Responses to External Peer-Review Comments

This document contains comments as well as responses to comments from the external peer-review process for the Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population.

In May, we obtained comments from four reviewers based on the report we sent out in April:

- NIH/ODS,

- FDA,

- USDA, and

- One academia person.

At that time, data on 24 fatty acids from NHANES 2003-2004 and data on 6 isoflavones and lignans from 2005-2006 were not yet available and couldn't be included for external peer-review.

In July, when that data became available for a government-only quality assurance review, we sent out data tables and figures for these new sections to three reviewers and obtained comments from all three reviewers:

- NIH/ODS,

- FDA, and

- USDA.

The footer on each page indicates which reviewer # the comments are from.

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

REPORT

APRIL 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1. **Are the report objectives clearly stated and appropriate?**
   Yes (X) No ( ) Unsure ( )

   Why?

2. **Is the overall report design appropriate for the report objectives?**
   Yes (X) No ( ) Unsure ( )

   Why?

   The authors should be lauded for the expansion of more than doubling the biochemical indicators detailed in this report compared to the one released in 2008.

   The overall design of the report provides extensive detail about the biochemical data presented. The overviews for indicators covered in the report including physiological function, health effects, intake recommendations, methods for assessing the indicators and specific methods used for collection in NHANES provide a comprehensive picture and invaluable reference.

   While the detail of data in the many data tables is excellent, of unique value is the selected observations and highlights section with inclusion of figures to illustrate major trends and health policy/concerns-related results. If time allows, expanding the highlights sections of the chapters

would be welcome. The knowledge and expertise of CDC's National Center for Environmental Health serves as a critical resource to accurately summarize these data.

Response: We thank the reviewer for the helpful comments. We added to each highlight section a summary of the most important observations and highlights to help readers retain a few salient points from the wealth of information presented.

To assure that users can quickly find data tables or figures, I would suggest adding a listing of all tables and figures with corresponding page numbers in either the Table of Contents or the Appendix.

Response: We created a table of contents which includes a listing of all tables and figures. The final report will include page numbers for each table and figure.

3.   **Are the methods and analyses appropriate for the report objectives?**
Yes ( ) No ( ) Unsure (X)

Why?

While my research background is not in biochemistry, I feel I am not qualified to judge the report on the methods used. However, the description and rich referencing in the report leads a reader such as me to have confidence in the methods used. Further, references and methods used are comprehensively referenced so further investigation/evaluation is possible of the methodologies employed.

4.   **Were the data analyzed in such a way to address appropriately the objectives of the report?**
Yes ( ) No ( ) Unsure (X)

Why?

I refer to my response in item 3.

5.   **Are the report results presented and interpreted appropriately and completely?**
Yes (X) No ( ) Unsure ( )

Why?

I answer yes overall but offer a comment on Chapters 2 and 3.

Chapter 2. Fat-Soluble Vitamins & Nutrients. In the Highlights section desribing Figure H.2.a., the statement that low dietary intake of vitamin E without widespread manifestation of deficiency suggest the need for further evaluations to determine whether improve estimates (either in dietary intake or nutrient composition) are necessary is well-appreciated. However, it is probable that the Estimated Average Requirement (EAR) for vitamin E may <u>also</u> need further evaluation. The dietary intake method used in NHANES, the Automated Multiple Pass Method (AMPM) has been validated for energy intake on a sample of 524 adults using the doubly labelled water technique. The results of that study show that reported energy intake using the AMPM was 11% less than total energy expenditure (http://ddr.nal.usda.gov/dspace/handle/10113/21951). This level of underreporting does not compensate to the disconnect between >95% of the population having

adequate serum concentrations of vitamin E and 93% of the population consuming less than the EAR for vitamin E.

Response: Thank you – this comment has been incorporated into the text as follows:

"Despite NHANES 2001–2002 dietary intake data demonstrating that 93% of the U.S. population consumed less than the Estimated Average Requirement (EAR) for vitamin E (Moshfegh 2005), for decades mean serum vitamin E concentrations have remained consistently adequate (Figure H.2.a), with less than 1% of the population vitamin E deficient (Table 2.4.d). Analyses of NHANES data showed that in 1999–2000 52% of adults (Rock 2007) and in 2003–2006 49% of the total U.S. population (Bailey 2011) used dietary supplements; thus, the intake data (food and supplements) for vitamin E seem to be inconsistent with the biomarker data. Several explanatory possibilities have been raised, including a suggestion that the intake of fats (and fat-soluble nutrients) is under-reported in overweight and obese subjects, the database of food values is not accurate, and/or that the EAR for vitamin E needs adjustment. Low intake without widespread manifestation of deficiency suggests the need for further evaluations to determine whether improved estimates are necessary, either in the nutrient tables or in dietary intake."

