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Food

Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food

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Executive Summary

Background

The Food Allergen Labeling and Consumer Protection Act of 2004 (P.L. 108-282) (FALCPA) amends the Federal Food, Drug, and Cosmetic Act (FFDCA) and requires that the label of a food product that is or contains an ingredient that bears or contains a "major food allergen" declare the presence of the allergen as specified by FALCPA. FALCPA defines a "major food allergen" as one of eight foods or a food ingredient that contains protein derived from one of those foods. A food ingredient may be exempt from FALCPA's labeling requirements if it does not cause an allergic response that poses a risk to human health or if it does not contain allergenic protein. FALCPA also requires FDA to promulgate a regulation defining the term "gluten-free."

This report summarizes the current state of scientific knowledge regarding food allergy and celiac disease, including information on dose-response relationships for major food allergens and for gluten, respectively. The report presents the biological concepts and data needed to evaluate various approaches to establish thresholds that would be scientifically sound and efficacious in relation to protection of public health. Each approach has strengths and weaknesses, and the application of each is limited by the availability of appropriate data. It is likely that there will be significant scientific advances in the near future that will address a number of the limitations identified in this report.

The Threshold Working Group expects that any decisions on approaches for establishing thresholds for food allergens or for gluten would require consideration of additional factors not covered in this report. Furthermore, one option that is implicit in the report's discussion of potential approaches is a decision not to establish thresholds at this time.

Approaches to Establish Thresholds

The report identifies four approaches that could be used to establish thresholds:

- Analytical methods-based-thresholds are determined by the sensitivity of the analytical method(s) used to verify compliance.
- Safety assessment-based-a "safe" level is calculated using the No Observed Adverse Effect Level (NOAEL) from human challenge studies and an appropriate Uncertainty Factor (UF) applied to account for knowledge gaps.
- Risk assessment-based-examines known or potential adverse health effects resulting from human exposure to a hazard; quantifies the levels of risk associated with specific exposures and the degree of uncertainty inherent in the risk estimate.
- Statutorily-derived-uses an exemption articulated in an applicable law and extrapolates from that to other potentially similar situations.

Any approach used to establish a threshold to protect consumers with food allergies or those susceptible to celiac disease should be reexamined periodically to consider new knowledge, data, and approaches.

Threshold Working Group Findings For Major Food Allergens

- Finding 1. The initial approach selected to establish thresholds for major food allergens, the threshold values, and any uncertainty factors used in establishing the threshold values should be reviewed and reconsidered periodically in light of new scientific knowledge and clinical findings.
- Finding 2. The **analytical methods-based approach** can be used to establish thresholds for those major food allergens for which validated analytical methods are available. However, if this approach is used, the thresholds should be replaced by thresholds established using another approach as quickly as possible.
- Finding 3. The **safety assessment-based approach**, based on currently available clinical data, is a viable way to establish thresholds for the major food allergens. If this approach is employed, the Lowest Observed Adverse Effect Level (LOAEL) or No Observed Adverse Effect Level (NOAEL) determinations used should be based on evidence of the "initial objective sign." Individual thresholds should be established for each of the major food allergens. If it is not feasible to establish individual thresholds, a single threshold based on the most potent food allergens should be established. In those instances where a LOAEL is used rather than a NOAEL to establish a threshold, an appropriate uncertainty factor should be used. Thresholds established using this approach should be reevaluated periodically as new data and tools become available.
- Finding 4. Of the four approaches described, the quantitative **risk assessment-based** approach provides the strongest, most transparent scientific analyses to establish thresholds for the major food allergens. However, this approach has only recently been applied to food allergens, and the currently available data are not sufficient to meet the requirements of this approach. A research program should be initiated to develop applicable risk assessment tools and to acquire and evaluate the clinical and epidemiological data needed to support the quantitative risk assessment-based approach. Thresholds established using this approach should be reevaluated periodically as new data and tools become available.
- Finding 5. The **statutorily-derived approach** provides a mechanism for establishing thresholds for allergenic protein in foods based on a statutory exemption. Potentially, this approach could be used to set a single threshold level for proteins derived from any of the major food allergens. This approach might yield thresholds that are unnecessarily protective of public health as compared with thresholds established using the safety assessment-based approach or the risk assessment-based approach. However, confirming this would require additional data. If this approach is employed to establish thresholds, it should be used only on an interim basis and should be reevaluated as new knowledge, data, and risk assessment tools become available.

Threshold Working Group Findings For Gluten

- Finding 6. The initial approach selected to establish a threshold for gluten, the threshold value selected, and any uncertainty factors used to establish the threshold should be reviewed and reconsidered periodically in light of new scientific knowledge and clinical findings.
- Finding 7. The **analytical methods-based approach** can be used to establish a threshold for gluten. However, if this approach is used, the threshold should be replaced by a threshold established using another approach as quickly as possible.
- Finding 8. The **safety assessment-based approach** is a viable approach to establish a threshold for gluten using currently available LOAEL data for celiac disease. An overall uncertainty factor should be estimated from the data and applied to the LOAEL to establish a threshold for gluten. Any threshold derived from this approach should be reevaluated as new research data become available. Available data are insufficient at the current time to use this approach to establish a threshold for oat gluten for those individuals with celiac disease who may also be sensitive to oats. However, it is likely that a threshold level based on wheat gluten would be protective for individuals susceptible t

oat gluten.

- Finding 9. Use of the quantitative **risk assessment-based approach** to establish a threshold for gluten does not appear to be feasible at the present time. However, considering the benefits that could be gained from using the risk assessment-based approach, priority should be given to establishing a research program to acquire the knowledge and data needed.
- Finding 10. There appear to be no suitable legal requirements or exemptions that would serve as the rationale for usin the **statutorily-derived approach** to establish a threshold for gluten. This approach is not viable.

Acknowledgements

Preface

In preparing this report, the Threshold Working Group conducted literature searches, gathered extensive scientific information about food allergy and celiac disease, and consulted technical experts. This information was used to identify approaches that could be used to establish thresholds, and to evaluate the strengths, weaknesses, and data needs of each approach. A notice of availability for the draft report was published in the Federal Register (70 FR 35258), and the report was made available through the FDA Docket and the CFSAN web site. The FDA requested that interested persons submit comments, scientific data, and information to FDA Docket No. 2005N-0231 during a 60-day period, ending August 16, 2005. Eighteen letters were received, including comments from consumer groups, the food industry, trade associations, experts on food allergens and gluten, and individual consumers.

In the Federal Register of May 23, 2005 (70 FR 29528), FDA announced a meeting of the Food Advisory Committee (FAC) to be held on July 13, 14, and 15, 2005. Members of the public were invited to participate in the meeting. The FAC was asked t consider whether the draft report was scientifically sound in its analyses and approaches and whether the report adequately considered available relevant data on food allergens and on gluten. The meeting included presentations on issues related to the diagnosis and treatment of food allergies and celiac disease, the quality of life for affected consumers, analytical method to measure allergens and gluten in foods, and clinical studies to characterize dose-response relationships. In seeking the Committee's advice, FDA posed a series of specific scientific questions. The transcript of the meeting is available at CFSAN

2005 Meeting Documents. The Committee's answers to the specific scientific questions is available (available in PDF, 460 Kb) A summary of the public comments [18] received at the Food Advisory Committee meeting and in the public docket with a brief indication as to how the revised report responds to each comment is available.

The Committee concluded that CFSAN's draft report includes a comprehensive evaluation of the currently available data and descriptions of all relevant approaches that could be used to establish thresholds for major allergens and gluten in food. The Committee suggested that, while the safety assessment-based and risk assessment-based approaches are distinct, they are not mutually exclusive. For example, statistical analyses could be incorporated into a traditional safety assessment by considering dose-response distributions. The Committee felt that the risk assessment-based approach is scientifically the strongest of the approaches, and that it should be used in a transparent manner with appropriate consideration of data uncertainties, when sufficient data become available. The Committee agreed that the criteria identified in the draft report for evaluating the available data were appropriate. The Committee also recommended that data from highly relevant, well designed studies be considered in establishing thresholds, even if they have not yet been published or peer-reviewed.

We wish to acknowledge and express our appreciation to those who provided written and oral comments. Both the public comments and recommendations and comments and recommendations of the FAC were considered in revising the report. These revisions addressed the use of technical terminology, clarification where needed, the inclusion of additional data, and minor editorial changes. Based on the comments and recommendations, FDA determined that it was not necessary to significantly revise the report or its findings. The specific comments made regarding the strengths and weakness of each approach will inform any decision as to whether to establish thresholds and, if so, which approach to use. The Agency also appreciates the suggestion that it may be possible to combine the safety assessment-based approach and the risk assessment-based approach to provide quantitative information on the uncertainties associated with thresholds established using the available published LOAELs and NOAELs. The Agency also takes note of the discussions that addressed issues beyond the scope of this report that may become relevant if a decision is made to establish thresholds.

