FDA's Responses to Comments on the Report Titled "Health Hazard Assessment for Gluten Exposure in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten."

December 2012

On August 3, 2011, the Food and Drug Administration (FDA or we) published a notice in the Federal Register (76 FR 46671) (the 2011 notice) announcing the reopening of the comment period for a proposed rule that we had published on January 23, 2007 (72 FR 2795) regarding the "gluten-free" labeling of food. We published the 2011 notice, in part, to invite comment regarding a report titled "Health Hazard Assessment for Gluten Exposure in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten" (the Gluten Report). We made the Gluten Report publicly available through the FDA Docket and the Center for Food Safety and Applied Nutrition's (CFSAN) web site.[1]

The Gluten Report consists of several components. The introductory hazard identification section examines and provides an overview of the nature and characteristics of the adverse effects associated with celiac disease found in susceptible individuals and also that of gluten proteins involved in inducing these effects. The hazard assessment section of the report first describes the nature of the evaluation performed on the available health effects data associated with celiac disease. This evaluation includes both a dose-response assessment and a safety assessment derived from data from individuals in this sensitive subpopulation. The former assessment describes and characterizes the dose-effect data examined for morphological and clinical adverse effects that are reflective of celiac disease, and the latter determines the tolerable daily intake (TDI) levels of exposure for each of these types of adverse effects in sensitive individuals. The hazard assessment section also includes an exposure assessment, in which a number of estimates of gluten consumption from food products are determined and presented. The final risk characterization section of the report addresses the uncertainty issues associated with the data available and the estimates derived, and identifies the TDI of primary focus for adverse morphological and clinical effects in this assessment. In addition, these TDIs,

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[1] United States Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Safety, "Health Hazard Assessment for Gluten Exposure in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten," May 2011, accessible at: http://www.fda.gov/downloads/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/UCM264152.pdf

along with the exposure estimates, were used to derive various levels of concern (LOC) for gluten in food for individuals with celiac disease. For purposes of this document, we use the term "the safety assessment" to describe the Gluten Report in its entirety.

In the 2011 notice reopening the comment period, we asked interested persons to submit comments, scientific data, and information regarding the safety assessment. The comment period ended on October 3, 2011. We also asked for comments regarding whether the safety assessment should affect our proposed definition of the term "gluten-free." Finally, we asked for comments on our tentative conclusion that a safety assessment-based approach for the purpose of defining the term "gluten-free" might lead to a conservative, highly uncertain estimation of risk to individuals with celiac disease associated with very low levels of gluten exposure, and that the final rule should adopt an analytical methods-based approach to defining the term "gluten-free."

We received several comments regarding the safety assessment from consumer groups, the food industry, trade associations, gluten experts, and individual consumers. We summarize and respond to the comments below.

Many comments we received addressed issues that are beyond the scope of our request for comments in our 2011 notice, and, therefore, we do not address them in this document. Finally, we do not intend to revise the safety assessment in light of the comments that we received.

General Comments Regarding the Safety Assessment

Some comments we received were general in nature. We summarize and respond to those comments in Table 1.

Table 1: General Comments Regarding the Safety Assessment

Topic	Summary of Comment	FDA Response
Overall quality of clinical studies used in the safety assessment	A few comments stated that we should use evidence-based research to substantiate our limits with double-blinded, placebo-controlled studies. Another comment observed that the safety assessment relied on open challenge studies, which the comment explained meant that everyone involved (i.e., the subjects, test administrators, etc.) were aware of the food (or placebo) being administered, and not on double-blind, placebo-controlled studies (i.e., studies in which everyone involved in the trial are unaware of the food, or placebo, being used). One comment asserted that a long-term, placebo-controlled study with an appropriate sample size and including very gluten- sensitive celiac disease patients should be conducted to define TDIs for that population.	We disagree with the comments suggesting that the methodologies and assumptions used in the safety assessment were not appropriate. We followed a standard hazard assessment approach to evaluate the available data on gluten sensitivity. The safety assessment was based on a comprehensive and systematic evaluation of all available clinical data from gluten challenge studies with gluten-sensitive individuals, and we considered the "weight" of those results as a body of evidence in considering its validity. We also reviewed prospective, open challenge studies, along with available single- or double-blind challenge studies, because they were available and provided a significant amount of quantitative data to assist in the determination of appropriate threshold levels. Most studies reviewed in the safety assessment were open challenge studies. Only a few studies were single-blind (only the subjects are unaware of the food or placebo being tested) or double-blind studies. As discussed in the safety assessment, the double-blind, placebo-controlled study is considered the "gold standard" with respect to study design, and the placebo control is particularly useful when evaluating clinical symptoms of a subjective nature in test subjects. However, when the challenge-induced adverse responses of subjects tend to be specific to each subject and to vary greatly between subjects, double-blind, placebo-controlled studies need a large number of subjects per treatment group to obtain measures of central tendency (means) that are representative of the treatment effects. Recruiting and maintaining the participation of a large number of subjects for challenges that may induce a disease state typically is very difficult. We also considered it appropriate, in characterizing the dose-response relationship between gluten exposure and adverse health effects, to consider all available open challenge study data in the safety assessment as well as any available data from single/double-blind, placebo controlled studies.

