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

Peer Review Report

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PEER REVIEW REPORT

I. Introduction

The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA)[1] requires the Food and Drug Administration (FDA) to develop and issue a rule that defines the term "gluten-free" on the labeling of foods and that delineates the conditions for its voluntary use in labeling to provide information to individuals with CD (CD) (P.L. 108-282, Section 202). In the FEDERAL REGISTER of January 23, 2007 (72 FR 2795), FDA proposed a regulation to establish a definition for gluten-free labeling for food. The proposal also indicated that this rulemaking would include FDA conducting a safety evaluation for gluten exposure in individuals with CD that encompassed a safety assessment-based approach[2]. FDA has completed a health hazard assessment for gluten exposure in the subpopulation of individuals with CD that includes a safety assessment for gluten. The FDA document is titled "Health Hazard Assessment for Gluten Exposure in Individuals with Celiac Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten[3]." This hazard/risk assessment subsequently underwent a scientific evaluation and critique by an external peer review panel of experts.

II. Peer Review Charge and Questions

In August 2008, FDA contracted Versar, Inc. to organize and conduct an external peer review of its draft document "Health Hazard Assessment for Gluten Exposure in Individuals with Celiac

[1] Information on abbreviations or acronyms used in this report is provided for and corresponds to the text in the report written by the FDA. The written responses and comments of the peer reviewers were not altered to provide this information in their text.
[2] This approach is addressed in the FDA report entitled "Approaches to Establish Thresholds for Major Food Allergens and for Gluten in Food (March 2006)."
[3] The name of the draft document at the time of the peer review process was "Safety Assessment of Gluten Exposure (April, 2008)."

Disease: Determination of Tolerable Daily Intake Levels and Levels of Concern for Gluten (HHA)." The independent expert peer reviewers were selected by Versar, Inc. and also deemed by Versar to have no conflicts of interest (see Section IV below).

The goal of the peer review was to provide FDA with a comprehensive appraisal of and feedback on the nature of the approach taken, the methodology used, and the judgments made in characterizing and assessing the hazards of gluten exposure in individuals with CD in the HHA[4]. The peer reviewers were first asked to evaluate and comment in a general way about the scientific basis and quality of the FDA HHA document (see "General Impressions" Section III, Part A below). Second, they were asked to respond to a list of specific charge questions that addressed various aspects of and the use of available scientific evidence within the context of the hazard/safety assessment, along with the weight-of-evidence, approaches employed in the HHA (see "Peer Reviewer Response to Charge Questions" in Section III, Part B below). Finally, the peer reviewers were asked to provide any additional comments, feedback or scientific information they had that would inform the HHA and assist FDA in refining the gluten assessment (see "Specific Observations" Section III, Part C).

III. Peer Reviewer Comments and FDA Response

Each peer reviewer's feedback and responses to the specific charge questions are provided below verbatim without attribution to the specific reviewer[5,6]. FDA considered and used this information to edit, clarify, supplement and improve the resulting final HHA document. FDA responded and/or commented in reply to the peer reviewers in instances when doing so was deemed warranted and appropriate but responses/comments were not provided in all instances.

A. General Impressions

Reviewer #1

I was impressed by this safety assessment, which involved many challenges. The authors should be commended for the transparency of the process they followed and for their open discussion of the assumptions they made, the limitations of their assessment, and outstanding issues for consideration. For the most part, the presentation is clear, although, as noted in responses to the charge questions, I do think that there are some specific issues that need to be addressed more explicitly, the most important of which are how the critical study was selected for each gluten exposure scenario and each class of outcomes.

Reviewer #2

I think the FDA scientists have done an admirable job of assessing the available literature on gluten response in CD. The information is accurate and although the presentation is necessarily legalistic at times, I would say that, for the most part, the presentation is clear. However, I do not understand how the authors derived the gluten levels of concern and their relationship to

[4] The focus of the charge given to the peer reviewers was to review of the nature of the scientific basis of the HHA. The reviewers were not to provide comment or advice on policy related to the use of the results of the HHA by FDA.
[5] To assist with delineation of the source of the different parts of this peer review report, the verbatim written feedback and responses of the peer reviewers are denoted in a different font type from the parts of the report written by the FDA.
[6] In some cases, the peer reviewers' feedback and responses were separated into parts by demarcations added by the FDA in this report subsequent to the peer review work because it allowed for the FDA comments/responses in this report to more clearly correspond to specific parts of the reviewers' written material.

consumption of wheat gluten foods. The soundness of the conclusions is difficult to assess because, as the authors of this position paper point out, the literature is extensive, yet inadequate. In my opinion, the most valid papers for consideration in relation to acceptable levels of gluten in foods are the papers by Catassi et al. 2007. Am J Clin Nutr 85:160-166 (note error in page citation in FDA draft) and the paper of Peraaho et al. 2003, Aliment Pharmacol Ther 17:587-594. These studies are impressive because this is such a difficult area of research and funding for truly adequate studies is virtually impossible to achieve. Nevertheless, the number of patients, 49 in Catassi et al. and 65 in Peraaho et al. is still rather small. I have a concern that there is no characterization of the of the gluten used in the study presented in this paper (no SDS-PAGE pattern, no HPLC pattern). In fact, I don't think there is any paper reviewed in the survey that shows adequate characterization of the gluten/gliadin samples used. Yet, because we do not have any really solid information about how toxicity of gluten proteins and peptides varies among the major gluten protein types (for example, alpha-gliadins vs gamma-gliadins vs omega-gliadins), characterization of the gluten/gliadin/peptide fraction used should be considered essential when studies involving feeding, instillation, or other in vitro studies are carried out. The problem is least serious when whole gluten was used, more of a problem when the challenge was with gliadin and a serious problem when Frazier Fraction III (FF3) or, presumed equivalent, peptic-tryptic digests are used. There has never been any proper molecular characterization of FF3 that I am aware of. The final mixture of peptides, in my opinion, is probably is different in every study, because medical people are not usually highly familiar with enzyme digestions of proteins. Without gel electrophoresis, or HPLC characterization of the mixtures, who knows what the investigators are testing? Good preparations may be much more toxic than gliadin or gluten because of the tendency to dissolve certain toxic peptides in the procedures more readily than less toxic sequences. In other words, there is a major disconnect between medical/physician researchers and protein characterization when it comes to patient or in vitro evaluation of toxicity. I will continue to point out such problems throughout my comments. Nevertheless, I would say that the Catassi et al. study is a valuable one and I would give it considerable weight even though it would be highly desirable to extend such studies to more patients and to different groups of patients from different geographical locations. I suggest that if Carlo Catassi and Pekka Collin (corresponding author of Peraaho et al.) could be brought together in a collaboration with adequate funding, we might actually get somewhere with this problem.

I will say that, personally, I somewhat favor making the FDA limits compatible with what the Codex Alimentarius is planning to approve (perhaps has already approved), that is, a 20 ppm level for intrinsically gluten-free foods, and a 100 ppm level for wheat starches. There may be some patients who cannot tolerate those levels, but I think such patients are very few, and if the FDA goes lower, it will be difficult to achieve lower levels in products and difficult to test them. And the USA will end up being in some conflict with the Europeans.

Reviewer #3

Overall, I agree with the values established for the TDIs and LOCs. The rational and approach (including UFs) used in the assessment are, for the most part, adequately discussed and justified. I would have like to seen a more developed discussion of the strengths and weaknesses of the clinical studies used in the assessment. The major strength of these data is that similar LOAELs were observed in multiple studies. The apparent weaknesses of the data are: that the number of subjects evaluated in any one study was small; few, if any, of the studies challenged with more

than one dose level and if a study used multiple doses, there is no discussion on the shape of the dose-response curve; the exposure designs varied – different vehicles/methods for exposure; there was no standard read-out (different clinical symptoms, even different histopath criteria) and there is no discussion on the potential of false negatives due to allowing insufficient time for a response to occur (time to response varies between individuals). Again, while I do not think the values that were derived would change, it might be useful to include a more detailed discussion on the strengths and weaknesses of the data set. It would also be useful to provide the rationale for a 10-fold intra-individual UF. It might be worth noting that if one was considering development of gluten sensitization then considerable more intra-individual variability might exist and probably necessitate a higher UF. For example, in addition to the usual age, gender, ethnicity issues associated with autoimmmune diseases, the need for a higher UF would be necessary to account for HL-A (risk alleles) and IgA deficiency. Individuals with the disease-associated HL-A alleles (HL-A DQ2 and DQ8) can have a 5 to 10-fold higher risk than those that do not (in fact I think there are data suggesting these alleles are a prerequisite for development of CD), while children with IgA deficiency may have up to a 10-fold higher risk. IgA deficiency is the most common form of primary immunodeficiency disease, effecting 1 in 700 children of European descent. Again, from the standpoint of existing disease (those already sensitized), I do not think these factors would have a significant impact on the assessment but it is worth discussing.

Reviewer #4

The FDA draft "Safety Assessment of Gluten Exposure," dated July 2008, is well written, carefully undertaken and, when considered as a whole, makes sound assumptions and takes a cautious approach to estimating the levels of gluten that are likely to be safe or not for people affected by CD. The assumptions and safety projections are largely aimed at the most sensitive patients with CD. The extrapolations that are made are based on the limited data available to estimate the NOAEL and LOAEL. I believe that this approach, which includes uncertainty issues of patient variability and factors such as that most data is based on wheat gluten not the prolamins of barley or rye, is justified given that the labeling as "Gluten-Free" should be safe for the vast majority of celiac patients. The ultimate aim of determining what is a safe level of contamination of foods labeled "gluten-free" is to provide patients (consumers of "gluten-free" foods) a level of assurance that they will not be exposed to an amount of gluten that will produce the following effects, listed below, when gluten containing foods are replaced in their entirety with "gluten-free" foods:

a. adverse symptoms; and
b. adverse damage to their bodies even in the absence of symptoms (which will protect them against long-term adverse consequences or complications of such gluten exposure).

Some discussion on the potential risk that subjects with wheat or even other grain allergies may try to rely on this "gluten-free" labeling as a way to avoid exposure to allergens contained with these grains, but not necessarily just the "gluten" proteins, is an issue of risk inherent in the "gluten-free" labeling process that implies that the source grains (rendered gluten-free) could be included in the foods.

The other issue will be a placebo effect. When individuals are aware of the possibility for tiny amounts of gluten to be present, they may anticipate an effect of the ingestion and hence may avoid “gluten-free” labeled foods if any detectable gluten is present or permitted to be present.

Ultimately, how the terms “gluten-free” or “low gluten” or even “ultra-low gluten” are used will be crucial in the utility that patients will have in improving their health and quality of living.

I think if the safety assessment is used as the basis for the "gluten-free label" then the most cautious and conservative approach to safety should and has been followed in the safety statement and hopefully would be applied in the labeling regulations. But I understand that this is an Agency issue.

Reviewer #5

This document is a nice review of the literature on threshold levels of tolerance for gluten. However, it does not appear to be a critical review of the evidence. It is unclear how critical low dose studies were chosen as they do not appear to have been chosen based on quality. Each study reviewed should have received a quality rating based on standard quality criteria. Quality criteria used, as well as the reports on each study, should be available for review. That the Ciclitira study, “Evaluation of a gliadin-containing gluten free product in celiac patients,” and the Chartrand study, ”Wheat starch intolerance in patients with CD,” were chosen as critical low dose studies calls into question the soundness of this report. That the subchronic TDI is so much lower than the chronic TDI should raise a red flag.

It is also concerning that only one study (and not a high quality study) was used to estimate the TDI for gluten for clinical effects. The Chartrand study was not blinded. Participant reporting of symptoms is subjective. Even though participants acted as their own controls, they knew they were consuming a product (i.e., wheat starch) that could cause gastrointestinal distress. Also concerning is the use of the Ciclitira study instead of the Catassi study, “A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with CD,” to estimate the TDI for morphological effects of subchronic exposure to gluten. The reason given for using the Ciclitira study was that the Catassi study may have excluded the most sensitive patients. Whether or not this is true, the Ciclitira study also may have excluded the most sensitive patients as it included, “ten adult celiac out-patients who had **clinically improved on treatment with a gluten-free diet** for at least a year.” The Catassi study is of higher quality than the Ciclitira study—it was for a longer duration, involved more study participants, used measured amounts of gluten, and was double-blind and placebo-controlled.

It is also unclear how (why) a NOEAL **only** would be used to determine a TDI in several instances in this report. For example, a NOEAL of 4mg/d is listed for subchronic exposure to gluten (morphological effects). No LOEAL is listed even though data is available from the Catassi study. Why is the NOEAL alone used to determine this TDI? The NOEAL is supposed to represent the largest amount of exposure observed to have no effect using reliable experimental data. Ideally we would be working from a NOEAL that was fairly close to a LOEAL.

Reviewer #6

The background information provided in the work assignment from Versar indicates that the results of the gluten safety assessment will be used by FDA to define "gluten-free" for voluntary use in the labeling of foods. From a consumer's perspective, the term "gluten-free" would likely be interpreted as "risk free." As such, the LOC derived based on this safety assessment will need to be interpreted as a threshold level below which no adverse reaction from gluten is expected. As one of the first safety assessments to establish a threshold for allergenicity, this document is precedent setting. Therefore, it is critical that the supporting evidence is adequately robust, the assessment approach is scientifically sound and data presentation is systematic so that a convincing scientific argument for threshold can be made. Unfortunately, as currently written, the report does not provide compelling support for an existence of threshold for gluten for CD individuals. Throughout the report, there are suggestions of the need for more uncertainty factors to account for variability in response, timing of response, and that the UF used in the current assessment may not be adequate. The lack of robust data to develop dose response for chronic low level of exposure to gluten is of concern. The weight-of-evidence that was reportedly undertaken by the Agency was not clearly summarized and does not convince readers that it was systematically undertaken. The report appears to be in its infancy draft state, as evidenced by the lack of table of contents, executive summary, and robust introduction. Additionally, tables are in the wrong order and not properly formatted, important scientific data are in footnotes (e.g., conversion of gliadin to gluten), incorrect terminology is used (e.g., intra-individual variability), etc…These deficiencies compromise the scientific integrity of the report and make the peer review challenging. There is also an issue regarding the purpose of this safety assessment. While the statement of purpose (page 2, lines 1-2) indicates that this "is a safety assessment of gluten exposure in a sensitive sub-population group, specifically individuals with CD (CD)," under the hazard assessment section (page 7, lines 1-7) it is stated that a health hazard assessment was performed to "determine a TDI of gluten in individuals susceptible to its adverse effects" and that the "adverse effect that was the primary focus of the assessment was CD." Lines 6-7 on page 7 further indicate that "because CD only occurs in sensitive individuals, this evaluation examines the effects of gluten on this sensitive sub-population." The "sensitive sub-population" in whom CD occurs was not defined. These inconsistent statements of purpose also compromise the integrity of this safety assessment. It is highly recommended that the report is edited and revised into a proper scientific document, including a detailed methodology section describing the literature search conducted (terms/search engine), summary of the available data (Appendix A is not readable and needs to be in tabular format so that comparison can be made across studies), method of reclassifying subjects (based on duration of exposure of interest), gluten dose conversion methods, etc… Specific comments are provided below under the Specific Observations section.

B. Peer Reviewer Response to Charge Questions

Charge Statement 1. **The adverse effects associated with gluten challenges in individuals with CD (CD) were delineated into clinical effects and morphological and/or physiological effects and assessed separately. Accordingly, No Observe Adverse Effect Level (NOAEL) and/or Lowest Observe Adverse Effect Level (LOAEL) values for each category of adverse effect were identified and evaluated separately.**

Charge Question 1a. **Is this recognition of these different types of adverse effects and their separate consideration a valid approach in assessing the resulting adverse responses associated with gluten exposure in CD?**

Reviewer #1
Yes, the substantial differences between the NOAELs/LOAELs for morphological/physiological and clinical effects suggests that the distinction between these categories of adverse effects must be maintained, at least at this stage of the safety assessment.

Reviewer #2
I do not think that clinical effects (such as abdominal discomfort) in the absence of morphological observations based on biopsy and physiological changes (such as antigen levels) can be given much credence. Because it is possible that clinical effects can be compounded by bacteria or viral infections, by psychological effects, and allergic reactions, I do not have much confidence in studies that are based only on clinical effects.

Reviewer #3
Yes, both morphological and clinical effects should be considered, as both can be adverse. Although clinical results, particularly when different studies are grouped together, would have less weight (particularly non-specific clinical indicators) than morphological effects, the clinical effects can occur at lower doses than morphological changes. Thus, I think it is appropriate to discuss them separately but establish the TDI on the most sensitive endpoint (clinical).

Reviewer #4
Yes. It is well recognized that patient reported symptoms may underestimate the degree of damage that can be ongoing in the intestine. Recent studies that recruited patients supposedly on the strict GF diet demonstrated a high frequency of injury suggestive of incomplete adherence or inadvertent intake of gluten, which may or may not be a cause of ongoing symptoms (Abdulkarim, 2002; Pyle, 2005). Therefore, it is crucial to consider both the symptomatic based (clinical) response and the morphological/physiological effects. However, the data for physiological effects are quite limited.

Abdulkarim AS. Burgart LJ. See J. Murray JA. Etiology of nonresponsive CD: results of a systematic approach. [Journal Article. Research Support, U.S. Gov't, P.H.S.] American Journal of Gastroenterology. 97(8):2016-21, 2002.

(1a.1)[7] Pyle GG. Paaso B. Anderson BE. Allen D. Marti T. Khosla C. Gray GM. Low-dose gluten challenge in celiac sprue: malabsorptive and antibody responses.[see comment]. [Journal Article] Clinical Gastroenterology & Hepatology. 3(7):679-86, 2005 Jul. UI: 16206501

FDA Response:
Part 1a.1
The findings of the Pyle et al. (2005) study was reviewed and included in the dose-response data summarized in the HHA.

Reviewer #5
Yes, this is a valid approach. However, using studies that were neither single nor double blind to draw conclusions about TDIs for clinical effects is very weak evidence. The strength of the conclusions (based on evidence analysis) should be discussed at length in this report.

Reviewer #6
Given the complex and multidimensional nature of associated health outcomes (immune-mediated enteropathy and abnormal morphology and an array of clinical signs and symptom) in individuals with CD, it is reasonable to separate the different types of adverse effects and develop separate dose responses for these effects.

Charge Question 1b. **Are the criteria used to define or characterize adverse responses to gluten as clinical and morphological toxic endpoints in this safety assessment reasonable and valid?**

Reviewer #1
Yes.

Reviewer #2
I think morphological endpoints, such as villus height to crypt depth ratios and IEL levels, are valid. I don't think that clinical endpoints are always valid.

Reviewer #3
The indicators are not what I would consider 'a validated test' but certainly are reasonable indicators. In using clinical data, I would think one would give more weight to overt physiological changes as a valid indicator for an adverse response vs. non-specific symptoms such as abdominal pain. One should note that even these physiological indicators could be misleading. In studies of children involving desensitization for peanut

[7] This demarcation of a section or part of a reviewer's written response or comments denoted by italic parentheses in a font different from the surrounding text was inserted by the FDA to delineate the part of the reviewer's statement to which the subsequent FDA response presented below it corresponds. The demarcations within the parentheses represent the charge question number and possibly question subpart, followed by the numbered FDA response part (e.g., Response 1a.1). In others words, these demarcations were not in the original written statements provided by the peer reviewers, but were inserted by the FDA for organizational purposes.

allergy, it has been noted that some children, even after successful desensitization, develop adverse clinical symptoms when they knowingly eat peanuts. Regarding biopsies of the small intestine, while this seems more straight forward and there is considerable discussion of criteria for establishing CD (degree of villous atrophy), it is not clear that it is any more valid than measuring physiological symptoms from the standpoint of a challenge test for use in a risk assessment.