I. Overview

A. Purpose

Accurate and informative labeling is critical for allergic consumers, individuals with celiac disease, and their families because they need to rely on strict avoidance of specific foods and ingredients to prevent potentially serious reactions. The Food Allergen Labeling and Consumer Protection Act of 2004 (P.L. 108-282) (FALCPA) amends the Federal Food, Drug, and Cosmetic Act (FFDCA) and requires that the label of a food product that is or contains an ingredient that bears or contains a "major food allergen " declare the presence of the allergen as specified by FALCPA. FALCPA defines a "major food allergen " as one of eight foods or food groups (milk, egg, fish, crustacean shellfish, tree nuts, wheat, peanuts, and soybeans) or a food ingredient that contains protein derived from one of those foods.

An important scientific issue associated with the implementation of FALCPA is the existence of threshold levels below which it is unlikely that a food allergic individual would experience an adverse effect. FALCPA provides two processes by which an ingredient may be exempted from the FALCPA labeling requirements, a petition process [21 U.S.C. 343(w)(6)] and a notification process [21 U.S.C. 343(w)(7)]. Under the petition process, an ingredient may be exempt if the petitioner demonstrates that the ingredient "does not cause an allergic reaction that poses a risk to human health." Under the notification process, an ingredient may be exempt if the notification contains scientific evidence that demonstrates that the ingredient "does not contain allergenic protein," or if FDA previously has determined, under section 409 of the FFDCA, that the food ingredient does not cause an allergic response that poses a risk to human health. Thus, understanding food allergen thresholds and developing a sound scientific framework for such thresholds are likely to be centrally important to FDA's analysis of, and response to, FALCPA petitions and notifications.

FALCPA also requires FDA to promulgate a regulation to define and permit the use of the term "gluten-free" on the labeling of foods. Such labeling is important to patients suffering from celiac disease, an immune-mediated illness. Strict avoidance of gluten at levels that will elicit an adverse effect is the only means to prevent potentially serious reactions. Thus, consumers susceptible to celiac disease need accurate, complete, and informative labels on food. Understanding thresholds for gluten will help FDA develop a definition of "gluten-free" and identify appropriate uses of the term.

Section 204 of FALCPA directs FDA to prepare and submit a report to Congress. The report is to focus principally on the issue of cross-contact of foods with food allergens, and is to describe the types, current use of, and consumer preferences with respect to advisory labeling. Cross-contact may occur as part of the food production process where residues of an allergenic food are present in the manufacturing environment and are unintentionally incorporated into a food that is not intended to contain the food allergen, and thus, the allergen is not declared as an ingredient on the food's label. In some cases, the possible presence of the food allergen is declared by a voluntary advisory statement. Understanding food allergen thresholds and developing a sound scientific framework for such thresholds is also likely to be useful in addressing food allergen cross-contact issues, including the use of advisory labeling.

Both as part of its ongoing risk management of food allergens and in response to FALCPA, CFSAN established an *ad hoc* internal, interdisciplinary group (the Threshold Working Group) to evaluate the current state of scientific knowledge regarding

food allergies and celiac disease, to consider various approaches to establishing thresholds for food allergens and for gluten, and to identify the biological concepts and data needed to evaluate the scientific soundness of each approach. This report is the result of the working group's deliberations.

This report summarizes the current state of scientific knowledge regarding food allergies and celiac disease, including information on dose-response relationships for major food allergens and for gluten, respectively. The ability to establish a threshold depends on understanding the dose-response relationship between the ingestion of an allergen or gluten and the elicitation of an adverse response. Implicit in establishing such dose-response relationships is the identification of susceptible populations and characterization of any exposure levels below which all, or part, of the susceptible population does not respond. There is no consensus in the scientific literature regarding thresholds for major food allergens or gluten. Therefore, the Threshold Working Group identified the biological concepts and data needed to evaluate various approaches for establishing thresholds that would be scientifically sound and efficacious in relation to protection of public health.

B. Definitions of Thresholds

The term "threshold" has been used to refer to a variety of different concepts (Table I-1) that apply either to individuals or populations. Thresholds can be measured experimentally in animals or humans [i.e., No Observed Adverse Effect Level (NOAEL) or Lowest Observed Adverse Effect Level (LOAEL)], derived from epidemiological data, estimated by modeling (statistical or simulation), established by statute, or arising as the result of the selection of an analytical method. The ability to measure or determine a threshold may be limited by the sensitivity and specificity of the methods available to measure either the stimulus or the response. Understanding the strengths and limitations of the data underpinning the different approaches is particularly important when dealing with adverse effects that have low probabilities of occurring.

Table I-1. Summary of Various Types of Thresholds

Type	Description
Etymological Definition	"The intensity below which a mental or physical stimulus cannot be perceived and can produce no response." (Webster's Dictionary).
Toxicological	The dose at, or below which, an adverse effect is not seen in an experimental setting.
Methodological	The limit of detection of an analytical method.
Statutory	The establishment of a limit by statute, below which no regulatory action will be taken.

C. FALCPA

As noted, FALCPA amends the FFDCA to prescribe the manner in which food labels must disclose that a food is, or contains a ingredient that bears or contains, a major food allergen. The law also requires the FDA to issue a regulation to define and permit use of the term "gluten-free."

FALCPA establishes a petition process through which a food ingredient may be exempt from FALCPA's labeling requirements i the ingredient does not cause an allergic response that poses a risk to human health. FALCPA also establishes a notification process under which a food ingredient described in section 201(qq)(2) of the FFDCA may be exempt from FALCPA's labeling requirements if the ingredient does not contain allergenic protein, or if FDA previously has determined, under section 409 of the FFDCA, that the food ingredient does not cause an allergic response that poses a risk to human health.

From the perspective of the Working Group, implementation of the FALCPA petition and notification provisions could present several key scientific issues. First, what is an "allergic response?" Second, do all allergic responses pose a risk to human health, or do some allergic responses pose more of a risk than others? Third, can allergens occur in a food either in a form o at a level that is too low to cause harm (i.e., either the allergen does not cause a biological response or the response is too mild to be considered hazardous)?

Under FALCPA, a "highly refined oil" derived from one of eight foods or food groups and "any ingredient derived from such highly refined oil" are exempt from the definition of "major food allergen " and from FALCPA's labeling requirements. As discussed further below, there is evidence that consumption of highly refined oils does not appear to be associated with allergic responses despite the potential presence of low levels of protein in these oils.

Section 202 of FALCPA requires FDA to issue a proposed rule to define and permit use of the term "gluten-free" on labeling o foods. Section 203 of FALCPA recognizes that "the current recommended treatment is avoidance of glutens in foods that are associated with celiac disease." FALCPA does not directly state how the term "gluten-free " should be defined.

II. Food Allergy

A. Adverse Reactions to Foods

Many consumers consider a wide variety of adverse reactions associated with the ingestion of foods to be "food allergies." While adverse reactions may occur for a variety of immunological, toxicological, or metabolic reasons only a small fraction of these are related to food allergies (figure II-1). The signs and symptoms associated with these reactions can range from oral irritation and swelling to cardiovascular collapse (Jackson, 2003). Although adverse reactions caused by microbial and toxicological agents can affect any most individual, immunological reactions only affect a small group of sensitive individuals. Reactions caused by the presence of toxic compounds such as histamine in seafood (e.g., scombroid poisoning) or from

metabolic (e.g., lactose intolerance) are not true food allergies. The nomenclature used to describe these well documented reactions in sensitive individuals is not consistent in the scientific literature. Generally, reactions not involving immune responses are termed food intolerances (Johansson *et al*., 2001; Sampson, 2004).