Chapter 3. Very minor comment -- In most of the figures presented, I noticed an order to the subgroups as listed in the x axis, based on what I believe is sample size from small to large. Figure H.3.d. changes that order with Mexican Americans and Non-Hispanic whites reversed.

Response: Thank you – this has been corrected.

6. **Are the report conclusions and recommendations appropriate and complete?**
Yes (X) No ( ) Unsure ( )

Why?

7. **Are there any overall comments on the manuscript? (How does the Report contribute to public health in ways not already noted? What persons/groups, in addition to those listed, will be interested in this Report? What channels of communication should be used to disseminate this Report?)**

Congratulations on a wonderful report. It will be an invaluable document in monitoring dietary and nutritional status.

Others that may be interested in the report: I would suggest that the National Collaboration for Childhood Obesity Research would be interested in this report. Their website: http://www.nccor.org/index.html.

Response: Thank you for suggesting this interest group. We will include them into our dissemination list.

8.   **Select the appropriate category below:**

        (List recommended changes or reasons for not recommending)

        A. Recommend (X)

        B. Recommend with Required Changes ()

        C. Not Recommended ( )

ADDITIONAL QUESTIONS:

1.   **Are there any comments on NCEH/ATSDR's peer review process? none**

2.   **Are there any other comments?**

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

REPORT

APRIL 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1. **Are the report objectives clearly stated and appropriate?**
    Yes (x) No ( )   Unsure ( )

    Why?

    NCHS is commissioning the report of data from the National Health and Nutrition Examination Survey (NHANES) in order to make this data available to the public. In this regard, the report is addressing a need to know this information. The stated purpose is to improve our understanding of the concentrations of biochemical indicators of diet and nutrition in the general U.S. population. Health professionals such as physicians, clinicians, scientists and public health officials may use the population reference levels to determine if a group of people has an unusually high or low level of a biochemical indicator of nutrients and/or compounds. There are other stated uses of the report including tracking trends in biochemical indicator levels over time, drawing comparison of nutritional status between special population groups, and stimulating more in-depth research on the NHANES data.

2. **Is the overall report design appropriate for the report objectives?**
    Yes (x) No ( ) Unsure (x)

    Why?

    The overall design is in the form of an easy to reach and follow report. It is organized by topic area and introduced by a complete table of contents section. The design supports the objectives and facilitates the reading and understanding of the report.

    Based on the objectives, there needs to be more biochemical indicators analyzed and included in the report. The most complete and comprehensive chapter is on the subject of Water-Soluble

vitamins. This chapter contains a more comprehensive discussion of the three essential water-soluble vitamins listed as problematic in the food supply. Biochemical indicators include measure of compounds in blood and urine which change in relationship to the nutritional status of folate and vitamins B6 and B12. While the report does not include other water-soluble nutrients such as choline, which has recently received attention related to perinatal brain development, the coverage of the three vitamins is complete and comprehensive. Perhaps, future analyses will include other water-soluble nutrients such as choline.

Response: We thank the reviewer for the helpful comments. The analytes covered in the report represent the diet-and-nutrition biochemical indicators for which information is available in NHANES during the time period covered. Some analytes have not been covered by NHANES (e.g., some of the vitamins, choline, some trace elements), while other analytes may not be monitored during each survey cycle (e.g., vitamin C, vitamins A and E and carotenoids). We are only able to present in the report on analytes for which information is available in NHANES.

The report misleads the reader by broad categories and headings. It is not a comprehensive report and the number of nutrients in a given is limited. One can find examples in each section where there is limited information. The most complete is the Fat Soluble vitamins chapter which includes data for vitamins A, D, & E, the major carotenoids in the diet, and fatty acids [data not included]. The chapter on water-soluble vitamins lists only folate, vitamins B6 B12 and C, metabolites homocysteine and methylmalonic acids. Vitamin C data is tucked in at the end of the Water Soluble vitamins chapter without being indexed in the beginning. This coupled with missing tables has made it difficult to evaluate the contents of this report effectively.