Reviewer #4
Yes. See above in Q1a. Frequency and amount of gluten are probably both important in determining toxicity, as are many other factors such as: age, genetic background, type of gluten (hexaploid vs. tetraploid), processing, healing of the intestine and stage of treatment (how long the patient has been on a gluten-free diet), and patient awareness of the symptoms.

Reviewer #5
They are valid. However, clinical effects should be reported from single and double blind studies only. If other studies are used, the evidence should be classified as weak.

Reviewer #6
No specific comment on the validity of the criteria used (not an expertise area of this reviewer).

Other comments:

(1b.1) One of the main challenges in reviewing this report is that CD is used to define both the sensitive sub-population and the outcome of interest. It was not until page 12, lines 24-33 of the report that it is made clear to the reader that because the subjects of the various studies evaluated in this hazard analysis were diagnosed with CD prior to their study, that the subsequent phases of pathogenesis elicited by these subjects upon a gluten test challenge were assessed as significant adverse effects with respect to the development (progression?) of CD. This specific definition of adverse effects that is the focus of the safety assessment should be made explicit in the introduction of the report.

(1b.2) The changing criteria for morphological effects were well described on pages 11-12 of the report. However, it is entirely unclear how FDA incorporated the changes in effect classification when reviewing and applying a weight-of-evidence approach to select the critical study for morphological dose response assessment.

FDA Response:
Part 1b.1
The introductory section of the document was an overall examination and assessment of the health hazards associated with and that characterize CD. It served to identify the health hazards and the toxic substance(s) that play a significant role in the development of the adverse CD effects. Some changes have been made to the title and introduction of and to the outline and organization of the document text to help clarify the focus and aims of the document for the reader as he/she begins reading it. In addition, the paragraph that begins the health hazard assessment section has been supplemented to

try to improve the reader's general understanding of the CD-associated adverse effects examined in this assessment at an earlier point in the document. However, because the available dose-response adverse effects data are very complicated and multi-faceted, we feel the detailed specifics of the definition of these effects for the safety assessment for gluten exposure in CD performed here is best described in the sections that address the specifics of the hazard and safety assessment.

Part 1b.2

As indicated in the main document, gluten-induced adverse effects in the small intestine morphology were assessed in each study by examining the various range of measures of aberrant morphological changes such as intraepithelial lymphocyte (IEL) count, villous height (Vh), crypt depth (Cd), Vh/Cd ratio, along with noted descriptions of enteropathic histopathological characteristics or grades, that were presented in study results (see subsection now under the subheading of "Basis of the Evaluation and Determination of Adverse Morphological Effects" in the "Dose-Response Assessment" section). The weight-of-evidence considerations made in evaluating these measures included analysis of whether the changes exhibited across the different individual morphological measures (e.g., IEL count, Vh, Cd) and/or enteropathic histopathological characteristics were reflective of and consistent with the adverse morphological effects that characterize CD. An example this type of weight-of-evidence consideration is the occurrence of increases in IEL levels in the small intestine were accompanied by decreases in Vh in contrast to an increases in Vh. The tables in Appendix B of the HHA that summarize the low-dose exposure data for morphological and/or physiological adverse effects extensively depict in the "Type of Adverse Effects" column the information from each study that was evaluated by FDA. This information is additionally described in the discussion of the each "critical" and "supporting" study in the "Safety Assessment" section. Also as indicated in the main FDA document and in Appendix B, aberrant changes in various physiological measures such as antibody levels, intestinal absorption and/or permeability and fecal fat content were examined to determine if they were consistent with and support the morphological changes found with a gluten challenge within a particular study, or possibly at a particular dose level. Finally, after the evaluation of key low-dose studies as described above and their subsequent comparison, the "critical" morphological study selected was based on the principles of the safety assessment approach.

***Charge Question 1c*. Is examining findings across different studies and employing weight-of-evidence considerations to assess and support the critical low dose study chosen a meaningful approach in selecting the critical study for each type of adverse effect?**

Reviewer #1

I endorse the application of a weight-of-evidence approach to consider all of the data available for a safety assessment, but this question asks whether such an approach is a meaningful way to "select the critical study for each type of adverse effect." To me, it is not a meaningful way to choose the critical study. The critical study should be selected based on a clear criteria established for evaluating the quality and applicability of a study to the issue under consideration. *(1c.1)* Pertinent to this, I think that a more explicit description of the criteria used to choose one study as the critical study and the others as supportive (or contributing to the weight-of-evidence) would be desirable. It is not always clear how the decisions were made in this assessment. There is useful general

material in the section "Dose-Response Assessment" (p.7-8) about the hierarchy of evidence, but detail on the application of the principles described in this section is generally lacking in the sections on specific adverse effects (i.e., "Tolerable Daily Intakes" section beginning on p.13). It is also stated that the weight given to a particular study "depended on the quality of the data" (p.9), but no indication is provided of what criteria were used to evaluate data quality. P.13 indicates that "the studies with the most significant low dose-response data…were identified." Again, it is not clear what "significant" means in this context and should be clarified. Is it the study with the lowest LOAEL/NOAEL or the study for which the P-value is most extreme?

FDA Response:
Part 1c.1
The "critical study" selected is based on the principles of the safety assessment approach, and the procedures and scientific judgments typical of this type of evaluation. The aim of the safety assessment is to identify the lowest level(s) of adverse response sensitivity. This translates to the identifying the overall NOAEL and/or LOAEL from the available data that best reflects the margin between no and lowest adverse effect levels. The study that characterizes the lower limits of this response sensitivity is termed the "critical study" in this approach and its associated low-dose values, the critical NOAEL and/or LOAEL. Additional information on criteria for study characteristics and weight-of-evidence evaluation has been included in the main text of the HHA and used to supplement discussion on the basis of the evaluation and determination of adverse morphological effects, adverse clinical effects and the weigh-of-evidence. In addition, the specific details and characteristics of the findings of each low dose-response study of relevance were presented in Table 1-3 and 5-7 of Appendix B (see columns labeled "Type of Adverse Effects" and "Other Information"). They included this information on the findings for each study that reflected these criteria. Last, feedback on the use of the term "significant" within the context of a safety assessment is presented in this report, along with the associated changes made, in FDA Response, Part 6 to Reviewer #5 and FDA Response, Part 4 to Reviewer #6, both under the "Specific Observations" section.

Reviewer #2
(1c.1) I think that most of the studies, other than Catassi et al. (2007) (with backup information from previous studies by Catassi and coworkers) and Peraaho et al. (2003), have serious flaws in them, or else have not been reproduced by other laboratories in order to warrant high confidence (e. g., Lavo et al. 1990, although the results do warrant further investigation).

FDA Response:
Part 1c.1
The hazard/safety assessment approach that includes evaluating all available dose-response data was employed, along with weight-of-evidence considerations, the latter of which is a common component of safety/risk assessments of this nature. The Catassi et al. (2007)[8] study was used in this assessment as part of the weight-of-evidence as a

[8] Catassi C, Fabiani E, Iacono G, D'Agate C, Francavilla R, Biagi F, Volta U, Accomando S, Picarelli A, De Vitis I, Pianelli G, Gesuita R, Carle F, Mandolesi A, Bearzi I, Fasano A. A prospective, double-blind, placebo-controlled trial to establish a safe gluten threshold for patients with CD. Am J Clin Nutr 85: 160-166, 2007

supporting study. The Peraaho et al. (2003)[9] work is not considered a dose-response study because it surveyed dietary intake and subjects' responses were not tied to a specific administered dose of exposure of gluten.

Reviewer #3
Most of the studies used only one dose level, small group sizes and different exposure designs (vehicle, length of exposure). However, it appears that the LOAELs reported were relatively consistent over different studies. Although this is not what I would consider a standard scientific approach to establish validity, it does provide a level of assurance that the correct answer was found. I also find solace in the fact that the immunological mechanisms for CD are well understood and as with other pathologies associated with immune recall, that while there is a threshold, the dose response curve is usually fairly steep and acute challenges could be as significant as low-level chronic exposure.

Reviewer #4
Yes. There are limited data with mostly small studies that are dispersed over several decades and continents where compliance and dietary guidelines have changed. Even the histological morphometry may have changed. Weight of evidence is also crucial especially in terms of sample size, as there appear to be a substantial variation in inter individual sensitivity. Also, the design used especially for clinical outcomes is crucial, as a placebo effect may be great in open label studies. Histological end points are not so likely to be a concern in the open label studies.

Reviewer #5
While this is a meaningful approach, it is not at all clear from the studies chosen how studies were evaluated. Each of the studies utilized should have a quality rating based on a standard quality criteria checklist. For example, it is unclear how the Ciclitira study (1985) would have been chosen over the Catassi study to determine a TDI for the subchronic intake of gluten (morphological).

Reviewer #6
It is reasonable to employ a weight-of-evidence approach to select the critical study upon which dose-response is derived. However, a systematic weight-of-evidence approach was not transparently outlined in the current report. The data summary in Appendix A does not allow the reader to easily read across studies for comparison purposes. It is highly recommended that the evidences (studies) are summarized in tabular format, with indication of degree of confidence for each study. The confidence rating should be based on various parameters including, but not limited to: study design (double blind, single blind or open challenge), inclusion/exclusion criteria, study population, test doses (and necessary dose conversion), subjective and/or objective outcome measures, etc…. The individual study parameter ratings can be used to form an overall confidence rating for

[9] Peraaho M, Kaukinen K, Paasikivi K, Sievanen H, Lohiniemi S, Maki M, Collin P. Wheat-starch-based gluten-free products in the treatment of newly detected celiac disease: prospective and randomized study. Aliment Pharmacol Ther 17: 587-594, 2003

each study. With this kind of a systematic analysis of the available evidence, the Agency can be more effective in providing rationale and support for the study(ies) that it chooses as the "critical studies," from which TDIs are derived. By using this systematic approach, if a critical study has deficiencies, a systematic approach to applying additional UFs to account for study deficiencies can be done. Without a systematic process, derived TDI and associated UF will appear ad-hoc and not scientifically compelling.

Charge Question 1d. **Given the availability of the studies that contain direct dose-response challenge data for gluten-induced adverse effects, what role should the findings of associational and/or epidemiological studies on CD and gluten exposure play in the determination of the critical low dose adverse effect levels?**

Reviewer #1
I think associational and/or epidemiological studies should be given relatively little weight compared to the trials that involve gluten challenge and in which subjects serve as their own control. If the results of these studies support the conclusions derived from the challenge trials, they can be considered to add to the weight-of-evidence. If they do not support the conclusions of the challenge trials, this can be mentioned but given that there are likely to be many possible explanations for the discrepancy and the methodological superiorities of the challenge trials, the discrepancy should not substantially reduce confidence in the conclusions drawn from the trials.

Reviewer #2
Once again, I maintain that with a few possible exceptions, the only studies worth serious consideration are those of Catassi et al. (2007) and Peraaho et al. (2003). However, I admit that my knowledge might be inadequate and I might be convinced otherwise in discussion with other reviewers and the FDA authors. I would have liked to discuss and collaborate on this review with an epidemiologist colleague of mine, Anneli Ivarsson (University of Umeå. Sweden), in order to make up for my deficiencies in the area of epidemiology.

Reviewer #3
I may have misunderstood this question, but useful clinical or epidemiological data would require having: individuals with established CD, controlled challenges with known quantities of gluten and an endpoint that is widely accepted. The latter would probably not include non-specific symptoms. The gluten antibody test is useful for diagnosing CD. However, in order for it to be useful to identify a challenge reaction, it would be necessary to have titers measured pre-challenge and then approximately 5-7 days post challenges – this being consistent with a secondary antibody response. I do not think this was done in many of the studies?

Reviewer #4
Adjunctive role in terms of understanding the broader population effect.

Epidemiological studies can only provide hints into long-term effects. The major confounders are likely issues of voluntary or inadvertent compliance and the difficulty in determining accurately the intake of gluten in patients over time. Selection bias in the

cohorts follows and the likelihood that many subjects are not diagnosed with CD until many years of active disease have elapsed, may predominate in terms of long-term outcome.

Reviewer #5
The lack of evidence should be cited.

Reviewer #6
Epidemiological studies can provide useful supporting information in an overall evidence-based approach. However, since quantitative exposure information is often lacking, their role in determination of the dose response information for risk assessment purposes will remain limited. Nevertheless, for the purpose of complete hazard identification, a systematic review and summary of the available epidemiological data should be provided in the revision of this report. More importantly, CD prevalence and incidence data in the US need to be summarized so that the full public health context/impact of gluten exposure can be understood by users of this document, presumably risk managers.

Charge Statement 2. **All available published studies (in English) with dose-response information on adverse health effects of gluten (or toxic protein derivatives of gluten) in individuals with CD were reviewed and their dose-effect data were examined. These studies included the data from different types of food challenge tests in humans---open, single-blind, double-blind challenge tests--- and included tests in which subjects were used as their own controls (i.e., pre-test measures, followed by experimental variables "test" measures, and possibly post-test measures).**

Charge Question 2a: **Is examining data from all types of challenge tests an appropriate approach in identifying significant dose-response data and assessing low dose adverse effects?**

Reviewer #1
Yes, all types of challenge trials should be considered, but an explicit hierarchy of evidence should be stated, for instance that double-blind trials are given more weight than single-blind, and single-blind given more weight than open, or that ABA trials are given more weight than AB trials.

Reviewer #2
I would have to say that many of the studies cited were not focused on assessing low-dose adverse effects and this makes them of limited value. The studies of Catassi et al. (1993 Gut 34:1515-1519) in children had clearly established that 100 mg of gliadin per day (28 days) produced both significant increase in the mean epithelial lymphocyte count and significant decrease in the villous height/crypt depth ratio. This study has served as a baseline for harmful effects, but left the question of whether or not less gluten could be tolerated unanswered. Any study involving higher levels of gluten than 100 mg /day seems to me to be impertinent. In the later studies of Catassi et al. (particularly the 2007 paper), it appeared that even 10 mg gluten per day caused a significant decrease in villous height/crypt depth ratio for about 2 of 9 patients in a 90-day test. No significant changes

in IEL counts were noted. What this means is not clear and further studies are needed. Nonetheless, I would conclude that probably about 80% of celiac patients can tolerate 10 mg of gluten per day and that at least 20% can tolerate 50 mg per day. Considering the study of Peraaho et al. (2003), it appears that 65 patients showed good response to a wheat-starch containing diet for 1 year. If I assume that the patients were eating at least 100 g of wheat starch per day and that the starches consumed contained 50-100 ppm gluten (based on findings of Kasarda et al. 2008: preprint on J. Ag. Food Chem. web site), then the patients were consuming approximately 5-10 mg of gluten per day. It is conceivable to me that the form of gluten given to patients in studies may be important. For example, in the studies of Catassi et al. (2007), the gluten was given in the form of a capsule and may have exposed the duodenum to higher concentrations of gluten peptides than if the patients had been consuming wheat starch products where the gluten was dispersed at very low concentrations on the surface of starch granules. Perhaps factors such as this might be considered in the experimental plan of future research.

Reviewer #3
Yes, all types of challenge tests should be evaluated, as there is no validated standard to assess a response. It might be worth noting that in challenge tests for skin sensitizers, the concentration to a given area of the skin surface is more relevant than the total concentration applied to the skin. Assuming a similar response would result in gluten challenge, if one administered equal amounts but in different dilutions, the more dilute (larger volume) would cause a lesser response than administering a concentrated solution in a small volume.

Reviewer #4
Yes. Due to the limited data available and the diverse nature of how gluten could contaminate foods, I think that all data, even data that use subfractions of gluten, should be included. The conversion of those fractions to "gluten" does introduce some uncertainty that cannot be avoided.

Reviewer #5
(2a.1) No. Open tests assessing clinical effects (symptoms) should not be used to estimate TDI. All of the studies should have a quality rating and overall evidence should be cited as strong, fair, or weak.

FDA Response:
Part 2a.1
The assessment of the hazard/safety of some toxic agents (such as gluten) and their associated toxic responses (such as CD) is not accompanied by requisite standard procedures or methodologies for the studies that investigate their health effects as is the case for some other agents (e.g. food additives, pesticides). The only data available to evaluate the adverse effects of a substance such as gluten are those of the various types and sources currently found in the published literature that may contain some applicable dose-response findings. To obtain and consider as many data points of reference and comparison as possible, FDA believes that the evaluation of all available types of challenge test data, including data of adequate quality from open challenge tests, is warranted even for clinical effects. This type of effect is a significant component of the detrimental effects exhibited in CD and thus needs careful examination. In the

section titled “Dose-Response Assessment,” now under the subheading “Nature of the Studies and Data Evaluated” (see in particular the subpart “Study Design and Related Data Characteristics”), we noted in a transparent manner the “issue” of the subjects in some studies being aware and not “blind” to the type of challenge substance administered. The role of the hazard/safety evaluation is to characterize and to assess the critical levels of gluten for clinical effects based on the best data available; whereas, the considerations associated with the subsequent application of the calculated clinical tolerable daily intake (TDI) value(s) is a component of the risk management stage. Several other factors suggest that it is a reasonable approach at this time to include the available open challenge data in this determination for clinical effects. These factors also provide a degree of confidence to the FDA on the use of those findings. First, as noted in subsection now titled “Study Design and Related Data Characteristics” (see “Dose-Response Assessment” section) of the main document, evaluating some clinical effects is potentially less problematic with respect to open challenges than others. For instance, clinical effects of an overt nature (e.g. diarrhea, vomiting, weight loss) are probably less attributable to spurious or biased responses than those responses of a subjective, covert nature alone (e.g., abdominal pain (AP), nausea, fatigue). Second, the nature and constellation of the clinical responses for each challenged individual subject is consistent over time and does not emerge as inconsistently varied and random over the test period. Third, in many open challenge studies, including the low-dose ones, a pattern of no clinical effects on a gluten-free diet (GFD) in individuals with CD, followed by clinical effects emerging with a gluten challenge, and then possibly including an extinguishing of the clinical effects upon removal of gluten (e.g., AB, or ABA experimental designs) is demonstrated. This temporal relationship between exposure and adverse effects provide support for the validity and reliability of the clinical effects exhibited. In addition, this type of “temporal” challenge test is often used for and/or incorporated into the procedure for diagnosis of CD (see subsection now titled “Nature and History of Diagnostic Definition of CD” under the “Dose-Response Assessment” section). Fourth, as revealed in Appendix B of the HHA, in several low-dose studies presented in Tables 5-7 for which clinical adverse effects are noted the reported responses are accompanied by significant abnormal morphological and/or physiological effects, thus buoying the validity and reliability of some clinical effects seen with gluten challenges. Fifth, several studies found in Tables 5-7 depicting CD-related clinical adverse effects included the challenging of different “control subjects” and no random or spurious clinical adverse effect responses were reported with these subjects which would be expected to some degree if the nature and manner in which the gluten was administered in the challenge test (e.g., “open” or lack of “blind” administration) played a significant role in the responses exhibited in all challenged subjects. The latter four factors were considerations made in evaluating the weight-of-evidence within a study as to the validity of the clinical responses exhibited by the subjects of a particular study and are thought by FDA to be reasonable ones in delineating the nature and basis of study findings. Sixth, the LOAEL for clinical acute, subchronic and chronic adverse effects was identified as approximately 1.5 mg/day and was derived from the study by Chartrand et al. (1997)[10]. This overall LOAEL identified at the low mg level is supported by a number of other investigations with study LOAEL values also in this range for both acute and subchronic responses and these findings also include results from double-blind placebo-

[10] Chartrand L, Russo PA, Duhaime AG, Seidmain EG. Wheat starch intolerance in patients with CD. J Am Diet Assoc 97(6): 612-618, 1997

controlled food challenge (DBPCFC) studies[11]. Thus, the weight-of-evidence across studies serves in this case to provide confidence in the identified critical clinical LOAEL in the low mg dose range based on an open challenge study.