Immunological responses to foods, including food allergies, occur in a sensitive population of individuals. The major immunological responses to foods, termed food hypersensitivities, can be divided into two major categories based on mechanism: (1) immunoglobulin E (IgE)-mediated hypersensitivity (e.g., oral allergy syndrome, anaphylaxis) and (2) non-IgE-mediated hypersensitivity (e.g., celiac disease, food protein-induced enterocolitis) (Johansson *et al*., 2001; Wershil *et al*., 2002, Sampson, 2004). A group of food-related disorders (e.g., allergic eosinophilic gastropathies, atopic dermatitis) may involve both IgE- and non-IgE-mediated immune mechanisms (Sampson, 2004). For the purposes of this report, the term "food allergy" will be used to describe IgE-mediated immune responses resulting from the ingestion of specific foods (Johansson *et al*., 2001; Jackson, 2003; Sampson, 2004). The most severe and immediately life-threatening adverse reactions to foods are associated with IgE-mediated hypersensitivity (Johansson *et al*., 2001; Jackson, 2003; Zarkadas *et al*. 1999).
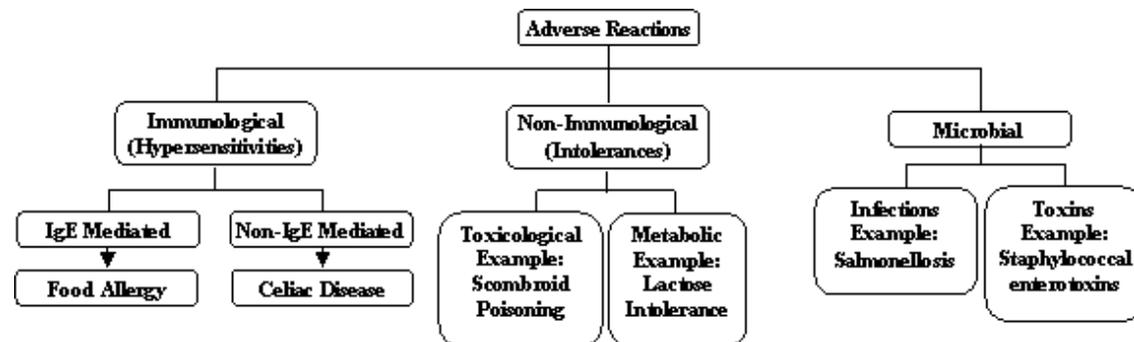
Figure II-1. Adverse Reactions to Foods

B. Mechanism of Allergic Reaction

An allergic reaction stems from an abnormal, or exaggerated, immune system response to specific antigens, which in foods are proteins (Sampson, 1999). This immune response occurs in two phases, an initial "sensitization" to an allergen and the "elicitation" of an allergic reaction on subsequent exposure to the same allergen. Sensitization occurs when a susceptible individual produces IgE antibodies against specific proteins in a food. Upon re-exposure to the same food, the allergenic proteins bind to IgE molecules on immune mediator cells (basophiles and mast cells), leading to activation of these mediator cells. This elicitation causes the release of inflammatory molecules (e.g., leukotrienes and histamine). The specific effects that are seen and the severity of an allergic reaction are affected by the concentration and type of allergen, route of exposure, and the organ systems involved (e.g., skin, GI tract, respiratory tract, and blood) (Taylor and Hefle, 2001).

Sensitization
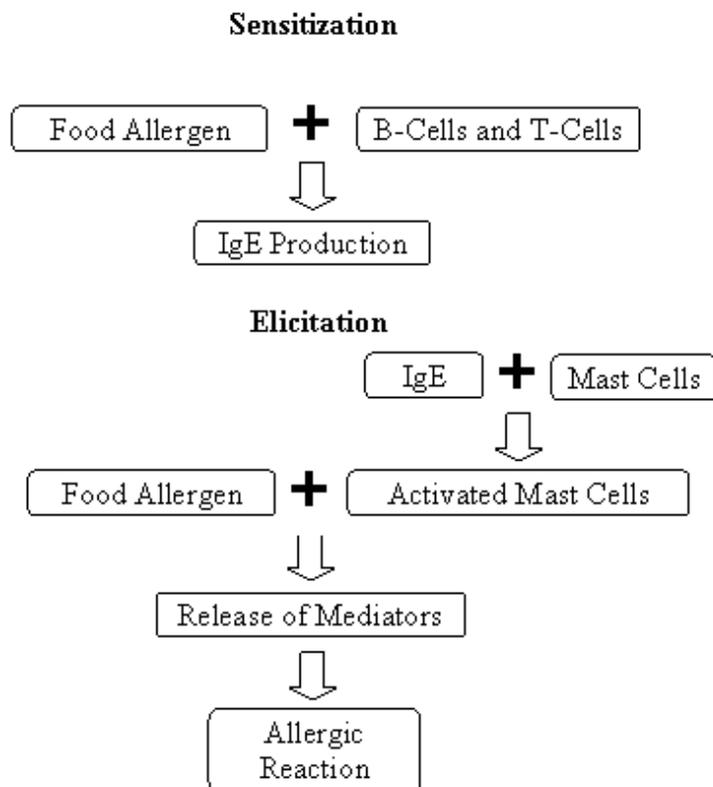
Figure II-2. Mechanism of Allergic Reactions

C. Range of Adverse Effects

The clinical manifestations of food allergic reactions range from mild irritation to severe, life-threatening respiratory distress and shock. Specific signs and symptoms may involve the skin (e.g., pruritis, erythema, urticaria, angiodemia, eczema), eyes (e.g., conjunctivitis, periorbital swelling), nose (e.g., rhinitis, sneezing), oral cavity (e.g., swelling and itching of lips, tongue, or palate), or gastrointestinal tract (e.g., reflux, colic, abdominal pain, nausea, vomiting, diarrhea). In more severe reactions involvement of the respiratory tract (e.g., cough, asthma, difficulty breathing, swelling around the larynx and vocal cords) and cardiovascular system (e.g., faintness, hypotension) can lead to loss of consciousness, asphyxiation, shock, or death. The term "anaphylaxis" is used to describe multisystemic severe reactions to an allergen requiring immediate medical intervention (Jackson, 2003).

Table II-1 provides a summary of the signs and symptoms that may be experienced during an allergic reaction. Allergic reactions usually occur within a few minutes to hours after ingestion of an offending food and often progress on a continuum from mild to severe, with higher doses causing more severe reactions (Sampson *et al*., 2005). Once exposure occurs, individuals may experience immediate numbness or pruritis at the site of contact or experience general uneasiness. These symptoms are characterized as "subjective" since they cannot be observed by others. As the effects progress, "objective" signs such as flushed skin, hives, or swelling of the lips and face may occur. These signs are often mild and short-lived. However, in some cases, they may be associated with more severe responses involving the respiratory and/or cardiovascular systems. Such responses can lead to hospitalization or death, even with appropriate medical intervention. Not all severe, or anaphylactic, reactions are necessarily preceded by milder signs and not all reactions are immediate. In some cases, anaphylactic reactions may be delayed by a few hours after the initial response (Sampson *et al*., 2005).

Anaphylaxis is a poorly defined condition representing a severe or multisystemic allergic reaction (Sampson *et al*., 2005). Allergic reactions described by objective signs involving the respiratory or cardiovascular systems would be considered sever and managed as an anaphylactic reaction by most clinicians. In some classifications, reactions involving two or more of the categories shown in Table II-1 (e.g., cutaneous, gastrointestinal, respiratory), would also be classified as anaphylaxis, if they are relatively mild. Anaphylactic "shock" denotes a consequence of anaphylaxis where heart irregularities and leakage of blood vessels leads to extreme blood volume loss (usually greater than 25% of resting blood volume) and extreme hypotension.

Table II-1. Signs and Symptoms of Allergic Reactions to Food

Subjective Symptoms	Objective Signs

CUTANEOUS	Skin	Pruritus (Itching)	Skin flushing or erythema (redness) Pilor erection ("goosebumps") Rash: Urticaria (hives) - acute Eczema (usually delayed, >6 hours) Angioedema (swelling, especially face)
	Oral cavity (lips, tongue, palate)	Pruritus (Itching), numbness, dryness	Edema (swelling, may also include the uvula)
	Eyes, conjunctiva	Pruritus (Itching)	Periorbital (around eyes) edema, redness of conjunctiva and tearing
GASTROINTESTINAL		Nausea, pain (except infants/young child)	Vomiting, diarrhea, abdominal pain (infants)
RESPIRATORY	Nose	Pruritus (Itching)	Nasal congestion or runniness, sneezing
	Larynx, throat	Pruritus (Itching), dryness/tightness	Swelling around the larynx and vocal cord, voice hoarseness stridor (inspiratory wheeze), cough
	Lungs	Shortness of breath, chest pain/tightness	Respiratory distress (i.e., ↑ breathing rate, difficulty catching breath, ↓ peak expiratory flow measurement), cough, wheezing
HEART and CARDIOVASCULAR		Chest pain/ tightness, feeling of faintness, dizziness	Syncope (fainting, loss of consciousness), hypotension (low) or shock (very low blood pressure), dysrhythmia (abnormal heart rhythm)
OTHER		"Sense of impending doom"	Uterine contractions (women)

The severity of an allergic reaction is affected by several factors that include genetic predisposition (atopy), age, type of food allergen, nature of any food processing, environment, and physiological conditions (Taylor and Hefle, 2001; Sampson, 2003; Maleki, 2004). For example, exercise, medications (e.g., non-steroidal anti-inflammatories), alcohol consumption, and asthma may enhance the severity of an allergic reaction (Sampson, 2005). Most severe and fatal allergic reactions to foods have occurred in adolescents and teens whom were highly atopic and had a history of asthma (Sampson, 2003; Pumphrey, 2004).

