March 2006

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

Prepared by
The Threshold Working Group

Table of Contents

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 heath 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 proteins 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 to 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 using the **statutorily-derived approach** to establish a threshold for gluten. This approach is not viable.

Acknowledgements

Threshold Working Group
Robert Buchanan (Lead)
Sherri Dennis (Associate Lead)

Core Writing Team
Steven Gendel (Lead)
David Carlson
Sherri Dennis

Working Group Members
David Acheson
Sue Anne Assimon
Nega Beru
Philip Bolger
Ricardo Carvajal
Catherine Copp
Kenneth Falci
Elizabeth Harden
Rhonda Kane
John Kvenberg
Stefano Luccioli
Douglas Park
Richard Raybourne
Terry Troxell
Katherine Vierk

The Threshold Working Group would also like to acknowledge the assistance provided by:

Curtis Barton for information on statistics and study designs,
Hugh Dallas for assistance with the reference database,
Eric Garber for information on methods of analysis,
Michael Landa for consultations on the FALCPA,
Lori Pisciotta in preparation of the report, and
Meredith Todd for assistance summarizing the public comments.

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 to 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 methods 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 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 an 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 if 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 or 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 of 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).

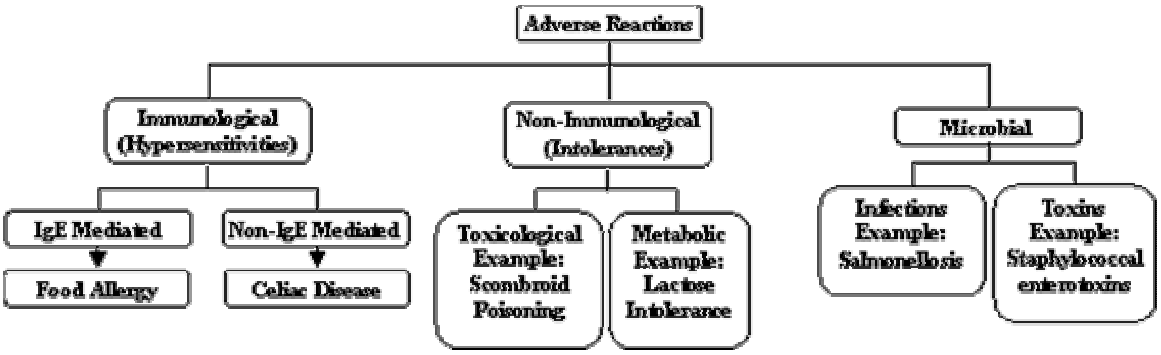

Figure II-1. Adverse Reactions to Foods

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).

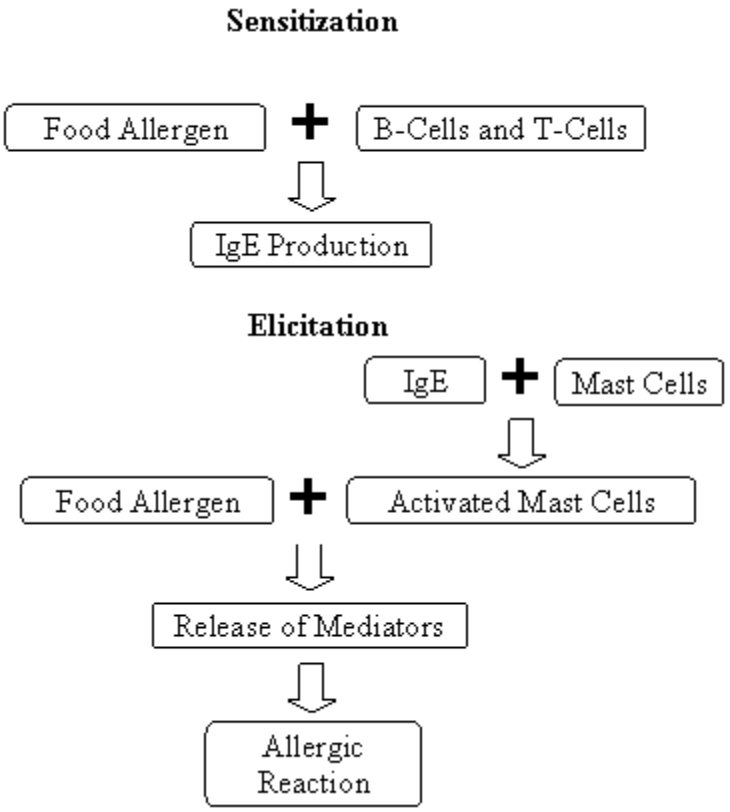

Figure II-2. Mechanism of Allergic Reactions

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 severe 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); Bousquet *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 exposures 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 a*l., 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 60 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 $\leq$ 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 immunologic 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.

III. Celiac Disease

A. Introduction

Celiac disease (also known as celiac sprue and gluten sensitive enteropathy) is a chronic inflammatory disorder characterized by mucosal damage to the small intestine leading to gastrointestinal illness, nutrient malabsorption, and a wide range of clinical manifestations (NIH, 2004; Shan, *et al.* 2002). There is a consensus opinion that celiac disease is caused by an aberrant (T lymphocyte) immune response to dietary glutens predominantly found in wheat, barley, and rye (NIH, 2004). However, there is evidence that at least some persons who have celiac disease may not tolerate oats (Lundin *et al*., 2003; Arentz-Hansen *et al*., 2004). Those individuals who have a genetic predisposition to celiac disease react to peptides within the proline- and glutamine -rich protein fractions of the grains (Dewar *et al*., 2004). For affected individuals, celiac disease is a lifelong condition and, if not treated, is associated with significant morbidity and increased mortality (Fasano, 2003; Corrao *et al*., 2001; Dewar *et al*., 2004). There is no cure for celiac disease (NIH, 2004). Strict avoidance of potentially harmful concentrations of glutens in the diet is the only known means of completely preventing the clinical and pathological complications of celiac disease (NIH, 2004; Fasano and Catassi, 2001).

B. Mechanism of Pathogenesis

Celiac disease is characterized by injury to the mucosa of the small intestine and specifically targets the fingerlike projections, called villi, where absorption of key nutrients takes place (Figure III-1). This injury is believed to be due to an autoimmune disorder involving modification of the antigenic presentation of gluten in the intestinal tract of genetically predisposed individuals expressing the major histocompatibility haplotypes HLA-DQ2 or HLA-DQ8 (Farrell and Kelly, 2002; Fasano, 2003). In these individuals, binding of the enzyme tissue transglutaminase (tTG) to wheat gluten (a glutamine rich protein) potentiates uptake and presentation by antigen-presenting cells in the lamina propria, triggering a vigorous T-cell response (Schuppan and Hahn, 2002), leading to production of IgG and IgA antibodies directed to wheat gluten peptides (i.e., gliadins and glutenins) and to tissue transglutaminase (tTG). The activated T-cells are responsible for the mucosal damage seen in celiac disease (Fasano and Catrassi, 2001). This immune-mediated damage occurs in two compartments, the epithelium and the lamina propria (Green and Jabri, 2003). Early intestinal disease is characterized by an increased number of intestinal intraepithelial lymphocytes (IELs). As the disease progresses, increasing numbers of lymphocytes and plasma cells infiltrate the lamina propria. This increase in the numbers of cells leads to elongation of intestinal crypts and shortening of villi, which eventually results in partial or total villous atrophy (James, 2005). Elimination of intestinal gluten results in modification of T lymphocyte and antibody responses and, in most cases, full mucosal recovery (Kaukinen *et al*., 1999; Fasano and Catassi, 2001).

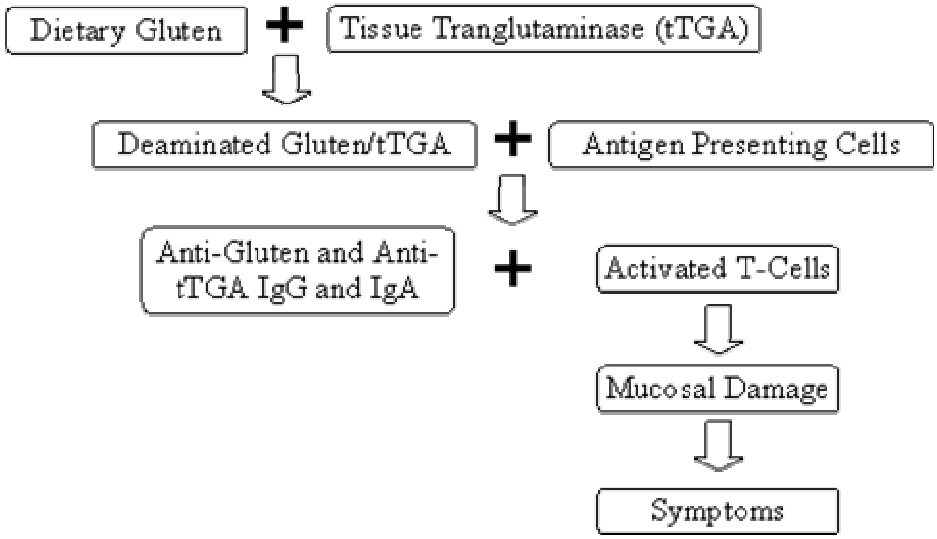

Figure III-1. Mechanism of Celiac Disease

C. Range of Adverse Effects

The clinical manifestations of celiac disease are highly variable in character and severity. The reasons for this diversity are unknown but may depend on the age and immunological status of the individual, the amount, duration, or timing of exposure to gluten, and the specific area and extent of the gastrointestinal tract involved by disease (Dewar *et al*., 2004). These clinical manifestations can be divided into gastrointestinal, or "classic," and non-gastrointestinal manifestations. Gastrointestinal manifestations usually present in children 4 to 24 months old and include abdominal pain and cramping, bloating, recurrent or chronic diarrhea in association with weight loss, poor growth, nutrient deficiency, and (in rare cases) a life-threatening metabolic emergency termed celiac crisis, characterized by hypokalemia and acidosis secondary to profuse diarrhea (Farrell and Kelly, 2002; Baranwal *et al*., 2003). Non-gastrointestinal manifestations are more insidious and highly variable and are the common presenting signs in older children and adults. These manifestations are frequently the result of long-term nutrient malabsorption, including iron deficiency anemia, short stature, delayed puberty, infertility, and osteoporosis or osteopenia (Fasano, 2003). In children, progressive malabsorption of nutrients may lead to growth, developmental, or neurological delays (Catassi and Fasano, 2004). Extra-intestinal manifestations such as dermatitis herpetiformis, hepatitis, peripheral neuropathy, ataxia, and epilepsy have also been associated with celiac disease (Fasano and Catassi, 2001). Individuals with untreated celiac disease are also at increased risk for potentially serious medical conditions, such as other autoimmune diseases (e.g., Type I diabetes mellitus) and intestinal cancers associated with high mortality (Farrell and Kelly, 2002; Peters *et al*., 2003; Catassi *et al*., 2002). For example, individuals with celiac disease have an 80-fold greater risk of developing adenocarcinoma of the small intestine, a greater than two-fold increased risk for intestinal or extraintestinal lymphomas (Green and Jabri, 2003) and a 20-fold greater risk of developing enteropathy-associated T cell lymphoma (EATL) (Catassi *et al*., 2005a). These are rare intestinal malignancies with a high mortality rate. In addition, the relative risk for developing non-Hodgkin's lymphomas, intestinal or extraintestinal, is three fold greater than in the general population (Catassi *et al*., 2002). These cancers contribute to nearly two thirds of deaths due to celiac disease and are a major reason for the nearly two-fold increase in overall mortality of adult patients with celiac disease compared to the general population (Corrao *et al*., 2001).

Currently, individuals with clinical manifestations, or "symptomatic" celiac disease, are believed to represent a small portion of the total affected population (Mäki and Collin, 1997). A larger number of individuals are believed to have "silent" celiac disease, characterized by positive serology and intestinal mucosal abnormalities in the absence of symptoms or nutritional deficiencies. Mäki and Collin (1997) also suggested that there is an even larger population with "latent" celiac disease, individuals who are positive for serological markers or genetic susceptibility to disease and are entirely asymptomatic. It is generally accepted that individuals with silent or latent disease, although asymptomatic, have the capability to manifest aberrant immune responses following exposure to dietary glutens and are, therefore, at increased risk for both acute and long-term complications of celiac disease (Fasano, 2003; Schuppan, 2000). However, the long-term benefit of strict gluten avoidance for these individuals is unproven (Green and Jabri, 2003).

D. Prevalence

Until recently, celiac disease was considered to be a rare disorder in the U.S., with an estimated prevalence rate of 1:5,000 (Talley, 1994). However, a large epidemiological study screened more than 13,000 people in 23 states and estimated a prevalence rate of 1:133 within the general U.S. population (Fasano *et al.*, 2003). The National Institutes of Health Consensus Development Conference Statement on Celiac Disease currently estimates that 3 million Americans, a little less than 1 percent of the population, may have celiac disease (NIH, 2004). Celiac disease occurs widely among North American and European populations, where wheat is a staple food, but is infrequent among native descendents of China and Japan and those with an African-Caribbean background, where wheat is not as widely consumed (Farrell and Kelly, 2002).

Precise prevalence data for celiac disease are not available. This disease is often misdiagnosed as another gastrointestinal malabsorptive disorder (e.g., irritable bowel syndrome) due to similarities in their symptoms (Sanders *et al.,* 2001). Due to the existence of silent or latent cases, it is assumed that the incidence of celiac disease is underreported (Mäki and Collin, 1997). These forms of celiac disease may go undetected in individuals for years before they develop symptoms causing them to seek medical attention (Green and Jabri, 2003). Mäki and Collin (1997) postulated that there are many more currently healthy individuals who are genetically predisposed to developing celiac disease in future years than there are individuals who are now affected by celiac disease. Only recently has the medical community become more aware of the need to screen for celiac disease when patients experience health problems that may be associated with the disease or when patients have family members, especially first- and second-degree relatives, who have celiac disease (NIH, 2004).

E. Celiac Foods of Concern

Celiac disease is caused by an immune response in genetically predisposed individuals to specific storage proteins, commonly referred to as "glutens," that occur naturally in cereal grains (Shan *et al.*, 2002). Technically, "gluten" is a term applied *specifically* to the combination of the prolamin proteins called "gliadins" and the glutelin proteins called "glutenins" found in wheat (Brown, 2004). However, the term "gluten" has been used generically to refer to prolamin and glutelin protein mixtures found in other cereal grains (Kasarda, 2005, personal communication). Although all cereal grains contain prolamin and glutelin proteins, these proteins are not identical in different grains. These proteins differ in their amino acid sequences in different grains, and not all have been shown to evoke an abnormal immune response that affects the intestinal lining of persons genetically susceptible to celiac disease (Kasarda, 2003). The term "gluten" will be used

in this report in the more general sense of the combination of both prolamin and glutelin proteins found in cereal grains.

The grains considered to be capable of producing adverse effects in individuals with celiac disease include the different species of wheat (e.g., durum, spelt, kamut), barley, rye, and their cross-bred hybrids (e.g., triticale, which is a genetic cross between wheat and rye) (Kasarda, 1994; Kasarda, 2004). There is also evidence that some individuals with celiac disease may react adversely to oats (Lundin *et al.*, 2003; Arentz-Hansen, 2004). These grains are all members of the grass family (*Gramineae*, also known as *Poaceae)* and are closely related taxonomically. The cereal grains assumed to be safe for persons with celiac disease include amaranth, buckwheat, corn, Indian ricegrass, Job's tears, millet, quinoa, ragi, rice, sorghum, teff (or tef), and wild rice (Kasarda, 2001; Johnson *et al*., 2002; Kasarda, 2004b; Kupper, 2004).

The grain prolamins of concern include gliadin in wheat, secalin in rye, hordein in barley (Thompson, 2001; Green and Jabri, 2003; Kagnoff, 2005) and possibly avenin in oats (Arentz-Hansen, *et al.* 2004; Lundin, *et al.*, 2003). There is substantial evidence that both prolamin proteins (i.e., gliadins) and glutelin proteins (i.e., glutenins) in wheat affect individuals with celiac disease (Shan *et al.*, 2002; Hausch *et al*., 2002; Vader *et al*., 2002; van de Wal *et al*., 1999; Molberg *et al*., 2003).

Wheat gliadin subtypes alpha, beta, gamma, and omega, have been shown to affect individuals with celiac disease (Ciclitira *et al*., 1984; EFSA, 2004). Rye, barley and triticale are taxonomically related to wheat, express peptides structurally similar to those found in wheat, and have been reported to affect individuals with celiac disease (Vader *et al.*, 2002; Kasarda, 2001; Kasarda, 2004b). In contrast, the prolamins in other cereal grains (e.g., zein in corn and orzenin in rice) have been shown not to affect individuals with celiac disease (EFSA, 2004; Kasarda, 2004b). However, much is still unknown about which proteins in the different grains can affect individuals with celiac disease (Kasarda, 2001).

Analytical information is not available on the actual amount of gluten proteins in different grain-derived food ingredients or finished foods. For single ingredient foods made from wheat, rye, barley, triticale, and oats, the simple presence of "protein" in that food may be used as an indicator that gluten proteins are present. The USDA *National Nutrient Database for Standard Reference, Release 17* (USDA, 2004), the major source of composition data for foods in the U.S., includes hundreds of food items that contain wheat, rye, barley, triticale or oats as an ingredient. Wheat, in particular, is used to manufacture a wide range of food ingredients and finished foods. Rye, barley, triticale, and oats are used to make substantially fewer food products.

Koehler and FDA (2005) estimated the average amount of total grain and individual types of grain available for consumption per person in the U.S., and the total exposure to gluten-forming proteins that would result from this grain consumption. The estimated mean daily consumption rate was approximately 250 grams of grain per capita. Wheat provided 180 of the 187 grams per person per day of grains that are of concern for individuals with celiac disease.

There is no consensus as to whether oats present a hazard for all individuals with celiac disease. Several studies, including one that lasted 5 years, have reported that most celiac study participants tolerated moderate amounts (e.g., 50-70 grams daily) of oats (Janatuinen *et al*., 1995; Janatuinen *et al*., 2000; Janatuinen *et al*., 2002; Lundin *et al*., 2003; Arentz-Hansen *et al*., 2004). The oats used by Lundin *et al.* (2003) and Arentz-Hansen *et al.* (2004) were tested to ensure that they did not contain any gluten proteins from wheat, rye, or barley.

F. Gluten Contamination of Grains

In the U.S., most commercially available oat products are believed to contain some gluten proteins from wheat, rye, or barley due to cross-contact with these grains during growth, harvest, transport, storage, or processing (Kasarda, 1999; Kasarda, 2001; AGA, 2001; Thompson, 2003). In a recent study, Thompson (2004) analyzed four lots of three brands of rolled or steel-cut oats commercially available in the U.S. for prolamins from wheat, barley, or rye. For one brand, all samples contained 338 to 1807 ppm gluten (expressed as the mean of duplicate determinations). For each of the other two brands, the level of gluten detected in all but one lot ranged from 12-725 ppm in one brand and 120-131 ppm in the other brand (expressed as the mean of duplicate determinations). Thus, only one lot of these two brands was negative for gluten. Thompson (2004) concluded that none of these three brands could be considered a reliable source of oats free of potentially harmful gluten proteins.

Grains that do not contain gluten can become contaminated with grains that contain gluten at any step in the farm-to-table continuum, particularly if shared equipment is not thoroughly cleaned between uses. It is difficult, if not impossible, to prevent all cross-contact situations, considering the tons of grain handled by farm equipment, bulk storage, and transport containers on a daily basis. In fact, the Official United States Standards for Grains (USDA, 1999) assume that most grains that have an established U.S. standard will contain a small percentage of other grains.

G. Gluten Challenge Studies

There is little information in the literature on minimal disease-eliciting doses of gluten for sensitive individuals. Gluten challenges have generally been performed in individuals where diagnosis is uncertain (e.g., infants, Laurin *et al*., 2002) or in individuals with unclear intestinal pathology results (Wahab *et al*., 2001). Challenges have also been performed to determine the time of disease relapse after a prolonged period of gluten avoidance (Mayer *et al*., 1989). In most cases, gluten challenges have been performed to elicit or confirm disease rather than to measure the level of sensitivity (Farrell and Kelly, 2002).

There is no standard protocol for gluten challenges, and challenge studies have varied greatly in amount and duration of gluten exposure. Although some studies have been designed to determine the acute effects (i.e., after 4 hours) of exposure to gluten (Sturgess *et al*., 1994; Ciclitira *et al*., 1984), most challenges consist of an open challenge to a fixed or incremental dose of daily gluten over a minimum period of 4 weeks. Many challenge studies use a high exposure ($\geq$ 10 g/day) to gluten, because this is believed to shorten time to disease confirmation or relapse and, therefore, to minimize discomfort to subjects (Rolles and McNeish, 1976). However, some studies have shown that low daily exposures to gluten also can elicit a disease response (Catassi *et al*., 1993; Laurin *et al*., 2002; Hamilton and McNeill, 1972).