Reviewer #6
Throughout the report, the Agency clearly indicated that the most sensitive individuals are not likely captured in the considered challenge studies and that responses may occur at doses lower than used in "critical studies." Hence, extra uncertainty factors (beyond conventional factors of 10 for inter-individual variability and 10 for extrapolating from LOAEL to NOAEL) may be necessary. This great uncertainty and the possibility of response well below the doses associated with the "critical studies," raise some real doubts whether these studies can be used to support the science-policy convention of a threshold approach (via development of TDI and LOC). This is of particular concern from a chronic exposure perspective where challenge data are extremely limited.

Charge Question 2b. **Is it reasonable to express the values for dose levels of exposure in terms of the amount of gluten exposure? Is the associated conversion(s) needed to do so in some cases (e.g. from gliadin to gluten) reasonable?**

Reviewer #1
(2b.1) Yes, estimating gluten exposure in those trials in which gliadin or FF3 was administered is necessary in order to conduct this safety assessment. As I am not an expert in this field, I cannot comment on the reasonableness of the conversion factor applied. Footnote 10 indicates many sources cite 100 mg gliadin is equivalent to 200 mg and that "various conversion factors for FF3" are cited in other sources, but I am not sure what factor(s) were actually used in this safety assessment, so this should be made clear.
(2b.2) In addition, I suspect that there is a range of estimates for the conversion, so, depending on how broad the range is, it might be useful, as a sensitivity analysis, to estimate TDIs using low, median, and high estimates of the conversion factors.

FDA Response:
Part 2b.1
In addition to the information in noted in "Footnote 10" of the original draft hazard/safety assessment document examined by the peer reviewers, Appendix A of the HHA lists each study reviewed for dose-response information under various assessed categories (e.g., age group, duration of exposure) and provides details about the relevant characteristics specific to each study including conversion factors used. A notation of the existence of Appendix A and of it containing study-specific information is made several times in the text of the main document prior to this point. The conversion factor(s) used for FF3[12] digest was specific to each study and was based only on the information available and/or references cited in each individual study about the methods used in their preparation of FF3. If the information allowing for this conversion from FF3 to gluten

[11] This refers to the results of a DBPC challenge study in addition to the separately published results of an associated preliminary study. The findings presented for each study do not reflect all the same subjects because subjects that exhibited clinical response to the gluten challenge were dropped out of each respective study.
[12] The abbreviation "FF3" represents Frazer's Fraction III or Frazer's peptic-tryptic digest of gluten.

is not noted in a particular study, then the dose-response gluten challenge data was not in the final assessment and comparison of adverse effects. The specific information on or source of the details concerning these conversion factors used for each study is noted in Appendix A. The information on conversion factor(s) that was originally noted in a single footnote (Footnote 10) in the main document has been edited and expanded in nature to include several footnotes and an added text paragraph in the HHA to clarify and better communicate the distinctions explained above.

Part 2b.2

The frequently noted quantitative value for the relationship between levels of exposure of gliadin and gluten (i.e., 100 mg gliadin : 200 mg gluten) was applied in this analysis and served as the basis of the conversion factor used to equate exposures of gliadin and gluten in this hazard/safety assessment. This reflects the 50:50 ratio for the two major fractions of gluten, gliadin and glutenin, that has been cited for many years. Reference to this gluten fraction ratio (gliadin:glutenin) and/or 2-fold conversion factor (gliadin:gluten) has been the predominant one found in a number and range of references and sources in the literature. This includes references that contain gluten-related challenge findings (e.g., Catassi et al., 1993 and 2005[13]), that examined the chemical composition of gluten (e.g., Pomeranz, 1987[14]), that to date have served as the basis of conversions involved in enzyme-linked immunosorbent assay (ELISA) gluten test kits calculations (e.g., Ridascreen Fast Gliadin, R-Biopharm AG; Prolamins Transia Plate, Diffchamb), and finally, the one stated and presumed by Codex in addressing their "gluten" considerations (e.g., Codex, 2006[15]). To be consistent with this information, all conversions performed in this assessment to express exposure levels as a uniform "amount of gluten" were based on these same ratios. Recently, the ratio of the gliadin:glutenin fractions of gluten of 65:35 has been put forth by Thompson and Mendez (2008)[16] based on their interpretation of the work of Weiser et al. (2007)[17] on the chemical analysis of gluten. A sensitivity analysis using a ratio based on this latter research as an estimated value for the equating ratio associated with the relationship between gliadin and gluten was performed and added to the HHA document. It was used to re-calculate the final TDI and level of concern (LOC) values if these values were based on a study with exposure data requiring conversion to gluten levels[18]. This analysis was added to the main text of the HHA document and served to examine the potential affect of this newer ratio on the final TDI and LOC values.

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[13] Catassi C, Rossini M, Ratsch I-M, Bearzi I, Santinelli A, Castanani R, Pisani E, Coppa GV, Giorgi PL. Dose dependent effects of protracted ingestion of small amounts of gliadin in CD children: a clinical and jejunal morphometric study. Gut 34: 1515-1519, 1993

Catassi C, Fabiani E, Mandolesi A, Bearzi I, Iacono G, D'Agate C, Francavilla R, Corazza GR, Volta U, Accomando S, Picarelli A, De Vitis I, Nardote G, Bardilla MT, Fasano A, Pucci A. The Italian study on gluten microchallenge: preliminary results. Chapter II, Clinical research reports. Proceedings of the 19th Meeting of the Working Group on Prolamin Analysis and Toxicity, edited by Martin Stern, Verlag Wissenschaftliche Scripten, Germany: Zwickau, pp 109-116, 2005

[14] Pomeranz Y. Chapter 4. Composition. In: Modern Cereal Science and Technology. New York, New York: VCH Publishing, Inc., pp 40-53, 1987

[15] Joint Food and Agriculture Organization of the United Nations, World Health Organization, Food Standards Program. Report of the 28th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses. Rome, Italy. pp 11-13, 2006

[16] Thompson T, Mendez E. Commercial assays to assess gluten content of gluten-free foods: Why they are not created equal. J Am Diet Assoc 108(10):1682-1687, 2008

[17] Wieser H. Chemistry of gluten proteins. Food Microbiol 24: 115-119, 2007

[18] An alternative gliadin:glutenin protein subtype ratio used in the sensitivity analysis for gluten conversion was 68:32 and was based on Wieser (2007). See the text of the sensitivity analysis added to the main document of the HHA for details.

Reviewer #2
I don't think so. If glutenin is not toxic and gliadin is, then a 2x conversion factor from gliadin to gluten would be valid. If glutenin is less toxic or more toxic, then a 2x conversion would not be appropriate. *(2b.1)* I think the best compromise at present (until we learn more about the relative toxicities of gluten sub-components: high-molecular weight glutenin subunits vs. low-molecular-weight glutenin subunits vs gamma-gliadins vs. alpha-gliadins vs omega-gliadins) is to use gluten in studies of dosage effects. Without further characterization of peptic-tryptic digests, chymotryptic digests, or other such digests (sometimes called FF3), I would not recommend that such digests be used in studies of dosage effects. I think these digests may be highly variable in composition from laboratory-to-laboratory and might thereby be more or less toxic than gluten itself. Gluten by the nature of the way it is prepared is least likely to vary in composition.

FDA Response:
Part 2b.1
With respect to the expression of the dose levels of exposure as gluten amount, it was indicated by Reviewer #2 that it is "best …to use gluten in studies of dosage effects." However, as discussed in the main document and also noted above (see FDA Response, Part 2a.1 to Reviewer #5), specific studies were not designed and executed to evaluate the "dosage effects" of gluten for this hazard/safety assessment. The effects of gluten (and related compounds) can only be based on all available published challenge studies that happen to contain dose-response data. In addition, most challenge studies only tested 1 dose of gluten (or gluten protein constituent). Hence, FDA feels the consideration of all available dose-response data across different studies and across different levels of exposure is the best approach available at this time to characterize the nature of the dose-effect relationship for gluten and CD-related responses. This includes the use of studies that administered gliadin or FF3, even if some uncertainty is introduced by the conversion of these exposures to gluten. We also recognize the uncertainty that is associated with the limitations in the knowledge about the relative nature of the contents and/or potency of the various sub-components comprising gluten and with the conversion factor used for gliadin. But, limitations and uncertainty are involved in all assumptions made while performing any safety/risk assessment and it is well-recognized that these assessments can only be made with and reflect the best data available at the time they are performed. Also, in the case of this analysis, because no information was located that would assist in equating exposure to subparts of gluten (e.g., B-fraction of gluten, high molecular weight (HMW)-GS glutenin) or of gliadin (e.g., α-gliadin) to those of gluten, the dose-response data for a study with such an exposure was not considered in the final hazard analyses (see also Appendix A of the HHA). As indicated above, the dose-effect data from challenge testing that administered FF3 digest was only assessed when specific information is available within each study about the nature of and methods used to prepare the digest and that could be employed to derive a factor for converting this exposure to a comparable gluten one (see above discussion and Appendix A). In addition, the administration of FF3 was only involved in one low-dose study identified as important. Again, the details about the specifics of the agent administered in a challenge test from each study and any associated conversion factors employed are summarized in Appendix A of the HHA.

Reviewer #3
YES – it is reasonable to express dose as gluten. Ideally, it would be best to define exposure in terms of the gliaden and gluten components. However, I doubt if the clinical studies conducted this type of analyses and there is a lack of understanding regarding the immunogenecity of the various wheat proteins.

Reviewer #4
Yes to both questions. All fractions can be related back to the "gluten" as a common "currency" in estimating thresholds. While this does introduce some uncertainty, as gluten is the amalgam of the toxic fractions, it seems reasonable to relate all tested fractions to this. What will be unclear is how one would detect those other grains in a "gluten-free" food, but the reviewer assumes that verification of gluten content will be the subject of a separate assessment document.

Reviewer #5
(2b.1) Yes, although the ratio of gliadins to glutenins may be closer to 65:35 than 50:50.

FDA Response:
Part 2b.1
A sensitivity analysis that addressed this possible difference in the ratio between gluten protein subfractions was performed and added to the main HHA document (See the subsection titled "Sensitivity Analysis Associated with Gluten Chemical Compositions" under the "Risk Characterization" heading). This analysis assessed the affects of the second alternative ratio on the estimated primary TDI and LOC values. See also the FDA Response, Part 2b.2 to Reviewer #1 above in this report.

Reviewer #6
(2b.1) While it is reasonable to convert different dose levels in the various studies to equivalent gluten exposure, the uncertainty associated with the conversion (i.e., observed effects may also be associated with aspects other than gluten or the conversion itself introduces uncertainty in the dose measures) should be accounted for in a systematic manner. For example, confidence in the dose-response data from studies subject to such conversion should be rated differently than studies from which direct dose-response data were available. A summary of such systematic evaluation will help reader to better appreciate the underlying data upon which the Agency will use to make risk management decisions.

FDA Response:
Part 2b.1
Information on the specific agent administered in a particular study, and the method and details associated with this administration, along with any related conversion factors employed, is provided in a systematic manner in Appendix A of the HHA. Also, in the text of the main document, the discussion of each "critical" study, and related "supporting" study or studies, that was identified for each type of toxic effect (morphological and clinical effects) and type of exposure duration (acute, subchronic and chronic) includes details about the relevant specifics and characteristics that pertain to that study. Additional description of the weight-of-evidence approach and associated general considerations that is involved in the assessment of aspects of study data has

now been included in the main text of the HHA document. Finally, procedures and scientific judgments typical of the safety assessment approach were employed in this evaluation.

FDA Charge Question 2c. **Are the relevant issues associated with the uncertainty of the available dose-response data adequately enumerated and discussed?**

Reviewer #1

Yes, this discussion is very good. *(2c.1)* Because individual subject data are available in some trials, I wondered whether it would be possible to derive an empirical estimate of inter-individual variability in response rather than using a default 10-fold factor. Qualitative arguments are made suggesting that a 10-fold factor might not be adequate, but attempting to address this on a more quantitative basis would be desirable. *(2c.2)* It seems that there is uncertainty as to whether the CD patients who were enrolled in the different studies available were comparable in terms of many factors, such as severity of background disease state, adherence to GFD, etc. This should result in selecting as the critical study the one that yields the lowest NOAELs and LOAELs since it might reasonably be assumed that this study focused on the most sensitive subgroup of patients. I endorse the proposal to include an additional UF because of the lack of dose-response data on gluten exposure and the risks of additional, long-term illnesses, as has been done in risk assessments of other food contaminants (e.g., U.S. EPA's derivation of a reference dose for methylmercury). Another source of uncertainty in the dose-response estimates issues from the fact that "most challenge studies only tested one dose…" Therefore the dose-response estimates are essentially based on the assumption that the response rates observed for different doses, in different studies, are comparable. Although it is difficult to quantify the uncertainty this might have introduced, it seems that it should be considered in the UFs. Finally, it might be appropriate to apply different UFs to acute, subchronic, and chronic TDIs depending on the amount of data on which each is based. For both morphological/physiological and clinical effects, far less data are available for chronic exposure than for either acute or subchronic. Adding an additional UF in calculating the chronic TDI would address this.

FDA Response:
Part 2c.1

A re-examination of the studies with individual data and with greater than one administered dose was performed to evaluate if any additional reliable information of a quantitative nature could be gleaned to support the qualitative observations noted about the great individual variability seen studies investigating in CD-related responses. In the study by Laurin et al. (2002),[19] children with CD were allowed to self-select their dietary exposure level to gluten in food and the individual data for each of the 24 subjects was available. The dose of reactivity (mean g gluten/day) for adverse effects associated with CD differed 22-fold across all challenged children. This information on a quantitative estimate of the nature of inter-individual variability in CD was added to the text of the main HHA document in the "Risk Characterization" section under the subsection

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

"Uncertainty Issues in the Hazard Assessment" in the subpart titled "Inter-Individual Variability and Related Uncertainty Issues".

Part 2c.2

For the present analysis, the traditional default 10-fold uncertainty factors typically used in the standard procedures for a safety assessment were employed. The goal of sections in the FDA HHA document that discussed additional uncertainty issues, particularly those pertinent to the nature of studies and associated data that is available for assessment of CD and to the nature of CD itself, was to describe and enumerate other noteworthy factors that may additionally play a role in or be unique to determination of a tolerable level of exposure of gluten in individuals with CD. FDA intended for these supplemental points and factors about the uncertainty issues to be addressed and possibly implemented in the risk management stage. The feedback, suggestions and "endorsements" of Reviewer #1 with respect to employing additional UF earlier at the safety assessment stage will be considered.

Reviewer #2

I doubt it given my concerns as stated in my previous comments (above).

Reviewer #3

The draft report indicates that in most studies, single challenge doses were conducted. *(2c.1)* I was left wondering whether some indication of the shape of the dose response curve could be obtained from those studies that used multiple challenge levels. As I indicated before, I assume the slope would be steep but data to support this would help support using a 10-fold UF for converting the LOAEL to the NOAEL.

FDA Response:

Part 2c.1

Only 3 of the low dose-response studies listed in Appendix B of the HHA had both NOAEL and LOAEL data values. Two studies administered gluten-related substances acutely with the challenge dose administered 1 time and the resulting data are such that any slope of curve (m) determination would probably not represent or provide meaningful information. The other study involved a subchronic exposure to a daily dose of gluten and expressed the resulting morphometric changes as a percentage change in group median values which also doesn't allow for an accurate determination or assessment of the slope of the curve. The study by Laurin et al. (2002) measured post-challenge IEL counts in subjects with CD exposed to different doses of gluten. The study authors graphed the correlation (best-fit) between gluten intake (expressed as g gluten/kg body weight/day) and the number of IEL in the post-challenge biopsy specimens. The slope of the "best fit" line of these measures in this particular reference was approximately m = 340, where slope m = Δ y/Δ x. In this case, the plotted dose-response data found in the Laurin et al. (2002) study suggest a steep slope and thus support the use of a 10-fold UF for converting the LOAEL to NOAEL for morphological effects as suggested by Reviewer #3. Finally, a safety assessment approach typically involves the derivation of point estimate(s) 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 and with limited information on "shape of the dose-response curve" an assumed default value of 10-fold for NOAEL to LOAEL extrapolation is usually considered reasonable.

Reviewer #4
Yes. There is a thorough discussion of the limitations of toxicology data. The studies reviewed include studies that were not done to detect toxic thresholds specifically, but rather to examine responses to specific fractions or to elucidate the pathobiology of the diseases.

Reviewer #5
No, as stated previously, the lack of data, poor quality of data, etc should be discussed at length.

Reviewer #6
Throughout the report, uncertainty associated with the available dose-response data was described. However, the systematic accounting for each source of uncertainty (dose conversion, age, timing, response, study design, etc…) was not summarized. Hence, the reviewer is left with an impression that uncertainty was not adequately enumerated.

Charge Statement 3. **Potential secondary health effects are described in the consideration of the potential hazards of gluten exposure.**

Charge Question 3a. **Have the significant issues and factors associated with secondary adverse health effects been adequately addressed?**

Reviewer #1
3a, 3b, and 3c: Not being a clinician who manages patients with CD, I cannot comment on these issues. It seems entirely appropriate, as stated above, to consider applying an additional UF to address the uncertainty regarding gluten's role in producing secondary health effects, many of which are more serious for one's health and well-being than some of the critical health effects used in this safety assessment. However, I do not feel that I can make a decision on this issue based on the material summarized on pp.3-4. I would want to know not just whether gluten exposure is associated with an increased risk of the diseases discussed but the magnitude of the risk (e.g., odds ratio), the quality of the data (i.e., whether confounding was adequately addressed), etc.

Reviewer #2
Yes, I think they have.

Reviewer #3
Yes, the secondary health effects are adequately discussed. The draft suggests that all the secondary health effects appear to be dependent upon the length of time the individual continues on wheat proteins. I would think this may be true for most of the diseases, particularly GI malignancies, but it may not be true for all of the secondary health effects. Some of these health effects may be related to genetic predisposition associated with an immune imbalance.

Reviewer #4
No. These are difficult issues that are not usually addressed in terms of acute studies of toxic effects of gluten. Epidemiological data on the risk of diseases, especially autoimmune diseases in CDs, in terms of compliance, are really the only source of data in this regard. Also, it is quite difficult to infer thresholds, as estimating the actual intake or exposure over years is difficult.

Reviewer #5
Yes, this section is comprehensive.