It is generally assumed that a history of previous serious allergic reaction(s) indicates an increased risk of future severe reaction(s). However, a history of mild reactions does not preclude the possibility of a future severe reaction. For example, Sicherer *et al.* (1998) observed that mild reactions to peanut in childhood tend to become more severe and unpredictable in later childhood and adulthood. This may be due to the fact that these children tend to develop asthma later in life (Sampson, 2005). Also, a recent review of anaphylactic fatalities in the United Kingdom showed that in 85% of fatal food reactions the patient had previously experienced a non-severe reaction (Pumphrey, 2004). Pumphrey (2004) stated that the severity of previous reactions is not a risk factor for fatal reactions in nut allergic patients. These data imply that any individual with a clinical history of IgE-specific food allergy may be predisposed to anaphylaxis or severe reaction.

D. Prevalence

Information on the prevalence of food allergies in the U.S. suggests that up to 6% of children and 4% of the total population have IgE-mediated food allergies (Sampson, 1997; Sampson, 2004; Sicherer *et al.*, 2003; Sicherer *et al.*, 2004). The estimated prevalence in the U.S. population of allergies to each of the food allergens identified by the FALCPA is given in Table II-2. Severe food-related allergic reactions result in an estimated 30,000 emergency room visits, 2,000 hospitalizations and 150 deaths per year (Sampson, 2004). Clinical data and surveys indicate that the prevalence of allergy, including food allergy, has been rising in recent years, though there are limited historical data to compare to more recent estimates (Sicherer *et al.*, 2003; Grundy *et al.*, 2002). Peanut allergy has received the most attention in the U.S., and data indicate an apparent doubling of peanut allergy in children under 5 years old from 1997 to 2002 (Sicherer *et al.*, 2003). An increase in peanut allergy has also been seen in the United Kingdom (Ewan, 1996; Grundy *et al.*, 2002). Peanuts and tree nuts are the most common cause for fatal reactions in the US, although seafood allergy is increasingly being recognized in adults (Yunginger *et al.*, 1988; Sampson *et al.*, 1992b; Bock *et al.*, 2001, Sicherer *et al.*, 2004, Ross *et al.*, 2006).

Table II-2. Allergy Prevalence in the United States

Age Group	Percentage of the Population								
	All Allergens	Milk	Egg	Peanut	Tree nuts	Fish	Shellfish[a]	Wheat	Soy
Children	6.0	2.5	1.3	0.8	0.2	0.1	0.0	UNK[b]	0.2
Adults	3.7	0.3	0.2	0.6	0.5	0.4	2.0	UNK[b]	UNK[b]

[a]Shellfish includes both crustaceans and mollusks. [b]UNK = unknown.

Sources: Cordle, 2004; Sampson, 1997; Sampson, 2004; Sampson, 2005; Sicherer *et al.*, 2003; Sicherer *et al.*, 2004.

E. Allergenic Foods of Concern

1. Whole foods

The FALCPA identifies eight major foods or food groups: milk, eggs, fish (e.g., bass, flounder, cod), crustacean shellfish (e.g.,

shrimp, crab, lobster), tree nuts (e.g., almonds, walnuts, pecans), peanuts, wheat, and soybeans. These eight foods are believed to account for 90 percent of food allergies and most serious reactions to foods (FALCPA section 202(2)(A); Bousque *et al*., 1998; Hefle *et al*., 1996). More than 160 other foods are known to cause food allergies; however, these allergies are relatively rare with prevalence rates ranging from a few percent of the allergic population to single cases (Hefle *et al*., 1996). Each of the eight major food allergens contains multiple allergenic proteins, many of which have not been fully characterized (Gendel, 1998).

2. Food Ingredients

Some food ingredients such as edible oils, hydrolyzed proteins, lecithin, gelatin, starch, lactose, flavors, and incidental additives (e.g., processing aids), may be derived from major food allergens (Taylor and Hefle, 2001). The role that these ingredients play in food allergy has not been fully characterized. For example, lecithin is a common food ingredient which is often derived from soybeans. It is possible that soy lecithin, which contains residual protein, could elicit an allergic reaction in sensitive individuals (Muller *et al*., 1998; Gu *et al*., 2001). Another example is protein hydrolysate, which is often made from major food allergens such as soybeans, wheat, peanuts, or milk protein. Partially hydrolyzed protein ingredients can elicit allergic reaction. For example, hot dogs formulated with partially hydrolyzed casein have elicited allergic reactions in children allergic to cow's milk (Gern, *et al*., 1991; Kocabas and Sekerel, 2003). Allergic reactions to partially hydrolyzed protein ingredients are more common than are reactions to extensively hydrolyzed protein ingredients (Bock and Atkins, 1989; Ellis *et al*., 1991; Saylor and Bahna, 1991; Kelso and Sampson, 1993; Niggemann *et al*., 1999).

Gelatins are ingredients derived from animals (e.g., cows, pigs) but also from the skin of various species of fish. A study of 10 fish allergic patients and 15 atopic individuals with eczema revealed that 3 and 5 individuals respectively had specific IgE to fish gelatin, suggesting the presence of allergenic protein (Sakaguchi *et al*., 2000). However, in a recent double-blind placebo-controlled food challenge (DBPCFC) study, all 30 fish allergic subjects in the study showed no response to a cumulative dose of 3.61 g of fish gelatin (Hansen *et al*., 2004).

Edible oils can be derived from major food allergens such as soybeans and peanuts, and they may contain variable levels of protein (Taylor and Hefle, 2001). The consumption of highly refined oils derived from major food allergens by allergic individuals does not appear to be associated with allergic reactions. For example, Taylor *et al*. (1981) and Bush *et al*. (1985) did not observe any reactions to refined peanut or soy oils in 10 and 7 allergic patients, respectively. On the other hand, unrefined or cold-pressed oils that contain higher levels of protein residues (Taylor and Hefle, 2001) may cause allergic reactions. For example, Hourihane *et al*. (1997b) reported that 6 of 60 peanut allergic individuals reacted to crude peanut oil but none responded to refined peanut oil. Similarly, Kull *et al*. (1999) reported that 15 of 41 peanut allergic children responded positively to crude peanut oil in skin prick tests, but none responded to refined peanut oil. The actual protein levels reported in various edible oils varies, probably due to differences in the oil, refining process, and the protein detection analytical method used. Crevel *et al*. (2000) reported that crude peanut and sunflower oils contained 100 to 300 µg/ml of protein, but that the most highly refined oils contained 0.2 to 2.2 µg/ml of protein. Intermediate protein concentrations were seen for partially processed oils. Teuber *et al*. (1997) showed that the amount of protein in both crude and refined gourmet nut oils varied both by type of oil and degree of processing; the reported values ranged from 10 to 60 µg/ml for various unrefined oils and from 3 to 6 µg/ml for the refined oils. Other investigators reported undetectable levels of proteins in refined edible oils (Hoffman *et al*., 1994; Yeung and Collins, 1996; Peeters *et al*., 2004) using assays with detection sensitivities of <0.3 ng/ml (Peeters *et al*., 2004) and 0.4 mg/kg (Yeung and Collins, 1996).

Starch, which is a widely used ingredient, is often derived from corn which is not a major food allergen. However, starch can also be derived from wheat, and may contain trace levels of wheat protein. For example, Lietze (1969) reported the presence of antibodies to wheat starch in several wheat sensitive individuals. However, the allergenicity of wheat starch for sensitive individuals has not been clinically evaluated (Taylor and Hefle, 2001).

A wide variety of flavoring substances are used in foods, but only a few are derived from known allergens (Taylor and Dormedy, 1998). As such, IgE-mediated allergic reactions to flavorings are rare, although a few cases have been documented involving hydrolyzed proteins. For example, several milk allergic individuals reacted to either hot dogs or bologna containing partially hydrolyzed casein as part of the natural flavoring used in the formulation of these products (Gern *et al*., 1991). Two other milk-allergic individuals reacted to milk protein in the natural flavoring used in a dill pickle-flavored potato chip (St. Vincent and Watson, 1994). The presence of peanut flour in the natural flavoring of a packaged soup elicited a reaction in a peanut-allergic individual (McKenna and Klontz, 1997).