Catassi *et al*. (1993) reported that children, whose celiac disease had previously been controlled on gluten-free diet, had evidence of intestinal mucosal or immunological changes (changes in intraepithelial lymphocyte counts and the villous height to crypt depth ratio) following 100 mg or 500 mg of daily gliadin over 4 weeks; this corresponds to 200 mg and 1000 mg of daily gluten respectively (Collin *et al*., 2004). The degree of inflammation was dose dependent. However, this study had several important limitations, which include the short-term follow up (4 weeks), testing in young children, the small number of subjects (n=20), and the lack of control groups. In addition, although gliadin is believed to be the major immunogenic portion of gluten, T cells from the small intestine of celiac disease patients have been shown to be responsive to peptides from the glutenin portion as well (Van de Wal *et al*., 1999). Thus, the Castissi *et al*. (1993) study

was also limited by the use of gliadin rather than gluten. Estimating potential harm by extrapolating from gliadin levels may not be representative of the harm from total gluten exposure.

A study currently in progress [The Italian Microchallenge Study] has extended the scope of these earlier findings by evaluating the effects of exposure to either 10 or 50 mg of purified gluten per day for 3 months with a population of 36 celiac disease individuals in a double-blind, placebo-controlled study (Catassi *et al*., 2005b). Preliminary unpublished results suggest that minimal mucosal abnormalities occur with a strict gluten-free diet, that both 10 mg and 50 mg daily gluten are well-tolerated, but that there is a trend for mucosal changes to occur at the 50 mg dose. These results can be compared to estimated gluten exposures from gluten-free diets containing various levels of gluten contamination (Table III-1, from Collin *et al*., 2004, reproduced below). Fasano (2005 personal communication) used these values to suggest that a conservative threshold for gluten exposure for sensitive individuals would lie between 20 and 100 ppm.

Table III-1. Estimated Daily Gluten Consumption from Combinations of Different Amounts of Food Containing Different Levels of Gluten

Gluten Content in Food (ppm[a])	Daily Amount of Gluten-Free Food Consumed (g)			
	50	100	200	300
	----------Daily Amount of Gluten Consumed (mg)----------			
200	10	20	40	60
100	5	10	20	30
50	2.5	5	10	15
20	1	2	4	6

Source: Collin *et al*., 2004.

[a] ppm=mg/kg

Note: Gluten content in food multiplied by food consumed equals gluten consumed. Six slices of bread is equivalent to approximately 100 g baking mix.

In an alternate approach, Collin *et al*. (2004) analyzed gluten levels in a number of different types of wheat starch (n=24) and naturally gluten-free (n=59) flours consumed by 76 individuals with celiac disease who had been on gluten-free diets for 1 to 10 years. These individuals had no reported evidence of mucosal deterioration or significant provocation of signs or symptoms while on this diet. The range of gluten found in these products was 0 to 200 ppm. Collin *et al*. (2004) then estimated that the total daily flour consumption for these individuals to be 10-300 gm (median 80 gm). Based on this estimate and the gluten content of the flour, a chart depicting estimated daily gluten exposures was devised (Collin *et al*., 2004). Collin *et al*. (2004) used this chart and data from low dose gluten challenge studies to suggest the use of a threshold of 100 ppm gluten. The main limitations of this study include lack of a prospective study design (for actual dose-response information) and the lack of information detailing diagnostic assessment (i.e., minimal mucosal involvement) for characterizing mucosal relapse in these individuals.

H. Measuring Gluten in Food

Currently, commercial immunology-based ELISA test kits for the detection of gluten in foods are manufactured by Immunotech (Czech Republic), Ingenasa (Spain), Morinaga (Japan), Diffchamb (Sweden), Neogen Corporation (U.S.), R-Biopharm (Germany), and Tepnel BioSystems (U.K.). All of these detect prolamins, the proteins found in soluble aqueous-alcohol

extracts from cereals. None is designed to detect all proteins associated with celiac disease. Five of the assays have separately undergone multi-laboratory validation studies (Skerritt and Hill, 1991; Akiyama *et al*., 2004; Gabrovsk´ *et al*., 2004; Immer *et al*., 2003). Each of these studies employed different target levels and matrices. The Tepnel kit was validated by AOAC at >160 ppm gluten (Skerritt and Hill, 1991). All the ELISA kits rely on the preparation of an aqueous-alcohol extracts as analytical samples, and four of the manufacturers include the use of reducing-denaturing conditions for the analysis of baked goods. During the 25th session of the Codex Committee on Nutrition and Foods for Special Dietary Uses in 2003, the R5-Mendez ELISA method, which entails the use of reducing/denaturing conditions, was forwarded to the Codex Committee on Methods of Analysis and Sampling for endorsement (Codex Alimentarius Commission, 2003). These ELISA test kits cross-react, to differing degrees, with prolamins derived from wheat, rye, and barley. None of the test kits cross-reacts with protein extracts from oats (Gabrovsk´ *et al*., 2004; Nonaka, 2004; Abouzied, 2004; Brewer *et al*., 2004). As such, the ELISA test kits do not provide protection to individuals with celiac disease who are sensitive to oats (Peraaho *et al*., 2004; Storsrud *et al*., 2003; Arentz-Hansen *et al*., 2004; Lundin *et al*., 2003). Proficiency testing studies conducted by the Food Analysis Performance Assessment Scheme (FAPAS®) have shown variability between the prolamin ELISA test kits (Central Science Laboratory, FAPAS Series 27 Round 05, Report No. 2705, 2003), indicating that further validation studies for these kits need to be carried out under comparable conditions. In addition to ELISA test kits, two of the manufacturers, Tepnel BioSystems and R-Biopharm, market lateral flow devices for the detection of gluten. To date, neither of these has been validated.

At this time there is no correlative information on the efficacy of using these tests to predict or help prevent adverse effects in individuals with celiac disease.

I. Gluten-Free Labeling

Although gluten-free diets are considered the only effective treatment for individuals with celiac disease, it has been recognized that it is difficult, if not impossible, to maintain a diet that is completely devoid of gluten (Collin *et al*., 2004). Therefore, several attempts have been made to define gluten-free in regulatory contexts. Efforts by the Codex Alimentarius to define an international standard for "gluten-free" labeling date back to 1981. At that time, due to the lack of sensitive, specific analytical methods, a threshold value of 0.05 g nitrogen per 100 g dry matter was set for wheat starch, on the assumption that wheat protein would be the only source of nitrogen in starch (Codex Standard 118-1981). The Codex Committee on Nutrition and Foods for Special Dietary Uses is developing a revised standard. The current draft proposal would define three categories of gluten-free foods: processed foods that are naturally "gluten-free" (≤ 20 ppm of gluten), products that had been rendered "gluten-free" by processing (≤ 200 ppm), and any mixture of the two (≤ 200 ppm). The Australia New Zealand Food Agency (ANZFA) defines gluten to mean "the main protein in wheat, rye, oats, barley, triticale and spelt relevant to the medical conditions, Coeliac disease and dermatitis hepetiformis." ANZFA recognizes two classes of foods, gluten-free foods (" ...no detectable gluten") and low-gluten foods (" ...no more than 20 mg gluten per 100 gm of the food") (ANZFA Food Code Standard 1.2.8). The Canadian standard for "gluten-free" is more general, simply stating that "No person shall label, package, sell or advertise a food in a manner likely to create an impression that it is a "gluten-free" food unless the food does not contain wheat, including spelt and kamut, or oats, barley, rye, triticale or any part thereof" (Canadian Food and Drugs Act Regulation B.24.018).

IV. Discussion and Recommendations

A. General Approaches

Four general approaches were identified that could be used to establish thresholds for allergens and glutens: analytical methods-based, safety assessment-based, risk assessment-based, and statutorily-derived. With any of these approaches, planned iterative reevaluation of threshold values should be carried out as new knowledge becomes available. These approaches are summarized in Table IV-1 and described in detail below.

Table IV-1. Approaches to Establishing Thresholds

Type of Approach	Examples
Analytical methods-based	Labeling of sulfiting agents "Zero" tolerance policy for *Listeria monocytogenes* in ready-to-eat foods
Safety assessment-based	Evaluation of food additive petitions
Risk assessment-based	Guidance levels for *Vibrio parahaemolyticus* in raw oysters
Statutorily-derived	Labeling exemption for highly refined oil in the FALCPA

1. Analytical Methods-Based Approach. In an analytical methods-based approach, thresholds are determined by the sensitivity of the analytical method(s) that can be used to verify compliance. This effectively establishes a "regulatory threshold," although this threshold is not necessarily correlated to biological effects. This approach has been used in food labeling. For example, the requirement to declare sulfiting agents on product labels when foods contain 10 ppm or greater is based on the limit of sensitivity of the analytical method used to measure these agents.

The issues that need to be considered when using an analytical methods-based approach to establish a threshold include:

- What are the sensitivity and specificity of the method?
- Has the method been adequately validated?
- How will the method be used?
- How will the threshold be modified when improved methods are developed?

The strength of this approach is that it is relatively simple, straightforward, and easy to implement. However, it is appropriate to use an analytical methods-based approach to establish thresholds for allergens or gluten only if analytical techniques are available for the food allergen and celiac-associated glutens.

2. Safety Assessment-Based Approach. Safety assessments are routinely applied to public health issues related to substances in foods, such as chemical contaminants or food additives, particularly when a biological threshold can be justified scientifically. The definition of "safe" varies according to the applicable legal provision. For example, for contaminants, the statutory definitions of safety are proscribed in section 402(a)(1). Food is considered adulterated if an added contaminant is in the food in a quantity"...which may render it [the food] injurious to health", or, if the substance is an inherent natural constituent of the food (i.e. "not an added substance") and is in the food in a quantity that would "ordinarily render it [the food] injurious to health". As another example, the phrase "reasonable certainty that no harm will result" is used in section 408 (a)(4) regarding the safety of tolerances for a pesticide chemical residue in or on a food.

For a safety assessment, the term "safety" has connotations involving both the degree of certainty and an assumption of "negligible risk." The prototype chemical safety assessment is the Acceptable Daily Intake (ADI) method which was first articulated by Fitzhugh and Lehman (1954) for use in considering the significance of available animal data. This approach or variations of it are used throughout the world (WHO, 1987). The ADI for a chemical is calculated from the No Observed Adverse Effect Level (NOAEL) and Uncertainty Factor (UF) using the following equation:

ADI = NOAEL / UF.

The same basic methodology can be used to derive other regulatory standards such as Tolerable Daily Intake (TDI), Reference Dose (RfD), and Minimal Risk Level (MRL). These values are derived from controlled animal studies, human clinical studies, or epidemiological studies that provide the exposure level for which there is no apparent adverse effect or which identify the lowest observable adverse effect level (i.e., NOAEL, LOAEL). These adverse effect levels are also considered in conjunction with one or more uncertainty factor(s). Uncertainty factors are applied to account for inter-species and inter-individual differences and other uncertainties in the data (WHO, 2004).

There have been consistent efforts to improve this process to make better use of scientific knowledge. These efforts have focused on both replacing the NOAEL approach and refining the development of uncertainty factors. One example is the development of the benchmark dose (BMD) concept (Crump, 1984; Kimmel and Gaylor, 1988). The BMD concept involves fitting a dose-response model to all the available data and to determine the statistical lower bound of the BMD (i.e., the BMDL). The major advantage of the approach is that the BMDLis not constrained to one of the experimental doses from a controlled study, as is the case with the NOAEL (Crump, 1994). The U.S. Environmental Protection Agency (EPA) uses the BMD method in health risk assessments (Filipsson *et al*., 2003).

3. Risk Assessment-Based Approach. A risk assessment is a systematic, scientific examination of known or potential adverse heath effects resulting from human exposure to a hazard. The generally accepted paradigm separates risk assessment into four components: hazard identification, exposure assessment, hazard characterization (dose-response), and risk characterization. This framework allows for organization of information, definition of uncertainties, and identification of data gaps. Risk assessments can describe the likelihood of adverse health effects either quantitatively or qualitatively depending on the extent of the knowledge available, the complexity of the problem, and the time available to conduct the assessment. In quantitative risk assessments, risk is expressed as a numerical estimate of the chance of illness or death after exposure to a specific hazard. This estimate represents the

cumulative probabilities of certain events happening and the uncertainty associated with those events. A qualitative risk assessment, on the other hand, uses verbal descriptors of the risk and uncertainties, and often involves the aggregation of expert opinions.

Of the four approaches, the quantitative risk assessment-based approach is the most scientifically rigorous and provides insight into the level of risk associated with specific exposures and the degree of uncertainty inherent in the risk estimate. An example of the use of a risk estimate and associated uncertainty is the current standard for hypoallergenic infant formulas, where there is 95% certainty that 90% of the sensitive population will not react (American Academy of Pediatrics, 2000). The risk assessment-based approach is preferred when a biological threshold cannot be justified scientifically. Several recent papers have discussed the application of the risk assessment-based approach to food allergens (Bindslev-Jensen *et al*., 2002; Moneret-Vautrin and Kanny, 2004; Cordle, 2004; Wensing *et al*., 2002a).

The issues that need to be considered when using a risk assessment-based approach include:

- What is the biological endpoint or biomarker of concern?
- Is the response measurable?
- What is the population (or sub-population) of interest?
- What are the exposure levels?
- What data and assumptions are needed for the assessment, and how do gaps in the existing data affect the level of uncertainty?

Other issues that should be considered in regard to understanding the relationship between the exposure level and nature of the response include:

- How sensitive and accurate are the available analytical methods?
- How do changes in individual sensitivities over time and within populations contribute to the overall uncertainty?
- What are the limitations of the clinical studies (e.g., small number of volunteers, not testing the most sensitive subpopulation) that are used to determine the dose-response relationship and how do these limitations contribute to the overall uncertainty?
- Which dose-response models (e.g., threshold, non-threshold) are appropriate?

It is not clear whether the data and modeling techniques available at the present time are sufficient to allow use of the risk assessment-based approach to establish thresholds for food allergens and for gluten. As an example of the complexity of this approach, the following describes the process of developing a dose-response model that can be used in a quantitative risk assessment:

Steps in Developing a Dose-Response Model

1. Determine the population of concern (e.g., infants, children, pregnant women).
2. Determine the endpoint or biomarker of concern (e.g., death, severe illness requiring hospitalization, subjective reactions such as tingling of lip).
3. Identify available relevant data including animal studies, human clinical studies, and epidemiological data that relate dose to frequency or severity of response.

4. Select the appropriate dose-response model(s) that characterize the shape of the dose-response curve.
5. Fit the selected model(s) to the data.
6. Characterize the uncertainty (i.e., curve weighting and/or use of alternative plausible models).

4. Statutorily-Derived Approach. The statutorily-derived approach establishes a threshold by extrapolating from an exemption established by Congress for another purpose. For example, the FALCPA defines "major food allergen " to include a food ingredient "that contains protein derived " from one of eight foods or food groups, "except... any highly refined oil " derived from one of those foods. If consumption of highly refined oils is not associated with allergic reactions, and if there is nothing unique about the proteins in highly refined oils, then consumption of another food containing levels of protein that result in an exposure that is equal to or less than the level in a typical serving of highly refined oils should not be associated with allergic reactions. Thus, a threshold could be established for all food allergen proteins based on the level of protein in highly refined oils. There is no comparable statutory standard for gluten.

B. General Criteria for Evaluating and Selecting Approaches to Establish Thresholds

The general criteria used to evaluate the four approaches to establish thresholds for allergens and gluten are shown in Table IV-2. Specific criteria related to food allergens are given in Section IV -C and gluten in section IV-D. The specific criteria should be weighted appropriately when implementing a particular approach. The general criteria focus on data availability and data quality. The Threshold Working Group recognizes that scientific knowledge is the product of a process which is inherently imperfect and often incomplete. As such, the degree of uncertainty in the data is a key consideration. It is expected that any decisions on approaches for establishing thresholds for food allergens or for gluten would require consideration of additional factors not covered in the current report. For example, ease of compliance and enforcement, stakeholder concerns (i.e., industry, consumers, and other interested parties), economics (e.g., cost/benefit analysis), trade issues, and legal authorities are all significant factors that are likely to influence the practicality of implementing any approach. One option that is implicit in the following discussion of potential approaches is a decision not to establish thresholds at this time, at least for food allergens.

Table IV-2. General Criteria for Evaluating and Selecting Recommended Approaches to Establish Thresholds

Criteria	Description
Data Availability	Identification and review of currently available data that can be used in any of the four approaches to establish a specific threshold.
Data Quality	Evaluation of the available data for utility, completeness, and scientific soundness. Evaluation of the degree of uncertainty associated with the data.

1. Feasibility. The published and unpublished literature summarized in Sections II and III of this report were reviewed to determine the availability of the specific types of data needed for each of

the approaches to establish thresholds. When necessary information was not available, the following questions were used to evaluate the existing information:

- Is there surrogate or alternate information available that could be used?
- Is the existing knowledge sufficient to support reasonable assumptions when specific data are not available?
- What is the level of uncertainty associated with these data and assumptions?

2. Uncertainty. Uncertainty is typically thought to arise from the lack of data or information. Other sources of uncertainty are often considered to be relevant to scientific evaluations such as subjective judgment, statistical variation, sampling errors, and inherent randomness (Byrd and Cothern, 2000). Techniques are available to account for or measure some of these uncertainties. For example, the uncertainty in a dose-response model can be characterized using advanced techniques, such as model weighting, that measure the degree of credibility associated with the model results (Carrington, 1997). State-of-the-art food safety risk assessment models, such as the HHS/USDA *Listeria monocytogenes* risk assessment for ready-to-eat foods (HHS/USDA, 2003) also used techniques that separate uncertainty from biological variability. It is important to note that uncertainty is different from variability. Uncertainty reflects incomplete knowledge about a system or population which can be reduced with additional study. Variability reflects the fact that all systems or populations have inherent, biological heterogeneity that is not reducible through further measurement or study (Voysey *et al*., 2002). Sufficient knowledge is needed to account for both variability and uncertainty in order to evaluate the four approaches for establishing thresholds.

As described above, uncertainty factors are used in safety assessment calculations. Fitzhugh and Lehman (1954) originally proposed a single safety factor of 100-fold applied to animal data. The justification for this factor included both scientific issues and social values. The scientific issues included the possibility that humans may be more sensitive to chemicals than the rodents used in laboratory tests and that there may be substantial variability among individuals in a population. In general, as uncertainty increases, the uncertainty factor employed in a safety assessment should increase proportionally. As a matter of practice, uncertainty is not characterized in a safety assessment, either formally or subjectively, as is done in a quantitative risk assessment. A minimum uncertainty factor of 10 is generally used to account for variation within the population when relying on human data and additional uncertainty factors may be included as appropriate. For example, the Food Quality Protection Act (FQPA) of 1996 requires, in certain cases, a 10-fold factor in addition to any other uncertainty factors to protect infants and children from exposure to pesticides. Similarly, the EPA uses uncertainty factors of 3 for inter-species differences,10 for variability among humans (intra-species variability), 10 for extrapolation from subchronic to chronic exposures, 10 for extrapolation from LOAELs to NOAELS, and 1 to 10 for data deficiencies in safety assessments related to continuous inhalation exposures (U.S. EPA, 2002; Jarabek, 2002). The assignment of uncertainty factors should be based on science but typically will include the application of expert judgment.

3. Data Quality. The FDA Information Quality Guidelines and the Agency for Healthcare Research and Quality (AHRQ) guidelines on systems for rating the strength of scientific evidence were used in evaluating the scientific data contained in this report (West *et al*., 2002). The FDA guidelines describe policies and procedures for ensuring the quality of the information disseminated by FDA. In these guidelines, data quality is defined in terms of utility, objectivity, and integrity. Utility is defined as the usefulness of the information to its intended users; objectivity as presentation of the data in an accurate, clear, complete, and unbiased manner; and integrity as protecting the information from unauthorized access or revision. In particular, the

guidelines provide transparency standards and ensure clarity. The AHRQ guidelines describe systems for evaluating the strength of scientific studies, including randomized clinical studies. In these guidelines, quality is defined as "the extent to which a study's design, conduct, and analysis has minimized selection, measurement, and confounding biases." In addition, the AHRQ guidelines suggest specific factors (called Domains and Elements) that should be considered in evaluating individual studies. These factors were considered in developing the criteria described below.

C. Allergen Thresholds: Evaluation and Findings

This section provides an evaluation of the data needed to establish thresholds for the major food allergens. Based on the availability and quality of the data, the Threshold Working Group provides findings that can be applied to establish such thresholds.

1. Evaluation of Data Availability and Data Quality

a. Sensitive Populations. Individuals within an allergic population express a wide degree of sensitivity to low dose allergen exposures. Moreover, the individuals who react to low dose allergen exposures may also have the most severe reactions following these exposures. Thus, there may be a distinct, highly sensitive population within the general population of food allergic individuals. Because most clinical studies exclude patients who have had previous anaphylactic reactions or who have high specific IgE titers, it is possible that the most sensitive individuals within the allergic population may be systematically excluded from these studies. Therefore, it is possible that the doses reported to elicit "initial objective signs" are higher than would be expected for the entire allergic population. The observed data may also not be representative of the allergic population in studies that use patient populations that are not known to be allergic to the food being tested (e.g., testing milk allergic patients for sensitivity to soy). In addition, individual sensitivity varies over time and "high sensitivity" may be a transient condition for an individual.