Topic	Summary of Comment	FDA Response
Number and size of studies used for the safety assessment	One comment stated that the scientific and clinical literature that we relied on for the low dose safety assessment contains relatively few studies involving only a small number of subjects with celiac disease. The comment argued that the safety assessment approach is overly conservative and is based on an uncertain scientific and clinical foundation and cannot be supported by available analytical methods.	We agree that the studies reviewed in the safety assessment evaluated the effects of gluten in a relatively small number of subjects. Recruiting subjects with celiac disease to participate in a study that may worsen or aggravate their disease presents difficult challenges. Studies with larger numbers of subjects, including a placebo-control group, would make it easier to study a disease that is manifested by highly individual responses and symptoms. Nevertheless, we followed a standard procedure for conducting the safety assessment, and the draft assessment underwent a scientific evaluation and critique by an external peer review panel of experts.[2] The peer reviewers identified the small number of subjects evaluated in the studies as a major weakness of the safety assessment and observed that more research is needed to truly assess the safety of low levels of gluten exposure in gluten-sensitive individuals. We agree that the assessment of the available published literature on adverse health effects of gluten in dose-response trials may have led to conservative estimates for TDIs because the goal of the safety assessment was to find the lowest dose which elicited no reaction in the most gluten-sensitive individuals. The 2011 notice stated that we had tentatively concluded that, based on the LOCs identified in the safety-assessment approach, we should not use that approach in defining "gluten-free" because the estimation of risk to individuals with celiac disease associated with very low levels of gluten exposure may be conservative and highly uncertain (76 FR 46671 at 46674).

[2] United States Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Food Safety, "External Peer Review of the FDA/CFSAN Draft Health Hazard Assessment for Gluten in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten," December 2010, accessible at: http://www.fda.gov/downloads/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/UCM264150.pdf%20accessed%20on%20September%2010,%202012.

Topic	Summary of Comment	FDA Response
Comparative quality criteria	One comment asserted that we did not summarize comparative quality criteria for the various studies used in the safety assessment. The comment noted, for example, that one study might have shown some effect at a dose that was shown to be safe in one or more other studies, but stated that we did not evaluate the conflicting studies to determine which had the best design or was the most relevant.	We disagree with this comment. We did summarize and present in detail the criteria that we used to evaluate and compare studies and their findings in the safety assessment. For example, we described the criteria for the type of route of exposure and the form or vehicle of exposure to gluten found in the studies and we described their contribution in evaluating and comparing the results of those studies. We also provided the details of the basis of our evaluation and determination of adverse morphological and clinical effects in individuals with celiac disease in response to gluten challenges. Because of the nature of the agent we were evaluating (gluten), the assessment of the toxicity and/or detrimental effects consisted of the evaluation of the entire body of available dose-response adverse effects data and consideration of the weight-of-evidence. A weight-of-evidence evaluation is a recognized approach in the area of toxicology and health effect risk assessment for evaluating a diverse data set, such as the data regarding reactions to gluten.[3] This approach provides evidential support for the likely presence of a direct or systematic relationship, and helps identify the lower limits of the dose levels of toxicologically relevant reactivity. We used the weight-of-evidence evaluations in our assessments of both "within study" data and "across study" data. We described, in detail, the criteria that we used in evaluating each of these types of evidence in the subsection of the safety assessment titled "Basis of Weight-of-Evidence Evaluations and Determinations." Taken together, these criteria enabled us to assess the reliability and validity, and therefore the "quality" of the findings of the studies that we included in our safety assessment. The specific studies and associated adverse effects of focus that we identified and selected as "critical" were based on established, well-characterized principles of the safety-assessment approach and the procedures and scientific judgments typical of this type of evaluation.

[3] See, e.g., World Health Organization, International Programme on Chemical Safety, Principles for the assessment of risks to human health from exposure to chemicals, Environmental Health Criteria 210, WHO, Geneva, 1999; World Health Organization, International Programme on Chemical Safety, "Chapter 2: Risk assessment and its role in risk analysis," Principles and Methods for the Risk Assessment of Chemicals in Food, Environmental Health Criteria 240, WHO, Geneva, 2009.