3. Cross-Contact

Allergens, or proteins derived from allergenic foods, may be present in foods as the result of cross-contact during processing and handling. The term "cross-contact" describes the inadvertent introduction of an allergen into a product that would not intentionally contain that allergen as an ingredient. Cross-contact may occur when a residue or other trace amount of a food allergen is present on food contact surfaces, production machinery, or is air-borne, and unintentionally becomes incorporated into a product not intended to contain, and not labeled as containing, the allergen. Cross-contact may also result when multiple foods are produced in the same facility or on the same processing line, through the misuse of rework, as the result of ineffective cleaning, or may result from customary methods of growing and harvesting crops, as well as from the use of shared storage, transportation, or production equipment. Cross-contact of foods with allergens has been shown to lead to

allergic reactions in consumers on numerous occasions (Gern *et al*., 1991; Jones *et al*., 1992; Yunginger *et al*., 1983). Much cross-contact can be avoided by controlling the production environment.

F. Measuring Thresholds

1. Design of Food Challenge Studies

A history of clinical reaction to a food and a positive skin prick test or the presence of food-specific IgE antibodies in serum are sufficient to establish that an individual has an allergy to that food. However, none of these reliably predicts the level of patient sensitivity to low doses of the food. At present, the level of individual sensitivity can only be determined using food challenge studies (including open, single-blind, and double-blind, placebo-controlled food challenges). The double-blind, placebo-controlled food challenge (DBPCFC) is the "gold standard" diagnostic measure for determining clinical reactivity to low concentrations of an allergen. In this type of study, neither the subject nor the researcher knows which test foods contain the allergen. Open (where both the subject and the researcher know which test foods contain the allergen) and single-blinded (where only the researcher knows which foods contain the allergen) challenges are used primarily for screening foods of low allergenic importance or for determining tolerance to food allergens. Single-blinded challenges can be placebo-controlled (SBPC). However, in open and SBPC challenges, experimenter bias may play a role in interpreting patient reactions.

The typical diagnostic food challenge protocol is a dose escalation study, usually with 15 to 30 minute dose intervals, which proceeds until a clinical effect is observed or the final dose is achieved. The test substance, starting dose and successive incremental doses vary between protocols. Because reactions are assumed to be less severe at lower doses, the starting dose for most diagnostic studies is generally in the milligram range for whole foods (Bindslev-Jensen *et al*., 2004). In the few studies designed to determine minimal eliciting doses, the initial doses are in the low microgram range for the whole food or whole food protein (Hourihane *et al*. 1997; Wensing *et al*. 2002a; Wensing *et al*. 2002b). Incremental doses are usually doubled or increased logarithmically, so that a reasonable number of incremental doses (i.e., 6 to 10) separate the starting dose from the end dose. This final dose is usually chosen to be the normal amount in a food serving, usually 8 to 10 gm of dried food or 60 to 100 gm of wet food (Bock *et al.*, 1988; Bindslev-Jensen *et al*., 2004). The ability to tolerate this amount, followed by a negative open challenge on a different day, is considered to be evidence that the individual is not allergic to that allergen (Taylor *et al*., 2004).

Most oral challenge studies are designed to establish a diagnosis of food allergy rather than to determine safety (Taylor *et al*., 2004). Consequently, these studies do not start at doses below a known LOAEL. Thus, individuals who react to the starting dose are not necessarily demonstrating a true LOAEL because it is not possible to know whether these individuals would have reacted to a lower dose without further testing. A NOAEL cannot be established as long as one or more study participants react to the starting dose.

Most elicited reactions occur within 3 to 15 minutes after a challenge (Bindslev-Jensen *et al*., 2004). Thus, an interval of 15 minutes between challenge doses may be sufficient to confirm a negative response. Most challenge studies report the dose that elicits the first objective sign. Because subjective symptoms may have preceded the first objective sign at lower doses, it is often difficult to ascertain whether the reported LOAEL truly represents the lowest dose to elicit a reaction. The measurement and interpretation of allergic reactions is discussed below.

2. Inclusion/Exclusion of Sensitive Populations

Individuals with a history of anaphylaxis to foods, infants and children are often excluded from challenge studies for ethical reasons (Taylor *et al*., 2002). Moreover, individuals with very high food allergen IgE serum titers are often excluded. Thus, food challenge studies may not include subpopulations of those allergic individuals who may be the most sensitive to allergen exposure.

Individuals with allergies to a specific food have different genetic backgrounds and express a wide distribution of sensitivity and reactivity. Studies have shown that there may be a range of as much as one-million-fold (10^6) in eliciting doses from the least sensitive to the most sensitive individuals (Leung *et al*., 2003; Wensing *et al*., 2002b; Bindslev-Jensen *et al*., 2002). Moreover, sensitivity and reactivity may change with age for individuals within a population. For example, unpublished challenge data described in Moneret-Vautrin and Kanny (2004) show that 83% of wheat allergic children reacted to less than 2 g of wheat flour compared to 18% of wheat allergic adults. Therefore, the inclusion or exclusion of data for highly sensitive individuals can greatly affect the NOAEL determination for the population. To add to this uncertainty, the most sensitive individuals also may have more severe reactions (Wensing *et al*., 2002b; Perry *et al*., 2004). The thresholds measured for populations that exclude these individuals may not apply to those with severe allergic disease.

3. Testing Materials

Food challenges vary in the type of testing material used (e.g., peanut flour versus ground peanut), oral challenge vehicle (e.g., whole food versus capsules), and in the efficacy of blinding. Differences in these variables could modify the distribution or concentration of allergen within the test material, affect digestibility and absorption, influence false-positive subjective reactions, and therefore, affect interpretation of the dose-response data.

The nature of the testing material is very important, as this can enhance or diminish the overall immunogenicity of the native

allergen (Beyer *et al*., 2001; Maleki *et al*., 2003). The matrix used (e.g., fatty substances) can delay absorption, thus affecting the time interval to a reaction, or may affect the intrinsic allergenic properties of the food. Also, gustatory differences in the challenge doses (because of the food matrix used) may influence subjective reactions due to poor taste or fear of consuming the allergen. The use of capsules eliminates problems caused by taste, but bypasses the oral cavity. Because the oral cavity plays an important role in the initial contact and metabolism of food allergens, this may affect the subsequent severity or character of response to the challenge dose.

4. Subjective Versus Objective Reactions

There are two types of physiologic reactions or effects that can occur during a food challenge - subjective symptoms, those reported by the subject, and objective signs, those observed by the researcher. Because subjective symptoms may be the result of non-immunological mechanisms, elicitation of objective signs is believed to be the more reliable indicator of clinical reactivity to the food allergen (Taylor *et al*., 2004).

The signs of a severe allergic reaction are associated with life-threatening conditions, e.g., anaphylaxis. However, there is no consensus as to which of the less serious signs or symptoms should be considered adverse effects. For example, can eczema be seen as a "safer" reaction than angioedema? Unlike well-defined toxicity endpoints, reactions to allergenic food ingredients are part of a wide spectrum of severity that includes trivial injury, objective systemic reactions, anaphylaxis, and death. Further, allergic reactions may involve multiple organ systems. For example, in Scibilia *et al*. (2006) 62% of responses involved more than one organ system.

Subjective symptoms may be good indicators of a subsequent objective reaction, i.e., subjective symptoms may precede or signal objective signs in a dose-dependent manner (Moneret-Vautrin, 2004). However, most challenge studies base their LOAEL determinations on the first objective sign rather than a subjective symptom. For example, although the Hourihane *et al*. (1997a) study reported a threshold for peanut proteins in the milligram range, mild subjective reactions were noted in two individuals at doses of 100 µg of peanut protein. Other studies do not report specific types of reactions but rather characterize reactions as mild, moderate, or severe. For example, a retrospective review of 253 failed challenges at one clinic showed that the initial reaction was severe in 72 (28%) and moderate in 88 (33%) of the challenges (Perry *et al*., 2004). There is only one published study (Wensing *et al*., 2002b) that evaluated reproducible subjective symptoms.

Currently, there is no universally accepted endpoint or response that can be used to predict significant harm from an allergic reaction. Anaphylaxis, a clearly significant endpoint, is a syndrome which is poorly described and subject to variable interpretation (Sampson *et al*., 2005). Moreover, anaphylactic reactions are at one extreme of a continuum of severity. There are a number of additional factors (e.g., use of medicine, alcohol consumption, anxiety) that can significantly reduce or potentiate the impact of exposure to an allergen. Given this combination of factors, a particular dose could result in mild symptoms one day and life-threatening reactions the next.