There are a number of case reports in the scientific literature documenting allergic reactions to incidental exposures to allergens. These reports are difficult to interpret because the level of exposure and potential influence of other factors (e.g., medications, exercise) are not known. Nevertheless, if these reports document true allergic reactions, this suggests that these individuals could be considered to be highly sensitive when compared to the general population of food allergic individuals.

Based on currently available data, the Threshold Working Group was unable to identify any scientifically-based studies that indicate that the standard 10-fold uncertainty factor used in safety assessments for inter-individual variability is not adequate to account for variation within the sensitive population. However, because of the limitations in the clinical studies and the case reports discussed above, this assumption should be reexamined as more data on the distribution of sensitivities within the population become available.

b. Biomarkers. Because there are no *in vitro* markers that can be used to assess the severity of an allergic reaction, and a number of different signs and symptoms are associated with allergic reactions, clinical symptoms elicited during challenge are currently viewed as the best indicators, or biomarkers, of an allergic response. The manifestations of an allergic reaction can be either subjective (reported by the patient but not overtly measurable) or objective (overt reactions that are observed or measured by another person). Objective signs vary on a continuum of severity from mild rashes to fatal anaphylaxis. Although each of these is an "adverse effect," there is no

consensus about where on this continuum they become "serious adverse effects." This makes it difficult to apply either risk assessment- or safety assessment-based approaches to establish thresholds for food allergens because both approaches require that the adverse end point be well defined.

Most clinical studies expose patients to increasing doses of an allergen until the first objective sign is observed. This is often, but not always, a relatively mild reaction. For ethical and technical reasons, few studies measure dose-response relationships for individual patients beyond the initial objective sign. Therefore, the currently available literature provides data based on the "initial objective sign." Although the "initial objective sign" is the biomarker measured in most available allergen clinical studies, it is unclear whether these signs are consistently considered across these studies. It is also not clear whether and when subjective reactions should be considered "adverse effects," or should influence the selection of a NOAEL or LOAEL for safety assessments.

Normally, the use of the "initial objective sign" would lead to threshold values that are "protective" in relation to the overall risk to food allergic consumers. However, it should be noted that severe reactions have been reported as the initial objective sign in some cases. For example, Perry *et al*. (2004) reported that almost 30% of initial reactions were severe and stated that "reaction severity did not increase as the amount of challenge food ingested increased." Likewise, the only severe reaction observed by Hourihane *et al.* (1997a) in a population of 100 patients occurred at the lowest dose tested. However, considering that the use of the "initial objective sign" does appear to be generally protective, and that such data would be used in conjunction with appropriate uncertainty factors, it may not be necessary to differentiate among "mild," "serious," or "life-threatening" signs when establishing a safety assessment-based threshold from existing clinical data.

c. Analytical Methods for Food Allergens. The criteria used to evaluate the available analytical methods for the major food allergens are shown in Table IV-3 and are applied in Appendix 1.

Table IV-3. Specific Criteria for Evaluating Analytical Methods for Food Allergens

Criteria	Comments
1. Has the method been validated?	Methods that have been validated (such as by AOAC) are preferred. Alternatively, the sensitivity, precision, and reproducibility of the method have been demonstrated in a peer-reviewed publication.
2. Is the method sufficiently sensitive?	The limit of detection and the limit of quantitation should be below the levels that appear to cause biological reactions.
3. Does the method detect both raw and processed food allergens?	The relevant processing methods (e.g., boiling, roasting, retorting) will depend on the food.
4. Has the species specificity of the method been determined?	This is most relevant to methods for allergens such as fish and tree nuts.
5. Has the protein target (or targets) for the method been determined?	This is relevant to determining whether the assay detects specific allergenic proteins or general biomarkers.

6. Is the method practical?	The method should use common laboratory equipment and supplies.

The response of sensitive consumers to exposure to an allergen is dependent on the levels of the allergen in the food and the amount of food consumed, two factors for which there is both variability and uncertainty. The levels of allergen in foods may not be known for a number of reasons, particularly when the presence of the allergen is the result of cross-contact. Even in highly controlled clinical studies, questions regarding the level of allergen arise due to differences in the methods used to process and prepare the test material, incomplete characterization of this material, variability in allergen levels among different sources of the food, lack of standardized reference materials, and differences in the analytical methods used to quantify the levels of the allergen.

The methods used to quantify and express the doses received during clinical studies and adverse event investigations are not consistent, and this increases the uncertainty associated with the available data. The amount of an allergen consumed has been described in terms of total weight of a food consumed, total protein from an allergenic ingredient, or amount of specific allergenic proteins. Although the last description is scientifically the most accurate, it is also the most difficult to use because not all individuals are allergic to the same proteins in a food allergen and all the allergenic proteins may not have been identified for a particular food. Measurements based on the whole foods are simple, but increase the level of uncertainty because the composition of the food may vary. For example, changes in water content of a food would change the relative amount of allergenic protein present in serving sizes of a specified mass. Further, the amount of protein present as a percent of the total weight of the food may vary due to maturation, environmental factors, seasonal factors, production variability, or between different cultivars or strains. The Threshold Working Group recognized that the scientifically most accurate means of assessing exposure would be to quantify individual allergenic proteins, but concluded that the most practical approach for evaluating the currently available data is to measure exposure in terms of the total protein from a food allergen. This is also consistent with current technology for detecting food allergens.

It should also be noted that, while clinical exposures are expressed in terms of doses (i.e., g, mg, or μg), allergen levels in foods are actually measured as concentrations (i.e., ppm, percent, or mg/kg). These values can be related by defining a standard serving size, usually 100 g. However, it is well documented that the actual serving eaten by consumers should be treated as a variable and a source of uncertainty when assessing exposures.

d. Challenge Studies. Clinical food challenge studies are recognized to be the most accurate way to diagnose allergies and to measure sensitivity to an allergen (Sampson, 2005). Unfortunately, the design of these food challenge studies varies widely. The lack of standardized protocols, variations in the dosing regimes (including number of doses, the interval between doses, and the relative size of the doses), and differences in the food sources (including differences in preparation and presentation) result in uncertainties when comparing the results of different studies. Double-blind placebo-controlled food challenges (DBPCFC) are considered the most robust clinical studies and data from these studies should be given preference whenever they are available. Food challenge studies are generally not designed to determine a lack of reaction (i.e., NOAEL). Instead, the doses that produce positive allergic reactions are generally reported, providing an estimate of the LOAEL for the population being studied. Despite the uncertainties associated with food challenge data from the literature, LOAELs from human clinical trials currently provide the best data for estimating population-based reactions to food allergens. In a safety assessment-based approach, the use of LOAELs instead of NOAELs would

introduce additional uncertainty. A standard DBPCFC protocol has been proposed to identify NOAELs for various food allergens, but few publicly available, peer-reviewed data of this nature are available at this time.

The specific criteria used to evaluate food challenge studies are shown in Table IV-4, and applied in Appendix 2.

Table IV-4. Specific Criteria for Evaluating Allergen Oral Challenge Studies

Criteria	Comments
1. Has the study been published in a peer-reviewed journal?	Published, peer-reviewed studies are preferred although unpublished studies may be considered.
2. Were the criteria for selecting the test population clearly and completely described, and are they appropriate?	This information is needed to evaluate how the study results apply to at-risk populations (i.e., was the tested population allergic to the tested food?).
3. Was the test material clearly and completely described?	This information is needed to determine the amount of allergenic protein in the test material.
4. Was the lowest tested dose of allergen described, or can it be calculated?	This information is needed to determine a NOAEL or LOAEL.
5. Were the total number and progression of dose levels described, or can they be calculated? (i.e., can the entire dose series be explicitly determined?)	This information is not needed for a safety assessment, but is needed for a risk assessment.
6. Did some of the test population respond to the lowest dose?	NOAELs and LOAELs cannot be determined in studies in which reactions occurred at the lowest dose tested.
7. Were the allergic reactions observed clearly described?	Objective reactions are preferred for both safety and risk assessments.
8. Were the data sufficient to describe the dose-response pattern for the population tested (e.g. for determining a cumulative dose-response curve)?	This information is needed for a risk assessment.

e. Differences Among Food Allergens. Allergens differ widely both in their potential to elicit allergic reactions and in the severity of these reactions. The simplest approach to dealing with these differences would be to establish a single threshold based on sensitivities to the most potent allergens. This threshold is likely to be unduly restrictive for many allergic consumers. Alternatively, separate thresholds could be established for each food allergen. However, the data needed for the separate threshold approach are not available for many allergens. The Threshold Working Group concluded that, to the extent possible, each food allergen should be treated independently but that a single threshold should be established if independent treatment is not possible. If a single threshold is established, it could be based on the allergenic food that elicits an allergenic reaction at the lowest total protein level.

Some of the major allergens identified in the FALCPA consist of multiple species (i.e., tree nuts, fish, crustacean shellfish). Because consumers who are sensitive to one species in a group are also likely to be sensitive to other members of the group, the Threshold Working Group

concluded that any thresholds established for these allergens should be based on the combined amount of protein from these species present.

f. Processing and Matrix Effects. Most of the food allergens identified in the FALCPA are eaten in a processed form. The existing data show that processing can increase, decrease, modify, or have no affect on allergenicity depending on the allergen, the process, and the matrix involved. A process that modifies the structure of an allergenic protein could reduce allergenicity for one population of susceptible individuals while simultaneously increasing allergenicity for a separate susceptible population.

Most clinical studies are conducted using test materials that have been processed, such as peanut butter prepared from roasted peanuts. Therefore, these studies are likely to mimic actual consumer exposure to the allergen. However, some uncertainty remains because consumers are exposed to food allergens processed in many different ways and in many matrices. It would not be practical to conduct the large number of clinical studies that would be necessary to reduce this uncertainty. Fish appears to be an important exception because raw fish is often used as a test material. Most people eat cooked fish and this should be taken into account when evaluating the results of these studies.

2. Options and Findings

There are four general approaches that could be used to establish thresholds for food allergens - analytical methods-based, safety assessment-based, risk assessment-based, and statutorily-derived. 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 was aware of several potentially important studies that are currently in progress, but was unable to fully consider them because the data or analyses were incomplete.

> **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.

a. Analytical Methods-Based Approach. The analytical methods-based approach could be used to establish thresholds if the available data are insufficient to establish thresholds using one of the other approaches. This approach requires that analytical methods be available to detect each major food allergen. Thresholds would be defined by the limits of detection of the available analytical methods, but there would be no relationship between these thresholds and the biological response thresholds. Currently, the lower detection limits for commercially available allergen ELISA or immunoassay test kits are in the range of 0.1 to 1.0 µg protein/g of food, but such kits are not available for all food allergens. Establishing thresholds at levels higher than the lower detection limits of the analytical methods would require the use of assumptions about the biological response thresholds. In that case, the thresholds are actually based on using another approach and should not be considered an analytical methods-based threshold.

Advantages. When accurate, validated methods are available to measure food allergens, determining a threshold based on these methods can be a straightforward way to establish that products are in compliance with this defined level.

Limitations. There are several disadvantages to using this approach in determining thresholds for food allergens:

1. The approach is not risk-based and it is likely that the appropriateness of any thresholds established using this approach will be questioned as existing methods are improved or new methods are developed. Further, in the absence of information on biological response thresholds, it is difficult to assess how well thresholds established using this approach protect public health.
2. Validated analytical methods are currently not available for all of the major food allergens. However, this is likely to change rapidly if there is a need for such analytical capability.
3. There is uncertainty as to the performance of the available analytical methods in the wide variety of food matrices that are likely to be encountered. Theoretically, the test methods should be validated for all foods and food matrices, but this is not practical.
4. Current methods, which are based on a food's total protein content, will not be sufficient in the future if techniques and technologies for reducing the levels of specific allergenic proteins are developed.

Presumably, the analytical methods used to establish thresholds in this approach could also be used to evaluate compliance with any applicable legal requirements. However, the ability to use these methods to help prevent the introduction of unlawful product into the market place would require that the methods be applied in a scientifically supportable manner. This would require the establishment of a statistically supportable sampling plan. The cost of the sampling to a degree sufficient to provide reasonable statistical confidence is potentially an issue.

> **Finding 2.** The analytical methods-based approach could be used to establish thresholds for those 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.

b. Safety Assessment-Based Approach. The safety assessment-based approach could be used to establish thresholds based on NOAELs or LOAELs reported in the literature in combination with appropriate uncertainty factors. Because very few publications report NOAELs or present results in a form that allows NOAELs to be calculated, this type of analysis would, for most food allergens, be based on LOAELs. NOAELs should be used when they are available or can be calculated (see Appendix 2).

As discussed previously, there are substantial differences in the relative potency of different food allergens (e.g., peanut vs. soy). As noted in Appendix 2 and summarized in Table IV-5, the reported LOAELs for peanuts are considerably lower (maximum of 10 mg protein) compared to soy (maximum 522 mg protein). A single threshold for food allergens, based on the most potent food allergens, could be employed if, as a matter of risk management policy, a single threshold is considered desirable. However, this could be considered overly protective, particularly in the case of soy.

Table IV-5. Summary of Published LOAELs for Food Allergens

Food	Range of LOAEL (mg protein)
Egg	0.13 to 1.0
Peanut	0.25 to 10

Milk	0.36 to 3.6
Tree Nuts	0.02 to 7.5
Soy	88 to 522
Fish	1 to 100

Advantages. Calculation of threshold levels based on NOAELs or LOAELs and the application of appropriate uncertainty factors to estimate exposure is relatively straightforward. When there are limited data in the literature, the application of appropriate uncertainty factors provides confidence that the majority of the sensitive populations will be protected. For a number of the major food allergens, there is reasonably good agreement among the reported LOAEL values. Establishing thresholds using the safety assessment-based approach and currently available clinical data has the advantage of being directly linked to biological effects.

Limitations. There are limited clinical trial data for most allergens and most available clinical food challenge studies have not been designed to identify a NOAEL. Furthermore, an inherent, but unexamined, assumption in all clinical studies is that the reactions seen in a clinical setting are representative of the reactions to food allergen exposure that occur in the real world. Most available clinical data are primarily limited to identifying LOAELs, and there is no way to know whether doses below the observed LOAEL would still elicit a reaction. Thus, the selection of appropriate factors to account for uncertainty and inherent variability is critical in using the safety assessment-based approach. Until there is a consensus as to whether subjective symptoms are acceptable biomarkers or which objective signs are considered harmful, it appears prudent to consider as adverse any objective reaction observed in a clinical trial.

We have identified several data gaps for allergens that add to the uncertainty associated with setting thresholds. Critical areas of uncertainty and variability include:

- Intraspecies differences. Safety assessments typically apply a 10-fold uncertainty factor to account for the variability both between individuals and variability in responses for a particular individual.
- Sensitive population of interest. The existence and size of highly sensitive subpopulations of allergenic individuals and their lack of participation in reported clinical trials is a potential data gap and should be included in the uncertainty factors. It is unclear whether the standard 10-fold uncertainty factor for variability within a species is sufficient to account for potential highly sensitive subpopulations. Because of the potential severity of reaction for this subpopulation it seems prudent to include an additional margin of safety (e.g., a 10-fold uncertainty factor) for this uncertainty. It is not unusual for safety assessments to provide additional protection for susceptible populations. For example, EPA uses an additional safety factor in reevaluating pesticides as per the Food Quality Protection Act (FQPA, 1996) to account for the greater susceptibility of children to certain pesticides.
- Adequacy of clinical trial data. Most of the available data from clinical trials report LOAELs. There is uncertainty associated with using LOAELs rather than NOAELs to establish a threshold. For peanuts, one of the few food allergens for which NOAEL values are available, the LOAELs for objective signs are approximately 2 to 3 fold greater than the NOAELs.
- Other. Additional data gaps have been identified by the Threshold Working Group; however, concluded that uncertainties associated with these factors were not sufficient to warrant additional uncertainty factors. These data gaps include the following: (1) the use

of total protein from a food as a surrogate for measuring the level of specific allergenic proteins in clinical trials; (2) variability in serving sizes and related exposure factors; and (3) the incompletely defined effects of food processing on the levels and reactivity of allergenic proteins.

The Threshold Working Group acknowledges that it is difficult to estimate uncertainty factors that apply in all situations for all allergen threshold determinations when using a safety assessment-based approach. We can, however, assume that a standard uncertainty factor of 10-fold should be applied for intraspecies differences in humans. Additional uncertainty factors could be added if justified from data gaps. In Table IV-6, we use peanuts, widely considered to be among the most potent food allergens, to illustrate how specific uncertainty factors may be developed for use in a safety assessment-based approach to set a threshold if that approach is adopted.

Table IV-6. Example of Uncertainty Factors for the Safety Assessment-Based Approach Using Peanuts.

Description	Uncertainty Factor	Justification
Intraspecies difference[1]	10	Standard factor for intraspecies variability
Estimation of NOAEL[2]	Not applicable	Two studies were identified that report NOAELs
Sensitive population[3]	10	Used to account for additional margin of protection for more susceptible populations not included in clinical trials
Overall Uncertainty Factor for Peanuts = 100		

[1] This includes both between- and within-individual variability.

[2] This includes both a factor for converting the LOAEL to a NOAEL and an additional factor for the uncertainty associated with that conversion. In this example for peanuts, there are data on both subjective and objective NOAELs and LOAELs. If the NOAEL values are used, the uncertainty factor is 1-fold (i.e., not applicable). If the LOAELs had been used, this value would have been higher. If subjective symptoms observed at lower levels are used, a different uncertainty factor may be considered.

[3] This includes uncertainty associated with an additional margin of protection to account for the potential severity of reaction (e.g., lethality) for the highly sensitive subpopulation.

Finding 3. The safety assessment-based approach, based on currently available clinical data, is a viable way to establish thresholds for food allergens. If this approach is employed, the LOAEL or 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.

c. Risk Assessment-Based Approach. The use of the risk assessment-based approach requires analysis of the population distributions of allergic sensitivities for each of the major food allergens. These distributions would then be used in conjunction with data on exposures to assess the probability of an adverse effect. These distributions could also be used to evaluate the likely efficacy of different risk reduction strategies.

Advantages. The quantitative risk assessment-based approach is the most scientifically rigorous approach and provides the most insight into both the level of protection and the degree of uncertainty associated with an exposure level. Several recent publications that present preliminary quantitative risk assessments based on data from clinical trials suggest that this approach shows promise (Bindslev-Jensen *et al*., 2002; Moneret-Vautrin and Kanny, 2004; Cordle, 2004; Wensing *et al.,* 2002a).

Limitations. Quantitative risk assessments require the most data of any approach to establish thresholds for food allergens, because they are based on determining the entire dose-response curve, not simply a NOAEL or LOAEL. The data currently available in the literature for food allergens are generally not detailed enough to be useful for quantitative risk assessment. Further, the underlying mathematical procedures and assumptions have not been fully described for the models that have been published. No consensus has been reached regarding the most appropriate mathematical model to use for analyzing allergen reaction data.

> **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.

d. Statutorily-Derived Approach. As discussed above, an allergen threshold could be extrapolated from a statutory exemption established by Congress for another purpose, such as the FALCPA exemption for "highly refined oils." Thus, a threshold could be established for all food allergen proteins based on the level of protein in highly refined oils.

There are surprisingly few data available in the published scientific literature reporting on the levels of proteins in highly refined oils. The criteria used to evaluate studies measuring protein levels in food oils are shown in Table IV-7 and applied in Appendix 3.

Table IV-7. Specific Criteria for Evaluating Protein in Oil Studies

Criteria	Comments
1. Has the study been published in a peer-reviewed journal?	Published, peer-reviewed studies are preferred, although unpublished studies can be considered.
2. Was the oil completely described, including all refining and treatment steps?	The level of processing must be known both to compare values among studies and because each processing step may change the level of protein in oil.
3. Was the method used to extract the protein completely described?	Extraction procedures should be described in sufficient detail to allow the extraction to be reproduced and, ideally, extraction efficiencies should be measured and reported.
4. Was the method used to quantify protein levels completely described?	The lack of these data increases the level of uncertainty.
5. Were replicate samples or batches tested, and was there a statistical analysis of these data?	The lack of these data and statistical analysis increase the level of uncertainty.

Based on the data presented in those studies that reported levels other than "not detected," the overall range of protein concentrations for highly refined oils was 0.014 to 16.7 µg protein/ml oil, with a mean of 2.35 µg/ml. The combined mean protein concentration for the two most widely used oils derived from food allergens, soy and peanut, is 0.74 µg/ml with a standard deviation (std) of 1.3 µg/ml. A threshold could be based on the mean protein concentrations or on the mean plus some multiple of the standard deviation. For example, using the mean protein concentrations for peanut and soy oils, protein levels for the mean, mean + 1 std, mean + 2 std, or mean + 3 std would be the 0.74, 2.05, 3.36, and 4.67 µg/ml, respectively.

Advantages. The primary advantage to the statutorily-derived approach is that it is derived from FALCPA's exemption for highly refined oils from labeling provisions in the FALCPA.

Limitations. The primary limitation of this approach is that it is based on an extrapolation of a level derived from a statutory exemption rather than a rigorous, systematic evaluation of all the available scientific data. Because not all the eight major food allergens are used to produce highly refined oil, the use of a statutorily-derived threshold for all food allergens would be based primarily on the protein levels in highly refined soy or peanut oil. Another current significant limitation is the lack of data on the levels of protein in highly refined oils. Based on the data that are currently available and estimates of the amount of oil consumed as a food or food ingredient, it is likely that a threshold based on this approach would be unnecessarily protective of public health.

