen thresholds in a paediatric population undergoing OFCs. The findings support the need for refined, population-specific cut-offs and highlight age-related differences in food allergy thresholds. Future research should focus on validating these findings and integrating them into clinical decision-making to enhance food allergy diagnosis and management.

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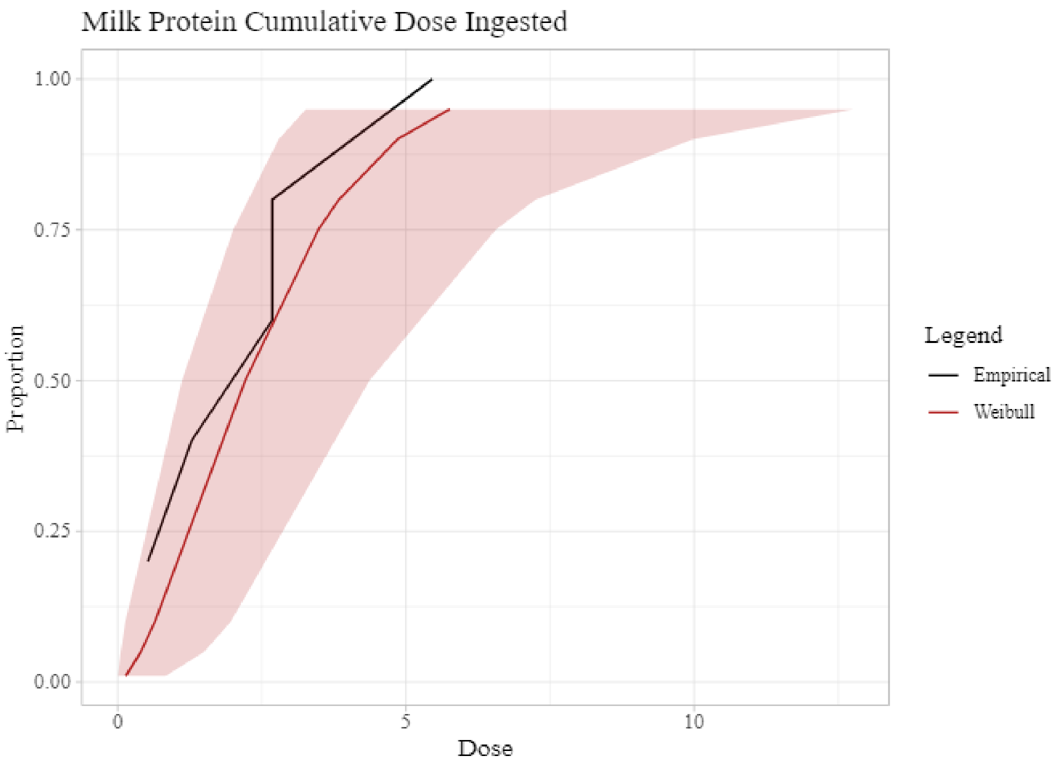
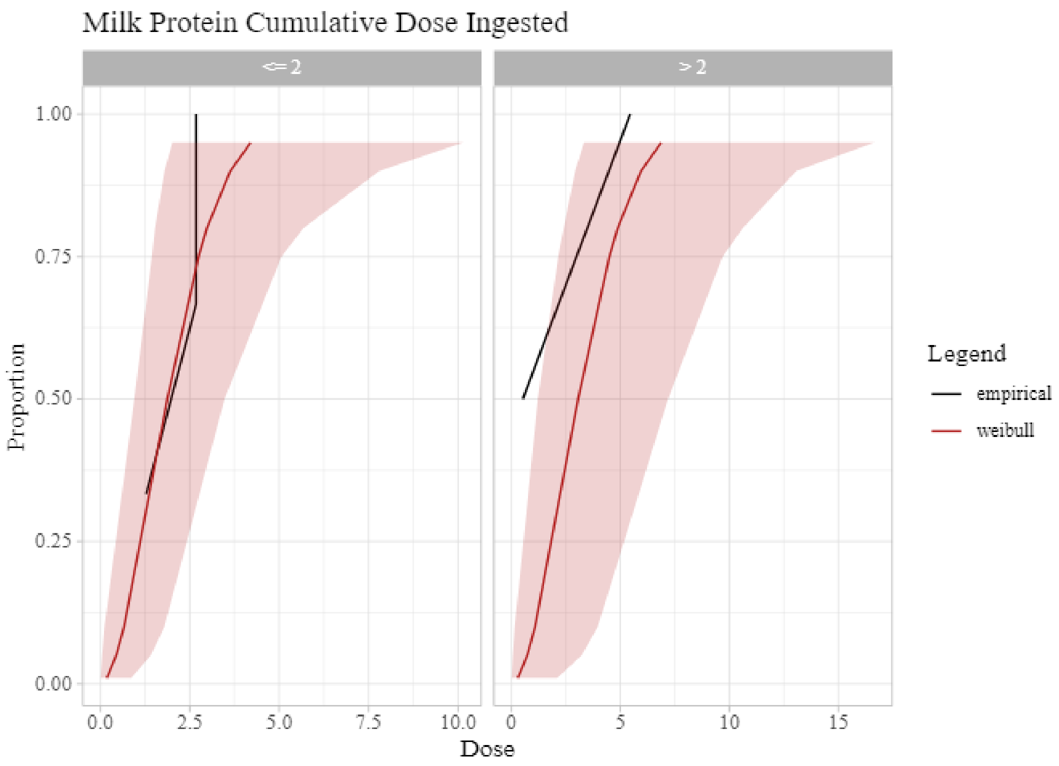
APPENDIX A: The estimated amount of protein according to the product information on the package

Food used	Amount of protein