5. Anecdotal Evidence

Although a great deal of attention has been focused on the use of challenge studies to determine threshold doses or reaction patterns for food allergens, anecdotal reports of individuals suffering life-threatening allergic reactions from minute exposure to food allergens suggests that there may not be a measurable allergen threshold level, especially for sensitive individuals. For example, literature reports have linked kissing (Hallett *et al*., 2002; Steensma, 2003; Eriksson *et al*., 2003) and exposure to airborne particles (Crespo *et al*., 1995; Casimir *et al*., 1997; Sackesen and Adalioglu, 2003) to allergic reactions. Although in many of these cases the amount of allergen exposure cannot be assessed, it is conceivable that the whole food exposure level needed to elicit a harmful reaction is extremely low. In this context, it should be noted that the statistical model developed by Bindslev-Jensen *et al*. (2002) suggested that concentrations as low as 700 ng for peanut and in the low microgram ranges for egg, soy flour, and cow's milk may elicit a reaction in one in a million allergic individuals. Although this model also suggests that a majority of allergic individuals would likely tolerate food allergen concentrations in the milligram range, it supports the anecdotal evidence that very low concentrations of allergen may, at some low but finite probability, elicit harm in highly sensitive individuals.

G. Exposure

1. Matrix Effects

Food allergens often occur as components of processed foods, and many allergic reactions occur following exposure to such allergens (Bock *et al*., 2001). Therefore, it is important to understand how the nature or composition of the food (i.e., the food matrix) affects the elicitation of a reaction.

Very little information exists on matrix effects for the majority of allergens. It has been reported that fat content can modify the reactions in a peanut DBPCFC (Grimshaw *et al*., 2003). Three of four subjects challenged with peanut flour in a matrix containing 31.5% fat reacted at a higher than expected dose, and had reactions that were more severe than expected, based on previous exposures to a standard recipe containing 22.9% fat. Upon rechallenge with the 22.9% recipe, their reactions returned to expected levels with respect to dose and severity. The cumulative dose of peanut protein required to elicit reactions was 12 to 31 times higher when using the higher fat recipe. The authors suggested that the peanut allergens in the higher fat recipe were not readily available to react with IgE on mast cells in the mouth. This was based on the observation

that radioallergosorbent test (RAST) inhibition assays and enzyme linked immonosorbent assay (ELISA) detection tests showed that peanut allergens in the higher fat mixture were less available *in vitro*. In addition, these three patients all had histories of an initial oral challenge response. The lack of an oral early warning with a high-fat food may have caused these patients to consume more allergen prior to the onset of other symptoms. By the time digestion of the fat took place in the stomach and intestine, the total dose consumed was higher, resulting in a more severe reaction.

Grimshaw *et al*. (2003) further reported that the slopes of RAST-inhibition curves did not change for peanut allergens in high-fat versus low-fat mixtures, indicating that there was no change in antibody-binding properties. Thus, it appears that the antigenic properties of the peanut flour were not altered by the higher fat matrix, and that the changes in apparent threshold may have resulted from a combination of physiological and behavioral factors.

Kato *et al*. (2001) also observed a matrix effect with the major egg allergen ovomucoid. The ability of ovomucoid to bind IgE was reduced in a model pasta composed of durum wheat and egg white. This decrease was attributed to changes in antigenicity associated with formation of disulfide bonds between the ovomucoid and wheat gliadins.

2. Processing Effects

Numerous studies have described alterations in allergens as a result of processing or cooking. Various types of processing (e.g., heating, milling, fermentation) may alter the antigenic properties of allergens because these processes can affect the three-dimensional structure of proteins and thus the IgE binding epitopes. The type and extent of structural alterations may vary depending on the processing method. This is especially true for conformational epitopes because they are dependant on tertiary structure (Cooke and Sampson, 1997; Vila *et al*., 2001). For many food allergens, processing effects are inherent in the data used to characterize thresholds because the test articles used in DBPCFCs are processed. For practical reasons, the test material must be concealed in some way for the study to be "blinded." For example, the taste of peanut butter or peanut flour must be disguised in DBPCFCs for peanut allergies. Preparation of the test material typically involves cooking or processing of the allergenic food. In addition to altering existing epitopes, processing might also induce chemical or structural changes that result in the formation of new antigenic epitopes, or neoantigens (Maleki, 2004).

Altered antigenic reactivity is most commonly assessed by measuring changes in the binding of antibodies to extracts of raw and processed foods. Reduced or enhanced IgE binding in such studies would suggest that the threshold for an allergic reaction could be affected by processing. However, definitive proof of an altered threshold requires DBPCFC testing.

The effects of processing on some specific major allergens have recently been reviewed, and are discussed below (Besler *et al*., 2001; Poms and Anklam, 2004). Variable patient responses make it difficult to conclude that a particular processing or cooking procedure affects allergenicity in all cases.

Peanuts. Extracts of roasted peanuts have been shown to bind IgE from patients at 90-fold higher levels than do similar extracts of raw peanuts in competitive, IgE-based ELISAs (Maleki *et al*., 2000). Using immunoblot techniques, two of the major allergenic proteins in peanut, Ara h 1 and Ara h 2, were shown to be highly resistant to heat and gastrointestinal digestion following treatment in the Maillard Reaction (which occurs during the processing or browning of foods in the presence of heat and sugars). Earlier studies also observed increased IgE binding and altered IgE epitopes in roasted versus raw peanuts (Nordlee *et al*., 1981). The allergenic proteins Ara h 1, Ara h 2, and Ara h 3 from fried or boiled peanuts bound significantly less IgE than the same proteins from roasted peanuts (Beyer *et al*., 2001), even though there were similar amounts of the allergenic proteins in peanuts processed by each method. These studies suggest that thresholds for boiled or fried peanuts may be higher than for roasted or raw peanuts, at least for the three major peanut allergens. In practical terms, the vast majority of peanuts consumed whole or in processed foods in the U.S. are roasted. Boiled or fried peanuts are an ethnic or regional specialty and are usually eaten whole, rather than as a component of processed foods.

Milk. Pasteurization and homogenization did not reduce allergenicity in skin prick tests or DBPCFC (Host and Samuelsson, 1988). However, boiling milk for 10 minutes reduced IgE binding of the allergenic proteins alpha-lactoglobulin and casein by 50 to 66% and eliminated beta-lactoglobulin and serum albumin reactivity in skin prick tests (Besler *et al*., 2001; Norgaard *et al*., 1996). Hypoallergenic infant formulas produced from heat denatured or enzymatically hydrolyzed caseins or whey proteins showed reduced allergic reactivity by immunoblot, RAST, and DBPCFC in most milk-allergic children. However, some severe reactions have been reported (Sampson *et al*., 1991; Saylor and Bahna, 1991). Maillard reaction products in milk are reported to have increased allergenicity in skin tests (Maleki, 2004). Allergic reactions have also been reported involving both hard and soft cheeses (Besler *et al*., 2001).

Egg. Both soft and hard boiling of eggs decreased, but did not eliminate, antigen binding of rabbit antiserum to ovomucoid and ovalbumin (Besler *et al*., 2001). Heated egg white showed a 58% decrease in IgE binding in RAST (Anet *et al*., 1985). A decrease in positive reactions was seen with heated egg white in 55% of egg allergic patients using DBPCFC (Urisu *et al*., 1997). There are reports of allergic reaction to egg contained in cooked meatballs or hamburger (Sampson *et al*., 1992b; Besler *et al*., 2001).

Fish. Boiling ten species of fish failed to eliminate allergenicity in DBPCFC (Bernhisel-Bradbent *et al*., 1992b). IgE binding to fish proteins in immunoblots was reduced, but not eliminated. Canning (presumably due to the heat processing) appears to reduce allergic reactions to tuna and salmon in allergic patients tested by DBPCFC (Bernhisel-Broadbent *et al*., 1992b). IgE binding of allergenic proteins from canned fish was reduced by 98 to 99% compared to boiled fish. IgE binding studies indicate that fish allergens are present in surimi (Mata *et al*, 1994).

Shellfish. Boiling does not reduce the allergenicity of shrimp allergens (Daul *et al*., 1988; Naqpal *et al*., 1989).