Topic	Summary of Comment	FDA Response
Clinical and morphological endpoints	One comment argued that, while we considered clinical and morphological endpoints, morphological endpoints should be more sensitive than clinical endpoints.	Significant components of an assessment that evaluates the health hazards of a toxic compound are hazard identification and characterization. The role of this evaluation is to identify the relevant adverse health effects and characterize their nature. In the safety assessment, both morphological and clinical adverse health effects were identified as distinct and significant responses to gluten for individuals diagnosed with celiac disease. Thus, the examination of the available dose-effect data and determination of the tolerable daily intake levels for gluten in the safety assessment for both types of adverse effects was warranted. The goal of this hazard/safety assessment was not to make a judgment about the relative value or importance of one adverse effect over another in those suffering with celiac disease; it was only to evaluate and characterize available data to determine a threshold level of reactivity for each type of effect.

Topic	Summary of Comment	FDA Response
Use of uncertainty factors	Some comments questioned the appropriateness of the safety assessment's use of a 100-fold uncertainty factor to determine the no observable adverse effect level (NOAEL). The comments stated that we employed methodologies generally used in toxicological safety assessments based on animal models, and thus may have used overly conservative uncertainty factors. One comment explained that animal and in vitro studies require larger safety factors for risk assessments due to the uncertainties of human extrapolations and bioavailability. A few comments asserted that, considering the vast body of evidence to support the safety of the gluten-free diet in current commercial gluten-free products (with levels of 20 ppm or more) and the fact that the threshold was not extrapolated from animal studies, we should not have applied uncertainty factors of 10 or 100 to account for the extrapolation of safety data from animals to humans. Some comments supported the use of a 10-fold safety factor alone to account for intra-species variation amongst humans.	Uncertainty, as it pertains to safety/risk assessments, refers to the inability to know for certain the level of a toxic agent that will not cause adverse health effects. It typically is due to the lack of relevant data. As the comments suggested, uncertainty factors are often applied to data from animal studies. However, they are not used strictly with animal studies. One of several, generally 10-fold, uncertainty factors is used experimentally and is intended to account for: (1) Variation in susceptibility among humans (inter-individual or intra-species variability); (2) Uncertainty in extrapolating animal data to humans (inter-species uncertainty); (3) Uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) Uncertainty in extrapolating from a lowest observable adverse effect level (LOAEL), rather than from a NOAEL; and (5) Uncertainty associated with extrapolating data from an incomplete database.[4] The use of uncertainty factors helps provide a margin of exposure (or safety) with respect to the effect levels identified as significant. In the safety assessment, we relied entirely upon human studies to obtain data on gluten toxicity in subjects with celiac disease. As we stated in the 2011 notice (76 FR 46671 at 46673), gluten dose-response data were divided based on age of the subjects (children and adults) participating in the studies. These different categorizations allowed for characterization and comparison of TDIs and other safety assessment determinations from a variety of studies based on adverse health response type (i.e., morphological and/or physiological or clinical), duration of gluten exposure (i.e., acute, subchronic, or chronic) and age (i.e., children or adults) of sensitive subjects with celiac disease. In cases where more than one appropriate study was available for a given assessment category (e.g., acute gluten exposures leading to morphological health effects in children), this assessment identified a ''critical study'' of high quality in line with the safety assessment procedure from which to estimate TDIs for the respective category. The final principal TDI identified for morphological effects was based on a NOAEL and thus only included a single 10-fold uncertainty factor to account for inter-individual variability. The principal TDI for clinical effects was based on a LOAEL value and thus included an additional 10-fold uncertainty factor to account for uncertainty of extrapolation from a LOAEL to a NOAEL. The application of uncertainty factors as used in the safety assessment was appropriate for the data that were available and followed established hazard assessment components and approaches used within FDA to determine TDIs for chemical and natural toxin contaminants in foods.

[4] See, e.g., United States Environmental Protection Agency Risk Assessment Portal, Glossary of Terms, accessible at: http://www.epa.gov/risk/glossary.htm.