Chicken`s egg	0.121 g (per 1 g)
Whole milk	0.035 g (per 1 ml)
Peanut butter	0.232 g (per 1 g)
Cashew butter	0.203 g (per 1 g)
Poached hake	0.178 g (per 1 g)

APPENDIX B: Eliciting doses for the overall population and by age




Milk Protein Cumulative Dose Ingested
<=2
>2
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0.75
0.50
0.25
0.00
0.0
2.5
5.0
7.5
10.0
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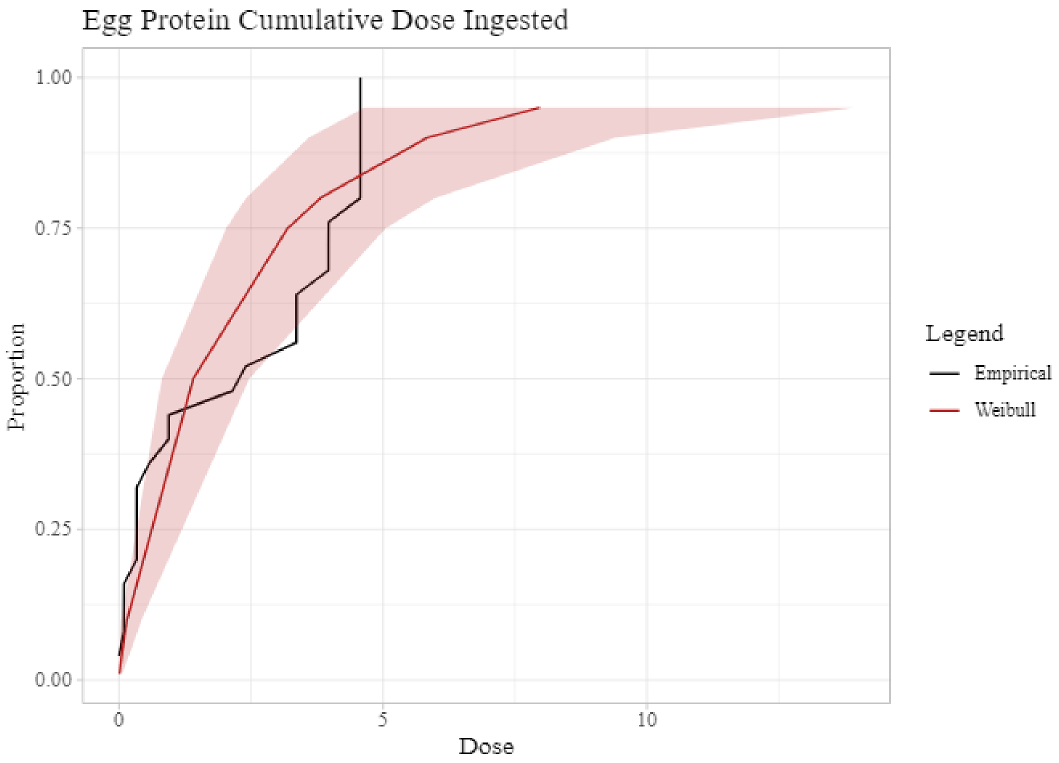
Dose
Legend
empirical
weibull


Egg Protein Cumulative Dose Ingested
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Dose