Soy. Heating soybeans at 100°C for 60 minutes does not completely eliminate IgE binding to allergenic soy proteins (Burks *et al*., 1992). Various soybean products including sprouts, soy sauce, hydrolyzed soy protein tofu, miso, and lecithin all retained IgE-binding activity (Besler *et al*., 2001). IgE binding proteins have been found in soy lecithin (Gu *et al*., 2001; Porras *et al*., 1985; Paschke *et al*., 2001). Allergic reactions to soy lecithin have also been reported (Renaud, 1996; Palm, 1999). The protein content of soy lecithin has been reported to vary between 2.8-202 mg per 100 g (Besler *et al*., 2001; Paschke *et al*., 2001). IgE binding proteins have been consistently detected in unrefined soybean oils (Paschke *et. al*., 2001), but inconsistently in refined oil (Awazuhara *et al*., 1998; Paschke *et al*., Errahali *et al*., 2002)

Tree nuts. Protein extracts of several hazelnut-containing products demonstrated less IgE binding than raw hazelnut aqueous extracts suggesting that heating reduced allergenicity. However, some IgE binding capacity remained (Wigotzki *et al*., 2001). Several cases of anaphylaxis have been described for a variety of processed nut-containing products, suggesting that tree nuts in general retain allergenic activity after heating (Besler *et al*., 2001).Roasting, blanching, autoclaving, or microwaving did not change the ability of animal antisera to bind almond proteins (Venkatachalam *et al*., 2002).

Wheat. Baking of wheat flour-containing foods results in the loss of IgE binding to one group of recognized wheat allergens, the alpha-amylase inhibitors. However, baking does not affect the ability of wheat prolamins to bind IgE from wheat allergic individuals (Simonato *et al*. 2001). The wheat allergen omega-5 gliadin also retains allergenic activity after cooking. For example, Daengsuwan *et al*. (2005) found IgE to omega-5 gliadin in seven children who had anaphylactic reactions to breads, buns, noodles, macaroni and pizza.

3. Detecting and Measuring Allergens

There are several factors that make it difficult to detect and measure food allergens. These include sampling problems and difficulties in quantifying proteins, particularly allergenic proteins, in a wide variety of foods. Further, an allergen may be a minor component of a highly complex, heterogeneous food. The food matrix can sequester allergens, hindering detection, while not significantly affecting allergenicity. It is also difficult to estimate the amount of a food allergen that may be present from the result of an assay that only measures protein, particularly when there is more than one allergenic protein.

The only commercial methods that have been shown to detect food allergens reliably use immunological techniques such as ELISA (Poms *et al*., 2004; Krska *et al*., 2003), although non-commercial PCR assays have been described (e.g., Popping *et al*., 2004). In some cases, these methods were designed to detect representative biomarkers, not necessarily a specific allergenic protein. Many kits contain polyclonal antibodies that detect both non-allergenic and allergenic proteins (e.g., Nogueira *et al*., 2004). For example, the peanut ELISA assays that have completed Multiple Laboratory Performance Tested validation are designed to detect multiple proteins indicative of the presence of the food (e.g., peanuts), not to detect or quantify specific allergenic proteins (Park *et al*., 2005). There are no validated detection methods or commercially available kits for most food allergens or allergenic proteins.

The FDA and AOAC investigated the ability of three commercial peanut test kits [BioKits Peanut Testing Kit (Tepnel), Veratox for Peanut Allergens (Neogen Corp.), and RiDASCREEN Peanut (R-Biopharm GmbH)] to accurately measure peanuts in four food matrices (cookies, ice cream, milk chocolate, and breakfast cereal) (Park *et al*., 2005). The validation study, requiring 6 analyses of test samples at the target level of 5μg peanut/g of food and 60 analyses of "peanut-free" controls, was designed to ensure that the lower 95% confidence limit on the true sensitivity and specificity rates exceeded 90% (Park *et al*., 2005). The results from this study showed that all the test kits correctly allocated the test samples at the target level. No comparable studies have been completed for any other food allergen.

Scientific practice is to calibrate, standardize, and validate assays and commercial test kits for each food product because minor differences in the matrix change the recovery and detection of specific food proteins. Standardization requires the preparation of samples identical to the test sample and containing known amounts of a specific food allergen. Nevertheless, because different antibody-based assays recognize different protein epitopes, variable results may be obtained using different test systems. This variability was evident in results obtained in the Food Analysis Performance Assessment Scheme (FAPAS® supervised proficiency studies of wheat (Central Science Laboratory, 2003a; Central Science Laboratory, 2004b), peanut (Central Science Laboratory, 2003b), egg (Central Science Laboratory, 2004a), and milk test kits (Central Science Laboratory, 2004a).

Highly variable food matrices and the nature of food production also create sampling challenges. The distribution of allergenic proteins within whole foods is not necessarily homogenous, and allergenic ingredients may not be evenly distributed throughout processed foods. In addition, cross-contact may result in a heterogeneous distribution of allergens within or on a food. For example, nuts may be introduced into chocolate on a production line where nut-containing and nut-free products are processed sequentially. In this case, cross-contact is most likely to occur at the beginning of a production run for the nut-free product. Thus, allergen testing using chocolate taken from the end of a production run might not adequately characterize the risk.

For a food product, development of a scientifically sound sampling plan that includes a statistical analysis of the probability that any allergens present are detected and measured accurately. Important sampling questions that need to be considered include whether the allergen is likely to be heterogeneously distributed within the batch; the number of samples per batch that should be tested; which batches should be tested; which portion of a run should be tested; and how to obtain a specific

degree of confidence (e.g., 95% confidence) that no allergen is present.

H. Collective Allergens

Three of the major food allergens identified in the FALCPA are actually groups of foods: crustaceans, fish, and tree nuts. It is possible that proteins from two or more species within each of these "collective allergens" might be present in a food and the available analytical methods are unable to distinguish between species in a group. Therefore, it may be necessary to consider total protein levels from all species in a group rather than the level of protein from each species. In addition, an individual allergic to one species is likely to also be allergic to other species in the group.

The ability of available test methods to distinguish different species within each group of "collective allergens" varies. To date there are no commercially available test kits for finfish proteins and only one for crustacean tropomyosin. Ben Rejeb *et al*. (2003) reported the development of an ELISA for shrimp that showed significant cross-reactivity with other crustaceans. There are three commercially available tree nut test kits (two for hazel nut, one for almond), but the species specificity of these kits is not clear. Hlywka *et al*. (2000) showed that an almond ELISA detected protein from seven other tree nuts. The hazel nut ELISA developed by Holzhauser *et al*. (2002) showed cross-reactivity with other nuts, and the walnut assay developed by Niemann and Hefle (2003) reacted with three other nut species. Wei *et al*. (2003) developed an ELISA for cashew that showed cross-reactivity with several other nuts. Ben Rejeb *et al*. (2003) developed a hazel nut-specific ELISA that did not cross-react with other nuts, and Clemente *et al*. (2004) developed a Brazil nut assay with "negligible" cross reactivity to five other nut species.

Although not likely to be useful for routine screening or testing, techniques such as liquid chromatography/mass spectrometry (LC/MS) are being used to identify specific allergenic proteins in complex food matrices (Shefcheck and Musser, 2004). These approaches may be useful either as confirmatory tests or for characterization of foods containing several allergens.

Crustacean Shellfish. Allergenic cross-reactivity among crustaceans is considered to be common. Sicherer (2001) estimated that there is a 75% probability that a shrimp-allergic individual will also react to at least one other crustacean. Waring *et al*. (1985) reported that 11 of 12 (92%) patients with skin prick reactions to shrimp also had positive skin prick reactions to at least one other crustacean. Similarly, Daul *et al*. (1987) showed that between 73 and 82% of shrimp allergic patients had positive skin prick tests to another crustacean. Chiou *et al*. (2003) showed that sera from 20 of 32 individuals with either shrimp- or crab-reactive IgE were reactive to both species. Further, inhibition studies with 15 of these cross-reactive sera showed relatively high affinity for both allergens. The basis for this high rate of cross-reactivity appears to be sensitivity to the highly conserved protein tropomyosin, which is considered to be a panallergen (Daul *et al*., 1993; Leung *et al*., 1999; Sicherer, 2001).

Fish. Allergenic cross-reactivity among fish species has been described in the clinical literature, but appears to be less common than among species of crustacea. Both Sicherer (2001) and Sampson (1999) estimate that there is a 50% probability that an individual allergic to one fish species will react to at least one other fish species. Helbling *et al*. (1999) reported that 4 of 14 (29%) fish allergic patients reacted to two or more species in DBPCFC tests. Bernhisel-Broadbent *et al*. (1992a) reported that 3 of 10 (30%) fish allergic patients responded to more than one fish species in oral challenges, but that skin prick tests were positive to multiple species for all of these patients. Similarly, Hansen *et al*. (1997) showed that eight cod allergic patients all had positive skin prick tests with two other fish species. The data presented in Pascual *et al*. (1992) suggest that at least 80% of a group of 79 fish allergic children had IgE antibodies to two or more fish species. In some cases, cross-reactivity has been shown to reflect the presence of one of more closely related allergenic proteins (e.g., paralbumins) in different species (Pascual, 1992; Hansen *et al*., 1997; Leung *et al*., 1999; Hamada *et al*., 2003).