Topic	Summary of Comment	FDA Response
Use of endpoints	One comment asserted that we used other health endpoints (autoimmune disease, malignancy, bone disease, etc.) in addition to celiac disease as a means to further justify the need to apply conservative assumptions. The comment asserted that such an approach was incorrect because the safety assessment was intended to focus on celiac disease, and so we should have addressed the other end points separately.	We disagree with the comment suggesting that we used other health endpoints as a means to further justify conservative assumptions (e.g., use of uncertainty factors). The purpose of a health hazard assessment is to characterize the risks and uncertainty associated with the exposure to a particular agent, which we did based on all available dose-response data. We evaluated and reported on the available data for morphological and clinical adverse reactions in individuals with celiac disease subsequent to acute, subchronic, or chronic exposure to gluten. When we discussed the use of uncertainty factors to account for inter-individual variability in determining TDIs, we suggested that the use of additional uncertainty factors to account for other adverse endpoints may be a consideration in the safety assessment. We reported that there has been no systematic investigation regarding potential links between long-term or chronic ingestion of trace amounts of gluten and development of cancer, autoimmune or other diseases.
Tolerable daily intake levels	One comment noted that, in the safety assessment, several TDIs are established for acute, subchronic, and chronic effects and questioned the rationale for establishing several TDIs for the same hazard. The comment also stated that the TDI is intended to cover the human population over a lifetime but managing the TDIs would be difficult, especially when the subchronic TDIs are much lower than the chronic ones.	One purpose of the health hazard analysis component of the safety assessment is to characterize the nature of all available low-dose response data. Morphological and/or clinical adverse reactions in individuals with celiac disease may occur subsequent to acute, subchronic, or chronic exposure to gluten. Therefore, we established the three TDIs to compare responses based on length of exposure to gluten. However, after further evaluation and analysis of the three resulting TDIs and the data sets on which each were based, the safety assessment focused primarily on the subchronic TDI from which to estimate the overall tolerable level of gluten intake for those with celiac disease. The comment is incorrect that the three TDIs were meant to be applied to the entire population of individuals with celiac disease. The TDI estimated for subchronic exposure, i.e., 0.4 mg gluten per day, represents a level that would be protective of the most gluten-sensitive individuals.

Topic	Summary of Comment	FDA Response
Use of daily gluten-containing / gluten-free food consumption estimates used in the safety assessment	One comment stated that we took a very conservative approach in the safety assessment by assuming that all food eaten on a daily basis by an individual with celiac disease would be food labeled as "gluten-free" and would be contaminated with gluten at the maximum level.	We disagree with this comment. In the exposure assessment section of the Gluten Report, we calculated estimates for consumption levels of gluten-free food for children and adults with celiac disease by using an established and recognized dietary survey database containing food consumption information for individuals in the United States. We based these estimates on the amount of food that is typically eaten by U.S. consumers and contain the grain, flour or germ of wheat, rye or barley. We used these estimates to determine the approximate amounts of food of this type that individuals with celiac disease would have to replace with gluten-free food to maintain similar caloric and nutrient intake levels. As we indicated in the Gluten Report's exposure assessment, the consumption estimates for the replacement gluten-free food in adults are consistent with the average daily consumption of commercially available gluten-free food products in adults found in a double-blind, placebo-controlled study in the literature in which the daily intake of gluten-free products by study subjects with celiac disease was recorded over 30 days.[5] We believe that our approach was appropriate and adequately accounts for, and thus protects, individuals with celiac disease who would use gluten-free food. We also note that the consumption estimates reflected only the gram weight of food eaten and that we did not consider or make any assumptions regarding the gluten content level of the replacement food products in the exposure assessment, as suggested by the comment.

[5] Catassi, C., Fabiani, E., Iacono, G., et al., "A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease," Am J Clin Nutr, 85:160-6, 2007.

Comments Regarding Our Use of Specific Studies for the Safety Assessment

We received a number of comments regarding the use and interpretation of various studies and data for the preparation of the safety assessment. Some comments criticized the use of specific studies and data to establish the gluten TDIs. Other comments criticized us for not relying more heavily on certain other studies and data for purposes of preparing the safety assessment.

Several comments criticized our reliance on a study by Chartrand et al. (1997) (the Chartrand study)[6] and on a study by Ciclitira et al. (1985) (the Ciclitira study)[7] as critical studies in determining the lowest exposure dose of gluten in a subpopulation of highly gluten-sensitive subjects.