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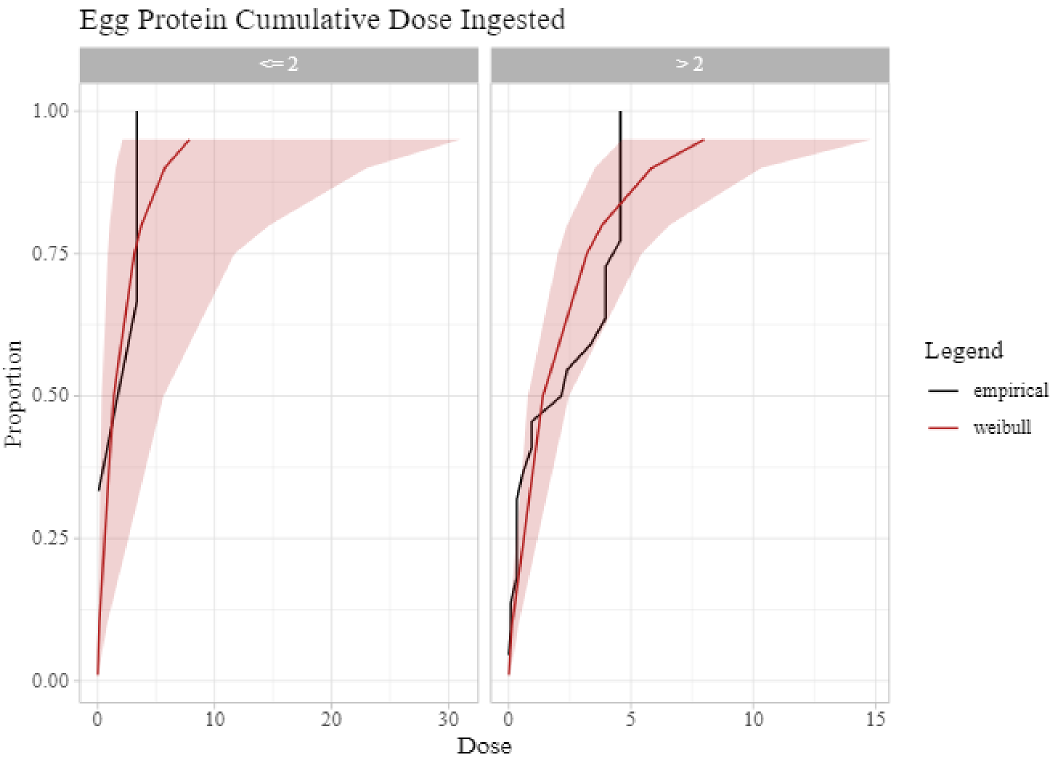
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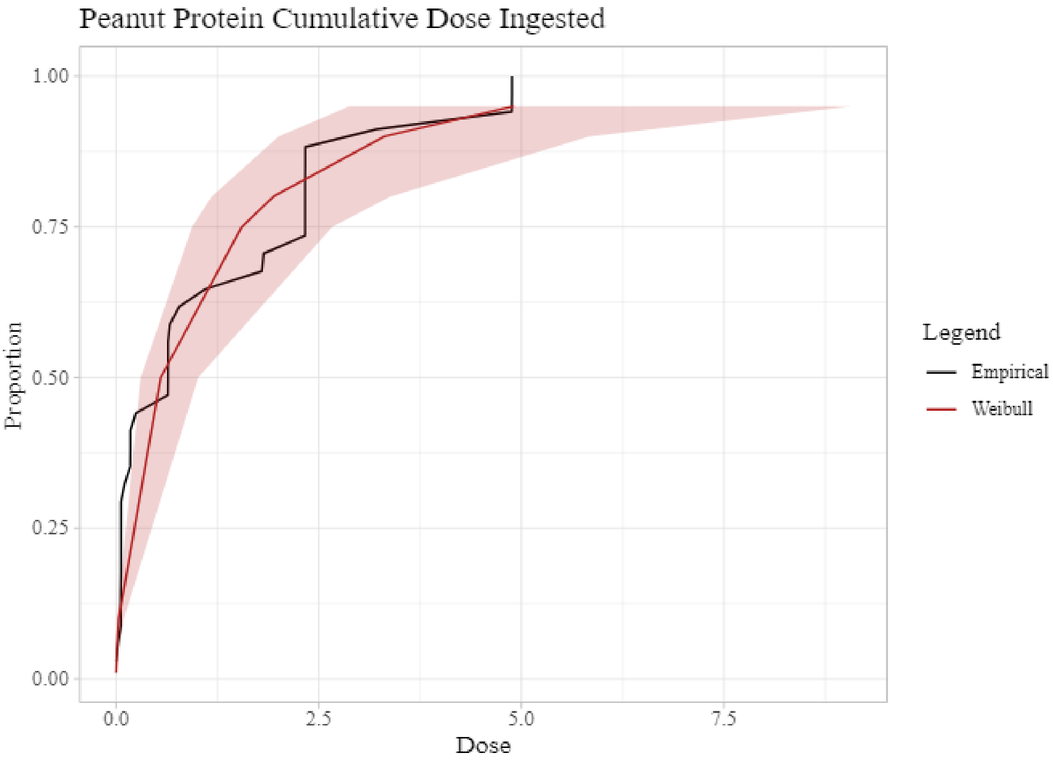
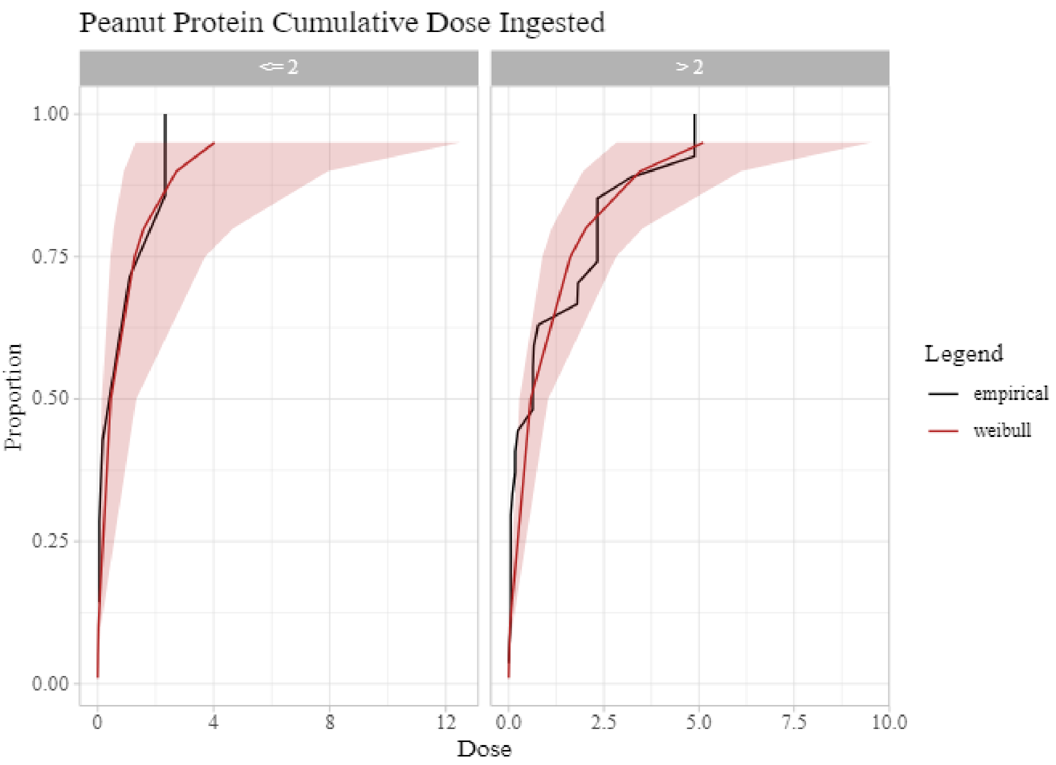
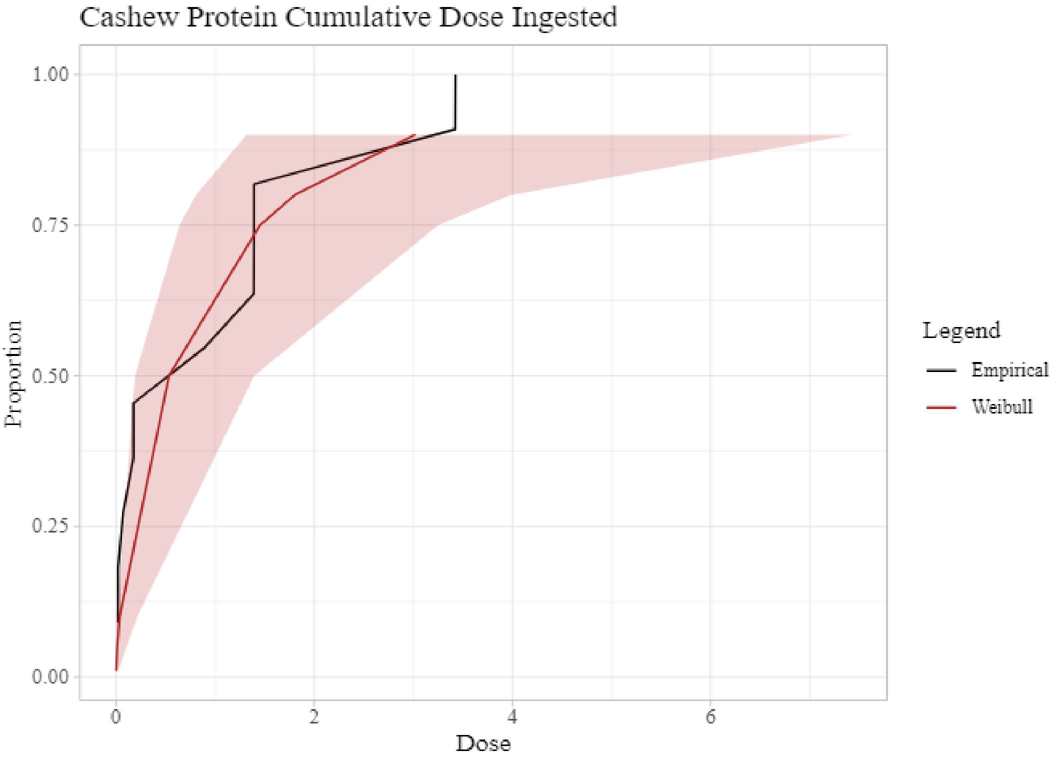
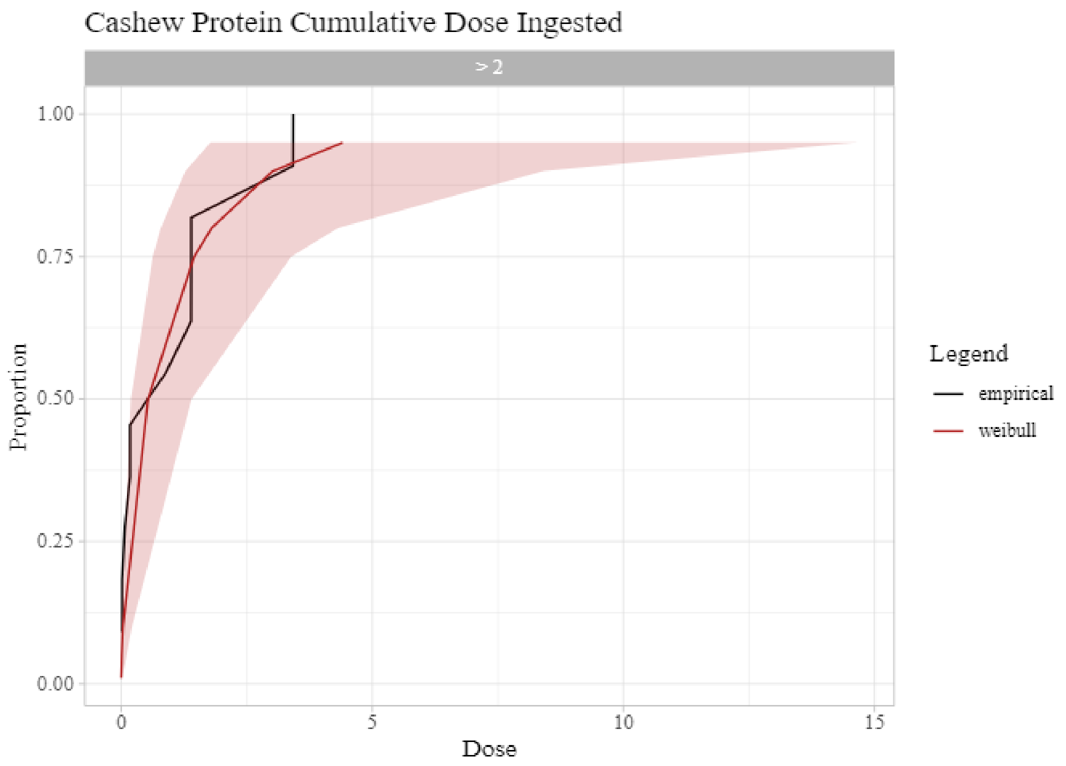