Reviewer #6
On pages 3-4, the available literature on the spectrum of clinical presentations associated with CD such as autoimmune diseases and GI cancers were briefly summarized. Increased risks in individuals with CD developing these diseases are proportional to the time (duration) of exposure to diets containing "relevant cereal protein" or the age at diagnosis (proxy of duration of exposure). Higher mortality rate was also reported among CD individuals with delayed diagnosis (reduced risk if diagnosed during childhood). However, no specific data (e.g., type of studies, OR/RR for various quintiles of exposures, if any) from the cited studies were presented. More detail from the reviewed literature should be summarized.

Charge Question 3b. **Is the uncertainty associated with the potential contributing factors of long-term exposure to trace amounts of gluten to the development of secondary disorders or diseases adequately addressed?**

Reviewer #1
See above

Reviewer #2
I think they have been addressed.

Reviewer #3
See response to question 3a above.

Reviewer #4
Yes. The document certainly reflects the lack of data on the likely threshold for gluten over the long term, as it relates to the increased risk of secondary disorders. The only real data, and these are largely observational, are on the circumstances of large quantities of gluten. These data are not informative in terms of the "very low gluten" exposure. It might be assumed that gluten would have to be present in sufficient quantities to cause histomorphological changes to result in secondary disorders such as osteoporosis, which are thought to be a consequence of the resulting malabsorption. Cancers such as lymphoma or adenocarcinoma, which are also thought to be secondary to the inflammation, are also measurable by histological examination. However, the assessment is quite right in pointing out the uncertainties inherent in extrapolating observational studies to long-term outcomes.

Reviewer #5
Yes, this section is comprehensive.

Reviewer #6
The report acknowledges that the association between long term ingestion of a very low level of cereal protein in an avoidance diet and the subsequent development of the secondary diseases has not been systematically investigated in any comprehensive fashion. The dose response assessment was based on challenge studies in which these secondary diseases in CD individuals were not addressed. Thus, as currently presented in the report, the developed TDIs and LOCs do not adequately address these potential secondary disorders among CD individuals.

Charge Question 3c. **Should the potential secondary adverse health effect of the development of osteopenia or osteoporosis that is associated with gluten exposure in CD also be considered along with the other secondary health effects in the hazard identification section, and if so, how should it be done?**

Reviewer #1
See above

Reviewer #2
(3c.1) I think osteopenia or osteoporosis should be considered along with other secondary health effects, but since I am neither a physician nor an epidemiologist, I don't have any suggestions as to how this should be done.

FDA Response:
Part 3c.1
A brief overview of the relationship between gluten exposure in CD and the development of osteopenia or osteoporosis was added to the main HHA document in the "Hazard Identification" section under the subsection titled "Other Health Effects."

Reviewer #3
(3c.1) Yes. Osteoporosis should be included as a potential health effect or at least how CD may contribute to the development of osteoporosis. May also want to state that it is probably due to GI damage - effects normal absorption of certain nutrients.

FDA Response:
Part 3c.1
A brief overview of the relationship between gluten exposure in CD and the development of osteopenia or osteoporosis was added to the main HHA document in the "Hazard Identification" section under the subsection titled "Other Health Effects."

Reviewer #4
(3c.1) Yes. See above. Also, the calcium intake in a GFD should be considered, especially if the individual is lactose intolerant or becomes lactose intolerant because of some low level dysfunction due to gluten induced injury.

FDA Response:
Part 3c.1
A brief overview of the relationship between gluten exposure in CD and the development of osteopenia or osteoporosis was added to the main HHA document in the "Hazard Identification" section under the subsection titled "Other Health Effects."

Reviewer #5
(3c.1) Yes, it should be done with a critical review of the literature.

FDA Response:
Part 3c.1
A brief overview of the relationship between gluten exposure in CD and the development of osteopenia or osteoporosis was added to the main HHA document in the "Hazard Identification" section under the subsection titled "Other Health Effects."

Reviewer #6
(3c.1) This information is not provided in the current draft. If the extent of the scientific evidence is comparable to that found with autoimmune diseases and GI cancers, then it would be prudent to consider these effects in the safety assessment of gluten, particularly when the stated focus of the assessment is the CD sub-population.

FDA Response:
Part 3c.1
A brief overview of the relationship between gluten exposure in CD and the development of osteopenia or osteoporosis was added to the main HHA document in the "Hazard Identification" section under the subsection titled "Other Health Effects."

Charge Question 4. **Should the lack of available findings from systematic investigation of long-term or chronic ingestion of trace amounts of cereal protein in an avoidance diet and the subsequent development of cancer or autoimmune diseases be considered a significant uncertainty in the safety assessment?**

Reviewer #1
Yes, possibly, as noted above.

Reviewer #2
I don't think such studies involving trace amounts are likely to yield measurable results. The study by GKT Holmes, P Prior, MR Lane, D Pope, and RN Allan, "Malignancy in coeliac disease—effect of a gluten free diet" Gut, 1989, 30, 333-338 (210 patients, 11 years+ of follow up), stated that "The results indicate that for coeliac patients who have taken a gluten-free diet for 5 years or more, the risk of developing cancer over all sites is not increased when compared with the normal population." The time period of this study (approximately 1970-1985), was a time when patients in the UK were including wheat starch in a gluten-free diet. This is not discussed in the paper, but in a personal discussion with Dr. Holmes, he agreed with me that such was likely the case. At that time, there was no good method for measuring the amount of gluten in wheat starch and the starches used were likely, in my opinion, to have anywhere from 100-300 ppm gluten. I conclude therefore that it is likely that small amounts of gluten protein do not increase

the risk of developing cancer for celiac patients. If my estimate of 100-300 ppm per day is correct and the patients were eating 100 g of starch per day, this would mean that the intake of gluten from wheat starch alone would have been 10-30 mg per day. In regard to cancer incidence, the paper by TR Card, J West, and Holmes GK (Risk of malignancy in diagnosed coeliac disease: a 24-year prospective, Aliment Pharmacol Ther. 2004 Oct 1;20(7):769-75) is of interest.

Reviewer #3
Several issues, in addition to lack of chronic exposure data, lead to uncertainties regarding development of long-term health effects. First, the disease is often undiagnosed for many years and not all GI effects from CD are considered reversible. Hence, while establishing a TDI is important, it will not necessarily protect for subsequent health effects that may have been initiated earlier. It certainly will prevent acute adverse effects associated with re-challenge. Secondly, as suggested earlier, it is not clear to me that all of the health effects attributed to long term CD are related to chronic gluten exposure. Some of these may be related to genetic predisposition or other non-gluten factors.

Reviewer #4
Yes. The risks of these consequences should be considered as substantial uncertainties in determining the NOAEL and LOAEL for gluten. Any amount of protein that could trigger an immune response in the intestine could trigger cytokine release, alter the gut immune homeostasis, and possibly alter gut permeability to macromolecules. All of these could potentially affect the chances of an autoimmune disorder or cancer if substantial inflammation occurs. See above.

Reviewer #5
Yes, this should be discussed.

Reviewer #6
Yes – this is also a consideration given that the available challenge data used by the Agency is limited in terms of chronic exposure. See comment 3b.

Charge Question 5. **Is it correct to assume that celiac patients have the same overall energy and nutrient requirements as the general population and that the foods "replaced" in celiac patient's diet are on a gram for gram basis nutritionally and energetically equivalent to the general population's foods?**

Reviewer #1
I have no expertise on this issue.

Reviewer #2
I am not a nutritionist, but I would guess that it is correct to make such an assumption.

Reviewer #3
Yes.

Reviewer #4
Not always. Celiac patients may have decreased absorption of iron, trace elements, and even calories, so their nutritional needs may vary from that of the general population in terms of intake. By and large, it is expected that intake will return closer to the average normal intake when the intestine heals. Gluten-free foods may differ substantially in terms of calorie density depending on the replacement strategies used for the preparation of gluten-free alternatives. The foods may have more fats, oils or sugars to compensate for taste differences or the need to reproduce a texture usually obtained in gluten containing foods. Also, there may be an issue with a general lack of micronutrient fortification in GF bread products, which is routine for mass produced bread. Higher content of fats and oils may also lead to increased calorie densities in GF foods.

Reviewer #5
An otherwise healthy person with CD once in remission appears to have the same energy and nutrient requirements as a person in the general population. Obviously this will not be the case if a person with CD has anemia, osteoporosis, or vitamin and mineral deficiencies brought on by malabsorption.

Gluten-free replacement foods are NOT nutritionally and energetically equivalent. Gluten-free cereal foods (breads, pastas, breakfast cereals, etc) tend to be made from refined flours and starches. Unlike refined wheat-based foods, the vast majority of refined gluten-free foods are not enriched with thiamin, riboflavin, niacin, folic acid, and iron.

Please see the following references:

Thompson T, Dennis M, Higgins LA, Lee A, Sharrett. Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? J Hum Nutr Dietet. 2005;18:163-169.

Thompson T. Folate, iron, and dietary fiber contents of the gluten-free diet. J Am Diet Assoc. 2000;100:1389-1396.

Thompson T. Thiamin, riboflavin, and niacin contents of the gluten-free diet: is there cause for concern? J Am Diet Assoc. 1999;99:858-862.

Reviewer #6
Common sense would lead one to assume that CD patients following the appropriate dietary guidance developed by nutrition professionals could maintain a diet that is nutritionally and energetically equivalent to the general population. The actual compliance with dietary recommendation among CD patients may be of a different situation.

Charge Question 6. **Do the calculations of the various resultant tolerable daily intakes (TDI) estimates include and reflect the necessary considerations, including the uncertainty associated with the significant intra-variability exhibited in those with CD in response to gluten. Is the uncertainty adequately reflected in the final TDI calculation?**

Reviewer #1

Morphological adverse effects: *(6.1)* It was not obvious to me why the study by Leigh et al. 1985) was chosen as the critical study for acute exposure and the others as supporting. The study by Ciclitira et al. (1984b), for example, suggested a much lower LOAEL than did the Leigh et al. study. Was the latter selected because the Ciclitira et al. study administered gliadin directly in the small intestine while Leigh et al. involved oral administration of a gluten digest? Although the findings of Ciclitira et al. (1984a) were mixed (involving oral administration of gliadin), the positive findings at low mg/d doses seem worrisome, especially in light of the findings of Ciclitira et al. (1984b). The potential problems with this study are noted, as well as the observation that a LOAEL based on this study is 2 orders of magnitude lower than the LOAEL based on Leigh et al. (1985). I'm not suggesting that the LOAEL shouldn't be based on Leigh et al., just that the rationale for selecting Leigh et al. as the critical study needs to be made more explicit.

(6.2) I found it problematic that the LOAEL for chronic exposure was based on children while the LOAELs for acute and subchronic exposure were based on adults, given the rather clear evidence that adults are more sensitive than children. In general, for acute and subchronic exposures, the LOAELs for adults seem to be lower by a factor of 3-10. I wondered if some such factor should be applied to the LOAEL for children's chronic exposure, estimated from the Laurin et al. (2002) study, to make the chronic exposure TDI more comparable to the acute and subchronic LOAELs for morphological effects. This would also help remedy the somewhat strange situation in which the subchronic LOAEL is far below the chronic LOAEL.

Clinical adverse effects: *(6.3)* As with the acute morphological effects, the basis for selecting the Chartrand et al. (1997) (which involved an open challenge) rather than, for example, the DBPCFC of Catassi et al. (2007) as the critical study for estimating the subchronic LOAEL is not clear. Presumably it was because adverse clinical effects were found at a lower gluten dose, but again the reasoning applied needs to be more explicit.

FDA Response:

Part 6.1

One primary reason that the Leigh et al. (1985)[20] study was chosen as the critical study for acute exposure over two of the other studies (Ciclitira et al., 1984b and Lavo et al., 1990a[21]) noted as being supporting studies is because the Leigh et al. (1985) administered the gluten-based compound via oral administration in contrast to the Ciclitira et al. (1984b) and Lavo et al. (1990a) studies that administered the substance via intraduodenal infusions and intrajejunal perfusions, respectively. It was clearly stated in the text of the HHA document under the section titled "Dose-Response Assessment" where it described the procedures used and approach taken to perform this assessment

[20] Leigh RJ, Marsh MN, Crowe P, Kelly C, Garner V, Gordon D. Studies of intestinal lymphoid tissue IX: Dose-dependent, gluten-induced lymphoid infiltration of coeliac jejunal epithelium. Scand J Gastroenterol 20: 715-719, 1985

[21] Ciclitira PJ, Evans DJ, Fagg NLK, Lennox ES, Dowling RH. Clinical testing of gliadin fractions in celiac patients. Clin Sci 66: 357-364, 1984b

Lavo B, Knutson L, Loof L, Hallgren R. Gliadin-induced jejunal prostaglandin E_2 secretion in CD. Gastroenterol 99(3): 703-707, 1990a

(now in the section subtitled “Nature of Studies and/or Data Evaluated” under the subpart “Routes of Exposure”). It indicated the following:

> “The challenge studies examined administered gluten (or related compound) via oral ingestion, or via infusion or perfusion directly into the small intestine. Dose-response information was considered from both types of routes of administration to obtain as many of data points of reference and sources of comparison as possible. However, the data from studies that used oral routes of administration were considered the most significant[22] and ultimately of primary focus in this assessment and in identifying critical dose-response studies.”

Other factors and considerations also contributed to the selection Leigh et al. (1985) as the critical study. For instance, in contrast to those performed by Ciclitira et al. (1984b) and Lavo et al. (1990), this study challenged a larger number of subjects doing so in an apparent single-blind fashion and also administered several different doses which resulted in the demonstration of a significant dose-dependent effect[23] and included identification of both a NOAEL and a LOAEL. In addition, the study included the challenge of two types of control subjects and of a control substance. These relevant study characteristics and other related details were noted in the description of the specific findings of this critical study in the main text in addition to in Table 1 of Appendix B and in Appendix A of the HHA. The characteristics enumerated about the nature of the studies and their results in the FDA HHA document reflect what are commonly recognized and identified as significant factors and considerations upon which expert scientific judgments and weight-of-evidence determinations are based in the area of toxicology and health assessment, along with other areas of scientific study of biological-based effects. Additional information on general weight-of evidence factors considered in evaluating studies has been included in the text of the main HHA document.

Part 6.2

As indicated in the text of the main HHA document, the assessment is based on the findings of all available published data that contains dose-response information. Thus, the resultant critical effect levels and associated TDIs are a function of the low-dose effects data currently available for factors such as duration of exposure, age groups of subjects, etc. This must be keep in mind when interpreting and/or deriving meaning from adverse effects level identified, especially for chronic exposure, in the safety assessment. It was stated in the main text of the HHA document (and also depicted Appendix A and Appendix B, Table 3) that limited low dose information is available for morphological and/or physiological effects of chronic exposure to gluten in sensitive individuals in general and for chronic durations of exposure in adults in particular. For example, there is only one study that evaluated the morphological

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[22] The footnote cited in the main document at this location in the text was as follows: “Exposure to gluten via the oral route of administration in studies was considered to best reflect the nature of exposure to gluten that would be experienced through normal dietary exposure. Some types of oral administration of gluten such as via capsules may not approximate dietary exposure as closely as other manners of oral administration.”

[23] Reference to a result being a “dose-dependent effect” reflects the demonstration in an experiment that the level of a “response” or “effect” (i.e. dependent variable) exhibited is related to the magnitude of the dosage administered (i.e., independent variable) over several different doses. Establishing the existence of this type of “dose-effect” relationship is considered support for the exhibited response(s) reflecting an underlying biological-based mechanism(s) and thus, being a “true” response or effect.

effects of chronic gluten exposure in adults and it did so at a relatively high range (e.g., 2500 - 5000 mg/day). Hence, great caution should be taken in this instance in drawing conclusions and attributing significance to the difference in TDIs between subchronic and chronic exposure and their apparent "problematic" (per Reviewer #1) nature. FDA feels the use of an additional UF as suggested by Reviewer #1 as a "remedy" for this discrepancy between subchronic and chronic estimates is a consideration for deliberation in the risk management stage. Lastly, the hazard/safety assessment sections of the HHA document was put forth to identify and characterize the critical studies and corresponding TDI estimates for each duration of exposure such as noted here by Reviewer #1 for chronic exposure (and just above in Part 6.1 for acute exposure). In the subsequent final HHA version of the document, a further analysis was performed and a "TDI of primary focus" was determined as a single overall representative TDI value for morphological adverse effects. The value was not based on the TDI or related data found for chronic (or acute) exposure to gluten which serves to alleviate to some degree the concerns expressed by Reviewer #1 on the less conservative nature of the resulting chronic (and acute) TDI estimates.

Part 6.3

The goal of the safety assessment approach is to protect the most sensitive individuals. To this end, when the data available from studies in general only provide estimates of LOAELs, the study exhibiting the lowest LOAEL (assuming the study is of sufficient quality) is selected as the critical study (which in this case is in Chartrand et al. (1997) for clinical effects) in accordance with the procedures of this approach. In addition, the Catassi et al. (2007) study administered gluten via capsules (see discussion of the significance of route and manner of gluten exposure pointed out above in FDA Response, Part 6.1 above to Reviewer #1 and in the FDA HHA document in the "Dose-Response Assessment" subsection now located under the subpart titled "Routes of Exposure" in this subsection) and appeared to exclude the most sensitive subjects with CD in their testing protocol (see several notations about this latter point in the main text of the HHA document in the "Safety Assessment" section, and in the associated Appendix A and B). These relevant study characteristics and other related details were noted in the description of the specific findings of these studies in the main text in section now titled the "Safety Assessment" in addition to in Table 6 of Appendix B and in Appendix A. Further indication of the nature of the selection of the overall critical NOAEL and/or LOAEL values in a safety assessment approach was added to the introductory section of the "Safety Assessment" subsection.

Reviewer #2

I had some difficulty in understanding how the estimates were arrived at and cannot comment on the uncertainties.

Reviewer #3

(6.1) Yes, but I think a brief discussion of why HL-A genetic variability and IgA deficiency (which are major risk factors for the development of disease) are probably not relevant to establishing a TDI for individuals with existing CD should be included.

FDA Response:
Part 6.1
A brief indication of genetic pre-disposition being a factor in the development of CD and of identification of HL-A now being suggested as possible component of the procedures for the diagnosis of CD was noted in the document (see, respectively, the newly added paragraph in the subpart titled "Celiac Disease" under the "Health Effects" subsection, and the discussion previously included and now found in the subsection titled "Nature and History of the Diagnostic Definition of CD" under the "Dose-Response Assessment" section). In addition, a notation of the possible role of the nature of HLA-DQ2 status in the large range in inter-individual differences seen in responsiveness to gluten exposure in those afflicted with CD was added to the subpart titled "Inter-Individual Variability and Related Uncertainty Issues" in the "Uncertainty Issues in the Hazard Assessment" section. However, because the dose-response data currently available for assessment for the most part were not from studies that included information on HL-A background or immunoglobulin A (IgA) status[24], an examination of the specifics of these factors was not included. Also because the sensitive population of individuals with CD was evaluated in this assessment, it is assumed that those with the propensity to develop CD due to HL-A genetic and IgA deficiency status are represented in the population of subjects analyzed.

Reviewer #4
Yes to both questions. Given the inherent variability between different patients to gluten, the TDIs seem to reflect the most conservative (safest) calculation for gluten other that an absolutely "zero gluten" possibility.