> **Finding 5**. The statutorily-derived approach provides a mechanism for establishing thresholds for allergenic proteins 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 compared to 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.

D. Gluten Threshold: Evaluation and Findings

Section 206 of the FALCPA requires that the term "gluten-free " be defined for use on food labels. The law neither describes how gluten-free should be defined nor states whether there is a safe level of gluten.

This section provides an evaluation of the available data to support various approaches for establishing a threshold for gluten. A threshold, if established, could be the basis for decisions on whether to use the term "gluten-free" on product labels.

1. Evaluation of Data Availability and Data Quality

a. Sensitive Populations. Like food allergies, celiac disease affects only a small proportion of the U.S. population (estimated at 1%) (NIH, 2004). Susceptibility to celiac disease is genetically determined and is linked to the presence of the DQ2 or DQ8 HLA alleles. However, carrying these alleles does not necessarily lead to celiac disease. Both acute and chronic morbidity have been well documented for individuals with symptomatic celiac disease. A gluten-free diet has been shown to greatly reduce the risk for cancer and overall mortality for these individuals. The

potential benefit of a gluten-free diet has not been established for individuals with silent or latent celiac disease.

b. Biomarkers. Unlike food allergies, clinical signs and symptoms do not appear to be reliable markers of disease activity because many individuals affected with celiac disease may be entirely asymptomatic. Furthermore, although biomarkers of genetic susceptibility (e.g., presence of DQ2 and/or DQ8 HLA alleles) and gluten exposure [e.g., antibodies for gliadin (AGA), endomysial (EMA), and tissue transglutaminase (tTG)] have been defined for use in noninvasive diagnosis of individuals with celiac disease, these biomarkers have not been shown to correlate with disease severity nor to be useful in assessing daily responses to gluten exposures. Rather, evidence of intestinal mucosal inflammation is the gold standard biomarker for diagnosis of celiac disease and for assessment of disease severity. Intestinal mucosal inflammation may occur long before the development of clinical signs or a rise in antibody titers following a gluten challenge. Intestinal inflammation is assessed by intestinal biopsy, which is an invasive procedure, associated with false negatives (due to sampling error), and is impractical for frequent monitoring of disease activity or severity.

c. Foods of Concern. The foods of concern for individuals with, or susceptible to, celiac disease are the cereal grains that contain the storage proteins prolamin and glutelin (commonly referred to as glutens in wheat), including all varieties of wheat (e.g., durum, spelt, kamut), barley (where the storage proteins are called hordiens), rye (where the storage proteins are called secalins), and their cross-bred hybrids (such as triticale). The proportion of individuals with celiac disease that are also sensitive to the storage proteins in oats (avenins) has not been determined but is likely to be less than 1% (Kelly, 2005).

d. Methods of Analysis. The criteria used to evaluate the available methods of analysis for gluten in food are shown in Table IV-8 and are applied in Appendix 4. A number of commercial immunology-based ELISA test kits for the detection of gluten in foods are available, and one has been validated by AOAC (the Tepnel kit, validated at 160 ppm). One limitation of these kits is that they only detect prolamins. This is not likely to limit the detection of gluten in foods because in most cases prolamins and glutelin occur together. However, it may lead to an underestimate of the level of gluten present. Also, none of the test kits cross-reacts with protein extracts from oats, which limits their efficacy for the small portion of celiac patients who are also sensitive to oats. Test kits suitable for the detection of oat proteins should be developed. .

Table IV-8. Specific Criteria for Evaluating Gluten Analytical Methods

Criteria	Comments
1. Has the method been validated?	Methods that have been validated (such as by AOAC) are preferred. Alternatively, the sensitivity, precision, and reproducibility of the method should have been demonstrated in a peer-reviewed publication.
2. Is the method sufficiently sensitive?	The limit of detection and the limit of quantitation should be below the levels that appear to cause biological responses in most patients with celiac disease.
3. Are extraction methods available for both raw and baked foods?	Different methods may be needed; each should be validated.
4. Does the method measure proteins from all relevant foods?	The cereal grains associated with celiac disease include wheat, barley, rye, and their cross-bred hybrids. Oats may be of concern for some celiac patients.

Criteria	Comments
5. Does the method measure both gliadins and glutenins?	The storage proteins in cereal grains (generally referred to as gluten) include both prolamin proteins (gliadins) and glutelin proteins (glutenins). Ideally, both of these should be measured.
6. Is the method practical?	The method should use common laboratory equipment and be reasonably priced.

e. Oral Challenge Studies. The criteria used to evaluate the available gluten oral challenge studies are provided in Table IV-9 and applied in Appendix 5. Only a limited number of gluten or gliadin challenge studies have been conducted. Of these, many have monitored the subjects' acute responses to a single high dose of gluten or gliadin. These acute studies were not designed to establish a NOAEL or (in most cases) a LOAEL, and the results may not be directly applicable to the chronic, low-level exposures that may lead to long-term consequences. Moreover, most clinical studies only test one or two dose levels and do not directly measure daily intestinal responses to gluten. Based on the criteria in Table IV-9, two currently available studies are considered to be of high utility. The data in these studies can be used to calculate LOAELs for short-term exposures. Although one study retrospectively assessed the effects of trace amounts of gluten consumption in diets of individuals for up to 10 years (Collin *et. al*., 2004), there are no prospective data on the impact of chronic or long-term consumption of lower gluten levels.

Table IV-9. Specific Criteria for Evaluating Gluten Oral Challenge Studies

Criteria	Comments
1. Has the study been published in a peer-reviewed journal?	Published, peer-reviewed studies are preferred although unpublished studies may be considered.
2. Were the criteria for selecting the test population clearly and completely described?	This information is needed to evaluate how the study results apply to the at-risk population.
3. Was the tested food material clearly and completely described?	It is important to know the level of gluten in the test material.
4. Was the dose regime clearly and completely described?	A study designed to measure chronic exposure (low doses over a long period of time) is preferable. Extrapolation of long-term effects from short-term studies increases the level of uncertainty.
5. Were the criteria for characterizing responses clearly described?	This information is needed to evaluate the relevance of the response measured. A definitive diagnostic assessment showing clinical signs or intestinal mucosal changes compared to controls is preferred.
6. Are response data available for each individual tested?	These data are needed to develop a risk assessment-based dose-response model.

2. Options and Findings

The feasibility of using each of the four methods to establish a threshold for gluten was evaluated in light of the available data. As with food allergens, it is likely 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 was aware of several potentially important studies that are

currently in progress, but we were unable to evaluate them because the data or analyses are incomplete.

In particular, the Threshold Working Group is aware of unpublished data from an ongoing clinical trial of the subchronic effects of gluten on celiac patients. The "Italian Microchallenge Study" is utilizing intestinal biopsies to relate changes in the intestinal mucosa to antibody biomarkers (Fasano, 2005 personal communication). Preliminary results indicate that daily consumption of both 10 mg and 50 mg of dietary gluten were well tolerated after three months of continuous consumption, but that minimal histological changes were seen in patients consuming 50 mg of gluten daily. Because these data have not yet been published, these results were not considered further.

> **Finding 6.** The initial approach selected to establish a threshold for gluten, the threshold value selected, and any uncertainty factors that were used to establish the threshold should be reviewed and reconsidered periodically in light of new scientific knowledge and clinical findings.

a. Analytical Methods-Based Approach. As with food allergens, an analytical methods-based approach could be used to establish a threshold for gluten if the available clinical and epidemiological data are insufficient to use one of the other approaches. This approach requires that analytical methods be available to detect all relevant glutens. Thresholds are defined by the limits of detection of the available analytical methods, but there is no relationship between these thresholds and the biological response thresholds. At the time of this report, the lower limits of detection for the commercially available gluten test kits are in the range of 10 µg gluten/g of food, and the ability to robustly quantify samples is in the range of 20 µg gluten/g of food. Establishing thresholds at levels higher than the lower detection limits of the analytical methods requires the use of assumptions about the biological response thresholds. In that case, the thresholds are actually based on using one of the other three approaches and should not be considered an analytical methods-based threshold.

Advantages. A threshold established using the analytical methods-based approach can easily be incorporated into any applicable FDA compliance programs that combine a specific standard method with a standardized sampling scheme.

Limitations. Several factors limit the applicability of the analytical methods-based approach to establish a threshold for gluten. At this time, only one commercially available analytical method has been AOAC validated, and that method was validated for detection at a relatively high concentration of gluten. In addition, there are limited data on the performance of the available methods in the wide variety of food matrices that could potentially contain gluten. Therefore, further characterization of available methods would be necessary before an analytical methods-based threshold could be established. Appropriate methods would need to be developed for the detection of oat gluten.

> **Finding 7.** The analytical methods-based approach could 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.

b. Safety Assessment-Based Approach. The safety assessment-based approach could be used to establish a threshold for gluten based on NOAELs or LOAELs reported in the literature in combination with appropriate uncertainty factors. Clinical data in the literature are limited, but a few studies are available that meet the Threshold Working Group's data quality criteria. The

currently available clinical studies do not report NOAELs. However, studies are available that could be used to establish a LOAEL from which a threshold could be derived.

Advantages. Establishing a threshold based on NOAELs or LOAELs and the application of appropriate uncertainty factors to estimated exposure levels is fairly straightforward. When there are limited data in the literature, the application of appropriate uncertainty factors can provide confidence that the majority of the sensitive populations will be protected. Establishing thresholds using the safety assessment-based approach and currently available clinical data has the advantage of being directly linked to biological effects.

Limitations. The primary limitation of this approach is the dearth of available prospective clinical data and the general lack of information about the impact of chronic low-level consumption of gluten on the emergence of symptomatic disease in individuals with latent or silent celiac disease. At the current time, the size of the combined uncertainty factors needed would be substantial due to the general lack of data; applying large uncertainty factors to the available data could lead to a gluten threshold that is not achievable, as a practical matter, in foods.

We have identified several data gaps for gluten that contribute to current uncertainty about setting gluten thresholds. The critical areas of uncertainty and variability are:

- **Intraspecies differences.** Safety assessments typically apply a 10-fold uncertainty factor to account for the variability both between individuals and variability in responses for a particular individual.
- **Chronic low-level exposure to gluten in "gluten-free " diets.** Data, from either prospective studies or long-term clinical trials, are severely limited on the effect of a long-term gluten-free diet on the manifestations of celiac disease.
- **Adequacy of clinical trial data.** There is uncertainty as to whether 4-week studies, or even 4-month studies, are of sufficient duration to predict the consequences of long-term ingestion of low levels of gluten. There is additional uncertainty as to whether currently available clinical trials include the most sensitive individuals. Accordingly, there is uncertainty as to whether the standard 10-fold uncertainty factor for variability within a species is sufficient to account for potential highly sensitive individuals. Additional uncertainty arises from the fact that the published clinical trials were designed to identify LOAELs rather than NOAELs.
- **Other.** Additional data gaps have been identified by the Threshold Working Group; however, the working group concluded that uncertainties associated with these factors were not sufficient to warrant additional uncertainty factors. These other data gaps include the following: (1) it is uncertain what percentage of individuals with celiac disease are sensitive to oat gluten and whether the levels to which they are sensitive are equivalent to those observed for wheat; (2) variability in serving sizes and related exposure factors; and (3) the incompletely defined effect of food processing on the levels of gluten tolerated by individuals with celiac disease.

The uncertainty associated with gluten thresholds arises primarily from the limited amount of clinical data. The critical knowledge gap about individuals with celiac disease is whether chronic, low-level exposure to gluten in a gluten-free diet will cause any harm over a lifetime. We are not aware of any prospective clinical trials that have examined the health of individuals with celiac disease on a gluten-free diet for more than a few months. There is uncertainty as to whether data from these short-term clinical trials will accurately predict reactions following chronic, low-level gluten exposure. Conversely, there appears to be only a small degree of

uncertainty as to whether the most sensitive celiac disease populations were included in the available clinical trials since most of the participants had evidence of disease.

As discussed in Section III, there may be an oat-sensitive subpopulation. The possible existence of this oat-sensitive subpopulation raises questions related to the definition of "gluten. " Because there are limited clinical data on the sensitivity of this subpopulation of individuals with celiac disease, the uncertainty related to the LOAELs or NOAELs for these individuals is high. Nevertheless, it is unlikely that theses individuals are substantially more sensitive to oat gluten than they are to wheat gluten.

Table IV-10 presents an example of how an overall uncertainty factor could be derived when estimating a threshold for gluten using the safety assessment-based approach. A standard uncertainty factor of 10 might be applied for intraspecies differences in human responses to gluten.

Table IV-10. Example of Uncertainty Factors for the Safety-Assessment-Based Approach.

Description	Uncertainty Factor	Justification
Intraspecies difference[1]	10	Standard for intraspecies variability.
Extrapolation from LOAEL [2]	10	Standard if NOAEL data not available. Supported by clinical trial data.
Chronic, low-level gluten exposure[3]	6	Estimate using data from gluten clinical trials.
Overall Uncertainty Factor[4] = 600		

[1] This includes both between- and within-individual variability.

[2] This includes both a factor for converting the LOAEL to a NOAEL and an additional factor for the uncertainty associated with that conversion factor. Preliminary NOAEL data from an unpublished clinical trial (Fasano, 2005 personal communication) support an approximate 10-fold difference between a NOAEL and published LOAELs (Catassi *et al*., 1993).

[3] Estimated by comparing published LOAELs in an acute, single dose exposure (Ciclitira *et al*., 1984) with repeated exposure over four weeks (Catassi *et al*., 1993).

[4] Uncertainty is likely to decrease as clinical trial data become available.

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 based on wheat gluten would be protective for individuals susceptible to oat gluten.

c. Risk Assessment-Based Approach. There are few data from human clinical trials that can be used to develop a dose-response model for gluten and celiac disease. In addition, limited data are available on exposure; for example, there are limited data on the actual levels of gluten in the diet of individuals on "gluten-free diets" and on the effects of low-level, chronic gluten exposure in individuals with silent or latent celiac disease. These limitations would lead to a very high level of uncertainty associated with models designed to predict the health effects of gluten in the

diet. Therefore, a scientifically defensible hazard characterization and exposure assessment are not possible at the current time.

> **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.

d. Statutorily-Derived Approach. The FALCPA does not include requirements or exemptions that could be used to establish a statutorily-derived threshold for gluten. Also, the law does not define the term "gluten-free. " Potentially, a threshold could be established using the international standards currently under review by Codex (Codex Alimentarius Commission, 2003. However, the proposed Codex standards do not appear to be based on either a scientific rationale for a distinction between naturally gluten-free foods and foods processed to be free of gluten, or a systematic evaluation of clinical data related to the effect of gluten on acute or chronic celiac disease etiology. The levels being considered by Codex seem to be based on anecdotal evidence and on the levels of gluten that are presumed to be historically present in foods that have been called "gluten-free."

> **Finding 10.** There appear to be no suitable statutory requirements or exemptions that would serve as the rationale for using for a statutorily-derived approach to establish a threshold for gluten. This approach is not viable.

Although the FALCPA directs FDA to establish a definition for the term "gluten-free" for food labeling, the quantity and quality of the data needed to accomplish this on a scientific basis are severely limited at the current time relative to all three of the potentially viable approaches. This was aptly summarized by the consensus statement published after a conference of experts convened by the National Institutes of Health which noted that "The strict definition of a gluten-free diet remains controversial due to the lack of an accurate method to detect gluten in food products and the lack of scientific evidence for what constitutes a safe amount of gluten ingestion " (NIH, 2004). These experts concluded that additional research is needed to "Define the minimum safe exposure threshold of gluten in the diet relative to celiac disease " (NIH, 2004).

In view of the consensus opinion stated in the NIH report, the Threshold Working Group concluded that Finding 6 should be reemphasized. Any approach used to establish a threshold for gluten to protect consumers with, or susceptible to, celiac disease should be used in an iterative manner and reexamined periodically to consider new knowledge, data, and approaches.

V. References

Abouzied, M. M., Carroll, M., Mozola, M. A., (2004). A Sandwich ELISA test for detection and quantitation of gluten in food products and ingredients. Abstract of presentation at AOAC Annual Meeting, September 19-23, 2004.

Akiyama, H., Isuzugawa, K., Harikai, N., Watanabe, H., Iijima, K., Yamakawa, H., Mizuguchi, Y., Yoshikawa, R., Yamamoto, M., Sato, H., Watai, M., Arakawa, F., Ogasawara, T., Nishihara, R., Kato, H., Yamauchi, A., Takahata, Y., Morimatsu, F., Mamegoshi, S., Muraoka, S., Honjoh, T., Watanabe, T., Sakata, K., Imamura, T., Toyoda, M., Matsuda, R., Maitani, T. (2004). Inter-

laboratory evaluation studies for development of notified ELISA methods for allergic substances (wheat). *Shokuhin Eiseigaku Zasshi,* 45(3):128-134.

American Academy of Pediatrics Committee on Nutrition (1989). Hypoallergenic infant formulas. *Pediatrics,* 83:1068-1069.

American Academy of Pediatrics Committee on Nutrition. (2000). Hypoallergenic infant formulas. *Pediatrics,* 106:346-349.

American Gastroenterological Association (AGA) (2001). Medical position statement: Celiac sprue. *Gastroenterology,* 120:1522-1525.

Ammar, F., de Boissieu, D., Dupont, C. (1999). Allergy to protein hydrolysates. Report of 30 cases. *Arch Pediatr,* 6:837-843.

Anet, J., Back, J. F., Baker, R. S., Barnett, D., Burley, R. W., Howden, M. E. (1985). Allergens in the white and yolk of hen's egg. A study of IgE binding by egg proteins. *Int Arch Allergy Appl Immunol,* 77(3):364-371.

Arentz-Hansen, H., Fleckenstein, B., Molberg, O., Scott, H., Koning, F., Jung, G., Roepstorff, P., Lundin, K. E., Sollid, L. M. (2004). The molecular basis for oat intolerance in patients with celiac disease. *Plos Med,* 1:84-92.

Asero, R., Lorini, M., Chong, S. U., Zuberbier, T., Tedeschi, A. (2004). Assessment of histamine-releasing activity of sera from patients with chronic urticaria showing positive autologous skin test on human basophils and mast cells. *Clin Exp Allergy,* 34:1111-1114.

Australian New Zealand Food Agency (ANZFA) (2005). Australian New Zealand food standards code: Chapter 1 - General food standards, Part 1.2 - Labeling and other information requirements, Standard 1.2.8 - Nutrition information requirements. p.3, 15, 16. Accessible December 2005 at http://www.foodstandards.gov.au/foodstandardscode.

Awazuhara, H., Kawai, H., Baba, M., Matsui, T., Komiyama, A. (1998). Antigenicity of the proteins in soy lecithin and soy oil in soybean allergy. *Clin. Exp. Allergy,* 28: 1559-1564.

Baranwal, A. K., Singhi, S. C., Thapa, B. R., Kakkar, N. (2003). Celiac crisis. *Indian J Pediatr,* 70:433-435.

Bellioni-Businco, B., Paganelli, R., Lucenti, P., Giampietro, P. G., Perborn, H., Businco, L. (1999). Allergenicity of goat's milk in children with cow's milk allergy. *J Allergy Clin Immunol,* 103 (6):1191-1194.

Ben Rejeb, S., Abbott, M., Davies, D., Querry, J., Cleroux, C., Streng, C., Delahaut, P., Yeung, J. M. (2003). Immunochemical-based method for detection of hazelnut proteins in processed foods. *J AOAC Int,* 86(3):557-563.

Bernhisel-Broadbent, J., Scanlon, S. M., Sampson, H. A. (1992a). Fish hypersensitivity. I. In vitro and oral challenge results in fish-allergic patients. *J Allergy Clin Immunol,* 89(3):730-737.

Bernhisel-Broadbent, J., Strause, D., Sampson, H. A. (1992b). Fish hypersensitivity. II: Clinical relevance of altered fish allergenicity caused by various preparation methods. *J Allergy Clin Immunol,* 90(4 Pt 1):622-629.

Besler, M., Steinhart, H., Paschke, A. (2001). Stability of food allergens and allergenicity of processed foods. *J Chromatogr B Biomed Sci Appl,* 756(1-2):207-228.

Beyer, K., Morrow, E., Li, X. M., Bardina, L., Bannon, G. A., Burks, A. W., Sampson, H. A. (2001). Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol,* 107(6):1077-1081.

Bindslev-Jensen C. (2001). Standardization of double-blind, placebo-controlled food challenges. *Allergy*, 56(Suppl.):75-77.

Bindslev-Jensen, C., Ballmer-Weber, B. K., Bengtsson, U., Blanco, C., Ebner, C., Hourihane, J., Knulst, A. C., Moneret-Vautrin, D. A., Nekam, K., Niggemann, B., Osterballe, M., Ortolani, C., Ring, J., Schnopp, C., Werfel, T. (2004). Standardization of food challenges in patients with immediate reactions to foods position paper from the European Academy of Allergology and Clinical Immunology. *Allergy*, 59**:** 690-697.

Bindslev-Jensen, C., Briggs, D., Osterballe, M. (2002).Hypothesis paper: Can we determine a threshold level for allergenic foods by statistical analysis of published data in the literature? *Allergy*, 57:741-746.