The Chartrand study was an open food challenge in which all subjects and investigators were aware of the test substance being administered (7.5 ppm gliadin/wheat starch). All subjects had celiac disease, but had followed a strict, gluten-free diet for at least one year prior to initiation of the study. The experimental group was asked to consume the equivalent of 4 to 6 slices of wheat starch-containing bread or comparable wheat starch-containing products each day for up to a year. A second group of wheat starch-tolerant celiac disease subjects who had been consuming wheat starch products for at least one year before the study started served as the control group for comparing clinical symptoms. The experimental group recorded any adverse gastrointestinal symptoms (e.g., diarrhea, abdominal pain, etc.) or extra-intestinal symptoms (e.g., change of appetite, fatigue, etc.) on patient diary forms. Subjects consumed an average of 1.5 mg of gluten per day. The subjects in the experimental group recorded symptoms as early as two weeks and as late as eight months after the start of the study and reported that the symptoms dissipated between ten days and three weeks after they stopped consuming the wheat starch-bearing foods. The control group consumed the same amount of wheat starch as the test group, but reported no symptoms. The Chartrand investigators did not perform biopsies to determine whether any of the subjects had experienced morphological changes during the wheat starch challenge. The investigators reported that it was difficult to determine whether any symptoms reported by the experimental group were psychosomatic because the study was not double-blinded. They also noted that, even though there was consistency and reversibility of the adverse symptoms observed in the study, the symptoms are not proof of clinical intolerance to the wheat starch product.

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[6] Chartrand, L.J., P.A. Russo, A.G, DuHaime, et al., "Wheat Starch Intolerance in Patients with Celiac Disease," Journal of the American Dietetic Association, 97(6):612-618, June 1997.

[7] Ciclitira, P.J., R. Cerio, H.J. Ellis, D. Maxton, J.M. Neluferr, J.M. Macartney, "Evaluation of Gliadin-Containing Gluten-Free Product in Coeliac Patients," Human Nutrition - Clinical Nutrition, 39C:303-308, 1985.

The Ciclitira study also was an open challenge study in which 10 adults with celiac disease who had shown improvement while on a gluten-free diet for at least a year consumed six slices daily of home-baked gliadin-containing gluten-free bread for 6 weeks. The subjects recorded any symptoms and graded the severity of those symptoms for purposes of determining a weekly composite symptom score. The mean score value was greater for the 6-week gluten exposure period than for a 6-week control period with no gluten-free bread. The investigators reported that wheat starch-based gluten-free products can cause persistent symptoms in patients with celiac disease. A LOAEL of 4 mg gluten per day, determined from this study, provided weight-of-evidence support for the subchronic LOAEL for clinical effects derived from the Chartrand study.

Several comments stated that we did not give sufficient weight in preparing the safety assessment to a study conducted by Catassi et al. (2007) (the Catassi study).[8] The Catassi study was a double-blind, placebo-controlled challenge trial that initially enrolled 49 celiac disease adult subjects who had been following gluten free diets for at least two years prior to initiation of the study. The subjects were instructed to ingest capsules daily containing 0, 10, or 50 mg of gluten for 90 days and 39 of the subjects completed the study protocol. Small intestine biopsies were performed to determine the morphological effects of exposure to gluten at these levels. No morphological adverse effects were detected at the exposure level of 10 mg per day but the protracted intake of 50 mg of gluten per day produced significant morphological damage. The study concluded that the ingestion of gluten by individuals with celiac disease should be kept lower than 50 mg per day.

We summarize and respond to the comments regarding our use and interpretation of specific studies and data for the safety assessment in Table 2.

[8] Catassi, C., Fabiani, E., Iacono, G., et al., "A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with celiac disease," Am J Clin Nutr, 85:160-6, 2007.

Table 2: Comments Regarding The Use of Studies for the Safety Assessment

Topic	Summary of Comment	FDA Response
The Chartrand study	Several comments criticized our reliance on the Chartrand study, stating that: (1) the study was poorly controlled in that the subjects could have been exposed to other sources of gluten during the study; (2) the investigators did not confirm symptoms; and (3) the investigators should have confirmed clinical effects at a dose that failed to produce morphological effects in other studies. The comments asserted that we did not resolve those differences but instead accepted the lowest exposure from the Chartrand study as the LOAEL.	The comments raise some legitimate criticisms of the Chartrand study. We acknowledged in the safety assessment that the subjects in the Chartrand study were not blinded. However, as we stated in the safety assessment, we considered the results of prospective, open-challenge studies, such as the Chartrand study, because they were available and provided a significant amount of quantitative data to assist in determining levels of toxicological importance with respect to gluten exposure and celiac disease.[9] We selected studies that estimated the LOAEL, or NOAEL when available, as the critical studies in accordance with the safety assessment approach because the goal of the safety assessment approach is to protect the most gluten-sensitive individuals. We identified the Chartrand study in the safety assessment as the critical study for clinical effects from which to derive TDIs because of the LOAEL values identified for acute, subchronic, and chronic exposure durations and because the validity of the reported symptoms associated with acute, subchronic, and chronic LOAELs of 1.5 mg gluten per day is supported by the fact that the onset of adverse clinical effects from a daily gluten challenge emerged after varying lengths of time of exposure for the individual celiac disease patients and the nature of those clinical effects for each subject was consistent over time and resolved after the challenge test terminated.[10] In considering the total body of evidence in the safety assessment, we conducted a weight-of-evidence evaluation because the assessment involved evaluating and comparing studies and data that varied in nature and differed in design and original purpose. The results of additional studies provide weight-of-evidence support for the LOAEL for clinical effects derived from the clinical study.[11] One important component of a weight-of-evidence evaluation is an examination of the findings of the body of studies, taken together, for consistency and biological plausibility of the effect and for evidential support for the likely presence of a direct or systematic relationship. The weight-of-evidence evaluation also includes a determination of the relevance, importance, and contribution of a particular study, such as the Chartrand study, and its findings to the overall body of work. We believe that it was appropriate to use the Chartrand study within the context of the safety assessment approach and to apply weight-of-evidence considerations to employ the use of uncertainty factors to account for variability in subject response and lack of data providing the NOAEL to estimate TDIs for clinical effects for gluten in the most gluten-sensitive individuals. The application of these findings is a risk management function as there are other factors to be considered in establishing a definition for "gluten-free" for labeling purposes.