Response: We corrected the omission of not having vitamin C indexed at the beginning of the water-soluble vitamins chapter. This analyte is tucked in at the end of the water-soluble vitamins chapter because we clustered the indicators within each chapter according to topic area; B vitamins and metabolites are the first section and vitamin C is the second section in this chapter. The fat-soluble vitamins and nutrients chapter has three sections: vitamins A and E and carotenoids, vitamin D, and fatty acids.

To the best of our knowledge, tables and figures for vitamin C were part of the materials included on the CD that each reviewer received (located inside the cover of the binder). Please accept our apologies if some tables or figures were missing from the CD. We provided a hard copy of the B vitamins section of the report to show reviewers how we expect the chapters to be organized in the final report. However, to be environmentally friendly, we chose not to print all chapters. The full report is almost 500 pages long. We regret if this led to any confusion.

To help readers familiarize themselves with the report content, we created a table of contents which includes a listing of all tables and figures for each analyte in each chapter.

3.   **Are the methods and analyses appropriate for the report objectives?**
        Yes (x) No ( )   Unsure (x)

        Why?

        The statistical analyses are quite appropriate for the report objectives. Statistical programs such as SUDAAN and SAS were used to analyze the data. The report explains and defines the statistical analyses that were used and how these methods provide the explanation of the data analyzed and reported.

        The biochemical and laboratory methods used for the selected biomarkers of nutritional adequacy in the report appear also to be appropriate. Individual variability is large for some of the parameters. Therefore, one wonders whether simply looking at serum and urine will provide sufficient information upon which to draw conclusions about nutrient status. For example, looking at serum fatty acids may not provide as much useful information as looking at red cell membrane

fatty acids. How reliable is looking at urinary excretion of isoflavones in determining status or intake? Individual variability in this area is also very large.

Response: We addressed the issue of measuring fatty acids in plasma versus RBC in the text as follows:

"Few studies have compared the fatty acid composition of plasma with red blood cells to assess which substrate best reflects dietary intake. In one study, fatty acid correlations with food frequency questionnaire data were only slightly stronger for erythrocytes than for plasma (Sun 2007)."

As stated in the background text of the isoflavones and lignans chapter, it has been shown before that urinary concentrations of these phytoestrogens are good biomarkers of dietary intake.

4.   **Were the data analyzed in such a way to address appropriately the objectives of the report?**
Yes (x) No ( )   Unsure ( )

Why?

The data were analyzed appropriately from a statistical viewpoint.

5.   **Are the report results presented and interpreted appropriately and completely?**
Yes ( ) No (x)   Unsure (x)

Why?

One cannot overlook the missing tables that were discussed in the text but not included in the report. There was no explanation as to which were missing and would be added later. There was no explanation as to why some nutrients in various categories were not included in the report. The 2010 Dietary Guidelines for Americans were recently published in which there is a list of nutrients that are of concern for the general public and various subpopulations. As a minimum, this report should include analyses of those nutrients from NHANES database.

Response: Please see also the response under #2. The CD (located in the inside cover of the binder) included six pdf files with the tables and figures for each chapter and one Word file with all the background text for each chapter, the observations and highlights. Tables and figures for the 24 fatty acids were not included on the CD because the data was not yet available at the time. This was noted at the end of the background text for fatty acids. A few months later when the data became available for a quality assurance review, the fatty acid data tables were shared with the governmental reviewers for comments.

The analytes covered in the report represent the diet-and-nutrition biochemical indicators for which information is available in NHANES during the time period covered. Not all nutrients mentioned in the 2010 Dietary Guidelines for Americans are part of the continuous or periodic NHANES monitoring and not all nutrients have reliable biochemical indicators that represent status.

6.   **Are the report conclusions and recommendations appropriate and complete?**
Yes ( ) No ( ) Unsure (x)

Why?

The conclusions and recommendations in the report were not found. Also not found was a section that would be most useful, i.e., an Executive Summary. There is an adequate introductory section

with background information. This information prepares the reader for the analyses that are in the report.

Response: We expanded the "Highlights" section to include a summary of the most important observations and highlights to help readers retain a few salient points from the wealth of information presented. We also developed an Executive Summary that will be a stand-alone document accompanying the report.

7. **Are there any overall comments on the manuscript? (How does the Report contribute to public health in ways not already noted? What persons/groups, in addition to those listed, will be interested in this Report? What channels of communication should be used to disseminate this Report?)**

This report is very spotty and has the appearance of being hastily pulled together. Some sections are well developed and complete and others have missing information. Much more work will be needed in order to get it ready for publication.