Tree Nuts. The prevalence of cross-reactivity among tree nuts is difficult to determine accurately for several reasons: the high proportion of severe reactions among nut-allergic patients makes it dangerous to carry out oral challenge studies, many published works test for reactivity to a small number (and variable assortment) of tree nuts, and studies often combine tests for tree nuts and peanuts. Nevertheless, Sicherer (2001) estimates that a tree nut allergic patient has a 37% chance of being allergic to two or more species of tree nut, and Sampson (1999) estimates that the probability of multiple tree nut sensitivities at greater than 50%. Ewan (1996) reported that 12 of 22 (55%) of tree nut allergic patients responded to multiple tree nuts by skin prick tests. Sicherer *et al*. (1998) and Pumphrey *et al*. (1999) both used *in vitro* IgE testing and found multiple sensitivities in 37% and 61% of tree nut allergic patients, respectively. There are a number of studies that report cross-reactions in one or a few patients (e.g., Teuber and Peterson, 1999; Ibanez *et al*., 2003; de Leon *et al*., 2003; Asero *et al*., 2004). The complex pattern of cross-reactivity among the tree nuts may reflect the fact that several different panallergens (lipid transfer proteins, profilins, Bet v1-related proteins) and evolutionarily conserved proteins (seed storage proteins) occur in various tree nuts (Roux *et al*., 2003).

I. Published Challenge Studies

An extensive literature review was conducted from November 2004 through April 2005 that included key word, author, and "related article" searches of the PubMed database and analysis of citations found in the published literature. Seventeen publications with quantitative dose-response data from DBPCFC testing were reviewed to identify those that contained data that could be used to estimate LOAEL levels for the major food allergens. These studies are described in more detail in Appendix 2. Fourteen (82%) of these report results from testing adults; the remaining three tested infants and children. In

four cases, the population being studied was not specifically chosen to be food allergic, and a large fraction of the individuals in these populations did not respond to the highest doses tested. In eight studies (47%), patients reacted to the lowest dose tested, and in three studies there was insufficient information to determine either the lowest dose used or the number of patients who responded to that dose. The most sensitive population was seen by Hourihane *et al.* (1997b), who reported that 67% of the patients tested reacted to "peanut rubbed on the lip," including one severe reaction.

Peanut. Hourihane *et al.* (1997b) observed the lowest measured dose of an allergen that provoked a reaction (i.e., a LOAEL). 0.1 mg of peanut protein provoked subjective reactions in two patients and 2 mg of peanut protein provoked an objective reaction in one patient. Objective reactions were observed in two other patients on exposure to 5 mg of peanut protein. Wensing *et al.* (2002a) also reported a LOAEL of 0.1mg for subjective reactions in two of 26 peanut allergic individuals tested. The LOAEL for the initial objective symptom was 10 mg. Several other papers reported LOAELs of 25-100 mg of peanut protein for objective reactions (May, 1976; Hourihane *et al.*, 1997a; Bock *et al.*, 1978).

Egg. A wide range of LOAELs have been observed for egg. Caffarelli *et al.* (1995) reported a LOAEL of 0.5 mg of dried whole egg (approximately 0.42 mg protein). Bock *et al.* (1978) reported observing an objective reaction with 25 mg of whole egg (approximately 1 mg protein), although the data are difficult to interpret as presented. In contrast, Eggesbo *et al.* (2001) report a LOAEL of 1 g of whole egg (approximately 260 mg of protein) for an objective reaction.

Milk. Relatively consistent LOAELs have been reported for milk. Bellioni-Businco *et al.* (1999) found a LOAEL of 1 ml of whole milk (approximately 362 mg of protein) with children, and Pastorello *et al.* (1989) found a LOAEL of 0.5 g of freeze-dried milk (approximately 187 mg of protein) with adults.

Soy. LOAELs of approximately 522 and 88 mg protein have been reported for soy (Zeiger *et al.*, 1999; Magnolfi *et al.*, 1996).

Tree Nut. Hazel nut is the most commonly studied tree nut. Wensing *et al.* (2002b) observed reactions to 1 mg of hazel nut protein in 4 of 29 patients, which was the lowest dose tested. Hansen *et al.* (2003) found a LOAEL of approximately 32 mg of hazel nut protein, although it is not clear whether this was the lowest dose tested.

Fish. Hebling *et al.* (1999) reported a LOAEL of 50 mg for catfish protein.

Wheat. Unpublished data described in Moneret-Vautrin and Kanny (2004) show that 83% of wheat allergic children reacted to less than 2 g of wheat flour while only 18% of wheat allergic adults responded at this level. Unpublished data described in Moneret-Vautrin (2004) on wheat flour challenges using 32 children and 32 adults with wheat allergy, reported a LOAEL of ≤ 1.8 mg protein for allergic children (the lowest tested dose) and 52.8 mg protein for allergic adults. Scibilia *et al.* (2006) reported that 2 of 13 responders reacted to the lowest dose of wheat flour tested (100 mg of a mix of bread and durum flour, approximately 15 mg protein) in DBPCFCs. In total, 31% of the patients who reacted did so to challenge doses less than or equal to 240 mg of wheat protein.

J. Food Treatments to Reduce Allergenicity

The best example of food products that are processed to render them less allergenic are hydrolyzed infant formulas derived from cow's milk proteins (i.e., casein and whey). Enzymatic hydrolysis of these proteins has been shown to significantly reduce the levels of both total and allergenic (e.g., b-lactoglobulin in whey) protein (Host and Halken, 2004). The degree of protein reduction depends on the method of hydrolysis. There is ample clinical evidence to suggest that both partially hydrolyzed formulas (PHF) and extensively hydrolyzed formulas (EHF) have reduced allergenicity in comparison to intact milk formulas (Amer. Acad. Ped., 2000; Host and Halken, 2004). Furthermore, there is preliminary evidence that the use of these hydrolyzed formulas may also delay or prevent the development of cow's milk allergy (CMA) in high-risk infants (Host and Halken, 2004).

Both PHF and EHF contain varying amounts of residual protein, including allergenic proteins, which can be detected using either *in vitro* or *in vivo* methods (Giampietro *et al.*, 2001; Docena *et al.*, 2002), that have been shown to retain immunological activity. Both PHF and EHF can cause allergic reactions, including anaphylaxis, in sensitive infants (Saylor and Bahna, 1991; Schwartz and Amonette, 1991; Tarim *et al.*, 1994; Ammar *et al.*, 1999; Giampietro *et al.*, 2001; Host and Halken, 2004). In general, the higher the level of residual protein, the higher the risk for an allergic response. Although the level of residual protein tends to be higher in PHF, the degree of hydrolysis cannot always be used as a predictor of the degree of allergenicity. Hydrolysis methods are not standardized, and formulas undergoing similar treatments may vary considerably in their residual protein levels. Additional processing, such as heat treatment and ultrafiltration, may further reduce residual protein levels in certain products (Host and Halken, 2004).

In 1989, the American Academy of Pediatrics (AAP) concluded that a formula could be considered "hypoallergenic" if challenge studies showed, at a minimum, 95% confidence that 90% of allergic infants would not react adversely to the formula (AAP, 1989). Since this time, a number of DBPCFC studies using various infant formula preparations have been performed in infants with CMA (Sampson *et al.*, 1991; Sampson *et al.*, 1992b; Giampietro *et al.*, 2001; Sicherer *et al.*, 2001), and a substantial number of infant formulas (most EHF) have met this criterion for hypoallergenicity. Even though they note that EHF contain residual proteins and may provoke allergic reactions in infants with CMA, the AAP currently recommends these formulas as alternatives for infants with CMA stating that at least 90% of these infants will tolerate the formula (AAP, 2000).

Newer technologies, such as genetic modification, are being developed to reduce allergenicity by removing, silencing, or

modifying the genes for specific allergenic proteins within foods (Tada *et al*., 1996; Herman *et al.*, 2003; Dodo *et al.*, 2005; Gilissen, 2005). To date, however, there is no example of a food allergen that has been rendered completely devoid of allergenic activity using these methods. This is due to the fact that each food contains a number of allergenic proteins, each with multiple allergenic epitopes. Unless these methods can eliminate all of these proteins, or modify all allergenic epitopes, the remaining proteins or epitopes could still elicit a reaction in sensitive individuals.

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