Bock, S. A. and Atkins, F. M.(1989). The natural history of peanut allergy. *J Allergy Clin Immunol*, 83:900-904.

Bock, S. A., Lee, W. Y., Remigio, L. K., May, C. D. (1978). Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol*, 62:327-334.

Bock, S. A., Munoz-Furlong, A., Sampson, H. A. (2001). Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol*, 107(1):191-193.

Bock, S. A., Sampson, H. A., Atkins, F. M., Zeiger, R. S., Lehrer, S., Sachs, M., Bush, R. K., Metcalfe, D. D. (1988). Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol*, 82(6):986-997.

Bousquet, J., Bjorksten, B., Bruijnzeel-Koomen. C. A. F. M., Huggett, A., Ortolani, C., Warner, J. O., Smith, M. (1998). Scientific criteria and the selection of allergenic foods for product labeling. *Allergy*, 53S:3-21.

Brewer, V. A., Thomas, F. S., Garber, A. E., Nguyen, J., X., Amato, S. P. (2004). The detection of wheat using commercial ELISA-based assays. Abstract of presentation at AOAC Annual Meeting, September 19-23, 2004.

Brown, A. (2004). Understanding Food Principles and Preparation, Second Edition, Wadsworth/Thomson Learning, Belmont, CA, USA, pp. 402-403.

Burks, A. W., Williams, L. W., Thresher, W., Connaughton, C., Cockrell, G., Helm, R. M. (1992). Allergenicity of peanut and soybean extracts altered by chemical or thermal denaturation in patients with atopic dermatitis and positive food challenges. *J Allergy Clin Immunol*, 90:889-897.

Bush, R. K., Taylor, S. L., Nordlee, J. A., Busse, W. W. (1985). Soybean oil is not allergenic to soybean-sensitive individuals. *J Allergy Clin Immunol*, 76(2 Pt 1):242-245.

Byrd, D. M. and Cothern, C. R. (2000). Chapter II: functions, models, and uncertainties IN: *Introduction to risk analysis: a systematic approach to science-based decision making*, Government Institutes, pp. 87.

Caffarelli, C., Cavagni, G., Giordano, S., Stapane, I., Rossi, C. (1995). Relationship between oral challenges with previously uningested egg and egg-specific IgE antibodies and skin prick tests in infants with food allergy. *J Allergy Clin Immunol*, 95(6):1215-1220.

Canadian Government: The Food and Drugs Act, Part 2 Food and Drugs Regulations, Part B Foods, Division 21 Foods for special dietary uses, Regulation B.24.018. Accessible December 2005 at http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/legislation/e_d-text-2.pdf .

Carrington, C. (1997). An administrative view of model uncertainty in public health. Accessible December 2005 at www.piercelaw.edu/risk/vol8/summer/Carringt.htm

Casimir, G. and Duchateau, J. (1997). Discriminant epitopes (EP) in allergy to cow's milk (CM). Characteristics of the immune response and clinical presentation. *Pediatr Pulmonol Suppl*, 16:22.

Catassi C., Bearzi, I., Holmes, G. (2005a) Association of celiac disease and intestinal lymphomas and other cancers. Gastroenterology; 128(Suppl 1):S79-86)

Catassi, C., Fabiani, E., Corrao, G., Barbato, M., De Renzo, A., Carella, A. M., Gabrielli, A., Leoni, P., Carroccio, A., Baldassarre, M., Bertolani, P., Caramaschi, P., Sozzi, M., Guariso, G., Volta, U., Corazza, G. R. (2002). Risk of non-Hodgkin lymphoma in celiac disease. *Jama*, 287**:**1413-1419.

Catassi, C., Fabiani, E., Iacono, G., Francavilla, R., D'Agate, C., Volta, U., Accomando, S., Picarelli, A., Corazza, G., De Vitis, I., Nardone, G., Bardella, M., Bearzi, I., Mandolesi, A., Fasano, A. (2005b). Toxicity of gluten traces in patients on treatment for celiac disease. Results of a prospective, placebo-controlled, double-blind, randomized study. Abstract of presentation at Digestive Disease Week, May 14-19, 2005.

Catassi, C. and Fasano, A. (2004). Celiac disease as a cause of growth retardation in childhood. *Curr Opin Pediatr*, 16**:**445-449.

Catassi, C., Rossini, M., Ratsch, I. M., Bearzi, I., Santinelli, A., Castagnani, R., Pisani, E., Coppa, G. V., Giorgi, P. L. (1993). Dose dependent effects of protracted ingestion of small amounts of gliadin in coeliac disease children: a clinical and jejunal morphometric study. *Gut*, 34(11)**:**1515-1519.

Central Science Laboratory, Food Analysis Performance Assessment Scheme (FAPAS) Series 27 Round 05 Report 2705 (2003a). Wheat.

Central Science Laboratory, Food Analysis Performance Assessment Scheme (FAPAS) Series 27 Round 08. Report No. 2708 (2003b). Peanuts.

Central Science Laboratory, Food Analysis Performance Assessment Scheme (FAPAS) Series 27 Round 10 Report No. 2710 (2004a). Eggs and Milk.

Central Science Laboratory, Food Analysis Performance Assessment Scheme (FAPAS) Supplement Report 2705 (2004b). Wheat.

Chiou, Y. H., Yuo, C. Y., Wang, L. Y., Huang, S. P. (2003). Detection of cross-reactivity for atopic immunoglobulin E against multiple allergens. *Clin Diagn Lab Immunol*, 10(2):229-232.

Ciclitira, P. J., Cerio, R., Ellis, H. J., Maxton, D., Nelufer, J. M., Macartney, J. M. (1985). Evaluation of a gliadin-containing gluten-free product in coeliac patients. *Hum Nutr Clin Nutr*, 39(4):303-308.

Ciclitira, P. J., Evans, D. J., Fagg, N. L., Lennox, E. S., Dowling, R. H. (1984). Clinical testing of gliadin fractions in coeliac patients. *Clin Sci (Lond)*, 66**:**357-364.

Clemente, A., Chambers, S. J., Lodi, F., Nicoletti, C., Brett, G. M. (2004). Use of the indirect competitive ELISA for the detection of Brazil nut in food products. *Food Control*, 15:65-69.

Codex Alimentarius Commission (2003). Report of the 25th session of the Codex Committee on nutrition and foods for special dietary uses. Bonn, Germany, 3-7 November 2003. Alinorm 04/27/26. Accessible December 2005 at http://www.codexalimentarius.net/ .

Collin, P., Thorell, L., Kaukinen, K., Maki, M. (2004). The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of coeliac disease? *Aliment Pharmacol Ther*, 19**:**1277-1283.

Cooke, S. K. and Sampson, H. A. (1997). Allergenic properties of ovomucoid in man. *J Immunol*, 159(4):2026-2032.

Cordle, C. T. (2004). Soy protein allergy: Incidence and relative severity. *J. Nutr*, 134:1213S-1219S.

Corrao, G., Corazza, G. R., Bagnardi, V., Brusco, G., Ciacci, C., Cottone, M., Sategna Guidetti, C., Usai, P., Cesari, P., Pelli, M. A., Loperfido, S., Volta, U., Calabro, A., Certo, M. (2001). Mortality in patients with coeliac disease and their relatives: a cohort study. *Lancet*, 358**:**356-361.

Crespo, J. F., Pascual, C., Dominguez, C., Ojeda, I., Muñoz, F. M., Esteban, M. M. (1995). Allergic reactions associated with airborne fish particles in IgE-mediated fish hypersensitive patients. *Allergy*, 50(3):257-261.

Crevel, R. W. R., Kerkhoff, M. A. T., Koning, M. M. G. (2000). Allergenicity of refined vegetable oils. *Food Chem Toxicol*, 38:385-393.

Crump, K.S. (1994). Overview of the benchmark dose method for use in determining acceptable exposures. Society for Risk Analysis Annual Meeting. Abstract.

Daengsuwan, T., Palosuo, K., Phankingthongkum, S., Visitsunthorn, N., Jirapongsananuruk, O., Alenius, H., Vichyanond, P., Reunala, T. (2005). IgE antibodies to omega-5 gliadin in children with wheat-induced anaphylaxis. *Allergy*, 60:506-509.

Daul, C. B., Morgan, J. E., Waring, N. P., McCants, M. L., Hughes, J., Lehrer, S. B. (1987). Immunologic evaluation of shrimp-allergic individuals. *J Allergy Clin Immunol*, 80(5):716-722.

Daul, C. B., Morgan, J. E., Lehrer, S. B. (1993). Hypersensitivity reactions to Crustacea and mollusks. *Clin Rev Allergy*, 11:201-222.

Daul, C. B., Morgan, J. E., Hughes, J., Lehrer, S. B. (1988). Provocation-challenge studies in shrimp-sensitive individuals. *J Allergy Clin Immunol*, 81(6):1180-1186.

de Leon, M. P., Glaspole, I. N., Drew, A. C., Rolland, J. M., O'Hehir, R. E., Suphioglu, C. (2003). Immunological analysis of allergenic cross-reactivity between peanut and tree nuts. *Clin Exp Allergy*, 33(9):1273-1280.

Dewar, D., Pereira, S. P., Ciclitira, P. J. (2004). The pathogenesis of coeliac disease. *Int J Biochem Cell Biol*, 36**:**17-24.

Diffchamb Transia Plate Gluten. (2005). Accessible December 2005 at http://www.diffchamb.com/ .

Diffchamb Transia Plate Prolamins. (2005). Accessible December 2005 at http://www.diffchamb.com/ .

Docena, G., Rozenfeld, P., Fernandez, R., Fossati, C. A. (2002). Evaluation of the residual antigenicity and allergenicity of cow's milk substitutes by in vitro tests. *Allergy*, 57:83-91.

Dodo, H., Konan, K., Viquez, O. (2005). A genetic engineering strategy to eliminate peanut allergy. *Curr Allergy Asthma Rep*, 5:67-73.

European Food Safety Agency (2004). Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the commission relating to the evaluation of allergenic foods for

labeling purposes. *EFSA J,* 32:1-197. Accessible December 2005 at http://www.efsa.eu.int/science/nda/nda_opinions/341/opinion_nda_04_en1.pdf.

Eggesbo, M., Botten, G., Halvorsen, R., Magnus, P. (2001). The prevalence of allergy to egg: a population-based study in young children. *Allergy,* 56:403-411.

Ellis, M. H., Short, J. A., Heiner, D. C. (1991). Anaphylaxis after ingestion of a recently introduced hydrolyzed whey protein formula. *J Pediatr,* 118:74-77.

Eriksson, N. E., Moller, C., Werner, S., Magnusson, J., Bengtsson, U. (2003). The hazards of kissing when you are food allergic. A survey on the occurrence of kiss-induced allergic reactions among 1139 patients with self-reported food hypersensitivity. *J Investig Allergol Clin Immunol,* 13(3):149-154.

Errahali, Y., Morisset, M., Moneret-Vautrin, D. A., Kanny, G., Metche, M., Nicolas, J. P., Fremont, S. (2002). Allergen in soy oils. *Allergy,* 57(7):648-649.

Ewan, P. W. (1996). Clinical study of peanut and nut allergy in 62 consecutive patients: new features and associations. *BMJ,* 312:1074-1078.

Farrell, R. J. and Kelly, C. P. (2002). Celiac sprue. *N Engl J Med,* 346**:**180-188.

Fasano, A. (2005). Celiac Disease. Personal communication.

Fasano, A. (2003). Celiac disease--how to handle a clinical chameleon. *N Engl J Med,* 348**:**2568-2570.

Fasano, A., Berti, I., Gerarduzzi, T., Not, T., Colletti, R.B., Drago, S., Elitsur, Y., Green, P.H.R., Guandalini, S., Hill, I,D., Pietzak, M., Ventura, A., Thorpe, M., Kryszak, D., Fornaroli, F., Wasserman, S.S., Murray, J.A., Horvath, K. (2003). Prevalence of Celiac Disease in At-Risk and Not-At-Risk Groups in the United States. *Acrh Intern Med,* 163:286-292.

Fasano, A. and Catassi, C. (2001). Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. *Gastroenterology,* 120(3)**:**636-651.

Filipsson, A.F., Sand, S., Nilsson J., and Victorin K. (2003). The benchmark dose method--review of available models and recommendations for application in health risk assessment. *Crit Rev Toxicol,* 33(5):505-542.

Fiocchi, A., Travainiw, M., D'Auriaw, E., Banderaliw, G., Bernardow, L., Riva, E. (2003). Tolerance to a rice hydrolysate formula in children allergic to cow's milk and soy. *Clin Exp Allergy,* 33:1576-1580.

Food and Agricultural Organization of the United Nations (FAO). 1995. Chapter 4: Chemical Composition. IN: Quality and Quality Changes in Fresh Fish. FAO Fisheries Technical Paper - 348. FAO, Rome, Italy. Accessible December 2005 at http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/V7180E/V7180E05.htm .

Food Quality Protection Act, FQPA (1996). Public Law 104-107. Accessible December June 2005 at http://www.epa.gov/oppfead1/fqpa/gpogate.pdf .

Gabrovsk´, D., Rysov´, J., Burkhard, M., Cuhra, P., Kubík, M., Barsov´, S. (2004). Collaborative study of a new developed ELISA kit for gluten determination. *J Food, Ag & Envir,* 2(1):113-115.

Gendel, S. M. (1998). Sequence databases for assessing the potential allergenicity of proteins used in transgenic foods. *Adv Food Nutr Res,* 42:63-92.

Gern, J. E., Yang, E., Evrard, H. M., Sampson, H.A. (1991). Allergic reactions to milk-contaminated "nondairy" products. *N Engl J Med,* 324:976-979.

Giampietro, P. G., Kjellman, N. I., Oldaeus, G., Wouters-Wesseling, W., Businco, L. (2001). Hypoallergenicity of an extensively hydrolyzed whey formula. *Pediatr Allergy Immunol,* 12:83-86.

Gilissen, L. J., Bolhaar, S. T., Matos, C. I., Rouwendal, G. J., Boone, M. J., Krens, F.A., Zuidmeer, L., Van Leeuwen, A., Akkerdaas, J., Hoffmann-Sommergruber, K., Knulst, A. C., Bosch, D., Van de Weg, W. E., Van Ree, R. (2005). Silencing the major apple allergen Mal d 1 by using the RNA interference approach. *J Allergy Clin Immunol,* 115:364-369.

Green, P. H. and Jabri, B. (2003). Coeliac disease. *Lancet,* 362:383-391.

Grimshaw, K. E., King, R. M., Nordlee, J. A., Hefle, S. L., Warner, J. O., Hourihane, J. O. (2003). Presentation of allergen in different food preparations affects the nature of the allergic reaction--a case series. *Clin Exp Allergy,* 33(11):1581-1585.

Grundy, J., Matthews, S., Bateman, B., Dean, T., Arshad, S. H. (2002). Rising prevalence of allergy to peanut in children: data from 2 sequential cohorts. *J Allergy Clin Immunol,* 110:784-789.

Gu, X., Beardslee, T., Zeece, M., Sarath, G., Markwell, J. (2001). Identification of IgE-binding proteins in soy lecithin. *Int Arch Allergy Immunol,* 126(3):218-225.

Hallett, R., Haapanen, L. A., Teuber, S. S. (2002). Food allergies and kissing. *N Engl J Med,* 346**:**1833-1834.

Hamada, Y., Tanaka, H., Ishizaki, S., Ishida, M,, Nagashima, Y., Shiomi, K. (2003). Purification, reactivity with IgE and cDNA cloning of parvalbumin as the major allergen of mackerels. *Food Chem Toxicol,* 41(8):1149-1156.

Hamilton, I., Hill, A., Bose, B., Bouchier, I. A., Forsyth, J. S. (1987). Small intestinal permeability in pediatric clinical practice. *J Pediatr Gastroenterol Nutr,* 6**:**697-701.

Hamilton, J. R., McNeill, L. K. (1972). Childhood celiac disease: response of treated patients to a small uniform daily dose of wheat gluten. *J Pediatr,* 81**:**885-893.

Hansen, K. S., Ballmer-Weber, B. K., Luttkopf, D., Skov, P. S., Wuthrich, B., Bindslev-Jensen, C., Vieths, S., Poulsen, L. K. (2003). Roasted Hazelnuts - allergenic activity evaluated by a double-blind placebo-controlled food challenge. *Allergy,* 58(2):132-138.

Hansen, T. K., Bindslev-Jensen, C., Skov, P. S., Poulsen, L. K. (1997). Codfish allergy in adults: IgE cross-reactivity among fish species. *Ann Allergy Asthma Immunol,* 78(2):187-194.

Hansen, T. K., Poulsen, L. K., Stahl Skov, P., Hefle, S. L., Hlywka, J. J., Taylor, S. L., Bindslev-Jensen, U., Bindslev-Jensen, C. (2004). A randomized, double-blinded, placebo-controlled oral challenge study to evaluate the allergenicity of commercial, food-grade fish gelatin. *Food Chem Toxicol,* 42(12):2037-2044.

Hausch, F., Shan, L., Santiago, N. A., Gray, G. M., Khosla, C. (2002). Intestinal digestive resistance of immunodominant gliadin peptides. *Am J Physiol Gastrointest Liver Physiol,* 283:G996-G1003.

Hefle, S. L., Nordlee, J. A., Taylor, S. L. (1996). Allergenic foods. *Crit Rev Food Sci Nutr,* 36:S69-S89.

Helbling, A., Haydel, R. Jr., McCants, M. L., Musmand, J. J., El-Dahr, J., Lehrer, S. B. (1999). Fish allergy: is cross-reactivity among fish species relevant? Double-blind placebo-controlled food challenge studies of fish allergic adults. *Ann Allergy Asthma Immunol*, 83(6 Pt 1):517-523.

Herman, E. M., Helm, R. M., Jung, R., Kinney, A. J. (2003). Genetic modification removes an immunodominant allergen from soybean. *Plant Physiol*, 132:36-43.

Hlywka, J. J., Hefle, S. L., Taylor, S. L. (2000). A sandwich enzyme-linked immunosorbent assay for the detection of almonds in foods. *J Food Prot*, 63(2):252-257.

Hoffman, D. R. and Collins-Williams, C. (1994). Cold-pressed peanut oils may contain peanut allergen. *J Allergy Clin Immunol*, 93(4):801-802.

Holzhauser, T., Stephan, O., Vieths, S. (2002). Detection of potentially allergenic hazelnut (corylus avellana) residues in food: a comparative study with DNA pcr-ELISA and protein sandwich-ELISA. *J Agric Food Chem*, 50:5808-5815.

Host, A. and Halken, S. (2004). Hypoallergenic formulas--when, to whom and how long: after more than 15 years we know the right indication! *Allergy*, 59 Suppl 78:45-52.

Host, A. and Samuelsson, E. G. (1988). Allergic reactions to raw, pasteurized, and homogenized/pasteurized cow milk: A comparison. A double-blind placebo-controlled study in milk allergic children. *Allergy*, 43:113-118.

Hourihane, J. O., Kilburn, S. A., Nordlee, J. A., Hefle, S. L., Taylor, S. L., Warner, J. O. (1997a). An evaluation of the sensitivity of subjects with peanut allergy to very low doses of peanut protein: A randomized, double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol*, 100**:**596-600.

Hourihane, J. O., Bedwani, S. J., Dean, T. P., Warner, J, O. (1997b). Randomised, double-blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts. *BMJ*, 314(7087):1084-1088.

Ibanez, M. D., Lombardero, M., San Ireneo, M. M., Munoz, M. C. (2003). Anaphylaxis induced by pine nuts in two young girls. *Pediatr Allergy Immunol*, 14(4):317-319.

Immer, U., Vela, C., Mendez, E., Janssen, F. (2003). PWG collaborative trial of gluten in gluten-free food through cocktail ELISA. Zwickau, Germany: *Verlag Wissenschaftliche Scripten*, pp. 23-33.

Jackson, W. F. (2003). Food allergy. ILSI Europe Concise Monograph Series, *International Life Sciences Institute*, 1-46.

James, S. P. (2005). Prototypic disorders of gastrointestinal mucosal immune function: Celiac disease and Crohn's disease. *J Allergy Clin Immunol*, 115**:**25-30.

Janatuinen, E. K., Kemppainen, T. A., Julkunen, R. J., Kosma, V. M., Maki, M., Heikkinen, M., Uusitupa, M. I. (2002). No harm from five year ingestion of oats in coeliac disease. *Gut*, 50 (3):332-335.

Janatuinen, E. K., Kemppainen, T. A., Pikkarainen, P. H., Holm, K. H., Kosma, V.M., Uusitupa, M.I., Maki, M., Julkunen, R. J. (2000). Lack of cellular and humoral immunological responses to oats in adults with coeliac disease. *Gut*, 46(3):327-331.

Janatuinen, E. K., Pikkarainen, P. H., Kemppainen, T. A., Kosma, V. M., Järvinen, R. M., Uusitupa, M. I., Julkunen, R. J. (1995). A Comparison of Diets with and without Oats in Adults with Celiac Disease. *N Engl J Med*, 333(16):1033-1037.

Jarabek, A.M. (1994) Inhalation RfC methodology; dosimetric adjustments and dose-response estimation of non-cancer toxicity in the upper respiratory tract. *Inhal Toxicol* 6:301-325.