Response: Hopefully this concern is primarily regarding the tables/figures that were not found, but included on the CD. We have addressed this in more detail under #2 and #5.

8. **Select the appropriate category below:**

(List recommended changes or reasons for not recommending)

A. **Recommend ( )**

B. **Recommend with Required Changes (x):**

Some of the URLs do not open appropriately and some do not open on the intended subject matter. Tables in chapters 2 and 5 are missing and will need to be included prior to publication. An explanation is needed as to why the broad chapter headings are used when there's just one nutrient analyzed. This is misleading. The chapter entitled "Trace Elements" has only a single nutrient, iodine, listed. Why not get rid of the broad heading "trace elements" in favor of simply listing "Iodine?" If that is not feasible, the author should include more than just a single trace element in this chapter. Additionally, iron is also a trace element but is listed in a separate chapter. Why not fold the "Iron Status" chapter into the "Trace Elements" chapter? That way, it could truly be listed as the plural "Trace Elements" chapter. The report needs an Executive Summary and an overall conclusion at the end.

Response: Some of the web addresses given were being used as placeholders until the report is finalized and the web elements are established. Once this process is complete, they will all be double-checked prior to publication.

We followed the reviewer's suggestion and folded the iron-status indicators and iodine into a Trace Elements chapter.

C. **Not Recommended ( )**

1.   Are there any comments on NCEH/ATSDR's peer review process?

I received the report well ahead of the deadline and was given adequate time to conduct the review. The fact that there are missing data made it difficult for me to give it a thorough evaluation. The portion that is intact made for an interesting review. Still, it was very interesting reading. Thank you for the opportunity to serve as one of the peer reviewers for this report.

2.   Are there any other comments?

PEER REVIEW COMMENT FORM

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

REPORT

APRIL 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1.   Are the report objectives clearly stated and appropriate?
Yes ( ) No ( ) Unsure (x)

Why?

I think it would be preferable to have an actual heading called "Objectives of this report". One of the objectives appears simply to be to provide data ("Addressing data needs"). The other set of objectives is provided under "Public Health Uses". Perhaps these could be subheadings.

Response: We thank the reviewer for the helpful comments. We modified the wording under "Public Health Uses" to clearly indicate that these are the objectives:

"This report's primary ==objective== is to improve our understanding of the concentrations of biochemical indicators of diet and nutrition in the general U.S. population and in selected subpopulations. These data will help assess inadequate or excess intake and will inform analyses on the relation between biochemical indicators and health outcomes. Other ==objectives== and potential public health uses of the information include…"

2.   Is the overall report design appropriate for the report objectives?
Yes (x) No ( )   Unsure ( )

Why?

3. **Are the methods and analyses appropriate for the report objectives?**
Yes ( ) No ( ) Unsure (x)

Why?

1. The report states that "a geometric mean provides a better estimate of central tendency for data distributions with a long tail at the upper end of the distribution…." But long tails at either end can distort the estimate. Perhaps this statement should be modified?

Response: As suggested by the reviewer, we modified the wording as shown below:

"The majority of the biochemical indicators reviewed in this report, with the exception of vitamin C and body iron stores, have a long upper tail (skewed right). For these biochemical indicators, a geometric mean provides a better estimate of central tendency because it is less influenced by high values than is the arithmetic mean."

2. Not all biochemical indicators are skew. One that usually is not skew is serum vitamin C. Log-transforming it actually introduces skewness that wasn't there before, and drags down rather than dragging up the mean. This could itself provide a distorted picture of the central tendency. For vitamin C, for example, the geometric mean for Total 6+ Years is 46.2 umol/L, while the median is 56.3, a huge difference.

Since you probably prefer to be consistent, you may not want to change use of geometric mean for specific indicators. However, I suggest you consider adding a sentence in some of the relevant indicators (specifically including vitamin C) to point out that for this indicator the median is a better estimate of central tendency than geometric mean due to the fact that log-transforming introduced rather than removed skewness.

Response: Thank you for this excellent comment. When we re-assessed the distribution for each indicator through the estimation of the box-cox transformation parameter and by using a combination of histograms and Normal probability plots, we found that vitamin C and body iron were the only two indicators that were not sufficiently right skewed to make a log transformation necessary. While the arithmetic means were already presented for body iron, we have changed the reporting for vitamin C from geometric to arithmetic means.

"However, the arithmetic mean is presented for vitamin C and body iron stores as the distributions for these biochemical indicators were reasonably symmetric."