Reviewer #5
Many of the TDI estimates are ridiculously low and do not make sense. The TDI for subchronic gluten exposure for morphological effects is listed as **0.4 mg/d**. The TDI for acute, subchronic, and chronic clinical effects is listed as **0.015 mg/d**. The FDA's proposed rule on gluten-free labeling would allow a food to be labeled gluten free if it contained less than 20 parts per million of gluten. Codex allows foods to be labeled gluten-free if they contain no more than 20 parts per million of gluten. If a person with CD ate just **one** ounce of a product containing 20 parts per million of gluten, they would be ingesting **0.57 mg** of gluten (greater than the TDI values listed above). If these TDI values are disseminated to the public, persons with CD will be afraid to eat.

Reviewer #6
Assuming the question is asking about inter-(between) individual variability – the report as written gives the impression that more UF is needed, particularly if the "target" sensitive sub-population are adults diagnosed with CD. See comment on TDI under the Specific Observations section below.

Charge Question 7. **Is the evidence/data in support of the different TDIs such that one TDI should be given more weight by the Agency in determining whether it can identify a safety assessment-based threshold for gluten? Why?**

[24] The HL-A background or IgA status was either not included as part of the characterization of subjects in the study or the study were performed prior to the development of the knowledge about or techniques to measure them.

Reviewer #1
(7.1) The assessment assigns much greater importance to morphological/physiological (particularly morphological) adverse effects than to clinical adverse effects (pp.12-13), and presumably the TDIs associated with morphological/physiological effects will contribute more significantly to the final TDI calculation. I would like to suggest that this be reconsidered. Although I understand the concern about the subjective nature of at least some of the clinical adverse effects considered (and most of the critical studies for this class of endpoint appeared to be open trials), it is apparent that the adverse clinical effects were very important in terms of the CD patients' well-being. It is notable that in most of the trials, participants who experienced these effects withdrew and refused to continue allowing themselves to be exposed. I conclude from this that recommendations made to CD patients about gluten exposure based on avoidance of morphological/physiological effects would result in many of them suffering clinical signs and symptoms sufficiently severe to affect their quality of life. To avoid such effects, it seems reasonable to me to give the clinical effects TDIs substantial weight in the final TDI calculation.

FDA Response:
Part 7.1
The FDA considers the importance of morphological (and supplemental physiological) effects and clinical effects associated with CD to be comparable. The enumeration and discussion of significant morphological/physiological adverse effects and related TDI derivations are presented on pp. 13 -15 and that of significant clinical effects and related derivations are presented on pp. 15 -18 in the original draft of the main document. Possibly because adverse morphological effects are comprised of a great number of potential measures (e.g., VH, Cd, epithelial surface cell count (E-SCH), Vh/Cd ratio) that might require presentation and discussion, it may appear that this aspect of the adverse effects of CD was the primary focus. However, the assessment included clinical effects of CD in this evaluation because it recognizes the significance of this aspect of the CD condition to its' suffers. This is the first identified attempt to comprehensively examine the nature and characteristics of CD-related clinical effects in a systematic way by evaluating the available dose-response data. Reviewer #1's concern about and support for the FDA's effort to include focus on clinical effects in a similar fashion as is done for morphological effects is noted as is recognition of their affect on the quality of life for individuals with CD, a concern also expressed by Reviewer #1.

Reviewer #2
I think that only a few studies should be considered in making the safety assessment. I don't feel that the Safety Assessment of Gluten Exposure document clearly explains how they arrived at the TDIs, although I think this may be my own ignorance rather than the fault of the authors of the FDA paper.

Reviewer #3
(7.1) No, considering the data available, the different TDIs represent a cautious but appropriate approach. It might be useful to discuss in more detail the overall quality of the clinical data that was reviewed. *(7.2)* For example, were there any studies with sufficient dose- response information that the shape of the dose-response curve could be

suggested?; *(7.3)* were there any concerns of false positive or false negatives in the critical studies due to missed diagnosis?

FDA Response:

Part 7.1

Considerations made in evaluating the quality of the clinical data available was discussed in detail in previous responses in this report (see detailed summary and discussion under Charge Question 2a, FDA Response, Part 2a.1 to Reviewer #5 and possibly also FDA Response, Part 1a to Reviewer #2 under "Specific Observations" in this report). Also FDA provided information on the relevant characteristics and other related details associated with the clinical studies in the description of the specific findings of the critical and accompanying supporting studies in the main text in addition to in Tables 5-7 in Appendix B and in Appendix A of the HHA. The key aspects of these studies were noted to reveal the basis of the scientific judgment and weight-of-evidence considerations made in evaluating the quality of a study and its findings. Examples are that CD subjects on a pre-test GFD and symptom-free exhibited symptoms with a gluten challenge, and the symptoms resolved with termination of the challenge test (e.g., Chartrand et al., 1997); and CD subjects that exhibited clinical effects to a gluten challenge demonstrated a time dependency between onset of symptoms and the peak level of the jejunal physiological measure of prostaglandin E_2 (Lavo et al., 1990), among a number of other possible factors that support the quality (e.g., reliability, validity) of the clinical findings (see peer review report responses noted above). The "weight-of-evidence" discussion in the text of the main HHA document has been supplemented to assist the reader in understanding the considerations made in evaluating the studies with adverse clinical effect(s) findings as has the description of the basis of the evaluation and determination of clinical adverse effects. In addition, as already indicated in the document text, all "studies identified as having dose-response adverse effect data for the acute, subchronic, and chronic categories of toxicity" were evaluated and listed in detail in Appendix A. From these references, only those studies that had relevant low dose-response data and that the FDA had a degree of confidence in the findings were included in the tables in Appendix B. This includes any study subsequently selected as a critical study or a supporting one.

Part 7.2

Reviewer #3 inquired about whether there was sufficient information available to delineate the shape of the dose-response curve for clinical effects. Because the "dependent measure(s)" for adverse clinical effects were essentially qualitative in nature (e.g., diarrhea, abdominal pain, dermatitis herpetiformis (DH)) and not quantitative responses, a dose-response curve can not be determined for the clinical signs and symptoms of CD. One study determined a "composite symptom score" which included a score for severity of each experienced symptom graded by the subjects. However, only one "dose" (or discrete range) of gluten was examined and administered in this study, and not several distinct such doses that could be plotted as a dose-response curve.

Part 7.3

Reviewer #3 also inquired about concerns of instances of false positive or false negative responses due to missed diagnosis being a factor in the adverse clinical effects associated with the critical studies identified. However, as indicated in the main HHA document, the gluten-challenged subjects considered in this dose-response evaluation were those with a confirmed diagnosis of CD. This diminished the likelihood of the

clinical effects exhibited being false negative or false positive responses as did the weight-of-evidence associated study criteria considerations and made in evaluating findings referred to elsewhere in this report (e.g., see above FDA Response, Part 2a.1 to Charge Question 2a under Reviewer #5 and below FDA Response, Part 1a to Reviewer #2 under the "Specific Observations" section).

Reviewer #4
Yes. The lowest TDI should be taken due to the uncertainties inherent in the long-term risk of exposure, and the term "gluten-free" should provide a level of safety to even the most sensitive people with CD. Other terms such as "low gluten," "very low gluten" or "ultra-low gluten" do convey a sense of some gluten content, which might serve to warn the most sensitive individuals.

Reviewer #5
As currently determined (based on the Chartrand study), the TDIs for clinical effects should not be used. The morphological TDIs are questionable as well as is evidenced by the large difference in subchronic and chronic amounts. The Catassi study should be revisited to help determine both NOAEL and LOAEL for morphological effects.

Reviewer #6
As described and summarized in the current safety assessment report, the levels of gluten that could trigger a clinical response in adults appear to be the lowest and thus should be given more weight in attempt to develop a threshold level. However, as noted earlier and below (see Specific Observations on the TDI section), in the face of uncertainty in the available dose response data from challenge studies and particularly the lack of robust long term chronic exposure information, additional uncertainty factors should be considered to account for uncertainty in the existing dose response information.

Charge Question 8. **Do you have additional comments that would assist FDA in refining the safety assessment? Are there additional scientific/technical studies available that were not considered?**

Reviewer #1
I do not know of additional studies that would be relevant or that should be considered as part of the safety assessment.

Reviewer #2
If Carlo Catassi and Pekka Collin (corresponding author of Peraaho et al. 2003) could be brought together as co-PIs in a collaboration with adequate funding, we might actually get somewhere with this problem. It would take some years to obtain results, but these two investigators have the experience and the knowledge to really make some progress in this challenging area. Catassi and Collin have somewhat different approaches, but bringing them together would facilitate a resolution of the approaches that would be highly beneficial. Good funding is the key—probably will never happen. Funding is made available for basic immunology (good in itself), but not for such important work as determining the lowest tolerable level of gluten for celiac patients.

Reviewer #3
No other comments. However, since the TDI was established using data with issues regarding data quality, it would be prudent to constantly review the CD literature in context of new data and adjust the values, if necessary.

Reviewer #4
The TDI should take into account the worst case scenario: the most sensitive patients replacing all gluten containing foods with "GF" foods that contain the maximum permitted concentrations, which are quantities that could be as much as 50% over their expected intake. The TDI may be different in those with persistent damage versus those with a previously healed intestine. There are also the imponderable issues of whether rye and barley prolamins are different from wheat in terms of toxic effects and the detectablity of these proteins, which could impact the verification of GF status of foods.

There may also be some impact on patients in terms of a placebo effect of even levels of gluten that are at NOAEL.

See Specific Observations for additional references.

Reviewer #5
No additional comments.

Reviewer #6
Please see comments on food consumption below in the Specific Observations section.

C. Specific Observations

Reviewer #1

1. *(1)* [25] In several places, reference is made to "intra-individual variability" when I think what is meant is "inter-individual" (p.19 line 26, p. 19 line 30, p.20 line 3)

2. *(2a)* I found Tables 10-13 somewhat difficult to interpret and was uncertain how the information in them, particularly the columns "LOC," would be used to establish gluten exposure recommendations. *(2b)* The Background section of the Work Assignment Authorization indicates that these LOC estimates pertain to gluten consumption. If I am interpreting them correctly, they pertain to the average gluten concentration (mg/kg) in the total food consumed that, if exceeded, would result in a gluten intake above the TDI. I don't think that the target average gluten concentration of all gluten-containing foods is very useful to the CD patient. What the patient needs to know in order to make choices that will maintain his or her gluten intake below the TDI is the gluten content (in mg/kg) of different foods.

FDA Response:

Part 1

Clarification and corrections on the "intra-species" versus "inter-individual" terminology was made in the main text of the document and in Appendix B.

Part 2a

Tables 10-13 were completely redone. The information communicated in them was recalculated and clarified. It was also condensed into two tables (Tables 10 and 11) which were moved to Appendix B.

Part 2b

The section in the HHA containing the discussion of the calculation of the LOC estimates has been rewritten and expanded. This section is now under the subheading "Levels of Concern for Gluten" and is located under the "Risk Characterization" section. In this assessment, the LOCs are concentrations of gluten in food that correspond to the identified TDIs of focus. The values are derived from various estimates of the level of exposure to "gluten-free" foods consumed per day in those with CD that were determined in the "Exposure Assessment" of the main document. This type of determination of the exposure estimates and subsequent LOC values was employed because it encompasses the nature and objective of the proposed rule for the labeling of gluten-free food and for defining the term "gluten-free" that was promulgated by the FDA in the Federal Register (72 FR 2795) in 2007 (see above, Part I, Introduction). The details of the context of the various aspects of gluten-free labeling reflects policy and risk management issues and is outside of the scope of the safety/risk assessment work performed in the HHA document. Finally, Reviewer #1 indicated that he/she doesn't "think that the target average gluten concentration of all gluten-containing foods is very

[25] This demarcation of a section or part of a reviewer's written response or comments denoted by italic parentheses in a font different from the surrounding text was inserted by the FDA to delineate the part of the reviewer's statement to which the subsequent FDA response presented below it corresponds. The demarcations within the parentheses represent a numbered subpart of the reviewer's comments/feedback inserted by the FDA for organizational purposes.

useful to the CD patient." This understanding conflicts with the intended interpretation of the LOC which in this case was to be associated with the "tolerable" gluten concentration for all gluten-free foods for those with CD and not "all gluten-containing foods" as suggested by Reviewer #1. The estimations performed were to inform the labeling of gluten-free food in a uniform way for individuals with CD and not to inform the different gluten contents of food for them.

Reviewer #2

(1a) The paper by Chartrand et al. 1997 J. Am. Diet. Assoc. 97:612-618 is cited frequently throughout the document. One problem with this study is the lack of any morphological data because no biopsies were taken. The symptomatic basis for concluding that patients were reacting to the wheat starch seems weak to me. These patients could be exhibiting psychologically-determined responses, or simply be exhibiting the symptoms of food allergy, bacterial or viral infection, or whatever. *(1b)* I doubt that the value of 0.75 mg/100 g given for the starch used is correct. On the basis of my experience with a few different commercial wheat starches analyzed by the R5 ELISA test (Kasarda et al., 2008, J. Ag. Food Chem.; on-line preprint available on journal web site), I feel moderately certain that this value is low by a factor of about 10-fold. The ELISA test used by the authors was based on an anti-omega gliadin antibody. I have suggested that omega-gliadins, lacking any cysteine or cystine, may be relatively more readily washed off starch granules during their separation than would be other gluten proteins, which would then result in a low value for the gluten analysis.

(2) p. 6, lines 30-35. It is true that information about the relative toxicities of wheat, barley, and rye proteins is lacking, but even more so information about the different types of gluten proteins in wheat is also lacking. Although it is often assumed that alpha-gliadins are the most toxic fraction—relative to gamma-gliadins, omega-gliadins, low-molecular-weight subunits, and high-molecular weight subunits, I would say that information on the relative toxicities of these components is almost nil. Furthermore, gliadin preparations and peptide digest preparations probably vary in the relative proportions of these sub-types and in the relative proportions of peptides derived from them in the case of peptide digests. Peptic-tryptic digests of gliadin or gluten, such as FF3, have never been characterized as to which specific peptides they contain and I suspect that some of the more toxic peptides are likely to predominate in such digests (partly because of solubility fractionation), which would then make the materials tested with patients much more toxic than simple gluten or gliadin preparations.

(3) p. 12, lines 39, 40. Chartrand et al. did carry out antibody testing, but found no changes.

(4a) p. 14, lines 1-7. The study by Ciclitira et al. (1984b) involved infusions of 1000 mg, so how were a NOAEL of 20 mg and a LOAEL of 24 mg of gluten derived from those experiments. *(4b)* In the case of the study by Lavo et al., a dose of 12 mg of crude gliadin was introduced into a perfused segment of intestine and allowed to remain in contact with the intestinal surface for 100 minutes. This may not be a very good model for the normal digestive exposure of the intestine to gluten where gluten peptides would be mixed with partially digested food products from substances other than gluten.

(5) p. 15, lines 4, 5. Subjects experiencing acute clinical symptoms in response to gluten challenge are odd in many ways. I have often wondered if such patients might be experiencing some sort of allergic reaction to gluten rather than a "true" CD type of reaction. Also, some patients seem to be highly susceptible to suggestion and this may be a contributing problem. As far as I know, this type of patient with an immediate, severe reaction to gluten has never been studied in isolation. This needs to be done. Celiac disease has always been classified as a delayed hypersensitivity and we don't know what to make of these immediate hypersensitivity types. They play havoc with tests because the assumption is made that there is extreme variability in type of reaction among celiac patients. Perhaps so, but objective studies need to be carried out to at least consider the possibilities of IgG-mediated, or IgE-mediated responses, along with hysterical responses to a gluten challenge.

(6a) pp. 18-19, lines 43, 44, and lines 1, 2. "TDIs for acute, subchronic, and chronic ingestion of gluten were determined and presented in Table 8 in Appendix B. The resulting tolerable daily intake levels for each of these exposures were 0.015 mg gluten/day." I have no idea how these conclusions were reached. I would need a much more detailed explanation than I found in the text for this result. It makes no sense to me. I suppose uncertainty factors were introduced, but I would have to be convinced that they were meaningful. *(6b)* All I can say is the authors might as well have said the tolerable daily intake is 0.00015 mg gluten/day. I don't believe that 0.015 mg gluten per day is meaningful. How would this be promulgated? Analytical methods are not suitable for measuring such low low levels and the average celiac patient attempting a naturally gluten-free diet is probably ingesting that much gluten every day.

FDA Response:
Part 1a
The FDA believes the findings of Chartrand et al. (1997) are such that they should be considered in combination with that of other study findings as part of the weight-of-evidence in determining the “threshold” of exposure associated with clinical effects. The diagnosis of CD for subjects in the Chartrand et al. (1997) reference was established by the well-established European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) criteria and then the subjects only consumed a strict GFD (which includes no GFD food products containing wheat starch) for at least 1 year prior to the challenge test. The constellation of responses reported in this study is reflective of CD and some such as DH could not be explained by the other states or conditions suggested by Reviewer #2 (e.g., food allergy, bacterial or viral infection). In addition, characteristics of the results found in Chartrand et al. (1997) also provide support for the adverse reactions being attributed to exposure to low doses of gluten exposure (see also discussion above in FDA Response, Part 2a.1 to Reviewer #5 under Charge Question 2a). For instance, adverse reactions only occurred in the experimental CD subjects when they were exposed to the food products containing wheat starch, not prior to or after this exposure[26], and no adverse reactions were ever exhibited in the “tolerant” CD control subjects during any time period. If the clinical adverse effects were attributed solely to “psychological” reasons or other illnesses as suggested by Reviewer #2, then

[26] For some subjects, it took time for some of the symptoms to diminish and resolve after the gluten challenge period was terminated, but all adverse clinical effects eventually ceased in the post-challenge period.

they should be exhibited at least to some degree during other time periods and to both group of subjects. The responses would also be expected to be more random, spurious and inconsistent in nature in each group of subjects and include instances of symptoms not associated with CD like fever, congestion, cough, urticaria, angioedema, rhinitis and possibly anaphylaxis. Moreover, apparently countering the "psychological," or the like, arguments made by Reviewer #2, the majority of the experimental CD subjects newly exposed to food products containing wheat starch in the challenge reported liking the more palatable options they provided their GFD. Because of this, several subjects despite experiencing symptoms tried to continue consuming the wheat starch food products until the adverse reactions became intolerable leading them to eventually withdraw from the challenge test. Next, a number of other different studies demonstrated LOAELs for clinical effects in the low mg range including one at the comparable level of 2.4 - 4.8 mg gluten/day for acute and subchronic exposure and two that administered gluten capsules in a DBPCFC fashion. Thus, there is weight-of-evidence support for a LOAEL in the dosage range of Chartrand et al. (1997), the study with the lowest LOAEL. In addition, CD-related morphological changes are associated with the emergence of adverse clinical effects and other studies listed in Appendix B suggest that they can emerge with exposure to gluten in the low mg range. However, a number of other biological-based changes occur in the aberrant state found in CD and can not be excluded as contributing factors to adverse clinical responses at this time. Some aspects of the clinical effects may be humorally- (e.g., cytokines), physiologically- (e.g., prostaglandins), or cell-mediated (e.g., Auricchio et al., 1991; van de Wal et al., 2000[27]). Also, gluten has been demonstrated to be an acute morphological toxin and that the morphological changes that occur after an acute intake episode can be transient in nature. Lastly, the safety assessment approach involves identification of the lowest margin of reactivity in its attempt to protect the most sensitive individuals and hence, in turn, all susceptible individuals from the development of an adverse health condition or disease state. To this end, the FDA believes consideration of the adverse clinical effects in the Chartrand et al. (2001) study that occurred at the very low mg dose levels (i.e., overall LOAEL for clinical effects), along with the nature and relevance of characteristics of this study and with supporting studies, is warranted.