Johansson, S. G., Hourihane, J. O., Bousquet, J., Bruijnzeel-Koomen, C., Dreborg, S., Haahtela, T., Kowalski, M. L., Mygind, N., Ring, J., van Cauwenberge, P., van Hage-Hamsten, M., Wuthrich, B.; EAACI (2001). A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy,* 56(9):813-824.

Johnson, D.L., Sands, D., Pilgeram, A. (2002). Indian RiceGrass: A Gluten-Free Cereal. (Abstract). Association for the Advancement of Industrial Crops Annual Meeting, August 25-28, 2002. Saskatoon, Saskatchewan, Canada. Accesible December 2005 at http://www.aaic.org/02progrm.htm.

Jones, R. T., Squillace, D. L., Yunginger, J. W. (1992). Anaphylaxis in a milk-allergic child after ingestion of milk-contaminated kosher-pareve-labeled "dairy-free" dessert. *Ann Allergy,* 68:223 -227.

Kagnoff, M.F. (2005). Overview and pathogenesis of celiac disease. *Gastroenterology,* 128(4 Suppl 1):S10-S18.

Kasarda, D. D. (1994). Toxic cereal grains in coeliac disease IN: Gastrointestinal immunology and gluten-sensitive disease. Proceedings of the sixth international symposium on coeliac disease, Trinity College, Dublin, July 1992. editors: Feighery and O'Farrelly. pp. 203-220.

Kasarda, D. D. (1999). U.S. Department of Agriculture, Agricultural Research Service. Grains in Relation to Celiac (Coeliac) Disease. Accessible December 2005 at http://wheat.pw.usda.gov/ggpages/topics/celiac.html .

Kasarda, D. D. (2001). Grains in relation to celiac disease. *Cereal Foods World*, 46(5):209-10.

Kasarda, D. D. (2003). Celiac Disease and Safe Grains. Accessible December 2005 at http://wheat.pw.usda.gov/ggpages/topics/Celiac.vs.grains.html .

Kasarda, D. D. (2004). What we know about grain safety. Paper presented at the Columbia University Celiac disease and other food intolerances conference, Columbia Medical Center, NY, NY, pp. 1-11.

Kasarda, D. D. (2005). Personal communication.

Kato, Y., Oozawa, E., Matsuda, T. (2001). Decrease in antigenic and allergenic potentials of ovomucoid by heating in the presence of wheat flour: dependence on wheat variety and intermolecular disulfide bridges. *J Agric Food Chem*, 49(8):3661-3665.

Kaukinen, K., Collin, P., Holm, K., Rantala, I., Vuolteenaho, N., Reunala, T., Maki, M. (1999). Wheat starch-containing gluten-free flour products in the treatment of coeliac disease and dermatitis herpetiformis. A long-term follow-up study. *Scand J Gastroenterol,* 34**:**163-169.

Kelly, C. (2005). Letter submitted to FDA Docket #2005N-0231 on July 28, 2005. Comment number EC4.

Kelso, J. M. and Sampson, H. A. (1993). Food protein-induced enterocolitis to casein hydrolysate formulas. *J Allergy Clin Immunol*, 92(6):909-910.

Kimmel, C.A. and Gaylor, D. W. (1988). Issues in qualitative and quantitative risk analysis for development toxicology. *Risk Anal*, 8:15-20.

Klurfeld, D. M. and Kritchevsky, D. (1987). Isolation and quanititation of lectins from vegetable oils. *LIPIDS*, 22(9):667-668.

Kocabas, C. N. and Sekerel, B. E. (2003). Cow's milk allergic patients should be informed of the sources of caseinate. *Turk J Pediatr*, 45(2):165-166.

Koehler, K. and U.S. Food and Drug Administration (2005). M*ean per capita consumption of selected foods and gluten-forming grain proteins in the U.S., 2000, based upon disappearance data*. Unpublished data.

Krska, R., Welzig, E., Baumgartner, S. (2003). Immunoanalytical detection of allergenic proteins in food. *Anal Bioanal Chem*, 378(1):63-65.

Kull, I., Hallner, E., Lilja, G., Ohman-Johansson, A. C., Oman, H., Wickman, M. (1999). Peanut oil in vitamin A and D preparations: reactions to skin test and manifestation of symptoms. *Pediatr Allergy Immunol*, 10(1):21-26.

Kupper, C. (2004). Dietary guidelines for celiac disease and implementation. (Abstract) *National Institutes of Health Consensus Development Conference on Celiac Disease Program & Abstracts*, pp. 91-95. Accessible December 2005 at http://consensus.nih.gov/2004/2004CeliacDisease118Program.pdf .

Laurin, P., Wolving, M., Falth-Magnusson, K. (2002). Even small amounts of gluten cause relapse in children with celiac disease. *J Pediatr Gastroenterol Nutr*, 34:26-30.

Lehman, A. and Fitzhugh, O. (1954). Ten-fold Safety Factor Studies. *Assoc. Food Drug Officials of the US Quarterly Bulletin XVIII* (1):33-35.

Leung, D. Y., Sampson, H. A., Yunginger, J. W., Burks, A. W., Jr., Schneider, L. C., Wortel, C. H., Davis, F. M., Hyun, J. D., Shanahan, W. R., Jr. (2003). Effect of anti-IgE therapy in patients with peanut allergy. *N Engl J Med*, 348**:**986-93.

Leung, P. S., Chen, Y. C., Chu, K. H. (1999). Seafood allergy: tropomyosins and beyond. *J Microbiol Immunol Infect*, 32(3):143-154.

Lietze A. (1969). The role of particulate insoluble substances in food allergy. 3. Heat labile antibody to wheat starch in sera of wheat sensitive patients. *Ann Allergy* 27; 9-12.

Lundin, K. E., Nilsen, E.M., Scott, H.G., Loberg, E. M., Gjoen, A., Bratlie, J., Skar, V., Mendez, E., Lovik, A., Kett, K. (2003). Oats induced villous atrophy in coeliac disease. *Gut*, 52(11):1649-1652.

Magnolfi, C. F., Zani, G., Lacava, L., Patria, M. F., Bardare, M. (1996). Soy allergy in atopic children. *Ann Allergy Asthma Immunol*, 77(3):197-201.

Maki, M. and Collin, P. (1997). Coeliac disease. *Lancet*, 349:1755-1759.

Maleki, S. J. (2004). Food processing: effects on allergenicity. *Curr Opin Allergy Clin Immunol*, 4 (3):241-245.

Maleki, S. J., Chung, S.Y., Champagne, E. T., Raufman, J. P. (2000). The effects of roasting on the allergenic properties of peanut proteins. *J Allergy Clin Immunol*, 106(4):763-768.

Maleki, S. J., Viquez, O., Jacks, T., Dodo, H., Champagne, E. T., Chung, S. Y., Landry, S. J. (2003). The major peanut allergen, Ara h 2, functions as a trypsin inhibitor, and roasting enhances this function. *J Allergy Clin Immunol*, 112:190-195.

Mata, E., Favier, C., Moneret-Vautrin, D., Nicolas, J., Ching, L., Gueant, J. (1994). Surimi and native codfish contain a common allergen identified as a 63-kDa protein. *Allergy* 49: 442-447.

May, C. D. (1976). Objective clinical and laboratory studies of immediate hypersensitivity reactions to foods in asthmatic children. *J Allergy Clin Immunol,* 58:500-515.

Mayer, M., Greco, L., Troncone, R., Grimaldi, M., Pansa, G. (1989). Early prediction of relapse during gluten challenge in childhood celiac disease. *J Pediatr Gastroenterol Nutr,* 8**:**474-479.

McKenna, C. and Klontz, K. C. (1997). Systemic allergic reaction following ingestion of undeclared peanut flour in a peanut-sensitive woman. *Ann. Allergy Asthma Immunol,* 79:234-236.

Molberg, O., Solheim Flaete, N., Jensen, T., Lundin, K. E., Arentz-Hansen, H., Anderson, O. D., Kjersti Uhlen, A., Sollid, L. M. (2003). Intestinal T-cell responses to high-molecular-weight glutenins in celiac disease. *Gastroenterology,* 125(2):337-344.

Moneret-Vautrin, D. A., (2004) Wheat Allergy. Third Food Allergy Research and Resource Program Threshold Conference, Mallorca, Spain, October 5, 2004.

Moneret-Vautrin, D. A. and Kanny, G. (2004). Update on threshold doses of food allergens: implications for patients and the food industry.*Current Opinion in Allergy and Clinical Immunology,* 4:215-219.

Moneret-Vautrin, D. A., Rance, F., Kanny, G., Olsewski, A., Gueant, J. L., Dutau, G., Guerin, L. (1998). Food allergy to peanuts in France--evaluation of 142 observations. *Clin Exp Allergy,* 28**:**1113-1119.

Montgomery, A. M., Goka, A. K., Kumar, P. J., Garthing, M. J., Clark, M. L. (1988). Low gluten diet in the treatment of adult coeliac disease: effect on jejunal morphology and serum anti-gluten antibodies. *Gut,* 29:154-1568.

Morisset, M., Moneret-Vautrin, D. A., Kanny, G., Guénard, L., Beaudouin, E., Flabbée, J., Hatahet, R. (2003). Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulinE-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy,* 33 (8):1046-1051.

Muller, U., Weber, W., Hoffmann, A., Franke, S., Lange, R., Vieths, S. (1998). Commercial soybean lecithins: a source of hidden allergens? *Z Lebensm Unters Forsch A,* 207:341-351.

Naqpal, S., Rajappa, L., Metcalfe, D. D., Subba Rao, P. V. (1989). Isolation and characterization of heat-stable allergens from shrimp *(Penaeus indicus). J Allergy Clin Immunol,* 83(1):26-36.

National Institutes of Health (NIH) (2004). Consensus statement on celiac disease, June 28-30. Accessible December 2005 at http://consensus.nih.gov/2004/2004CeliacDisease118PDF.pdf.

Nelson, H. S., Lahr, J., Rule, R., Bock, A., Leung, D. (1997). Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol,* 99(6 Pt.1):744-751.

Neogen Alert for Gliadin. Food allergens. Available December 2005 at http://www.neogen.com/pdf/FS_CatalogPages/AlertGliadin.pdf .

Niemann, L., and Hefle, S. L., (2003). Validated ELISA for detection of undeclared walnut residues in food. *Journal of Allergy and Clinical Immunology,* 111(Suppl.):248. Abstract.

Niggemann, B., Binder, C., Klettke, U., Wahn, U. (1999). In vivo and in vitro studies on the residual allergenicity of partially hydrolyzed infant formulae. *Acta Paediatr,* 88(4):394-398.

Nogueira, M., McDonald, R., Westphal, C., Maleki, S., Yeung, J. (2004) Can commercial peanut assay kits detect peanut allergens? *J AOAC,* 87(6): 1480-1484.

Nonaka, I., Kamiya, K., Takashi. (2004). Development of food allergen rapid detection kits by immunochromatography. Abstract of presentation at AOAC Annual Meeting, September 19-23, 2004.

Nordlee, J. A., Neimann, L. M., Hefle, S. L., Taylor, S. L. (2002). Determination of proteins in soybean oil from distinct processing steps. Abstracts of the IFT Annual Meeting.

Nordlee, J. A., Taylor, S. L., Jones, R. T., Yunginger, J. W. (1981). Allergenicity of various peanut products as determined by RAST inhibition. *J Allergy Clin Immunol,* 68(5):376-382.

Norgaard, A., Bernard, H., Wal, J. M., Peltre, G., Skov, P. S., Poulsen, L.K., Bindslev-Jensen, C. (1996). Allergenicity of individual cow milk proteins in DBPCFC-positive milk allergic adults. *J Allergy Clin Immunol,* 97:237(abstract).

Olszewski, A., Pons, L., Moutete, F., Aimone-Gastin, I., Kanny, G., Moneret-Vautrin, D. A., Gueant, J. L. (1998). Isolation and characterization of proteic allergens in refined peanut oil. Clin. Exp. *Allergy,* 28:850-859.

Ortolani, C., Ballmer-Weber, B. K., Hansen, K. S., Ispano, M., Wuthrich, B., Bindslev-Jensen, C., Ansaloni, R., Vannucci, L., Pravettoni, V., Scibilia, J., Poulsen, L. K., Pastorello, E. A. (2000). Hazelnut allergy: a double-blind, placebo-controlled food challenge multicenter study. *J Allergy Clin Immunol,* 105:577-581.

Osterballe, M. and Bindslev-Jensen, C. (2003).Threshold levels in food challenge and specific IgE in patients with egg allergy: Is there a relationship. *J. Allergy Clin Immunol,* 112(1):196-201.

Palm, M., Moneret-Vautrin, D. A., Kanny, G., Denery-Papini, S., Fremont, S. (1999). Food allergy to egg and soy lecithins. *Allergy,* 54:1116-1117.

Park, D. (2005). FDA and AOAC peanut allergens test kits performance validations. Memo to record. U. S. Food and Drug Administration.

Park, D. L., Coates, S., Brewer, V. A., Garber, A. E., Abouzied, M., Johnson, K., Ritter, B., McKenzie, D. (2005). Performance tested method: Multiple laboratory validation study of ELISA-based assays for the detection of peanuts in food. *J AOAC Intern,* Vol.88(1):156-160.

Paschke, A., Zunker, K., Wigotzki, M., Steinhart, H. (2001). Determination of the IgE-binding activity of soy lecithin and refined and non-refined soybean oils. *J Chromatogr B Biomed Sci Appl,* 756(1-2):249-254.

Pascual, C., Esteban, M. M., Crespo, J. F. (1992). Fish Allergy: Evaluation of the importance of cross -reactivity. *J of Pediatrics,* 121(5:2)S29-S34.

Pastorello, E. A., Stocchi, L., Pravettoni, V., Bigi, A., Schilke, M. L., Incorvaia, C., Zanussi, C. (1989). Role of the elimination diet in adults with food allergy. *J Allergy Clin Immunol,* 84:475-483.

Peeters, K. A. B. M., Knulst, A. C., Rynja, F. J., Bruijnzeel-Koomen, C. A. F. M., Koppelman, S. J., (2004). Peanut allergy: sensitization by peanut oil-containing local therapeutics seems unlikely. *J Allergy Clin Immunol,* 113:1000-1001.

Peraaho, M., Collin, P., Kaukinen, K., Kekkonen, L., Miettinen, S., Maki, M. (2004). Oats can diversify a gluten-free diet in celiac disease and dermatitis herpetiformis. *J Am Diet Assoc,* 104 (7):1148-1150.

Perry, T. T., Matsui, E. C., Conover-Walker, M. K., Wood, R. A. (2004). Risk of oral food challenges. *J Allergy Clin Immunol,* 114**:**1164-1168.

Peters, U., Askling, J., Gridley, G., Ekbom, A., Linet, M. (2003). Causes of death in patients with celiac disease in a population-based Swedish cohort. *Arch Intern Med*, 163:1566-1572.

Poms, R. E. and Anklam, E. (2004). Effects of chemical, physical and technological processes on the nature of food allergens. *J AOAC Int*, 87(6):1466-1474.

Poms, R.E., Klein, C.L., Anklam, E. (2004). Methods for allergen analysis in food: a review. *Food Addit Contam*, 21(1):1-31.

Popping, B., Pardigol, A., Dan, A., Guillet, S., Schneede, A., Koelln, S., Pinero, D. (2004). Development and in-house validation of a DNA-based rapid and cost-effective multi-allergen screening system. Abstract of presentation at AOAC Annual Meeting, September 19-23, 2004.

Porras, O., Carlsson, B., Fallstrom, S. P., Hanson, L. A. (1985). Detection of soy protein in soy lecithin, margarine and, occaisionally, soy oil. *Int Arch Aller Appl Immunol*, 78:30-32.

Public Law 108-282 (2004). Title II-Food allergen labeling and consumer protection. Accessible December 2005 at http://www.cfsan.fda.gov/~dms/alrgact.html .

Pumphrey, R. S., Wilson, P. B., Faragher, E. B., Edwards, S. R. (1999). Specific immunoglobulin E to peanut, hazelnut and brazil nut in 731 patients: similar patterns found at all ages. *Clin Exp Allergy*, 29(9):1256-1259.

Pumphrey, R. (2004). Anaphylaxis: can we tell who is at risk of a fatal reaction? *Curr Opin Allergy Clin Immunol*, 4:285-290.

R-Biopharm Ridascreen Fast Gliadin (2004). Enzyme immunoassay for the quantitative analysis of gliadins and corresponding prolamines. Accessible December 2005 at http://www.r-biopharm.com/foodandfeed/pdf/fast_gliadin_03_06_04_%20k.pdf .

R-Biopharm Ridascreen Fast Peanut. (2005). Enzyme immunoassay for the quantitative analysis of peanut. Accessible December 2005 at http://www.r-biopharm.com/foodandfeed/ridascreenfast_peanut.php?act=foodandfeed&p_nav=allergens .

R-Biopharm Ridascreen Gliadin R7001 (2005). Enzyme immunoassay for the quantitative analysis of gliadins and corresponding prolamines. Accessible December 2005 at http://www.r-biopharm.com/foodandfeed/pdf/R7001%20Gliadin%2005-01-11.pdf .

Reeves, R. M. (1999). Letter from Institute of Shortening and Edible Oils to Joint FAO/WHO Expert Committee on Food Additives, Food and Nutrition Division. Unpublished data analytical results for protein in oil.

Reindl, J., Anliker, M. D., Karamloo, F., Vieths, S., Wuthrich, B. (2000). Allergy caused by ingestion of zucchini (Cucurbita pepo): Characterization of allergens and cross-reactivity to pollen and other foods. *J Allergy Clin Immunol*, 106:379-385.

Renaud, C., Cardiet, C., Dupont, C. (1996). Allergy to soy lecithin in a child. *J Pediatric Gastroenterology and Nutrition* 22(3): 328-329.

Rolles, C. J. and McNeish, A. S. (1976). Standardised approach to gluten challenge in diagnosing childhood coeliac disease. *Br Med J*, 1**:**1309-1311.

Ross, M., Street, D, Ferguson, M, Klontz, K, and Luccioli, S. (2006). Emergency Department Visits for Food Allergy Reactions from theNational Electronic Injury Surveillance System. Unpublished data. Submitted to the Society for Epidemiology Research Conference.

Roux, K. H., Teuber, S. S., Sathe, S. K. (2003). Tree nut allergens. *Int Arch Allergy Immunol*, 131 (4):234-244.

Sackesen, C. and Adalioglu, G. (2003). Hidden fish substance triggers allergy. *J Investig Allergol Clin Immunol*, 13(3):216-217.

Sakaguchi, M., Toda, M., Ebihara, T., Irie, S., Hori, H., Imai, A., Yanagida, M., Miyazawa, H., Ohsuna, H., Ikezawa, Z., Inouye, S. (2000) IgE antibody to fish gelatin (type I collagen) in patients with fish allergy. *J Allergy Clin Immunol*, 106(3):579-584.

Sampson, H. A. (1997). Food allergy. *JAMA*, 278(22):1888-1894.

Sampson, H. A. (1999). Food allergy. Part 1: Immunopathogenesis and clinical disorders. *J Allergy Clin Immunol*, 103(5):717-728.

Sampson, H. A. (2003). Anaphylaxis and emergency treatment. *Pediatrics*, 111 (6 Pt 3):1601-1608.

Sampson, H. A. (2004). Update on food allergy. *J Allergy Clin Immunol*, 113(5):805-819.

Sampson, H. A. (2005). Food allergy - accurately identifying clinical reactivity. *Allergy*, 60 Suppl 79:19-24.

Sampson, H. A., Bernhisel-Broadbent, J., Yang, E., Scanlon, S. M. (1991). Safety of casein hydrolysate formula in children with cow milk allergy. *J Pediatr*, 118(4:Pt 1):520-525.

Sampson, H. A., James, J. M., Bernhisel-Broadbent, J. (1992a). Safety of an amino acid-derived infant formula in children allergic to cow milk. *Pediatrics*, 90:463-465.

Sampson, H. A., Mendelson, L., Rosen, J. P. (1992b). Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med*, 327(6):380-384.

Sampson, H.A., Munoz-Furlong, A., Bock, S.A., Schmitt. C., Bass, R., Chowdhury, B.A., Decker, W.W., Furlong, T.J., Galli, S.J., Golden, D.B., Gruchalla, R.S., Harlor, A.D., Hepner, D.L., Howarth, M., Kaplan, A.P., Levy, J.H., Lewis, L.M., Lieberman, P.L., Metcalfe, D.D., Murphy, R., Pollart, S.M., Pumphrey, R.S., Rosenwasser, L.J., Simons, F.E., Wood, J.P., Camargo, C.A . (2005). Symposium on the definition and management of anaphylaxis: summary report. *J Allergy Clin Immunol*,115(3):571-574.

Sanders, D.S., Carter, M.J., Hurlstone, D.P., Pearce, A., Ward, A.M., McAlindon, M.E., Lobo, A.J. (2001). Association of adult celiac disease with irritable bowel syndrome: a case-control study in patients fulfilling ROME II criteria referred to secondary care. *The Lancet*, 358:1504-1508.

Saylor, J. D. and Bahna, S. L. (1991). Anaphylaxis to casein hydrolysate formula. *J Pediatr*, 118 (1):71-4.