4. **Were the data analyzed in such a way to address appropriately the objectives of the report?**
Yes ( ) No (x)   Unsure ( )

Why?

I'm actually going to say 'no' on this one, to indicate the strength of my concern on this issue.

1. For a number of these indicators, use of vitamin/mineral supplements is the single most important factor influencing serum concentrations. I realize you don't want to get into interpretation or modeling, etc., but I think not presenting some data by vitamin-mineral supplement use greatly weakens the usefulness of the data for at least one of your stated objectives, "Assessing the effectiveness of public health efforts to improve diet and nutritional status …." Improvements in dietary intake can be masked by vitamin supplement use. And we can see that in many cases African Americans have poorer nutrient status that whites, but our resulting public health efforts to promote better dietary intake will be off base if a large part of the ethnic differences result from differences in supplement use rather than dietary intake.

Observations that are likely to have been influenced by vitamin/mineral supplement use: a) U-shaped curve by age (older people take more such supplements than e.g. adolescents and young adults. See B vitamins; b) lower concentrations in men (women take more such supplements than men); lower concentrations in African Americans and Hispanics (supplement use is less common in these groups).

I know you don't want to greatly multiply the number of tables or to give too much space and emphasis to something that is not age-sex-race, but I think my concern could be handled fairly simply and minimally, as follows. For the figures that present graphs by age (e.g., Figure 1.1.a), just add one more pair of figures, on the same page: users of vitamin-mineral supplements, non-users of vitamin-mineral supplements. This wouldn't increase the number of pages, and would provide very valuable information to aid in the interpretation of the gender and race-ethnicity graphs and tables.

This observation applies mainly to water-soluble indicators and to iron indicators. And for purposes of this concern, I am specifically referring to vitamin-mineral supplements, not 'supplements' in general. So I'm talking about multivitamin/mineral supplements, and single supplements of A, B, C, D, E and iron.

Response: We agree with the reviewer that use of dietary supplements is in many cases the main determining factor of blood concentrations. Supplement use as well as other influencing factors such as BMI, smoking status, and socio-economic factors are of great interest to a large audience. Addressing these factors in this report would exceed the scope of the report. However, we are undertaking special data analyses that include the above mentioned factors and we intend to produce a series of research papers presenting on this important topic.

2. I consider that the first line in tables (e.g., in Table 1.1.a.1), that represents the whole population aged 1 year and older (or in some tables 6 & older), is uninformative. I use tables like these all the time, and I never even look at that line because it isn't useful. I think it would be MUCH preferable to instead have two summary lines, 1-19 years and 20 and older. Because of their smaller bodies, children almost always have higher nutrient concentrations. Including them in a summary line with adults provides no real information about typical status of "the population".

Similarly, in the same table and tables like it, including children in with adults in the race/ethnicity lines may give misleading information about race-ethnic differences. We need to be able to compare how children are doing and how adults are doing. In addition, Mexican Americans and non-Hispanic Blacks tend to have a higher proportion of their populations as children. Unless the sample-weighting already takes care of this, this could result in higher-appearing concentrations of nutrients in Mexican American and non-Hispanic Blacks than would be seen if only their adult concentrations were shown. It's not clear to me whether or not sample-weighting already takes care of this, but if not, I would urge again that the race-ethnicity lines be split into 1-19 and 20+ years.

Response: We agree with the reviewer that the 3-level stratified tables are most informative because they show information by a combination of age, gender, and race/ethnicity. For this reason we present such tables for each indicator, even if data is only available for one survey cycle or for a subpopulation. To compensate for the smaller sample size, however, we only present the 10th and 90th instead of the 5th and 95th percentiles in those cases. The main reason for including the 1-level stratified tables for each indicator is to have a large enough sample size to present 2.5th and 97.5th percentiles. Those percentiles reflect the central 95 percent interval, which is commonly defined as the reference interval. One major objective of the report is to provide reference intervals. NHANES provides a unique opportunity to assess the central 95 percent reference intervals and we do not want to miss that opportunity.

3. The vitamin D section reports the prevalence of both 'at risk for deficiency' and 'at risk for inadequacy'. In addition, Figure H.2.c presents the prevalence by ethnicity of those two levels of potential risk. In contrast, the vitamin C section reports that there are two previously-defined levels, 'deficiency' and 'low', but reports on the prevalence of only one of those. I would urge that the two levels in vitamin C be handled as they were in vitamin D, and that prevalence of both be reported in the General Observations and Highlights, and particularly that both levels be shown in figure H.1.e as they are shown in H.2.c. We don't have much scurvy in this country, but we do have a lot of 'at risk of inadequate' levels of vitamin C.