Part 1b

Reviewer #2 questions the 7.5 ppm gliadin content level that was reported for wheat starch that was administered to CD patients in the "critical" Chartrand et al. (1997)[28] study. He/she believes it is low by "a factor of about 10-fold" and provides a reference of Kasarda et al. (2008)[29] as the basis for this determination. Chartrand and colleagues measured the level of gliadin in the certified gluten-free wheat starch mix commercial food product using an ELISA analysis that employed an anti-ω-gliadin antibody. The Kasarda et al. (2008) paper examined the nature of 4 commercial wheat starch food products rendered gluten-free. These authors speculated that the washing process involved in removing gluten surface-associated proteins from wheat to produce

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[27] Auricchio S, Troncone R. Effects of small amounts of gluten in the diet of celiac patients. Panminerva Med 33: 83-85, 1991

van de Wal Y, Kooy Y, van Veelen P, Vader W, Koning F. Coeliac disease: it takes three to tango! Gut 46: 734-737, 2000

[28] Chartrand L, Russo PA, Duhaime AG, Seidmain EG. Wheat starch intolerance in patients with CD. J Am Diet Assoc 97(6): 612-618, 1997

[29] Kasarda DD, Dupont FM, Vensel WH, Altenbach SB, Lopez R, Tanaka CK, Hurkman WJ. Surface-associated proteins of wheat starch granules: suitability of wheat starch for celiac patients. J Agric Food Chem 56: 10292-10302, 2008

commercial wheat starch products is more prone to eliminate ω-gliadin because of its chemical structure than other gliadin subfractions (α-, β-, γ-gliadin). They and, in turn, Reviewer #2 suggested that the ELISA analysis for gluten based on monoclonal antibodies for ω-gliadin (like the one Chartrand et al., 1997 used) probably results in low estimates of gluten content. First, it is not clear from the analyses performed and the findings reported in the Kasarda et al. (2008) paper how the suggested 10-fold lower estimate was concluded by Reviewer #2. This work characterized surface-associated gluten proteins present in the different commercial gluten-free wheat starch products by two-dimensional electrophoresis (2DE) and mass spectrometry (MS/MS)-based identification. These analytical procedures identified differences in the presence of various gluten proteins in the different wheat starch products but the investigators say in the paper itself that they "were not able to quantify our results." They also indicated that "[i]t is conceivable that results are skewed" because it "is recognized that some proteins have peptide sequences that are more likely to provide good signals during MS analysis than others." For example, there is "a tendency to identify LMW[30] glutenin subunits preferentially to α- and γ-gliadins" because these "gliadins provide few tryptic peptides amenable to identification by MS/MS." In addition, an inconsistency in the results from these two different analytical methodologies was seen as no ω-gliadin proteins were identified in MS/MS analysis of the various starches but they were evident in the 2DE analysis of at least 1 of the same starch products. Hence, the findings from these 2 analytical approaches do not provide quantitative information on the degree, such as a 10-fold factor, or on the nature of lowered levels of ω-gliadin in gluten-free wheat starch products, or if this occurs in the processing of wheat or is a factor in any significant way. Next, Kasarda and colleagues also measured the gluten content of the 4 wheat starch food products in this study by an ELISA methodology that employed a R5 monoclonal antibody to a celiac gluten epitope binding site (QQPFP amino acid sequence). It was suggested that this ELISA methodology would not result in an underestimation of gliadin levels in contrast to the one based on an ω-gliadin antibody noted above because it recognizes epitopes on all gliadin subfractions. However, no direct comparison of the resulting gluten measurements of the same wheat starch samples (and/or spiked controls) from each ELISA methodology was made to determine if the ω-gliadin antibody-based ELISA approach appeared to underestimate gliadin content levels. Also the gluten-free wheat starch product administered in the Chartrand et al. (1997) study was not one of the brands evaluated in the Kasarda et al. study, and furthermore, one of the brands analyzed in the Kasarda et al. study had a lower gluten content (5-8 ppm gluten) than was reported for the wheat starch product administered in the Chartrand et al. paper (15 ppm gluten), the latter of which is purported by Kasarda et al. and Reviewer #2 to represent an underestimation. Moreover, when measured by the R5 ELISA method alone in the Kasarda et al. study, differences of approximately 30-fold (R5 ELISA test #1, range: 8 - 212 ppm gluten) and 70-fold (R5 ELISA test #2, range: 5 - 363 ppm gluten) in gluten content of the 4 wheat starch products examined was found, suggesting other relevant factors play a role in the varying gluten content of wheat starch than only the type and detection characteristics of the ELISA test used. Also, no data on direct comparisons of gluten-free wheat starch products known to undergo differing washing procedures to remove gluten and their corresponding gluten content were provided in the Kasarda et al. paper to support their suggestion of its role in differentially removing the gliadin subfractions.

[30] The abbreviation "LMW" represents the term "low molecular weight." This footnote was inserted by the FDA and was not part of the original quoted statement presented here.

Other data is available that contrasts the premise of the argument made by Reviewer #2 that use of the ω-gliadin antibody ELISA test results in a 10-fold lower gluten level estimate of the actual content value. The ELISA analysis that employs the monoclonal antibody ω-gliadin is not (mono-)specific solely to the ω-gliadin subfraction in wheat. It also recognizes and cross-reacts with other wheat gliadin subfraction types and thus, includes them in its quantification of a gluten content estimate. Recent evidence also suggests that it appears to bind to some degree to traces of the HMW glutenin fraction of gluten found in the gliadin fraction in at least some commercial ELISA kits[31]. Also ELISA analytical procedures typically involve employing various standards (e.g., calibrants), and possibly correction factors that make considerations for the characteristics of antibody detection within each protocol or kit to arrive at a representative value of the total gliadin and/or glutenin, and in turn, gluten levels. Next, some studies have evaluated and directly compared the findings from the analyses of the same wheat (alone) samples for gluten by both the ω-gliadin and R5 antibody based ELISA methods.[32,33] They found that the ELISA based on the ω-gliadin monoclonal antibody demonstrated measurement accuracy and did not significantly underestimate the gluten levels when wheat content of the food (not contaminated by other grains such as barley) was in the lower ppm range (e.g., <50 - 100 ppm). In addition, the results of analysis of wheat samples by R5 ELISA were comparable to and/or were not found to be more accurate (at $\leq$ 50 ppm) than ω-gliadin ELISA based method.

Finally, the performance of any safety/risk assessment for the detrimental health effects from exposure to a toxic agent involves employing "estimates" of a number of factors. This includes estimates of the amount of this agent administered or in a food. The variability and uncertainty associated with measurement techniques, analytical procedures, certain methodologies for determining these "contents" is recognized as an element in the resultant "estimate" derived. It is understood that the health hazard assessment approach can only use, and thus, be based on the "best" estimates derived and/or available at the time they are performed. This concept is well-recognized and accepted in the field of safety/risk assessment.

Part 2

As revealed in Appendix A, a majority of all studies with dose-response data that were evaluated administered gluten. Of the low-dose studies of relevance presented in the tables of Appendix B that administered gliadin, the substance administered was unfractionated gliadin[34] which would consist of all gliadin subfractions. No study that administered a subfraction of gliadin were found in the tables in Appendix B and thus involved in low-dose exposure determinations because no clear conversion factors

[31] Diaz-Amigo C, Yeung JM. Critical evaluation of uncertainties of gluten testing: Issues and solutions for food allergen detection. In: Pathogens and Toxins in Foods: Challenges and Interventions, edited by JK Juneja and JN Sofos, Washington, DC: ASM Press, 2010

[32] Gelinas P, McKinnon CM, Mena MC, Mendez E. Gluten contamination of cereal foods in Canada. Internat J Food Sci Tech pp 1-8, 2007

[33] Westphal CD, Jupiter JM. Detection of gluten by commercial test kits: Effects of food matrices and extraction methods. In: Food Contaminants—Mycotoxins and Food Allergens, edited by D Siatar, MW Trucksess, PM Scott and E Herman,. ACS Symposium Series 1001, pp 462-475, 2007

[34] Some studies specifically stated that "unfractionated gliadin" was administered. Others indicated that "gliadin" was administered. It was assumed that it was unfractionated gliadin when no reference to subfractions was made as was typically indicated in studies that specifically investigated the effects of subfractions (e.g., α-gliadin, γ-gliadin).

specifically for them were identified to estimate a comparable gluten value. Again, this information is provided in Appendix A. Next, the possibility of differences in relative proportions of the different subfractions found within unfractionated gliadin preparations made in different laboratories or at different times (as suggested by Reviewer #2) is an issue that falls under the variability and uncertainty recognized as a part of deriving "best" estimates in any safety/risk assessment. Not every aspect, factor or component of such an assessment is "knowable" in the absolute nor does the FDA believe it is reasonable or feasible to wait for such a remote possibility of obtaining "complete" knowledge before conducting an assessment (see also discussion above in FDA Response, Part 1b to Reviewer #2 comments under "Specific Observations" about using the "best" available data and making the "best" estimates). Moreover, the subfraction classifications of gluten (e.g., α- and β-gliadin, LMW subunits of glutenin) referred to by Reviewer #2 are based on chemical distinctions (e.g., solubility properties, molecular weight units) and not biological-based distinctions such as associated with biological functions (e.g., digestion) and sensitivities (e.g., IgA-related responses) and the related constituent amino acid sequences or peptides or so-called "epitopes" that are found within the subfractions that are based on chemical classifications. As indicated in the main text of the HHA document in the "Dietary Effects" section under the subsection "Gluten" in the subpart now titled "Nature and Characteristics of the Components of Gluten," similarities between and overlap in the primary amino acid peptides structures of the different gluten-related proteins and their subfractions exist. Hence, the presence of similar repetitive amino acid sequences suggests a significant degree of homology between the heterogeneous and different types of proteins within wheat gluten probably exists.

Other issues that were related to the use of the FF3 digest in particular were already addressed in this report in FDA Response, Part 2b.1 both to Reviewer #1 and to Reviewer #2 to Charge Question 2b. Again, of the studies that administered FF3, the results of only 2 total were represented in the 6 different low-dose adverse effects tables in Appendix B and only 1 study was involved in a single critical effect determination (again, also see Appendix A for specific details provided for each study). So the great focus on this point by Reviewer #2 throughout the peer comments appears to exceed its relevance with respect to the whole safety assessment.

Lastly, the statements by Reviewer #2 in this response that first indicate that no "information on the relative toxicities of these components," meaning gluten subfractions, is available, versus a second later statement in the same paragraph that indicates the specific peptides that comprised FF3 digest are "some of the more toxic peptides" of gluten seem to be conflicting points, and the FDA finds them confusing and difficult to address. Also, references supporting these two arguments made were not provided.

Part 3

In this assessment, physiological measures were considered as supplemental findings that could support the existence of morphological changes found in a study. They are part of the weight-of-evidence considerations possibly involved in evaluating all the findings on different measures within a study. Certain antibody level changes are suggestive of the presence of the sensitivity to gluten in CD but are not considered absolute, definitive measures of this disease. Correspondingly, the measure of changes in antibody levels are only used as an adjunct measure or a screening tool in the diagnosis of CD that accompanies the primary morphological evaluations made. Thus, the lack of significant changes in antibody testing, especially at the very low doses of

gluten exposure involved in the Chartrand et al. (1997) study, does not in itself result in excluding consideration of the findings of this study. For instance, Catassi et al. (2007), a DBPCFC study, reported changes in various measures of morphological effects with a low-dose gluten challenge in CD-diagnosed subjects which was not accompanied by changes in IgA anti-tissue transglutaminase (anti-tTg) and IgG class antigliadin antibody (AGA) levels. During a 3-week gluten challenge test, Pyle et al. (2005)[35] demonstrated an increase in symptoms and gluten-related changes in physiological measures such as intestinal xylose absorption and excretion and fecal fat content without accompanying changes in antibody (transglutaminase IgA and IgG and antigliadin IgA) levels. Others have also indicated that endomysial antibodies are not reliable markers of slight dietary transgressions while on a GFD, or their absence (or disappearance) on a GFD does not necessary correspond to complete histological recovery.[36]

Part 4a

Careful reading of the methods and procedures of the study by Ciclitira et al. (1984b) reveal that two different of challenge tests were performed in this work. One set that administered 1000 mg of different gliadin subfractions to subjects. The exposure data from these subfraction challenge tests were not considered in the hazard/safety assessment of low-dose data. The second set of challenge tests in this study was the one evaluated in the hazard/safety assessment. They administered different doses of unfractionated gliadin, the lowest dose being 10 mg unfractionated gliadin (i.e., 20 mg gluten as a NOAEL). Next, revealed in the main HHA document (at pp 14, line 1-7 of the draft hazard/safety assessment referred to by Peer Reviewer #2) a reference to "respectively" was made in discussing the studies Ciclitira et al. (1984b) and Lavo et al. (1990a), the 24 mg gluten corresponds to a dose administered in the study by Lavo et al. (1990a) and not Ciclitira et al. (1984b) as thought by Reviewer #2.

Part 4b

As indicated in the main text of the HHA in a clear and transparent manner, dose-response information from both oral ingestion, and infusion or perfusion into the small intestine was considered to obtain as many data points of reference and sources of comparison as possible. Data from studies that used oral routes of administration were considered to best reflect the nature of exposure to gluten that would be experienced through normal dietary exposure, and thus, to best serve as the basis of identifying critical dose-response studies. Subsequently, data from studies that used infusion or perfusion of gluten compounds into the small intestine were not given primary focus in this assessment, but considered to be supportive evidence (see also in this report FDA Response, Part 6.1 to Reviewer #1 under Charge Question 6). This is indicated in the manner in which findings of studies were described and presented in the "Safety Assessment" section in main HHA document.

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[35] Pyle GG, Paaso B, Anderson BE, Allen D, Marti T, Khosla C, Gray GM. Low-dose gluten challenge in celiac sprue: Malabsorptive and antibody responses. Clin Gastroenterol Hepatol 3(7): 679-686, 2005

[36] For example: Troncone R, Mayer M, Spagnuolo F, et al. Endomysial antibodies as unreliable markers for slight dietary transgression in adolescents with CD. J Pediatr Gastroenterol Nutr 21: 69-72, 1995

Dickey W, Huges DF, McMillan SA. Disappearance of endomysial antibodies in treated CD does not indicate histological recovery. Am J Gastroenterol 95: 712-714, 2000

Part 5
The development of CD may be a "delayed hypersensitivity" condition in that an immune mechanism needs to be initiated for the disease state to emerge in susceptible individuals. However, the subjects analyzed in this assessment were already diagnosed with CD, so the gluten challenge tests were characterizing the nature of the toxic response itself. The gluten-induced responses associated with these challenges should not be assumed to result in delay reactions. In addition, the analysis of gluten challenge test data in the FDA assessment revealed that gluten is an acute morphological/physiological toxin with aberrant changes sometimes occurring within hours to days which make the possibility of it also being an acute clinical toxin a reasonable one. Hence, the FDA considers clinical effects for those diagnosed with CD of significance and their assessment warranted. The nature and characteristics of CD-related clinical effects was comprehensively examined in this assessment in a systematic way by evaluating all available dose-response data and its "weight" as a body of evidence in considering its validity. The FDA feels that a systematic examination such as this is a preferable analysis of the nature of the effect than judging their nature and basis as being "psychological", "hysterical" or un-"true."

Part 6a
FDA feels the information provided in the subsection (now titled "Tolerable Daily Intake Levels for Clinical Effects" under the "Safety Assessment" subheading) describing the calculation of the TDI for clinical effects along with that provided in Table 8 of Appendix B adequately indicated the derivation of the resultant clinical TDIs based on the safety assessment approach.

Part 6b
The role of a hazard health assessment is to identify and assess the hazards or adverse effects of exposure to an agent and to characterize the risks and uncertainty associated with this exposure which this HHA has done based on all available dose-response data. The promulgation of the findings of this assessment is outside the scope of a health hazard assessment such as this. The promulgation of the resultant TDI values is a risk management decision, and as indicated in the work task order charge to peer reviewers, they were not to provide comment on policy related to the use of the HHA by FDA but just on the nature of the scientific basis of the HHA. In other words, the findings of the hazard/risk assessment serve to inform, but is separate from, the next step, that being the risk management stage.

Reviewer #3
(1) The suggested TDIs for children and adults are the same. I think this is appropriate considering the weakness of existing data available. However, I have some concern regarding the comment that adults may be more sensitive than children (see pg 22, summary & conclusions). As recently reviewed by C Hischenhuber et al., (Alimentary Pharmacol & Therapeutics, 23:559, 2006), they discuss studies which suggests that CD is more common in children (although less severe and tend to remiss in children) compared to adults and the lowest doses necessary to provoke a reaction is also larger in adults.

Just as a note: Overall, I think this is a well written assessment based upon a difficult question. Like allergic hypersensitivity reaction, one can assume that in CD there is a sensitization phase followed by an elicitation response. The latter evokes pathology and usually occurs at lower doses than required for sensitization. The assessment for gluten

can only address this so-called secondary response. Unfortunately, there is little mechanistic information available for the secondary/memory/elicitation step in terms of risk assessment, particularly regarding differences between acute and chronic exposure. In fact low dose chronic exposure has been used as a form of treatment to induce tolerance. For environmental chemicals the assessment would be based upon the sensitization phase.

FDA Response:
Part 1
This section of the "Summary and Conclusions" noted by Reviewer #2 addresses the ways that exposure to gluten in CD varies between individuals. It indicates a "suggestion" in the findings available that age differences in CD-related sensitivity to gluten may exist. Two earlier sections in the main HHA document address this point, so its mention as a possible factor in the variability found in responsiveness to gluten seems appropriate. Briefly, first, the evaluation of available studies in this assessment for critical adverse effects associated with gluten exposure in CD suggested that age is "a factor in the responsiveness of individuals with CD," but also included caveats that may play a role such as the possibility that age is "a function of the existing studies available in the published literature and/or to the year that the study was performed." Second, in the paragraph beginning on pp. 9, line 32 in the original draft hazard/safety assessment document evaluated by peer reviewers (now under the subsection titled "Nature and Characteristics of the Toxic Responses Evaluated" under the subpart "Age Groups of Subjects Evaluated"), various references were cited that support the notion that the differences between children and adults exist in a number of factors associated with CD. These include morphological changes to treatment, occurrence of other CD-related diseases, and age-related variables used to examine responsiveness and outcomes associated with these secondary diseases. Finally a sentence was added to the main HHA text under the discussion in the "Age-Related Effects" subsection (now located under the "Safety Assessment" section) indicating that further investigation of age differences are needed.

Finally, an examination of the Hischenhuber et al. (2006)[37] paper noted by Reviewer #3 reveals that the differences in sensitivity between children and adults to wheat discussed in the text of this paper was referring to their response to wheat as a food allergen and not as an inducer of CD. In fact, under the "Gluten challenge studies" category in Table 1 of the Hischenhuber et al. (2006) reference, the listed available data suggests that adults appear to be more sensitive to gliadin or gluten (depicted as dosage per day) with respect to CD development than children.