Schuppan, D. (2000). Current concepts of celiac disease pathogenesis. *Gastroenterology*, 119**:**234-242.

Schuppan, D., Hahn, E. G. (2001). IgA anti-tissue transglutaminase: Setting the stage for coeliac disease screening. *Eur J Gastroenterol Hepatol*, 13:635-637.

Schuppan, D., Hahn, E. G. (2002). Biomedicine. Gluten and the gut-lessons for immune regulation. *Science*, 297:2218-2220.

Schwartz, R. H., Amonette, M. S. (1991). Cow milk protein hydrolysate infant formulas not always "hypoallergenic ". *J Pediatr*, 119:839-840.

Scibilia, J., Pastorello, E.A., Zisa, G., Ottolenghi, A., Bidslev-Jensen, C., Pravettoni, V., Scovena, E., Robino, A., Ortolani, C. (2006) Wheat allergy: A double-blind, placebo-controlled study in adults. *J Allergy Clin Immunol*, 117 (2):433-439.

Shan, L., Molberg, O., Parrot, I., Hausch, F., Filiz,F., Gray, G. M., Sollid, L. M., Khosla, C. (2002). Structural basis for gluten intolerance in celiac sprue. *Science*, 297:2275-2279.

Shefcheck, K. J. and Musser, S. M. (2004). Confirmation of the allergenic peanut protein, Ara h 1, in a model food matrix using liquid chromatography/tandem mass spectrometry (LC/MS/MS). *J Agric Food Chem*, 52:2785-2790.

Sicherer, S. H. (2001). Clinical implications of cross-reactive food allergens. *J Allergy Clin Immunol*, 108:881-890.

Sicherer, S. H., Burks, A. W., Sampson, H. A. (1998). Clinical features of acute allergic reactions to peanut and tree nuts in children. *Pediatrics*, 102:1-6.

Sicherer, S. H., Noone, S. A., Koerner, C. B., Christie, L., Burks, A. W., Sampson, H. A. (2001). Hypoallergenicity and efficacy of an amino acid-based formula in children with cow's milk and multiple food hypersensitivities. *J Pediatr*, 138:688-693.

Sicherer, S. H., Morrow, E. H., Sampson, H. A. (2000). Dose-response in double-blind, placebo-controlled oral food challenges in children with atopic dermatitis. *J Allergy Clin Immunol*, 105:582-586.

Sicherer, S. H., Munoz-Furlong, A., Sampson, H. A. (2003). Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: A 5-year follow-up study. *J Allergy Clin Immunol*, 112(6):1203-1207.

Sicherer, S. H., Munoz-Furlong, A., Sampson, H. A. (2004). Prevalence of seafood allergy in the United States determined by a random telephone survey. *J Allergy Clin Immunol*, 114(1):159-165.

Simonato, B., Pasini, G., Giannattasio, M., Peruffo, A., DeLazzari, F., Curioni, A. (2001). Food allergy to wheat products: the effect of bread baking and in vitro digestion on wheat allergenic proteins. A study with bread dough, crumb, and crust. *J Agric Food Chem* 49; 5668-5673.

Skerritt, J. H. and Hill, A. S. (1991). Enzyme immunoassay for determination of gluten in foods: collaborative study. *J Assoc Off Anal Chem*, 74(2):257-264.

Skinner and Haynes. (1998). Seed crushers and oil processors association report as reported in: WHO Food Additive Series 44 and in EFSA Notification.

St. Vincent, J. C. M., Watson, W. T. A. (1994). Unsuspected sources of cow's milk protein in food. *J Allegy Clin. Immunol*, 93:209. Abstract.

Steensma, D. P. (2003). The kiss of death: a severe allergic reaction to a shellfish induced by a good-night kiss. *Mayo Clin Proc*, 78(2):221-222.

Storsrud, S., Hulthen, L.R., Lenner, R. A. (2003). Beneficial effects of oats in the gluten-free diet of adults with special reference to nutrient status, symptoms and subjective experiences. *Br J Nutr*, 90(1):101-107.

Sturgess, R., Day, P., Ellis, H. J., Lundin, K. E., Gjertsen, H. A., Kontakou, M., Ciclitira, P. J. (1994). Wheat peptide challenge in coeliac disease. *Lancet*, 343**:**758-761.

Tada, Y., Nakase, M., Adachi, T., Nakamura, R., Shimada, H., Takahashi, M., Fujimura, T., Matsuda, T. (1996). Reduction of 14-16 kDa allergenic proteins in transgenic rice plants by antisense gene. *FEBS Lett*, 391(3):341-345.

Talley, N. J., Valdovinos, M., Petterson, T. M., Carpenter, H. A., Melton, L. J.(1994). Epidemiology of celiac sprue: a community-based study. *Am J Gastroenterol*, 89(6):843-846.

Tarim, O., Anderson, V. M., Lifshitz, F. (1994). Fatal anaphylaxis in a very young infant possibly due to a partially hydrolyzed whey formula. *Arch Pediatr Adolesc Med,* 148:1224-1229.

Tattrie, N. and Yaguchi, M. (1973). Protein content of various processed edible oils. *Journal of the Institute for Cancer, Science Technology and Alimentation*, 6:289-290.

Taylor, S. L., Busse, W. W., Sachs, M. I., Parker, J. L., Yunginger, J. W. (1981). Peanut oil is not allergenic to peanut-sensitive individuals. *J Allergy Clin Immunol,* 68(5):372-375.

Taylor, S. L. and Dormedy, E. S. (1998). The role of flavoring substances in food allergy and intolerance. *Adv Food Nutr Res,* 42:1-44.

Taylor, S. L. and Hefle, S. L. (2001). Ingredient and labeling issues associated with allergenic foods. *Allergy*, 56 Supple 67:64-69.

Taylor, S. L., Hefle, S. L., Bindslev-Jensen, C., Atkins, F. M., Andre, C., Bruijnzeel-Koomen, C., Burks, A. W., Bush, R. K., Ebisawa, M., Eigenmann, P. A., Host, A., Hourihane, J. O., Isolauri, E., Hill, D. J., Knulst, A., Lack, G., Sampson, H. A., Moneret-Vautrin, D. A., Rance, F., Vadas, P. A., Yunginger, J. W., Zeiger, R. S., Salminen, J. W., Madsen, C., Abbott, P. (2004). A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? *Clin Exp Allergy*, 34:689-695.

Taylor, S. L., Hefle, S. L., Bindslev-Jensen, C., Bock, S. A., Burks, A. W., Jr., Christie, L., Hill, D. J., Host, A., Hourihane, J. O., Lack, G., Metcalfe, D. D., Moneret-Vautrin, D. A., Vadas, P. A., Rance, F., Skrypec, D. J., Trautman, T. A., Yman, I. M. and Zeiger, R. S. (2002). Factors affecting the determination of threshold doses for allergenic foods: How much is too much? *J Allergy Clin Immunol*, 109**:** 24-30.

Tepnel - BioKits food allergen protein additive kits. (2005). Accessible December 2005 at http://www.tepnel.com/ag_bio_and_food_testing/allergen_products.asp .

Tepnel - BioKits product information summary. (2005). Accessible December 2005 at http://www.tepnel.com/ag_bio_and_food_testing/allergens_product_summary.asp

Tepnel Biosystems. Bio Kits peanut assay kit. (2005). For the detection of peanut content in foods. Accessible December 2005 at http://www.tepnel.com/ag_bio_and_food_testing/downloads/LA0035%20BK%20P%20Peanut%20Flyer%20v01.pdf .

Teuber, S. S., Brown, R. L., Haapanen, L. A. D., (1997). Allergenicity of gourmet nut oils processed by different methods. *J Allergy Clin Immunol*, 99:502-507.

Teuber, S. S. and Peterson, W. R. (1999). Systemic allergic reaction to coconut (Cocos nucifera) in 2 subjects with hypersensitivity to tree nut and demonstration of cross-reactivity to legumin-like seed storage proteins: new coconut and walnut food allergens. *J Allergy Clin Immunol*, 103 (6):1180-1185.

Thompson, T. (2001). Wheat starch, gliadin, and the gluten-free diet. *J Am Diet Assoc*, 101(12):1456 -1459.

Thompson, T. (2003). Oats and the gluten-free diet. *J Am Diet Assoc*, 103(3):376-379.

Thompson, T. (2004), Gluten contamination of commercial oat products in the United States. *N Engl J Med*, 351(19):2021-2022.

United States Department of Agriculture (USDA) (1999). Official United States Standards for Grain. Subpart A: General Provisions. Accessible December 2005 at http://151.121.3.117/reference-

library/standards/810101.pdf. [Note standards for specific grains are also available at http://151.121.3.117/reference-library/standards/standards.htm].

United States Department of Agriculture (USDA) (2004). Agricultural Research Service (2004), *USDA National Nutrient Database for Standard Reference, Release 17*. Accessible December 2005 at http://www.nal.usda.gov/fnic/foodcomp.

United States Department of Health and Human Services and United States Department of Agriculture (DHHS/USDA). (2003).Quantitative assessment of the relativerisk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. College Park, Maryland. Accessible on December 2005 at http://www.foodsafety.gov/ .

United States Environmental Protection Agency (EPA). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington DC, EPA/600/8-90/066F.

United States Food and Drug Administration (FDA). (2001).Draft risk assessment on the public health impact of *Vibrio parahaemolyticus* in raw molluscan shellfish. Washington, D.C.Accessible December 2005 at http://www.foodsafety.gov/ .

Urisu, A., Ando, H., Morita, Y., Wada, E., Yasaki, T., Yamada, K., Komada, K., Torii, S., Goto, M., Wakamatsu, T. (1997). Allergenic activity of heated and ovomucoid-depleted egg white. *J Allergy Clin Immunol*, 100(2):171-176.

Vader, W., Kooy, Y., Van Veelen, P., De Ru, A., Harris, D., Benckhuijsen, W., Pena, S., Mearin, L., Drijfhout, J. W., Koning, F. (2002). The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. *Gastroenterology*, 122(7):1729-1737.

van de Wal, Y., Kooy, Y. M., van Veelen, P., Vader, W., August, S. A., Drijfhout, J. W., Pena, S. A., Koning, F. (1999). Glutenin is involved in the gluten-driven mucosal T cell response. *Eur J Immunol*, 29(10):3133-3139.

Venkatachalam, M., Teuber, S. S., Roux, K. H., Sathe, S. K. (2002). Effects of roasting, blanching, autoclaving, and microwave heating on antigenicity of almond (Prunus dulcis L.) proteins. *J Agric Food Chem*, 50(12):3544-3548.

Vila, L., Beyer, K., Jarvinen, K. M., Chatchatee, P., Bardina, L., Sampson, H. A. (2001). Role of conformational and linear epitopes in the achievement of tolerance in cow's milk allergy. *Clin Exp Allergy*, 31(10):1599-1606.

Voysey, P., Jewell, K., and Stringer, M. (2002). Risk Characterization. IN: *Microbiological risk assessment in food processing*. Ed. M. Brown and M. Stringer. Woodhead Publishing Limited, Cambridge England.

Wahab, P. J., Crusius, J. B., Meijer, J. W., Mulder, C. J. (2001). Gluten challenge in borderline gluten-sensitive enteropathy. *Am J Gastroenterol*, 96:1464-1469.

Waring, N. P., Daul, C. B., deShazo, R. D., McCants, M. L., Lehrer, S. B. (1985). Hypersensitivity reactions to ingested crustacea: clinical evaluation and diagnostic studies in shrimp-sensitive individuals., *J Allergy Clin Immunol*, 76(3):440-445.

Wei, Y., Sathe, S. K., Teuber, S. S., Roux, K. H. (2003). A sensitive sandwich ELISA for the detection of trace amounts of cashew (*Anacardium occidentale L.*) nut in foods. *J Agric Food Chem*, 51(11):3215-3221.

Wensing, M., Penninks, A. H., Hefle, S. L., Akkerdaas, J. H., van Ree, R., Koppelman, S. J., Bruijnzeel-Koomen, C. A., Knulst, A. C. (2002a). The range of minimum provoking doses in

hazelnut-allergic patients as determined by double-blind, placebo-controlled food challenges. *Clin Exp Allergy,* 32**:**1757-1762.

Wensing, M., Penninks, A. H., Hefle, S. L., Koppelman, S. J., Bruijnzeel-Koomen, C. A., Knulst, A. C. (2002b). The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *J Allergy Clin Immunol,* 110**:**915-920.

West, S., King, V., Carey, T., Lohr, K., McKoy, N., Sutton, S., Lux, L. 2002. Systems to rate the strenght of scientific evidence. Evidence Report/Technology Assessment No. 47. AHRQ Publication No. 02-E016. Agency for Healthcare Research and Quality, Rockville, MD.

Wigotzki, M., Steinhart, H., Paschke, A. (2001). Determination of the allergenicity of various hazelnut products by immunoblotting and enzyme allergosorbent test inhibition. *J Chromatogr B Biomed Sci Appl,* 756(1-2): 239-248.

Wirshil, B., Butzner, D., Sabra, A., Savilahti, E., Seidman, E., Strobel, S., Yamashiro, Y. (2002) Allergy and Immunologic Disease: Working Group Report of the First World Congress of Pediatric Gastroelterologu, Hepatology, and Nutrition. *J Pediatric Gastroenterology and Nutrition*, 35: S74-S77.

Wood, R. A. (2002). Food manufacturing and the allergic consumer: accidents waiting to happen. *J Allergy Clin Immunol*, 109(6):920-922.

World Health Organization (1987). Principles for the Safety assessment of food additives and contaminants in Food Environmental Health Criteria 70, International Programme on Chemical Safety, Geneva. Accessible December 2005 at http://www.inchem.org/documents/ehc/ehc/ehc70.htm .

World Health Organization (2004). Principles for modeling dose-response for the risk assessment of chemicals. Accessible December 2005 at http://www.who.int/en/ .

Wuthrich, B., Ballmer-Weber, B. K. (2001). Food-induced anaphylaxis. *Allergy,* 56:102-104.

Yeung, J. M., Collins, P. G. (1996). Enzyme immunoassay for determination of peanut proteins in food products. *J AOAC Int,* 79(6):1411-1416.

Yunginger, J. W., Ahlstedt, S., Eggleston, P. A., Homburger, H. A., Nelson, H. S., Ownby, D. R., Platts-Mills, T. A., Sampson, H. A., Sicherer, S. H., Weinstein, A. M., Williams, P. B., Wood, R. A., Zeiger, R. S. (2000). Quantitative IgE antibody assays in allergic diseases. *J Allergy Clin Immunol,* 105:1077-1084.

Yunginger, J. W., Gauerke, M. B., Jones, R. T., Dahlberg, M. J. E., Ackerman, S. J. (1983). Use of radioimmunoassay to determine the nature, quantity and source of allergenic contamination of sunflower butter. *J Food Protection,* 46:625-628.

Yunginger, J. W., Sweeney, K. G., Sturner, W. Q., Giannandrea, L. A., Teigland, J. D., Bray, M., Benson, P. A., York, J. A., Biedrzycki, L., Squillace, D. L., Helm, R. M. (1988). Fatal food-induced anaphylaxis. *JAMA,* 260(10):1450-1452;

Zarkadas, M., Scott, F. W., Salminen, J., Ham Pong A. (1999). Common allergenic foods and their labeling in Canada - A review. *Canadian Journal of Allergy & Clinical Immunology*, 4(3):118-141.

Zeiger, R. S., Sampson, H. A., Bock, S.A., Burks, A.W. Jr., Harden, K., Noone, S., Martin, D., Leung, S., Wilson, G. (1999). Soy allergy in infants and children with IgE-associated cow's milk allergy. *J Pediatr,* 134(5):614-622.

Appendices

Appendix 1: Evaluation of Commercially Available Analytical Methods for Proteins from Major Food Allergen

Allergen	Manufacturer[a]	Method	Validation[b]	Sensitivity (LOD[c]) (ppm)	Quantitation (LOQ[c]) (ppm)	Raw and Processed Foods?	Species Specificity	Protein(s) Detected	Practicality
Peanut	Abkem Iberia	Peanut DiagnoKit	No	Not reported	Not reported	Yes (for specified foods)	Not reported	Not reported	Yes
	Elisa Systems	Peanut	JRC	0.5	1	Yes	Peanut	Ara h2	Yes
	Elisa Systems	Total Peanut	No	0.5	1	Yes	Peanut	Total	
	Neogen	Alert for Peanut	No	5	No	Not reported	Not reported	Not reported	Yes
	Neogen	Reveal for Peanut	No	5	No	Not reported	Not reported	Not reported	Yes
	Neogen	Veratox for Peanut	AOAC MLPT	Not reported	2.5	Yes (for specified foods)	Peanut	Not reported	Yes
	R-BioPharm	RiDASCREEN Peanut	No	2.5	Not reported	Yes	Not reported	Total	Yes
	R-BioPharm	RIDASCREEN FAST Peanut	AOAC MLPT	1.5	2.5	Yes (for specified foods)	Peanut	Total	Yes
	Tecra	Peanut Visual Immunoassay	No	0.5	2.5	Yes	Not reported	Not reported	Yes
	Tepnel	BioKits Peanut	AOAC MLPT	0.1	1	Yes (for specified foods)	Peanut	Conarachin (Ara h 1)	Yes
	Tepnel	BioKits Rapid Peanut	No	Not reported	No	Not reported	Not reported	Not reported	Yes
Milk	Abkem Iberia	Casein DiagnoKit	No	Not reported	0.16	Yes (for specified foods)	Not reported	Casein	Yes
	Elisa Systems	Beta-lactoglobulin	No	0.5	1	Yes	Not reported	Beta-Lactoglobulin	Yes
	Elisa System	Enhanced Beta -lactoglobulin	No	1	1	Yes	Not reported	Beta-Lactoglobulin	Yes
	Elisa Systems	Enhanced Casein	No	1	1	Yes	Not reported	Casein	Yes
	Neogen	Alert for Total Milk	No	5 whole milk10 dry nonfat milk	No	Yes	Not reported	Not reported	Yes
	Neogen	Veratox for Total Milk	No	Not reported	2.5	Yes (for specified foods)	Not reported	Not reported	Yes
	R-BioPharm	RIDASCREEN Beta-Lactoglobulin	No	0.2	5	Not reported	Not reported	Beta-Lactoglobulin	Yes
	SafePath	Milk Residue	No	Not reported	Not reported	Not reported	Not reported	Beta-Lactoglobulin	Yes
	Tepnel	BioKits BLG	No	7.5	25	Yes	Not reported	Primarily Beta-Lactoglobulin	Yes
	Tepnel	BioKits BSA	No	10	25	Yes	Not reported	Bovine Serum Albumin	Yes

	Tepnel	BioKits Casein	No	1	2	Yes	Not reported	Primarily Alpha-Casein	Yes
	Tepnel	BioKits Casein Rapid	No	Not reported	Not reported	Yes (for specified foods)	Not reported	Casein	Yes
Egg	Elisa Systems	Egg	No	0.5	1	Yes	Ovalbumin, Ovamucoid	Yes	Not reported
	Neogen	Alert for Egg	No	5	N	Yes (for specified foods)	Not reported	Not reported	Yes
	Neogen	Veratox for Egg	No	Not reported	2.5	Yes (for specified foods)	Not reported	Not reported	Yes
	R-BioPharn	RIDASCREEN Egg Protein	No	2	Not reported	Yes (for specified foods)	Not reported	White	Yes
	SafePath	Egg Residue	No	Not reported	Not reported	Not reported	Not reported	Ovomucoid	Yes
	Tecra	Egg Visual Immunoassay	No	0.5	0.6	Yes		Total	Yes
	Tepnel	BioKits Egg	No	0.1	0.5	Yes	Not reported	Ovomucoid	Yes
Tree Nuts	Abkem Iberia	Almond DiagnoKit	No	Not reported	0.06	Not reported	Not reported	Not reported	Yes
	Abkem Iberia	Hazelnut DiagnoKit	No	Not reported	0.08	Not reported	Not reported	Not reported	Yes
	Elisa System	Almond	No	0.5	1	Yes	Not reported	Not reported	Yes
	Elisa System	Hazelnut	No	0.25	0.5	Yes	Not reported	Not reported	Yes
	Neogen	Alert for Almond	No	5	No	Not reported	Not reported	Not reported	Yes
	Neogen	Veratox for Almond	No	Not reported	2.5	Not reported	Not reported	Not reported	Yes
	R-BioPharm	RIDASCREEN Hazelnut	No	3.3	Not reported	Not reported	Not reported	Total	Yes
	R-BioPharm	RIDASCREEN FAST Almond	No	1.7	2.5	Not reported	Not reported	Total	Yes
Soy	Elisa System	Soy	No	1	1	Yes (for specified foods)	Not reported	Trypsin Inhibitor	Yes
	Elisa Systems	Enhanced Soy	No	1	2.5	Yes (for specified foods)	Not reported	Soy flour proteins	Yes
	SafePath	Soy Residues	No	Not reported	Not reported	Not reported	Not reported	Trypsin Inhibitor	Not reported
	Neogen	Alert for Soy Flour	No	5	Not reported	Yes	Not reported	Not reported	Yes
	Neogen	Veratox for Soy Flour	No	2.5	2.5	Yes	Not reported	Not reported	Yes
	Tepnel	Soya Protein	No	0.5% soy protein in food sample	0.5% soy protein in food sample	Not reported	Not reported	Not reported	Yes
Crustaceans	Abkem Iberia	Crustacean DiagnoKit	No	Not reported	0.005	Not reported	shrimp, crab, lobster and scampi.	Tropomyosin	Yes
	Elisa Systems	Crustacean (18 species)	No	0.05	0.05	Yes (for specified foods)	Not reported	Tropomyosin	Yes

Fish	No commercial methods are available.								
Wheat	See Appendix 4 for gluten methods. No other commercial methods are available.								