Response: We followed the reviewer's suggestion and included a new figure H.1.f that shows the prevalence of deficient (< 11.4 umol/L) and low vitamin C concentrations (11.4-23 umol/L) by age group.

4. See comment above re skewness.

Response: Addressed under #3.

5.   **Are the report results presented and interpreted appropriately and completely?**
Yes ( ) No ( ) Unsure (x)

Why?

We were emailed detailed tables and figures for the fat-soluble vitamins/nutrients section. However, my book was missing comparable detailed tables and figures for Iron status, Trace elements, Isoflavones, and acrylamide. I assume that the same sets of tables found for the water-soluble and fat-soluble vitamins/nutrients will be presented for the other sections? I'm sure this was intended, as otherwise this publication will be much less valuable.

Response: Indeed, tables and figures for each indicator are presented in the report. However, to be environmentally friendly, we chose not to print all chapters. The full report is almost 500 pages long. The CD (located in the inside cover of the binder) included six pdf files with the tables and figures for each chapter and one Word file with all the background text for each chapter, the observations and highlights. We provided a hard copy of the B vitamins section of the report to show reviewers how we expect the chapters to be organized in the final report. We regret if this led to any confusion.

6.   **Are the report conclusions and recommendations appropriate and complete?**
Yes ( ) No ( ) Unsure (x)

Why?

I didn't see a conclusions/recommendations section, either overall or for specific biochemical indicators.

Response: We added to each highlight section a summary of the most important observations and highlights to help readers retain a few salient points from the wealth of information presented.

7. **Are there any overall comments on the manuscript? (How does the Report contribute to public health in ways not already noted? What persons/groups, in addition to those listed, will be interested in this Report? What channels of communication should be used to disseminate this Report?)**

The Report is very well written, and will be a very important contribution to understanding about concentrations of these indicators.

8. **Select the appropriate category below:**

    (List recommended changes or reasons for not recommending)

    A. **Recommend ( )**

    B. **Recommend with Required Changes (x)**

    Obviously these are not "required" changes. They are strongly recommended.

    C. **Not Recommended ( )**

ADDITIONAL QUESTIONS:

1. **Are there any comments on NCEH/ATSDR's peer review process?**

2. **Are there any other comments?**

    *Typo, last line of paragraph "Biochemical Indicators and Methods": 'functional indicator OF folate…."
    *Typo?: in Water-soluble write-up, I've usually seen it shown as "radio-protein binding" rather than "radio protein-binding". This would also mean "non-radio-protein binding".
    *Typo, Caption for figure H.2.c.

    Response: Thank you; these have been corrected.

PEER REVIEW COMMENT FORM

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

REPORT

APRIL 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1. **Are the report objectives clearly stated and appropriate?**
   Yes (X) No ( ) Unsure ( )

   Why?

2. **Is the overall report design appropriate for the report objectives?**
   Yes (X) No ( ) Unsure ( )

   Why?

3. **Are the methods and analyses appropriate for the report objectives?**
   Yes (X) No ( ) Unsure ( )

   Why?

4. **Were the data analyzed in such a way to address appropriately the objectives of the report?**
   Yes (X) No ( ) Unsure ( )

   Why?

5. Are the report results presented and interpreted appropriately and completely?
Yes ( ) No ( ) Unsure (X)

Why?

I believe that a discussion of the interpretation of the geometric mean and age-adjustment would have been useful for the reader not familiar with those statistical methods.

Response: We thank the reviewer for the helpful suggestions. The "Data Analysis" section in the Introduction provides information on how geometric means and corresponding confidence intervals were calculated:

"Geometric means were calculated by taking the log of each concentration, calculating the mean of those log values, then taking the antilog of that mean (the calculation can be done by use of any log base, such as 10 or e). The confidence interval uses the standard error and mean on the log scale and the appropriate critical value from the t-distribution to calculate upper and lower confidence limits on the log scale. The confidence intervals of geometric means in this report are based on taking the antilog of those upper and lower confidence intervals."