[9] United States Food and Drug Administration, Center of Food Safety and Applied Nutrition, Office of Food Safety, "Health Hazard Assessment for Gluten Exposure in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten," at 13, May 2011; accessible at: http://www.fda.gov/downloads/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/UCM264152.pdf.

[10] Id. at 33.

[11] Id. at 32.

Topic	Summary of Comment	FDA Response
Analytical methodologies and the gluten content of food used in the Chartrand study	Some comments argued that the analytical method used in the Chartrand study to measure the gluten content of test food administered to the study subjects was not validated, thereby raising questions about the accuracy of the level of gluten consumed by the study subjects. Another comment stated that clinical findings in human studies of this nature will depend, in large part, on the ability to accurately determine gluten content in test foods. The comment noted that rapid methods are available today to determine residual gluten in foods and also noted that such methods have evolved significantly over the past several years. The comment asserted that the newer technologies call into question the accuracy of some data generated in past clinical studies and the need to factor in complexities of species-to-species variations and extrapolations.	The comments correctly raise the question of the importance of the analytical accuracy of methods used to determine levels of gluten in test foods. While validation is important, the issue of a method's analytical accuracy is of greater concern. The only formally recognized method available in 1997 for detecting gluten in food was AOAC method of analysis 991.19, which was a sandwich enzyme-linked immunosorbent assay (ELISA) validated to a sensitivity of 160 ppm. At the time, the effects of fermentation, hydrolysis, or covalent modification of proteins in grains on gluten detection and celiac disease were not fully understood. In addition, detailed cross-reactivity studies demonstrating the inadequacy of AOAC method 991.19 to recognize barley were not yet done. Accordingly, we are unable to evaluate the accuracy of the method used to determine the gluten content in the food samples used in the Chartrand study or any conclusions made based on the method used. In recent years, research has focused on developing analytical methods to detect all grains that might contain gluten, detecting lower concentrations of gluten (i.e., 20 ppm), and on developing methods to address the problems associated with protein hydrolysis and modification.