[a] Information from manufacturers web sites, except for information on the Elisa System Crustacean test kit from FDA Docket #2005N-0231, comment number EC1.

[b]MLPT - Multiple Laboratory Performance Tested; JRC- European Commission Joint Research Centre; AOAC = AOAC International.

[c]LOD = Limit of detection, LOQ = Limit of quantiation.

Appendix 2: Evaluation of Available Allergen Oral Challenge Studies.

Study	Published	Test Population	Food Allergen Tested	Test Material	Lowest Dose Tested (mg protein[a])	Dose Progression	Responses at lowest dose tested?	LOAEL Observed (mg protein[a, b])	Sign(s)s or symptom (s) used to determine LOAEL	Population Dose/Response Data
May, 1976	Yes	38 asthmatic children, 8 reacted to peanut, 1 to milk, 4 t o egg	Peanut,	Raw peanut	25	2-10 fold increase	Yes	25	Objective	Not reported
			Milk	Whole	Not reported	2-10 fold increase	No	Not reported	Objective	Not reported
			Egg	Whole	Not reported	2-10 fold increase	No	Not reported	Objective	Not reported
			Eggs	Dried	Not reported	2-10 fold increase	No	250	Objective	Not reported
Bock *et al*., 1978	Yes	68 children with suspected allergy, 12 reacted to peanut, 10 to milk, 10 to egg, 5 to soy, 2 to cashew, 1 each to pecan, filbert, pistachio	Peanut	Unroasted	Not reported	Not reported	Not reported	25	Objective	No
			Milk	Dried nonfat	Not reported	Not reported	Not reported	280	Objective	No
			Egg	Dried whole	Not reported	Not reported	Not reported	1	Objective	No
			Soy	Protein isolate	Not reported	Not reported	Not reported	Not reported	Objective	No
			Cashew	Not reported	Not reported	Not reported	Not reported	Not reported	Objective	No
			Pecan	Not reported	Not reported	Not reported	Not reported	Not reported	Objective	No
			Filbert	Not reported	Not reported	Not reported	Not reported	Not reported	Objective	No
			Cashew	Not reported	Not reported	Not reported	Not reported	Not reported	Objective	No
			Pistaschio	Not reported	Not reported	Not reported	Not reported	Not reported	Objective	No
Pasterello *et al*., 1989	Yes	23 adults with suspected allergy, 4 reacted t milk, 2 to hazelnut, and 1 each to egg and wheat	Milk	Dried	Not clear - differed for different foods	Dose doubling	Not reported	187	Objective	No
			Egg white	Dried			No	1500	Objective	No
			Hazelnut	Ground			No	2775	Objective	No

Bernhisel-Broadbent *et al*., 1992b	Yes	11 fish allergic children and adults	Fish	Raw and cooked extracts of 9 species	Not reported	Not reported	Not reported	Not reported	Not reported	No
Caffarelli *et al*., 1995	Yes	21 infants and children with no previous egg exposure, 14 reacted	Egg	Dried egg	0.042	Not reported	No	0.42	Objective	No
Magnolfi *et al*., 1996	Yes	131 skin prick positive children, 8 reacted	Soy	Formula	"1 drop" for infants	6 defined doses	No	360	Objective	No
					88 mg soy protein for older children	7 defined doses	Yes	88	Objective	No
Hourihane *et al*., 1997a	Yes	14 peanut allergic adults, 8 reacted	Peanut	Peanut flour	0.01	12 defined doses	No	0.1 2	Subjective Objective	Yes
Hourihane *et al*., 1997b	Yes	60 peanut allergic adults	Peanut	Whole peanut	? (Labial challenge)	4 defined doses	Yes	Not reported	Objective	No
Nelson *et al*., 1997	Yes	12 peanut allergic adults	Peanut	Defatted peanut	0.45	12 defined doses	Not reported	Not reported	Subjective	No
Bellioni-Businco *et al*., 1999	Yes	26 milk allergic children	Milk	"Fresh" whole milk	"1 drop"	Not reported	3.65		Objective	No
Hebling *et al*., 1999	Yes	9 fish allergic adults	Fish	Cooked meat from 3 species of fish	50	4 specified levels	Yes	50	Subjective and Objective	Yes
Zeiger *et al*., 1999	Yes	93 milk allergic infants and children, 13 reacted to soy	Soy	Formula	1 drop to 5 ml	6 to 7 doublings	No	522	Objective	No
Otolani *et al*., 2000	Yes	86 hazelnut allergic adults	Hazelnut	Ground nuts	224	Dose doubling, possibly 4 levels	Not reported	Not reported	Not reported	No
Sicherer *et al*., 2000	Yes	196 children with a variety of food allergies[c]	Peanut	Not reported	400 or 500 mg of food	6 or 7 specified levels	Yes	Not reported	Not reported	No
			Milk	Not reported				Not reported	Not reported	No
			Egg	Not reported				Not reported	Not reported	No
			Soy	Not reported				Not reported	Not reported	No
			Fish	Not reported				Not reported	Not reported	No
			Wheat	Not reported				Not reported	Not reported	No
Eggesbo *et al*., 2001	Yes	41 children with reported allergy, 5 tested by DBPCFC	Egg	Pancakes	260	Dose doubling until reaction or maximum dose	Yes	260	Objective	No

Bindslev-Jensen and Hansen in Taylor *et al.*, 2002	No	14 patients, not clear whether all were challenged with each fish	Fish	Cod	Not reported	Not reported		5 mg of fish		
				Mackerel	Not reported	Not reported	Not reported	500mg of fish		
				Herring	Not reported	Not reported	Not reported	5mg of fish		
				Plaice	Not reported	Not reported	Not reported	6000mg of fish		
Bindslev-Jensen and Mortz in Taylor *et al.*, 2002	No	5 patients	Peanut	Ground	Not reported	Not reported	Not reported	40	Not reported	No
Bindslev-Jensen and Norgaard in Taylor *et al.*, 2002	No	3 milk allergic, 7 egg allergic patients	Milk	Whole	Not reported	Not reported	Not reported	180	Not reported	No
			Egg	Whole raw	Not reported	Not reported	Not reported	0.65	Not reported	No
Bock in Taylor *et al.*, 2002	No	69 peanut allergic, 66 milk allergic, 91 egg allergic, 8 fish allergic patients	Peanut	Ground	Not reported	Not reported	Not reported	1.25	Not reported	No
			Milk	Nonfat dried	Not reported	Not reported	Not reported	67	Not reported	No
			Egg	Whole or dried	Not reported	Not reported	Not reported	Not reported	Not reported	No
			Fish	Minced	Not reported	Not reported	Not reported	200 mg of fish	Not reported	No
Burks and Christie in Taylor *et al.*, 2002	No	10 peanut allergic, 21 milk allergic, 25 egg allergic patients	Peanut	Peanut butter	Not reported	Not reported	Not reported	100	Not reported	No
			Milk	Nonfat dried	Not reported	Not reported	Not reported	140	Not reported	No
			Egg	Whole dried	Not reported	Not reported	Not reported	200	Not reported	No
Hill in Taylor *et al.*, 2002	No	100 patients each for peanut, milk, egg	Peanut	Peanut butter	Not reported	Not reported	Not reported	6	Not reported	No
			Milk	Whole	Not reported	Not reported	Not reported	0.6	Not reported	No
			Egg	Raw white	Not reported	Not reported	Not reported	2	Not reported	No
Host in Taylor *et al.*, 2002	No	15 milk allergic patients	Milk	Forumula	Not reported	Not reported	Not reported	75	Not reported	No
Lack in Taylor *et al.*, 2002	No	6 peanut, 6 milk, 18 egg allergic patients	Peanut	Ground	Not reported	Not reported	Not reported	125	Not reported	No
			Milk	Whole	Not reported	Not reported	Not reported	150	Not reported	No
			Egg	Cooked white	Not reported	Not reported	Not reported	10	Not reported	No
				Raw white	Not reported	Not reported	Not reported	20	Not reported	No
Moneret-Vautrin #1 in Taylor *et al.*, 2002	No	28 peanut allergic, 6 milk allergic, 19 egg allergic, 4 fish allergic patients	Peanut	Ground	Not reported	Not reported	Not reported	1.25 (single blind)2.5 (double blind)	Not reported	No
			Milk	Whole	Not reported	Not reported	Not reported	30 (double blind)150	Not reported	No

								(single blind)0.2		
			Egg	White	Not reported	Not reported	Not reported	15mg of fish (single blind)	Not reported	No
			Fish	Minced	Not reported	Not reported	Not reported	65mg of fish (double blind)	Not reported	No
Moneret-Vautrin #2 in Taylor *et al*., 2002	No	9 peanut allergic, 8 egg allergic patients	Peanut	Ground	Not reported	Not reported	Not reported	66	Not reported	No
			Egg	White	Not reported	Not reported	Not reported	26.5	Not reported	No
"National Jewish" in Taylor *et al*., 2002	No	3 peanut allergic patients	Peanut	Ground	Not reported	Not reported	Not reported	2	Not reported	No
Rance in Taylor *et al*., 2002	No	74 peanut allergic, 31 milk allergic, 38 egg allergic, 6 fish allergic patients	Peanut	Ground	Not reported	Not reported	Not reported	0.25	Not reported	No
			Milk	Whole	Not reported	Not reported	Not reported	15	Not reported	No
			Egg	Whole raw	Not reported	Not reported	Not reported	0.13	Not reported	No
			Fish	Minced	Not reported	Not reported	Not reported	16mg of fish	Not reported	No
Zeiger in Taylor *et al*., 2002	No	56 milk allergic patients	Milk	Formula	Not reported	Not reported	Not reported	1.5	Not reported	No
Wensing *et al*., 2002a	Yes	31 hazelnut allergic adults	Hazelnut	Raw nuts	1	7 specified doses	Yes	1	Subjective and Objective	Yes
Wensing *et al*., 2002b	Yes	26 peanut allergic adults	Peanut	Roasted peanut meal	0.030	10 specified doses	No	0.1	Subjective and Objective	Yes
Fiocchi *et al*., 2003	Yes	18 children with allergy to both milk and soy	Milk	Whole	43.2	4 specified doses	Yes	43.2	Objective	Yes
			Soy	Formula	21.8			21.8	Objective	
Hansen *et al*., 2003	Yes	17 hazelnut allergic adults	Hazelnut	Raw and roasted nuts	Not reported	Not reported	Not reported	32 - roasted16 - raw	Oral allergy syndrome	No
Morisset *et al*., 2003	Yes	Undefined	Peanut	Crushed	1.25	5 specified levels	Yes	1.25	Objective	No
				Oil	Not reported		No	Not reported	Objective	No
			Milk	Lactose free	0.36		Yes	0.36	Objective	No
			Egg	Raw white	0.2		Yes	0.2	Objective	No
			Soy	Oil	Not reported		No	Not reported	Objective	No
Osterballe and Bindslev-Jensen, 2003	Yes	56 egg allergic children	Egg	Pasteurized whole egg	2.9	8 specified doses	Yes	2.9	Objective	No
Perry *et al*., 2004	Yes	Not reported	Milk, egg, peanut, soy, wheat	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported	No

Scibilia *et al*., 2006	Yes	27 wheat allergic adults	Wheat	Flour, raw and cooked	15	7 specified doses	Yes	15	Objective	Yes

Note: Question marks (?) in the table indicate either that the information was not given or could not be determined.

[a] Calculated based on the following estimate protein levels: 16% in raw hazelnuts, 20% in fish meat, 3.6% in whole milk, 37.5% in dried milk, 25% in whole peanut, 45% in defatted peanut flour, 10% in egg white, 84% in dried whole egg , 26% in raw egg, 1.8% in soy formula (FAO, 1995; Wensing *et al*., 2002; Bindslev-Jensen *et a*l., 2002). In studies involving fish, the amount of fish is given when there is insufficient information to calculate protein levels.

[b] When responses are observed at the lowest dose tested, the reported LOAEL may not represent the lowest dose at which a reaction could occur.

[c] It is not clear if all children were tested with all allergens.

Appendix 3: Evaluation of Published Measurements of Protein Concentrations in Oils.

Oil Type	Reference [a]	Protein Concentration (ug/g)	Description of Oil	Published	Protein Separation Method	Protein Quantitation Method
Soy	Tattrie and Yaguchi, 1973	0.96	Refined, deodorized	Yes	Chromatography	Amino Acid Analysis
	Klurfeld and Kritchevsky, 1987	1.93 0.72	Crude "Processed"	Yes	Aqueous Extraction	Commercial Bradford Assay
	Awazuhara *et al.*, 1998	0.014 0.017 0.018 0.023 0.027 0.040	Uncharacterized, commercial	Yes	Aqueous Extraction	Lowery Assay
	Reeves, 1999	0.033 0.042 0.049 0.057 0.082 0.114 0.222	Fully refined, commercial	No	Unknown	Amino Acid Analysis
	Paschke *et al*., 2001	0.0332 0.0353 0.0898 0.1010	Refined Unrefined	Yes	Acetone Precipitation	Bradford Assay

		0.1380				
	Errahali *et al*., 2002	0.32 1.80	Deodorized Cold pressed	Yes	Aqueous Extraction	Unknown
	Nordlee *et al*., 2002	0.16 - 20.8 0.043 - 6.8 0.033 - 3.1 0.021 - 0.443	Degummed Refined Bleached Deodorized	No	Aqueous Extraction	Amino Acid Analysis
Peanut	Klurfeld and Kritchevsky, 1987	0.120 0.154 0.204 0.206 0.580	Processed	Yes	Aqueous Extraction	Bradford Assay
	Hoffman and Collins-Williams, 1994	0.2 0.6 3.3 3.3	Cold pressed	Yes	Aqueous Extraction	Commercial Coomassie Dye Assay
	Teuber *et al*., 1997	3.0 ± 0.3 5.7 ± 1.2 10.5 ± 0.4 10.7 ± 0.8	Refined, bleached, deodorized Unrefined	Yes	Aqueous Extraction	Commercial Bradford Assay
	Olszewski *et al*., 1998	0.10 0.13 0.15 0.16 0.20	Refined, commercial	Yes	Aqueous Extraction	Commercial Bicinchoninic Acid (BCA) Assay
	Skinner and Haynes, 1998	187	Crude	No	Aqueous Extraction	Lowery and Commercial BCA Assay
		60	Alkali refined, neutralized			
		15	Alkali refined, neutralized, bleached			

		2.2	Alkali refined, neutralized, bleached, deodorized			
	ISEO, 1999	0.047 0.049 0.063	Fully refined, commercial	No	Unknown	Amino Acid Analysis
		0.828	Partially refined, commercial			
	Crevel *et a*l., 2000 [b]	48 91	Refined, neutralized, bleached, deodorized	No	Aqueous Extraction	Commercial BCA Assay
		220	Crude			
	Peeters *et al*., 2004	0.09 6.4	Crude, noncommercial	Yes	Unknown	ELISA (not described)
		2.55	Cold pressed			
Tree Nut	Teuber *et al.*, 1997 (Almond)	2.2 ± 0.7 16.7 ± 0.8	Refined, bleached, deodorized	Yes	Aqueous Extraction	Commercial Bradford Assay
		12.7 ± 2.8	Blend			
		62.2 ± 2.2	Unrefined			
	Teuber *et al*., 1997 (Walnut)	7.0 ± 2.5 7.0 ± 0.8 9.2 ± 3.1	Refined, bleached, deodorized	Yes	Aqueous Extraction	Commercial Bradford Assay
		16.5 ± 2.4	Unrefined			
		20.4 ± 1.8	Blend			

Note: Protein levels too low to detect or measure were reported by Tattrie and Yaguchi (1973), Hoffman and Collins-Williams (1994), Yeung and Collins (1996), Peeters *et al*. (2004) for peanut oils and by Tattrie and Yaguchi (1973), Porras *et al*. (1985) for soy oils. These values were not included due to the lack of methodological information.

[a] None of the publications provide sufficient information to evaluate the overall extraction efficiency, accuracy, reproducibility, or precision of the method used. In addition, in most cases, it was not clear whether replicate samples were tested or whether replicate measurements were carried out for individual samples.

[b] Crevel *et al*. (2000) is a review paper that includes previously unpublished data. These data are given here, but are considered unpublished because the research that generated these values has not specifically been peer-reviewed.

Appendix 4: Evaluation of Gluten Testing Methods.

Method [a]	Validation	Sensitivity (LOD) (ppm gluten)	Quantitation (LOQ) (ppm gluten)	Raw and Baked Foods?	Species Specificity	Protein(s) Detected	Practicality
Diffchamb Transia Plate Gluten	No	10	Not reported	Not reported	Wheat, triticale, rye, barley	Gliadin	Yes
Diffchamb Transia Plate Prolamins	Working Group on Prolamin Analysis and Toxicity	3	Not reported	Yes	Wheat, triticale, rye, barley	Gliadin	Yes
Ingensa Gluten EIA	No	3	Not reported	Not reported	Wheat, rye, barley	Gliadin	Not reported
Neogen Alert for Gliadin	No	10	No	Not reported	Wheat, rye, barley	Gliadin	Yes
Neogen Veratox for Gliadin	No	5	5	Not reported	Wheat, rye, barley	Gliadin	Yes
R-BioPharm RIDASCREEN Gliadin	Prolamin Working Group Ring Study [b]	3	5	Yes	Wheat, rye, barley	Gliadin	Yes
R-BioPharm RIDASCREEN FAST Gliadin	No	10	10	Yes	Wheat, rye, barley	Gliadin	Yes
R-BioPharm RIDAQUICK Gliadin	No	5	No	Yes	Wheat, rye, barley	Gliadin	Yes
Tepnel BioSystems Wheat Gluten	AOAC	160 2 - not validated	16	Yes	Wheat, triticale, rye	Omega gliadin	Yes
Tepnel BioSystems Gluten Rapid Test Kit	No	50 - breads, etc 200 - "highly processed flour	No	Yes	Wheat, triticale, rye	Omega gliadin	Yes

[a] Information from manufacturers web sites:
Ingensa
Neogen Food Allergen Test Kits
R-BioPharm Food and Feed Analysis RIDASCREEN® Gliadin
Tepnel BioSystems

[b] Immer *et al*., 2003.

Appendix 5: Evaluation of Gluten Oral Challenge Studies.

Study	Published	Test Population	Test Material	Dose Level(s)	Duration	Diagnostic Assessment (Biomarker)	Individual Response Data?
Fasano, 2005; Abstract: Catassi *et al.*, 2005b	No; analysis is ongoing	33 of 46 adults completed study	Not reported	0, 10 or 50 mg gluten/day	3 months	Intestinal biopsy, symptoms	Not reported
Catassi *et al.*, 1993	Yes	20 children (10 each dose level)	Commercial crude gliadin	100 mg or 500 mg gliadin/day	4 weeks	Intestinal biopsy, symptoms	Yes
Ciclitera *et al.*, 1984	Yes	1 adult	White flour milled from Kolibri strain of wheat	10, 600, and 1000 mg gliadin	Intraduodenal infusion over 2 hr period; 3 doses on separate days	Intestinal biopsy	Yes
Ciclitera *et al.*, 1984	Yes	3 adults	White flour milled from Kolibri strain of wheat	1000 mg of 4 gliadin subfractions	Intraduodenal infusion each subfraction at variable intervals of 3 to 11 days	Intestinal biopsy, symptoms	Yes
Ciclitera *et al.*, 1985	Yes	10 adults	Not reported; article states that gluten-free bread usually contains 0.2 to 0.4 mg gliadin/30-g slice	6 slices/day Juvela gluten-free bread; oral	6 weeks	Intestinal biopsy,symptoms	Yes
Montgomery *et al*, 1988	Yes	12 adults on strict gluten-free diet and 13 adults on low-gluten diet	Not reported	Gluten-free diet Low-gluten diet (2.5 to 5 gm gluten/day)	Gluten-free diet: 6 to 27 months (mean 13 months); Low-gluten diet: 3 to 14 months (mean 6 months)	Intestinal biopsy, symptoms, anti-gluten Ab	Yes, graphs
Sturgess *et al*, 1994	Yes	4 adults	Undigested gliadin prepared from Kolibri wheat flour by standard method; oligopeptides synthesized and analyzed	1 gm gliadin or 200 mg of synthetic peptides/dose	2 hours by infusion	Intestinal biopsy hourly for 6 hrs after start infusion	Yes; as percentage enteropathic change

March 2006 FDA's Responses to Public Comments on the Draft Report

---

Food Allergens

CFSAN Home | CFSAN Search/SubjectIndex | CFSAN Disclaimers&PrivacyPolicy | CFSAN Accessibility/Help
FDA Home Page | Search FDA Site | FDA A-Z Index | Contact FDA

FDA/Center for Food Safety & Applied Nutrition
Hypertext updated by acr/ear May 24, 2006