The "Data Presented for each Biochemical Indicator" section in the Introduction provides information on why geometric means were selected over arithmetic means for most biochemical indicators. We have reworded and expanded this section:

"The majority of the biochemical indicators reviewed in this report, with the exception of vitamin C and body iron stores, have a long upper tail (skewed right). For these biochemical indicators, a geometric mean provides a better estimate of central tendency because it is less influenced by high values than is the arithmetic mean. However, the arithmetic mean is presented for vitamin C and body iron stores as the distributions for these biochemical indicators were reasonably symmetric."

The "Data Analysis" section in the Introduction provides information on how age standardization was carried out and how age standardized data should be interpreted:

"Figures in the highlight section that present age-adjusted geometric mean concentrations from NHANES III (1988–1994), NHANES 1999–2002, and NHANES 2003–2006 or age-adjusted prevalence estimates by demographic subgroups have been generated in SUDAAN by use of age-standardizing proportions from the 2000 U.S. Census population (using direct standardization). Statistically significant differences between age-adjusted geometric means and age-adjusted prevalences were assessed through pairwise comparisons. A reader should take care when interpreting these age-adjusted figures in isolation. The magnitude of an age-adjusted geometric mean or age-adjusted prevalence is completely arbitrary, and it depends upon the chosen standard population. Additionally, age-adjusted geometric means or age-adjusted prevalences can mask important information about trends if age-specific rates do not have a consistent relationship."

6. Are the report conclusions and recommendations appropriate and complete?
Yes (X) No ( )   Unsure ( )

Why?

7. Are there any overall comments on the manuscript? (How does the Report contribute to public health in ways not already noted? What persons/groups, in addition to those listed, will be interested in this Report? What channels of communication should be used to disseminate this Report?)

It is unclear to me how widely disseminated is this report. "Health US" is a know product but this report is relatively new. It is clear that this report adds a great deal not included in "Health US" but not if this report has much of an impact. I suggest that if you do not already do so that you advertise it in, say, AJCN and the EpiMonitor. Even better would be a short article in AJCN

announcing the release of the 2nd edition and discussing the key findings.

Response: Thank you for the suggestion. We are currently working with the communication team on release strategies.

8.   **Select the appropriate category below:**

(List recommended changes or reasons for not recommending)

A. **Recommend ( ).**
I have made a number of relatively minor suggestions which I will leave to the Editors to decide if they wish to incorporate them or not.

B. **Recommend with Required Changes (X)**
The suggested change to the Vitamin D section is, I believe, an important change that should be made. In addition, I have made a number of other relatively minor suggestions which I will leave to the Editors to decide if they wish to incorporate them or not.

C. **Not Recommended ( )**

ADDITIONAL QUESTIONS:

1.   **Are there any comments on NCEH/ATSDR's peer review process?**

None, except to thank you for considering me qualified enough to act as a reviewer.

2.   **Are there any other comments?**

Let me start with that the overall the 2nd Report is well thought out and prepared. Everyone involved with it should be proud of their work.   My comments are:

General Comments;

1. I suggest that you provide a definition for a "Geometric Mean" including how they are calculated and how the back transformation is performed.

Response: Please see response to #5.

2. In addition, I suggest that you describe in detail including providing an example of how the age adjustment was performed.

Response: Please see response to #5.

3. Tables 1.1.a.1 and 1.1.a.2 are very similar are you sure you need both of them? Given that the limits of the sampling design for NHANES are at the 5th and 95th percentiles I believe that you could drop – throughout the volume – tables similar to 1.1.a.1.

    Response: In the NHANES III documentation, recommended sample sizes are given for any reported percentiles assuming a specific design effect. As stated in the Data Analysis section of the Introduction, we assumed a design effect of 1.4. Using this, at least 112 persons were required to estimate the 10th and 90th percentiles, 224 persons for the 5th and 95th percentiles, and 448 persons for the 2.5[th] and 97.5[th] percentiles. The 3-level stratified tables (Total, Mexican Americans, non-Hispanic blacks, and non-Hispanic whites) meet these requirements; in the few cases when they don't, the estimates have been flagged and footnoted.

    However, one major objective of the report is to provide reference intervals, which are most commonly defined as the central 95 percent intervals. That is why we have included the 1-level stratified tables for each indicator. In these tables the sample size is large enough to present 2.5[th] and 97.5[th] percentiles. NHANES provides a unique opportunity to assess the central 95 percent reference intervals and we do not want to miss that opportunity.

Page Specific Comments:

4. "1. Water Soluble B-Vitamins: Figure H.1.c: I believe that the years for NHANES III should be 1988-94.

    Response: The vitamin B12 status of the population started being monitored in the second phase of NHANES III, beginning in 1991.