Topic	Summary of Comment	FDA Response
Hazard characterization, the Chartrand, Ciclitira, and other studies	One comment stated that the quality criteria used in the safety assessment are unclear because the assessment omitted some key studies without explanation. The comment questioned the suitability for hazard characterization of the Chartrand and Ciclitira studies. The comment argued that the Chartrand study was not blinded, the choice of the control group was inappropriate, and participants self-reported their symptoms. The comment asserted that we should consider the results of this study in context of other arguably better studies, e.g., a study conducted by Kaukinen et al (1999) (the Kaukinen study).[12] The comment noted that other expert and research groups have reviewed the scientific literature available on the dose-response relationship between gluten intake and symptoms of celiac disease, but did not consider the Ciclitira and Chartrand studies to be pivotal studies for hazard characterization.	We conducted the safety assessment following the principles of the health hazard assessment approach and the procedures and scientific judgments typical of this type of evaluation. This includes weight-of-evidence considerations and determinations as discussed elsewhere in this document. The goal of the assessment was to identify the overall NOAEL and/or LOAEL from the available data that best reflects the margin between no and lowest adverse effect levels. We evaluated all available published literature that included dose-response data on the adverse health effects (clinical symptoms and morphological adverse effects) of gluten (or toxic protein derivatives of gluten) in individuals with celiac disease. We also used weight-of-evidence evaluations, which is common practice in the execution of assessments of this nature. The studies we evaluated were primarily food challenge tests given to the gluten-sensitive subpopulation. The gluten was the test food and was administered orally, for example in the form of capsules or as a constituent of a food product. Almost all food challenge studies we evaluated were open challenge tests. Although the open studies were not blinded, the majority of these studies collected pre-challenge data with the subjects on a gluten-free diet that served as a baseline. Some studies also collected post-challenge data after the subjects had returned to a gluten-free diet. These two study designs allowed the subjects to serve as their own controls, which can be of value because subject responses to gluten tend to be individualistic in nature. From the open challenge and available single- and double-blind studies reviewed, we collected quantitative data to help determine TDIs of gluten in gluten-sensitive individuals. We selected the Chartrand study as a critical study because, in our view, it provided the best clinical data on adverse responses to a gluten challenge from which to determine a TDI for gluten. We also characterized the Ciclitira study as a critical study because it identified a subchronic low-dose gluten content (i.e., from the low gluten content in gluten-free bread) that caused some adverse clinical symptoms in patients with celiac disease but no adverse morphological effects. From this data, we were able to derive a NOAEL for gluten. The Kaukinen study is not a dose-response study, but rather is a cross-sectional study in which subjects with celiac disease were surveyed over a period of a year for their intake of gluten and wheat starch. Their dietary gluten intake was evaluated and followed-up with small-bowel biopsy to evaluate the effects of their diet. Results from studies like this can provide insight into long-term effects from gluten exposure, but their role in evaluating a dose-response to gluten remains limited.

[12] Kaukinen, K., P. Collin, K. Holm, et al., "Wheat Starch-Containing Gluten-Free Flour Products in the Treatment of Coeliac Disease and Dermatitis Herpetiformis. A Long Term Follow-Up Study," Scand J of Gastroenterol, 34:163-169, 1999.

Topic	Summary of Comment	FDA Response
Use of uncertainty factors and the Chartrand study	One comment said that we used a standard safety assessment approach to identify the NOAEL and LOAEL and then applied standard uncertainty factors. The comment noted that we applied a ten-fold uncertainty factor to the LOAEL estimated in the Chartrand study to obtain the NOAEL, but said that such a value could not be determined from the study. The comment argued that, while use of ten-fold uncertainty factor is relatively standard when only the LOAEL is available, the level of gluten representing the LOAEL was not confirmed in subsequent studies. Therefore, the comment said, the absence of such a confirmation could justify the use of no uncertainty factor or at least a much smaller one. The comment also asserted that we used a highly conservative approach when we applied another ten-fold uncertainty factor to account for possible differences among a subset of the human population (sensitive subjects with celiac disease). Again, the comment said that we should have considered use of a much smaller uncertainty factor.	Uncertainty, as it pertains to safety or risk assessments, refers to the inability to know with certainty the level of a particular agent that will not cause adverse health effects, typically because of a lack of relevant data. There was insufficient data in the dose-response studies reviewed in the safety assessment from which to develop a dose-response curve that would have provided meaningful information, such as extrapolation of an uncertainty factor that reflects the change in response to incremental changes in gluten exposure. In the peer review document associated with the safety assessment, we noted that one study (Laurin et al 2002[13]) showed a plot of dose-response data that suggests a steep slope and thus support the use of a 10-fold uncertainty factor.[14] Finally, a safety assessment approach typically involves the derivation of point estimates of "safe" levels of exposure to a toxic agent and is reflective of the overall NOAEL and/or LOAEL exhibited after exposure to the agent. We believe that an assumed default value of a 10-fold uncertainty factor is reasonable because of the lack of sufficient information on the shape of the dose-response curve.

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

[14] United States Food and Drug Administration, Center of Food Safety and Applied Nutrition, Office of Food Safety, "External Peer Review of the FDA/CFSAN Draft Health Hazard Assessment for Gluten Exposure in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten," at 22, December 2010; available at: http://www.fda.gov/downloads/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/UCM264150.pdf%20accessed%20on%20September%2010,%202012.