Reviewer #4
(1) Page 2, lines 23 and 24: Primary data is McDonald 1964 and Murray et al. CGH 2008

Murray JA. Rubio-Tapia A. Van Dyke CT. Brogan DL. Knipschield MA. Lahr B. Rumalla A. Zinsmeister AR. Gostout CJ. Mucosal atrophy in CD: extent of involvement,

[37] Hischenhuber C, Crevel R, Jarry B, Makis M, Moneret-Vautrin DA, Romano A, Troncone R, Ward R. Review article: safe amounts of gluten for patients with wheat allergy or CD. Aliment Pharmacol Ther 23: 559-575, 2006

correlation with clinical presentation, and response to treatment. Clinical Gastroenterology & Hepatology. 6(2):186-93; 2008 Feb.

W.C. MacDonald, L.L. Brandborg and A.L. Flich *et al.*, Studies of celiac sprue IV: the response of the whole length of the small bowel to a gluten free diet, *Gastroenterology* 47 (1964), pp. 573–589.

(2) Page 2: There is no data to support the assumption that clinical responses are due to the length of involvement of the small intestine with CD. See Murray et al 2008 above

(3) Page 3, lines 16 and 17: The statement that "fewer new cases exhibit the typical classical gastrointestinal symptoms" may not be strictly true. A greater proportion of new cases exhibit atypical clinical features, however, there has been such an increase in the numbers of new cases that there may actually be an increase in new cases with classical symptoms.

Murray JA. Van Dyke C. Plevak MF. Dierkhising RA. Zinsmeister AR. Melton LJ 3rd. Trends in the identification and clinical features of CD in a North American community, 1950-2001. [Journal Article. Research Support, U.S. Gov't, P.H.S.] Clinical Gastroenterology & Hepatology. 1(1):19-27, 2003)

(4) Page 3, line 29: DH may have an abrupt onset.

Page 4, lines 1-10: This area is controversial. Some studies were negative.

(5) Sategna-Guidetti C., Solerio E., Scaglione N., et al: Duration of gluten exposure in adult coeliac disease does not correlate with the risk for autoimmune disorders. *Gut* 49. (4): 502-505.2001;

Biagi F., Pezzimenti D., Campanella J., et al: Gluten exposure and risk of autoimmune disorders. *Gut* 51. (1): 140-141.2002;

(6) There are many times that CD develops after the development of Type one diabetes mellitus, when testing done at the time of Dx of DM was negative. This suggests that silent CD did not precede that of T1DM (Glastras et al. Diabetes Care, 28, 2170-2175, 2005). For a more detailed discussion of this issue see:

Barton SH. Murray JA. Celiac disease and autoimmunity in the gut and elsewhere. [Review] [79 refs] [Journal Article. Research Support, N.I.H., Extramural. Review] Gastroenterology Clinics of North America. 37(2):411-28, vii, 2008

(7) Page 11, lines 43 to 47: Several updated guidelines for the diagnosis of CD have been published. The AGA in 2006 and NASGHAN in 2005.

Hill ID. Dirks MH. Liptak GS. Colletti RB. Fasano A. Guandalini S. Hoffenberg EJ. Horvath K. Murray JA. Pivor M. Seidman EG. North American Society for Pediatric

Gastroenterology, Hepatology and Nutrition. Guideline for the diagnosis and treatment of CD in children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. [Guideline. Journal Article. Practice Guideline] Journal of Pediatric Gastroenterology & Nutrition. 40(1):1-19, 2005

Rostom A. Murray JA. Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of CD. [Review] [303 refs] [Journal Article. Research Support, N.I.H., Extramural. Review] Gastroenterology. 131(6):1981-2002, 2006

FDA Response:

Part 1

Reviewer # 4 provided two references to include as citations noting that they were the primary data source that supports a statement made in the first paragraph under the subpart heading "Celiac Disease" in the "Health Effects" subsection. These references were reviewed and included as references as suggested by Reviewer #4.

Part 2

Reviewer #4 suggested a reference that describes the relationship between clinical responses and the extent of the small intestine involvement in CD. The data in it counter a statement about this relationship found in the original health hazard assessment draft document in the second paragraph under the subpart heading "Celiac Disease" in the "Health Effects" subsection. Information from the reference noted by Reviewer #4, in addition to other references, was used to insert a new, more accurate statement in its place.

Part 3

Reviewer #4 provided information to supplement statements about the sign and symptoms exhibited by those with CD over time that were located in the last paragraph under the subpart heading "Celiac Disease" in the "Health Effects" subsection. These statements in the original of the hazard/safety assessment document was edited to reflect this feedback and/or supplemented with additional information.

Part 4

Numerous references with information on DH were examined to locate a source of reference for the point that "DH can have an abrupt onset." No citation for this was found, so because it was indicated in the original FDA document that it "usually has a gradual onset" meaning most often but not always the case, the statement was not changed.

Part 5

In contrast to some points made in the original gluten hazard assessment draft document, the findings of the references noted by Reviewer #4 suggest that the duration of exposure to gluten and/or age of CD diagnosis in those diagnosed as adults are not related to the development of autoimmune diseases. However, it is difficult to interpret results and draw definitive conclusions about the role of the total and/or cumulative amount of exposure to gluten prior to disease occurrence when this exposure is assessed in all cited references by indirect measures such as those related to "time" of gluten exposure, or assessed over many decades of ages

grouped together (e.g., subpart, 2002[38]) when eating patterns and exposure levels (and body weights) tend to vary over this time. Also individuals subsequently diagnosed with CD (or other related diseases) may alter their intake of gluten-containing foods over time and/or age prior to diagnosis because of clinical signs or symptoms, feelings of malaise, etc. or for other reasons (e.g., weight loss dieting) and thus, their consumption levels may be lower to some relative degree with increased age. Examination of the references noted by Reviewer #4, along with those cited in the original gluten hazard assessment document, appear to suggest that differences in the relationship of "age at diagnosis" or an index of "duration of gluten exposure", and the development of autoimmune disease exist in children and young adults versus found in older adults, or between the first half life and the second half. Notation of the differing results found in the literature with respect to the factors of age and/or duration of gluten exposure in CD and the development of autoimmune disease have been added to the discussion in the "Autoimmune Disease" subpart under the subsection heading of "Other Health Effects."

Part 6

Reviewer #4 provided additional information and supporting references about the timing of the development of CD and of an autoimmune disease. This information was used to supplement the discussion of the associations between these conditions in this "Autoimmune Diseases" subpart of the "Hazard Identification" section of the HHA document.

Part 7

Reviewer #4 provided 2 references that are recently published guidelines for the diagnosis of CD. These references, along with information from them, were included in the subsection now titled "Nature and History of the Diagnostic Definition of CD" under the "Dose-Response Assessment" section to update its contents.

Reviewer #5[39]

(1a) Page 3, line 4 "have" misspelled

(1b) Page 3, line 4 "atrophy" not "agrophy"

(1c) Page 5, line 9 "groups" not "group"

(2a) Page 7, line 23 Then why wasn't the Catassi study used as the "critical study?

(2b) Page 7, lines 36-37 This is precisely why the Chartrand study should be viewed with some skepticism.

[38] Biagi F, Pezzimenti D, Campanella J, Corazza GR. Gluten exposure and risk of autoimmune disorders. (Letter) Gut 50: 140-142, 2002

[39] In this section of comments by Reviewer #5, he/she often refers to papers by the lead author's name only and no reference to an associated date. Because some reference authors published several papers, the specific paper to which Reviewer #5 was referring was not always confidently known to the FDA responder to these comments. Also Reviewer #5 often just refers to effects, TDIs, and types of exposure (e.g., chronic) without indicating whether he/she meant those associated with morphological or clinical effects. Thus, the FDA responded to the Reviewer #5 comments by interpreting intended meaning as best as possible or by providing a general response.

(3) Page 8, lines 7-9 Based on the studies identified as "critical" (e.g., Ciclitira, Chartrand), these criteria do not seem to have been used to any great degree.

(4a) Page 8, lines 14-16 This appears to be one reason the Ciclitira study was identified as critical and the Catassi study was not. *(4b)* However, it is important to keep in mind that the actual commercial mixes used by study participants in the Ciclitira study were not assessed for gluten content. This mix was assumed to contain a certain amount of gluten based on testing from a prior study. As is commonly known, wheat-starch based gluten-free products vary greatly in gluten content even among different lots of the same product.

(5) Page 9, lines 28-30 If this is indeed the case, then why was a 23 year old study identified as a critical study (i.e. Ciclitira)?

(6) Page 13, lines 6-8 How was "significant data" defined? Certainly some of the studies chosen (Chartarand, Ciclitira) are not considered to contain "significant data."

Page 14 (last paragraph) There is a big difference between a 4 mg NOEAL and a 10 mg NOEAL when you are talking about such small amounts of gluten exposure.

(7) Page 15 (second paragraph, Laurin study) The TDI for chronic exposure was based on the findings from only 2 children??

(8) Page 15, line 40 "The TDI for subchronic exposure is 0.4 mg gluten/day and for chronic exposure is 7.0 mg gluten/day." This finding does not make sense and suggests there is something wrong with this analysis.

FDA Response:
Part 1a-c
Typographical errors noted on pp. 3, line 4 and pp. 5, line 9 were fixed.

Part 2
Reviewer #5 does not appear to understand the concept of a "critical study" in a safety assessment approach. "Critical study" is terminology in this approach that refers to the identified study that reflects the NOAEL and/or LOAEL on which the TDI, or the like (e.g., Reference Dose), is based. These are the NOAEL and/or LOAEL that best reflect the lowest level of response sensitivity and its practical "threshold". The description and discussion on the types of studies examined (pp. 7 of the main draft document reviewed and referred to by Reviewer #5) was presented to be transparent about the issues involved in evaluating the studies available in the published literature. The issues that are related to the decisions made in the use of the "Catassi study" and the "Chartrand study" are discussed a number of times throughout this peer review report (e.g., see FDA Response, Part 6.3 to Charge Question 6 for Reviewer #1, FDA Response, Part 1a and 3 to Reviewer #2 under the "Specific Observations" section, and also FDA Response, Part 4a to Reviewer #5 under this same section) and in the main HHA document.

Part 3

Under the section now titled "Safety Assessment" in the main text of the HHA document, significant characteristics and details were included in the descriptions of each "critical" and "supporting" study that was examined and selected. The "critical" study selected is based on the principles of the safety assessment approach and its procedure in addition to the scientific judgments that are typical of this type of assessment and are made in evaluating biologically-based experimental findings of toxicological effects. However, the weight-of-evidence considerations more specific to this assessment and made in evaluating findings found within a study and those found between studies has been expanded in several earlier sections of the main HHA document (e.g., "Basis of Weight-of Evidence Evaluations and Determinations" under the "Dose-Response Assessment" section) to assist the reader in better understanding the criteria used and decisions made. See also the FDA Response, Part 1b.2 to Charge Question 1b for Reviewer #6, FDA Response, Part 2a.1 to Charge Question 2a for Reviewer #5, and FDA Response, Part 1a to Reviewer #2 under the "Specific Observations" section.

Part 4a

The procedures, interpretation of data and related criteria typically associated with selecting critical studies in a safety assessment approach were employed in this evaluation. FDA believes that the information on the route of exposure as a data criterion (i.e., pp 8, line 14-16 in the original draft document examined by peer reviewers) was communicated in a clear and transparent manner in the text of the original health hazard draft document. Information on the weight-of-evidence considerations including route of exposure considerations made in this assessment has been supplemented in the text of the main final HHA document and in Appendix A and B to assist the reader. Next, several reasons for the selection of Catassi et al. (2007) as a supporting study versus a critical study and are noted in a number of locations in the main HHA document and appendices, along with the responses to the other peer reviewers noted above (e.g., see FDA Response, Part 10a to Charge Question 6 for Reviewer #1). Some included the fact that Catassi et al. (2007) administered gluten via capsules, and subjects were excluded from analysis if they had enteropathy on the pre-challenge GFD or they exhibited clinical sign and symptoms to the gluten challenge. These factors indicate that the Catassi et al. (2007) most likely does not reflect the response levels of the most sensitive individuals. The goal of the safety assessment approach is to account for and protect individuals that are identified as adversely responding at the lowest levels of exposure, in other words, the most sensitive individuals.

Part 4b

Reviewer #5 noted that the gliadin content of the commercial gluten-free bread mix in "the Ciclitira study" was not measured directly for the gluten content of the exact mix involved in the study's challenge test. The studies by Ciclitira et al. (1984a and 1985)[40] served as "critical" and/or "supporting" studies for both morphological and clinical effects for acute and subchronic durations of exposures in the safety assessment. The value (expressed as a range[41]) for the gluten content of the test food prepared from the bread

[40] Ciclitira PJ, Ellis HJ, Fagg NLK. Evaluation of gluten free product containing wheat gliadin in patients with coeliac disease. Br Med J 289: 83, 1984a

Ciclitira PJ, Cerio R, Ellis HJ, Maxton D, Nelufer JM, Macartney JM. Evaluation of gliadin-containing gluten-free product in coeliac patients. Hum Nutr Clin Nutr 39C: 303-308, 1985

[41] In these studies, Ciclitira and colleagues reported the gliadin content of the commercial gluten-free bread mix as a "range" of values which was interpreted as their accounting for variability in gliadin content measured in this gluten-free food product.

mix was cited by these authors from other analyses they performed (and published or cited as an “unpublished observation”). Several characteristics of the gliadin/gluten content values used in these studies suggest that they would provide reasonable estimates of gliadin/gluten exposure and serve to minimize the variability of these estimates. For instance, the analysis of the gliadin content of the food product was performed by the same investigators as executed the challenge tests and was done so in the same laboratory and medical center on the same brand (i.e., manufacturers) of the gluten-free wheat product as was used in challenge tests. Also, this content analysis appeared to be performed and the associated results were reported within the same year or so as the 2 challenge studies were performed.

Finally, discussion about the variability and uncertainty associated with the gluten content levels of challenge substances and related assumptions made has been presented earlier in this report (e.g., see above in last paragraph of FDA Response, Part 1b to Reviewer #2 under the “Specific Observations” section). The potential for consumers to encounter varied gluten content of similar gluten-free products supports the selection of the lowest identified adverse effect levels demonstrated in available dose-response data in determining the TDI.

Part 5

The statement on pp. 9, lines 28-30 in the original draft HHA main document, inquired about by Reviewer #5, refers in part to the differences between early physiological measures such as fecal fat levels and gastrointestinal absorption studies versus more recently assessed measures such as antibody levels. Also in the beginning of the process of the assessment of dose-response morphological data it was thought this factor of “improved and more current knowledge” (e.g., Marsh-Oberhuber rating system, computerized histopathological analysis) might possibly emerge as a distinguishing “weight” factor to consider in the evaluation of morphological measurements and characterization these types of responses if a very large number of low dose studies, particularly recent ones, were identified. However, as revealed in the examination of the all dose-response studies listed in Appendix A and the ones subsequently presented in tables in Appendix B, a limited number of recently performed studies administered gluten or related compounds in a low dosage range, thus, leaving this potential distinction not a relevant “weight” factor in the final assessment of morphological effects compared to the other ones that were considered. The “lines” or sentence noted by Reviewer #5 was edited to better depict the general considerations made at the end in comparing morphological studies.

Part 6

This reviewer does not appear to understand what is meant by “significant” within the context of a safety assessment approach and thus which data is “significant” within the assessment process. The term “significant” here refers to the findings of studies that contain information on the no and/or low dose adverse effect level that are relevant to this approach and in determining TDIs. Also, NOAEL data, by definition, would not be expected to reflect statistically “significant data” with respect to changes or “effects.” However, if Reviewer #5 is referring to significance determined by statistical methods, then the findings (mean values of morphological and clinical measures of 10 subjects) of Ciclitira et al. (1985) were analyzed by these methods. Also Chartrand et al. (1997) used statistical methods to analyze “anthropometric data, biochemical analyses, and AGA and EmA tiers” and described the percentages of GFD-treated CD subjects affected by introduction of a gluten source in the challenge period. Finally, statistical significance

was a consideration in assessing the weight-of-evidence within a study to identify adverse effect levels. To eliminate the possibility of confusion about the nature of the use of the term “significant” within the context discussed here, the term was replaced by a more direct reference to identifying the margins of the low dose-response effects of gluten in the location in the HHA referred to by Reviewer #5, and also later in this same paragraph by the term “relevant.”

Part 7

The study on which the overall chronic “critical” LOAEL for morphological effects, and thus TDI, was based challenged 13 children with CD with differing amounts of gluten (children self-selected levels of intake in accordance with their comfort). Of these, 2 subjects reacted at the 700 mg/d level of gluten exposure, and thus, identified as representing the most sensitive of this group of subjects with CD. This study also included one subject that demonstrated some morphological changes (increase in IEL count) at 200 mg/d. As indicated in the main text of the HHA in the discussion of chronic morphological effects, limited chronic exposure data is available. The safety assessment involves the derivation of point estimate(s) of the “safe” level(s) of exposure to a toxic agent that is based (in this case because no NOAEL is available) on the identified overall LOAEL from the best available data that reflects this for chronic morphological effects (see also FDA Response, Part 1c.1 to Charge Question 1c to Reviewer #1 and FDA Response, Part 6.2 to Charge Question 6 to Reviewer #1). Finally, Reviewer #5’s concerns of the selection of the critical chronic LOAEL (with n=2) noted here seems to conflict with his/her preferred selection of a study with 1 subject that exhibited clinical responses after subchronic gluten exposure duration over a study with 11 subjects reacting in this timeframe (see under “Specific Observations” Reviewer #5’s 2 comments with respect to “page 7” of the original draft main hazard/safety assessment document and the first comment with respect to “page 8” of this draft main document). So his/her concerns here about selection of this chronic study noted here with 2 subjects responding at the LOAEL for morphological effects is difficult to reconcile (see FDA Response, Part 2 and 3 associated with the comments by Reviewer #5 noted just above in this report). Lastly, the role of the hazard/safety assessment sections of the HHA document was to identify and characterize the critical studies and corresponding TDI estimates for each duration of exposure including that for chronic exposure from all dose-response data presently available. The subsequent determination of a “TDI of primary focus” for morphological adverse effects was not based on the TDI or related data found for chronic exposure to gluten (see also FDA Response 6.2 to FDA Charge Question 6 to Reviewer #1).

Part 8

As indicated in the main text (see the “Morphological Adverse Effects” subsection under the heading now titled “Safety Assessment” where the morphological effects results and TDI determinations for chronic exposure were discussed), limited dose-response data is available in the published literature for morphological and/or physiological effects for the chronic duration of gluten exposure. Thus, the finding of a higher TDI for chronic exposure than for subchronic exposure may be a function of the limited low-dose effects data currently available (see also discussion in FDA Response, Part 6.2 to Charge Question 6 to Reviewer #1 in this report). One role of a health hazard analysis such as this is to first characterize the nature of all available low dose-response data. Hence, a characterization and assessment of chronic exposure data was included. Separate delineation in the data set of the onset of morphological and/or clinical adverse reactions

that may occur subsequent to long-term exposure to gluten, in addition to acute and subchronic durations of exposure, in individuals with CD was important. An additional section has been added to the main HHA document that indicates that upon further evaluation the subchronic gluten exposure data provides the best information on lower response levels and thus, better estimates of TDI values for morphological effects, and suggests that at this time the subchronic TDI should be considered of primary focus in the final analysis of tolerable levels of gluten that serve to protect for those with CD (see the subsection titled "Analysis and Determination of the TDIs of Primary Focus" in the "Risk Characterization" section).