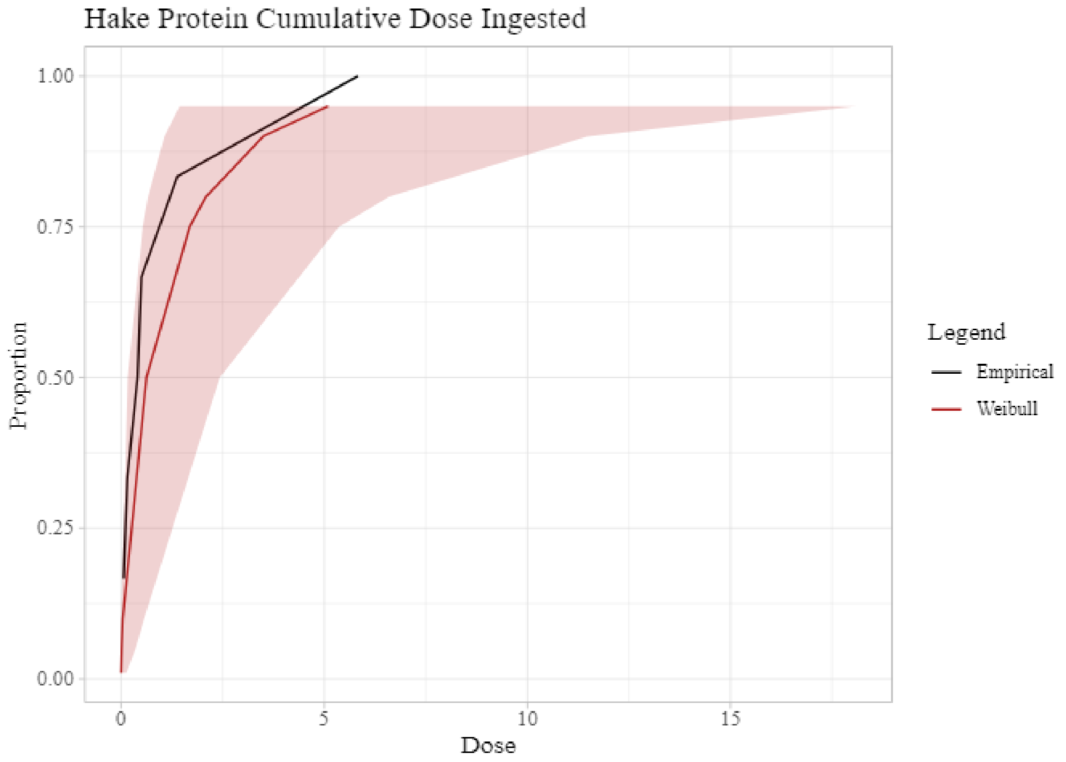
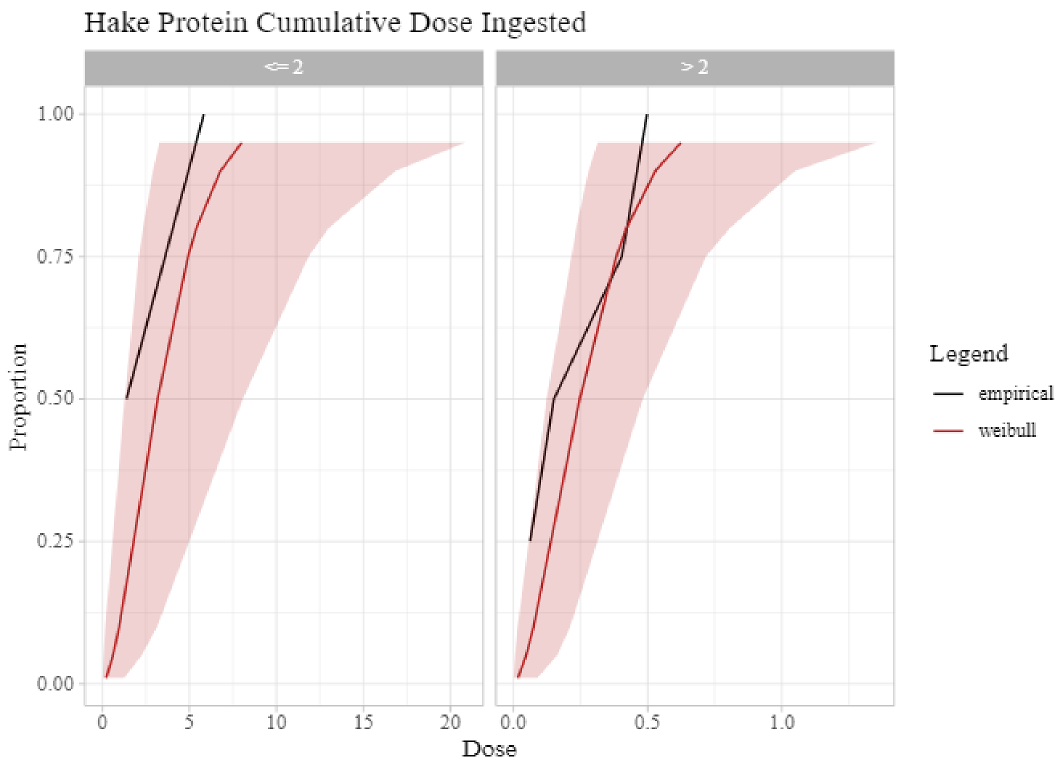

Peanut Protein Cumulative Dose Ingested


Peanut Protein Cumulative Dose Ingested
<=2
> 2
Proportion
1.00
0.75
0.50
0.25
0.00
0
4
8
12
0.0
2.5
5.0
7.5
10.0
Dose
Legend
empirical
weibull


Cashew Protein Cumulative Dose Ingested
Proportion
1.00
0.75
0.50
0.25
0.00
0
2
4
6
Dose
Legend
Empirical
Weibull


Cashew Protein Cumulative Dose Ingested


Hake Protein Cumulative Dose Ingested


Hake Protein Cumulative Dose Ingested
<=2
> 2
Proportion
1.00
0.75
0.50
0.25
0.00
0
5
10
15
20
0.0
0.5
1.0
Dose
Legend
empirical
weibull