The Catassi study	One comment stated that the Catassi study is a pivotal study and its diagnostic pathology criteria are well accepted in the scientific community. The comment stated that the overall scientific literature supports the view that adults may be more responsive to gluten adverse effects than children. The comment asserted that a TDI of < 10 mg per day of gluten is a safe limit for most celiac individuals and a gluten threshold of < 20 ppm would allow for 500 g of gluten-free food per day. Another comment described the Catassi study favorably in the context of stating that we should use evidence-based science to establish a safety level for gluten, with safe limits established through double-blind, placebo-controlled studies. The comment stated that although one can measure gluten to 5 ppm, it does not mean that we should determine safety based on the sensitivity of the analytical assay. The comment also asserted that there are no evidence-based studies that demonstrate toxicity with exposure to 20 ppm gluten or safety with 5 ppm exposure. Another comment stated that the Codex Alimentarius Commission established < 20 ppm gluten as the recommended guideline for gluten-free labeling and relied heavily on the Catassi study. The comment described the Catassi study as a well-designed study that evaluated very sensitive morphological endpoints (median villous height/crypt depth, intraepithelial lymphocyte count). The comment observed that the Codex Alimentarius Commission used the Catassi study without applying uncertainty factors and stated that this is appropriate because the subjects experienced only morphological alterations and not symptomatic changes with intakes up to 50 mg per day. The comment stated that we reported that the Catassi study may not have involved the most sensitive celiac patients because we had said that subjects were excluded if they had morphological damage on the pre-challenge evaluation. The comment asserted that this factor only means that the subjects in the Catassi study had well controlled celiac disease before the study began and could have included patients with varying degrees of gluten sensitivity.	We agree with these comments' factual descriptions of the Catassi study. However, we disagree that the Catassi study should be considered a pivotal or critical study or given greater weight than other studies. We considered the results of the Catassi study in the safety assessment, but it is considered a supporting study for several reasons. These reasons include the fact that the study might not have included the most gluten-sensitive individuals, given that subjects with any initial mucosal abnormalities at the time of the gluten challenge were excluded from the study and given the number of subjects who dropped out of the study because of adverse responses to the challenge dose. The Catassi study also administered gluten to subjects orally in a capsule which was noted as a factor in the criteria used to evaluate studies. We note that the comment mischaracterizes the Codex Alimentarius Commission's gluten level guidelines. The Codex Alimentarius Commission's standard states that the gluten level may not exceed 20 ppm for purposes of "gluten-free" labeling, as opposed to establishing a limit of <20 ppm. We considered the Catassi study as a supporting study as part of the weight-of-evidence in our safety assessment. As we have noted elsewhere in this document, we believe it was appropriate to give greater weight to available open challenge studies in the safety assessment in part because very little data is available from single- or double-blind, placebo-controlled studies, as evidenced by the Catassi study. We concluded that a greater reliance on the open challenge studies for the purpose of establishing TDIs is reasonable and provides insight into the health challenges of highly-sensitive individuals with celiac disease because there are some who may experience adverse effects after consuming foods containing more than 0.01 ppm gluten, but less than 20 ppm gluten. We agree in part with the comment regarding the use of analytical methods in establishing regulatory definitions of safety. As we discussed in the proposed rule and the 2011 notice, our decision in defining the term "gluten-free" involved consideration not only of the availability of validated analytical methods but other factors, such as 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, in addition to the results of the safety assessment. Any future changes to the level of gluten allowed in food that is voluntarily labeled as "gluten-free" should be supported by scientific evidence about new analytical methods, as well as updated epidemiological and clinical data about the effects of exposure to levels of gluten by individuals with celiac disease. We believe that additional data are needed from long-term trials with a large group of gluten-sensitive subjects to confirm the level of gluten that would be safe for all gluten-sensitive individuals, including the most sensitive sub-group, and to evaluate sensitivity in children as well as in adults.

Topic	Summary of Comment	FDA Response
Variability, uncertainty, and the Catassi study	One comment discussed the sources of variability and uncertainty in defining a threshold of toxicity for gluten. These sources of variability and uncertainty include the fact that the relatively short time frames of administration of gluten in studies may not extrapolate to a lifelong exposure, and those who are the most sensitive to gluten are the least likely to meet enrollment criteria for an effective study. The comment cautioned against over-interpreting the results of the Catassi study because of the study's small sample size and warned of the dangers of trying to extrapolate from such a small study to the entire population of gluten sensitive individuals. The comment noted that, in one arm of the study, 13 patients consuming 50 mg per day gluten exhibited a statistically significant degree of mucosal damage, but without overt clinical symptoms. The comment questioned what the margin of safety should be for such patients to prevent such damage. The comment stated that morphological changes were observed in a few patients consuming 10 mg per day gluten but those changes did not reach statistical significance. The comment suggested that it is important to take such individual responses into consideration because they may reflect real differences in gluten sensitivity.	We agree that one limitation of the Catassi study is the small number of subjects to reach statistically significant effects. We considered the results of the Catassi study as part of the body of evidence in the safety assessment, but we did not select it as the critical study from which to derive a TDI. The open challenge studies evaluated and considered in the safety assessment examined the individual responses of challenged subjects which the commenter noted are "important to take . . . into consideration."