Reviewer #5 also indicated that the adverse effects level data for subchronic and chronic exposure "does not make sense." In addition to the issues addressed in the above in the first paragraph of FDA Response, Part 8, it should be kept in mind that the delineation of the nature of acute, subchronic and chronic adverse responses to gluten in the case of individuals with CD differs from such an evaluation of the effects of other toxic agents in a population, and suggests that the CD-associated data set is unique in some ways. Typically, a specific toxic substance is associated with elicitation of a reaction within a particular time period (e.g., acutely, sub-chronically or chronically). Or possibly, all (or most) subjects challenged with the same dose react in approximately the same timeframe depending on the dosage level administered of the toxic agent, with reactions generally taking longer to emerge at lower dose levels. In contrast, when a population of subjects with CD is challenged with the same dose level, the onset of adverse reactions in individual subjects greatly varies. They can occur within an acute, subchronic and chronic timeframe for a subject and in a fashion that appears not to be solely a function of the dose of exposure but also appears to be a function of the sensitivity of the individual with CD being challenged. In fact, some evidence suggests the possibility that those that react to gluten in a shorter timeframe may tend to do so at a lower dose than those with CD who tolerate gluten exposure for a longer duration before adverse effects emerge (e.g., Laurin et al., 2002).

Reviewer #6

- *Defining adverse effects for dose response is problematic:*

  (1) Page 2, lines 1-4: the introductory paragraph stated the following: "….safety assessment of gluten exposure in a sensitive sub-population group, specifically individuals with CD (CD)….." Then, on page 7, lines 2 – 7, the following is stated: "A health hazard assessment was performed to determine a TDI of gluten in individuals susceptible to its adverse effects. The adverse effect that was the primary focus of this assessment was CD…Because CD only occurs in sensitive individuals, this evaluation examined the effects of gluten on this sensitive subpopulation."

  Question: If the sensitive sub-population group is defined as individuals with CD, then how is CD defined as an adverse effect? The logical premise upon which this safety assessment is based is very difficult to follow.

- *Health effects section:*

  (2a) The description of gluten and gluten dose estimation is included in the health effects section and should be a separate section. The potential for exposure

misclassification and source of uncertainty is described on page 5 and should be clearly highlighted in its own section.

(2b) Page 6, lines 29-35: "many of the specific findings and conclusions of the research on "gluten" can only be assumed to apply to wheat gluten and its protein components. Information on the nature and effects of the relevant proteins of wheat, rye and barley in relation to each other is lacking (e.g., toxic equivalency factors). Thus, without information on the relative potency of the respective protein derivatives of these different cereals, the appropriateness of extrapolation of quantitative data derived from wheat gluten studies, for instance, dose response effects, to other toxic grains is problematic at this time."

Given these statements, the calculated LOCs for all gluten foods (Tables 10-13) are misleading.

- *Hazard assessment section:*

(3) The purpose of this section is not clear. The purpose statement in this section is confusing. The dose-response assessment section here is not an assessment, but rather a summary of available dose response data, considerations (variabilities/uncertainties) and treatment of available data. Issues of exposure duration (referred to as variability in timing of response in the report on page 8, lines 18-33) were discussed and data from original studies were apparently reorganized to fit the "exposure duration" of interest. No data summary were provided on how many studies and subjects were re-organized/re-classified in this fashion and what implications there may be regarding interpretation of results. Better explanation of the methodology used is needed.

- *Tolerable daily intake:*

(4) Page 13, lines 7 and 14: the term "most significant low dose-response data" and "most
significant lowest" overall effect level values were used.

Question: How was "most significant" determined? Was it by statistical test methods?

(5) Page 14, lines 9-32: the discussion in this paragraph on the low dose acute exposure from studies by Ciclitira et al. (1984a) and Vh/Cd ration as sensitive effect measures (Catassi et al., 2007) clearly raises doubt about the adequacy of the key dose effect used to develop the TDI for morphological effect from acute exposure to gluten (NOAEL of 125 mg gluten, Leigh et al., 1985). Based on the data reported here, the acute LOAEL for gluten could instead be as low as 2.4 - 4.8 mg gluten (Ciclitira et al, 1984a), which is -2 orders of magnitude (~100 fold) lower than the critical NOAEL chosen by FDA. If a 100-fold UF is used (10-fold to account for the LOAEL and 10-fold for inter-individual variability), the TDI for morphological effect from acute exposure to gluten could be as low as 0.02 – 0.05 mg gluten (in comparison to the 12.5 mg gluten TDI listed in Table 4 of the report).

- *Uncertainty issues:*

  (6) Page 19, lines 26 and 30 and Page 20, line 3: the term intra-individual variability is used; however, this section indicates that the 10x UF is to account for differences between individuals. Discussion in this section also suggests that great variability exists across individuals in terms of timing of the development and degree of severity upon exposure to gluten. The reviewer assumed that this is a typo and the Agency meant to say inter-individual variability?

  Page 20, lines 5-7: "….Additional uncertainty factors are a consideration in the derivation of tolerable intake levels that reflect the variability issues discussed here. In this case in particular, it is a consideration of significance in addressing uncertainty at the risk management stage."
  Question: What does this mean? More UF should be considered at the RM phase? Why not during the TDI development phase and provide explicit rationale as to why additional UF is needed to account for the significant variability between individuals? Why at the RM phase?

- *Food consumption estimates (pages 20-21):*

  Lines 31-38 on page 20: indicate that food consumption from CSFII 94-96,98 was used to identify foods that list as an ingredient any of the grains or gluten containing ingredient. Foods in the CSFII are reported as foods consumed at the dinner table (e.g., pizza, spaghetti, etc…). *(7)* How were foods with gluten containing ingredient identified? What ingredient codes (nutrient databank code or raw agricultural commodity (RAC), or other ingredient codes/names) were used? How were the amounts of "grain" and "gluten containing ingredients" in food consumed determined?

  (8) Line 7: How was chronic consumption (i.e., consumption estimates over a lifetime) estimated based on 2-days of intake from CSFII 94-96, 98. Was this based on "per capita", was other "usual" intake modeling method applied to develop chronic intake? How was the person-day estimate derived? Was it a 2-day average per user or any one day intake for all reporting users? These estimates were used to derive the acute LOC (Table 10), so the reviewer assumed that this per day estimate is based on any single day intake and per user basis. The report should explain these estimates explicitly. Were statistical weights (associated with the CSFII survey design) used in the intake assessment?

  (9) The current assessment used CSFII 94-96, 98 consumption data. There are more recent intake data from NHANES that could be used to assess US consumption. Footnote #26 (page 20) indicates that FDA was unable to use an alternate source of exposure data, NHANES 03-04, due to software problems and the use of either dataset would likely indicate very similar population average consumptions, given

that these foods represent high percentage of the average diet. Using the NHANES 1999-2004 consumption data, this reviewer conducted an intake assessment for the same population sub-groups (1-18 and >18) and showed slightly lower intake of all gluten foods and wheat gluten foods on a per day and per-user basis than what is being presented in Table 9. The difference may be due to the fact that FDA included a broader list of CSFII foods containing "grain" and "gluten ingredients" than this reviewer. However, it could also be a reflection of changes in US diet (if this reviewer included the same foods as FDA). While the observed differences may not have material impact on the LOCs, for the reason of comprehensiveness, it is highly recommended that newer consumption data are utilized and assessment methodology are more thoroughly described in future edits of this document.

- *(10)* Table 9: need to indicate units (g/day?), need to indicate per capita and/or per user.

- *(11)* Tables 10-13: columns and headers are not lined up, very difficult to read and need to be fixed.

- *(12)* Appendix A: This appendix is very difficult to read. It should be put in tabular format so that comparison of study type, routes of exposure, challenge agent, etc… can be read across studies for comparative purposes.

FDA Response:
Part 1
Aspects of the nature and occurrence of CD and related adverse effects are complicated and somewhat unique in comparison to most other toxic reactions. Exposure to dietary gluten in a "normal," healthy individual causes no detrimental health effects. Only individuals who are sensitive to gluten ingestion because of CD[42] react adversely to exposure to this substance. Thus, they are the subgroup of concern within the general population, in other words, the "sensitive subgroup." However, CD-diagnosed subjects on a GFD, for the most part, do not exhibit the adverse effects associated with active CD. The dietary challenge of gluten-sensitive CD-diagnosed individuals previously on a GFD and thus, in an apparent, "inactive" state of the disease leads to the occurrence of adverse effects associated with CD, these being various morphological changes (measured by, e.g., Vh, Cd, Vh/Cd, E-SCH), physiological effects (e.g., increased fecal fat, antibody levels), and clinical signs and symptoms (e.g., diarrhea, abdominal pain). The dose-response data examined in this assessment was on CD-diagnosed subjects on a GFD and thus with "inactive" CD who were challenged with doses of gluten resulting in the development of "active" CD. Thus, in this case, the sensitive subgroup is individuals with CD and the adverse effect is the development of CD. An attempt to better clarify the distinctions discussed above was made in the main document at the locations noted by Reviewer #6.

[42] Individuals with DH also react adversely to gluten exposure. However, the gluten-induced occurrence of the DH condition alone (without the development of CD) was not the primary focus of the health effects assessment conducted in this FDA document.

Part 2a

The organization of some of the subject headings has been changed by FDA in the main HHA document from the original hazard/safety assessment document read by the peer reviewers, along with some subheadings being added, to improve classification of the discussion or information in these different sections and subsections of the HHA document, and thus, leading to better understanding for the reader. This includes the section that discussed gluten on page 5 referred to by Reviewer #6.

Part 2b

The LOC section of the main document was corrected to more appropriately and accurately address the issues associated with the lack of direct adverse effects dose-response data on gluten-like proteins in rye and barley and of information on the relative potency of these grain proteins compared to wheat "gluten" proteins. To calculate LOC concentration values for gluten and gluten-like proteins for the combined grains of wheat, barley and rye, the assumption was made that the gluten-like proteins in rye and barley are comparable to that found with wheat gluten. This underlying assumption made in the derivation of LOC values for exposure to "all grains" was added to and described in the LOC section of the main text. It was noted in Tables 10-13.

Part 3

A hazard/risk assessment is a well-defined process that consists of several components, these being hazard identification, dose-response assessment, exposure assessment and risk characterization.[43] The dose-response assessment is recognized as a step of this process and examines the quantitative nature of the toxicological response associated with the dosage of the agent of interest. It includes consideration of factors that play a role or further affect the "response" exhibited such as age, sex, and exposure type, pattern or duration. This assessment is a reflection of the best quantitative data that is available for analysis for the agent of interest. Hence, the analysis performed in the health hazard assessment by the FDA is a dose-response assessment with the associated findings depicted in the tables in Appendix B, and described and discussed them in the text of the main HHA document. In Appendix A, each of the studies from which data for the different subject age groups and durations of exposure were derived and assessed are listed under the respective category that corresponds to their findings. From these studies, the ones that were identified as having low dose-response data were depicted in detail in tables in Appendix B as studies of focus. These studies are denoted in Appendix A with an asterisk symbol (see Appendix A). In addition, the FDA believes that the re-organization or re-classification of the results of available studies with consideration of the age of the subjects and the duration of gluten exposure along with types of adverse effects exhibited allows for delineation of potential relevant distinctions in the data, and thus, it better refines interpretation of study results.

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[43] National Research Council. Risk Assessment in the Federal Government: Managing the Process. National Academy Press, Washington, DC, 1983

Merrill RA. Chapter 30: Regulatory Toxicology. Casarett and Doull's Toxicology: The Basic Science of Poisons, Fourth edition. MO Ambur, J Doull, CD Klassen, Editors. New York: Pergamon Press, Inc., pp. 1011-1023, 1996

Faustman EM, Omenn GS. Chapter 4: Risk Assessment. Casarett and Doull's Toxicology: The Basic Science of Poisons, Fourth edition. MO Ambur, J Doull, CD Klassen, Editors. New York: Pergamon Press, Inc., pp. 75- 88, 1996

Information has been added to the text of the main hazard/safety assessment document to provide more details on this approach taken with study data and a summary of the subjects and/or studies involved.

Part 4

As indicated in the text of the main draft HHA document, it refers to the subset of studies, derived from all the studies reviewed, evaluated and listed in Appendix A, that have low dose-response data within each category of data type that were considered for further assessment in determining the "critical" no and/or low dose adverse effect levels. The "significance" of a study is meant within the context of a safety assessment approach and its methodology (see also FDA Response, Part 6 to Reviewer #5 under "Specific Observations"). It includes what are commonly recognized and identified as significant factors, considerations and study characteristics in low-dose effect determinations made in the area of toxicology and health effects assessment. The use of this terminology in reference to the nature of low dose-response studies and their relevance within a safety assessment approach was edited in this paragraph of the HHA to avoid the possibility of confusion of its meaning to a reader of the document.

Part 5

The FDA recognizes the relevance of the low dose acute exposure of the findings of Cicliteria et al. (1984a). This is why they were noted and discussed at length in the main document. However, although the change in Vh/Cd ratio after a gluten challenge that is seen in this study was statistically significant, it was a change in a single morphological measure of CD. It was not accompanied by changes in the mean IEL and E-SCH as there were no statistically significant changes found in these measures. Thus, a consistent response in the CD-related direction of changes typically associated with a number of different gluten-induced morphological measures was not found in this study. Also, IEL typically is expected to precede changes in Vh or Cd (e.g., see subsection "Nature of Morphological Adverse Effects that Characterize CD" under the "Dose-Response Assessment" section; also Marsh, 1992, Dickson et al., 2006). A possible explanation for this deviation in the progression of abnormal morphology is presented in the FDA assessment. Although the data suggests that morphological changes are associated with this dose of gluten, the FDA felt that the weight-of-evidence of the findings within this study was such that it could not be chosen as the "critical" study for low-dose acute exposure. It was presented as part of the data available that characterizes the nature of relationship between acute gluten exposure and CD-related morphological effects and was well-described in the document, along with its implications, for possible consideration as a factor at the risk management stage.

Part 6

Clarification and corrections on the "intra-species" versus "inter-individual" terminology was made in the main text of the document and in Appendix B.

Part 7

Foods with gluten containing ingredients were obtained by searching the available food codes in the database for foods with ingredients containing "wheat germ, flour, oil, bran, or rough". All of these food codes were used in the analysis.

There was no attempt to determine the amount of grain or gluten in any of the foods. Total consumption of these foods was estimated.

Part 8
Two-day average intakes are used to model chronic food consumption. No attempt to normalize the averages to usual intake was made. The software used to compile the average intakes is capable of separating the population average from person-day estimates. Acute intakes are based on one day of reported consumption of foods for all users. Statistical weights were used in the analysis.

Part 9
The NHANES data from 2005-6 will be available shortly and these data can be used to update the estimates. The crude nature of the estimates used in the analysis, which was meant to broadly estimate the amount of gluten that might be consumed unknowingly, make it unlikely that a significant difference will be detected using the newer data.

Part 10
Grams per day (g/day) and per user (everyone consumes one food or another that might contain a gluten containing ingredient, so per-capita and per user are identical) are the correct units.

Part 11
Tables 10-13 were completely re-done and this included the correction of problems noted by Reviewer #5 in addition to other corrections. These four tables were replaced by two tables now labeled Tables 10 and 11 which were moved to Appendix B.
The information communicated in these tables was clarified and also improved by employing the "TDIs of primary focus." These principal TDIs were determined and discussed in a newly added subsection titled "Analysis and Determination of the TDIs of Primary Focus" under the "Risk Characterization" section of the main text of the HHA document and were used to estimate various LOC values.

Part 12
As indicated in the introduction to Appendix A, this appendix lists all identified gluten-related studies with dose-response data associated the adverse effects of CD. From the dose-response studies characterized in Appendix A, the relevant low-dose gluten (or related substance) exposure studies were further evaluated and detailed in the tables of Appendix B. This latter set of studies are the ones from which the relevant "critical" and "supporting" studies addressed in the HHA were derived. FDA does not feel that it is necessary to put the specific characteristics and results of all reviewed studies in tabular form. Only presenting the relevant low-dose studies in great detail in the tables in Appendix B as has been done by the FDA seems warranted. Page headers were added to Appendix A to improve the communication of the experimental categories and the transition from page to page for the reader of the information in this HHA document.

IV. External Peer Reviewers

David C. Bellinger, PhD
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Dr. Bellinger holds a joint appointment as Professor in the Department of Environmental Health, Harvard School of Public Health and the Department of Neurology, Harvard Medical School. He is a neuroepidemiologist and an expert on the functional consequences of early metabolic and chemical insults to the developing nervous system. Much of the focus of his research is on the neurotoxic and neurobehavioral effects of heavy metals such as lead, mercury and cadmium on development and the associated risks of this exposure. He has extensive experience serving in the United States and internationally on expert science or technical advisory committees or workgroups in addition to those that address public health initiatives. Dr. Bellinger has published together over 200 original research papers, review articles or meeting proceedings, and books.

Donald D. Kasarda, PhD
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Dr. Kasarda is a food protein and cereal analytical chemist, and held a Research Leader position at the Agricultural Research Service, Western Regional Research Center of the United States Department of Agriculture in Albany, CA until 1999. He currently holds a position in the Department of Agronomy and Range Science at the University of California at Davis. He has published over 20 scientific journal articles on his research on aspects of the nature of gluten proteins associated with CD, and has received several awards for his work in this area of study.

Michael I. Luster, PhD
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Dr. Luster was a research scientist in the area of molecular biology and immunotoxicology for over 25 years. He has held senior positions in the National Institute of Environmental Health Sciences (NIEHS) of the National Institute of Health (NIH), and the National Institute for Occupational Safety and Health (NIOSH) along with several adjunct university professor appointments. He has served as an expert member of several national and international scientific advisory boards, committees and panels in the areas of immuno-toxicology, -pathology, and -genetics. He also has published over a total of 300 original research papers, and review articles or book chapters in these areas of study. This work includes examination of methods and approaches in assessing immunotoxic effects and risks.

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Dr. Murray is a board certified internist and gastroenterologist, and a Professor of Medicine at the Mayo Clinic. He is an expert in the area of gluten-induced sensitivity and enteropathy. He is extensively involved in the research of many aspects of CD and the related condition of DH. The focus of this research includes the clinical epidemiology of CD, the characteristics and outcome of disease development in CD, the nature of the immunogenetics of gluten sensitivity enteropathy, and the development of animal models of CD and DH. Dr. Murray also has published together over 50 original research papers, review articles and book chapters on a range of areas of study or topics in CD.

Tricia Thompson, MS, RD
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Ms. Thompson is a registered dietician and an independent nutrition consultant that specializes in issues associated with CD. She has published articles and guides on different aspects of the gluten-free diet in nutrition and medical journals along with general resources (e.g., newsletters) and books. The professional associations of which Ms. Thompson is affiliated include the American Dietetic Association and Dietitians in Gluten Intolerance Diseases.

Nga L. Tran, DrPH, MPH, CIH
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Dr. Tran has extensive experience, having held numerous positions, in the area of health risk evaluation and assessment with particular expertise in exposure assessment. She currently is a Senior Managing Scientist at the Center for Chemical Regulation and Food Safety within Exponent, and an Adjunct Assistant Professor in the Bloomberg School of Public Health at Johns Hopkins University. Her professional experience includes service on various advisory committees and peer review panels. She also has conducted or been involved with a range of exposure and/or risk assessments in the field of food safety and nutrition. Dr. Tran has authored many journal articles, book chapters and reports on varied topics in the areas of health, safety and exposure assessment along with the topic of regulatory risk management and policy.