5. Vitamin D: I would like to suggest that the authors include data on the prevalence of persons with a serum 25(OH)D level < 40 nmol/L in Figure H.2.c. That cut-point was defined as the Estimated Average Requirement (EAR) by the recent DRI Committee on Calcium and Vitamin D. I suggest that the order of presentation in the Figure be: < 40 nmol/L, < 30 nmol/L and 30-49 nmol/L. I would use as the upper value for the last group 49 nmol/L as persons with levels of 50-75 were defined by the DRI Committee as having sufficient levels.

    Response: As suggested, we added the discussion of the < 40 nmol/L cut-point in the text as follows:

    "Of interest to public health scientists, the [IOM] report indicated that a serum 25(OH)D level consistent with the Estimated Average Requirement for dietary intake (EAR) lies between 30 and 50 nmol/L and that 40 nmol/L was selected from the middle of the range to serve as the targeted level for median dietary requirements (Institute of Medicine 2011)."

    We included a separate figure on race/ethnic differences in serum 25(OH)D < 40 nmol/L (*Figure H.2.d.)* and described it as follows:

    "The Institute of Medicine (2011) report concluded that serum 25(OH)D levels of 40 nmol/L are at a level consistent with a desirable median intake of vitamin D. Non-Hispanic blacks had significantly higher prevalence of serum concentrations < 40 nmol/L (Figure H.2.d) which corresponds to the targeted level for the median dietary requirement. The median meets the requirement of approximately half the population thus individuals with levels < 40 nmol/L are at increased risk of adverse health outcomes."

6. For the chapter on "Iodine" I would like to suggest that the authors consider including an additional reference which gives a very nice summary of the WHO guidelines. The citation for the article is:

Andersson M, de Benoist B, Delange F, Zupan J. *Prevention and control of iodine deficiency in pregnant and lactating women and in children less than 2-years-old: conclusions and recommendations of the Technical Consultation.* Public Health Nutrition 2007;10(12A):1606-1611

WHOSec.Prev.Iodine
.Def.Preg.PHN.2007.

Response: Thank you; the reference has been added.

PEER REVIEW COMMENT FORM

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006

REPORT

July 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1. **Are the methods and analyses appropriate for the report objectives?**
   Yes (X) No ( )   Unsure ( )

   Why?

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 1 of 6
Reviewer #1

2. Were the data analyzed in such a way to address appropriately the objectives of the report?
Yes (x) No ( ) Unsure ( )

Why?

3. Are results presented and interpreted appropriately and completely?
Yes (x) No ( ) Unsure ( )

Why?

4.   Are the conclusions and recommendations appropriate and complete?
    Yes (x) No ( )   Unsure ( )

    Why?

5.   Are there any overall comments on the data analysis and presentation?

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 3 of 6
Reviewer #1

6.   Select the appropriate category below:

(List recommended changes or reasons for not recommending)

    A. Recommend (x)

    B. Recommend with Required Changes ( )

    C. Not Recommended ( )

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 4 of 6
Reviewer #1

1.   Are there any comments on NCEH/ATSDR's peer review process?

2.   Are there any other comments?

These data are an enormous contribution to the scientific literature, particularly for blood concentrations of fatty acids since no data of this type exist in NHANES prior to 2003.

Under the discussion of dietary sources of saturated fatty acids in the diet, I question if French fries are a primary source of saturated fatty acids in the diet today since most fast food restaurants are not using a saturated fat source for the frying of French fries. The reference used for the food sources is not very current. Suggest supplementing it with the results from analysis conducted by the National Cancer Institute using dietary data from What We Eat in America, NHANES 2005-2006. It can be found at: http://riskfactor.cancer.gov/diet/foodsources/sat_fat/#figure.

Response: We thank the reviewer for their helpful comment and show below the changes we will make.

Original
"In the American diet the main sources of SFA are dairy products such as full-fat cheese, milk and ice cream; other sources include French fries, meat and chocolate candy (Lichtenstein 2005). The chief dietary sources of MUFA are French fries, meat, cookies, snacks, salad dressing, and cheese. The major foods containing n-6 PUFA are salad dressing, bread, mayonnaise, French fries, cakes and cookies, meats, snacks and fried fish; corn, safflower, soybean, and sunflower oils are the primary sources. The main sources of n-3 PUFA are soybean and canola oils (C18:3n-3) and fatty fish (C20:5n-3 and C22:6n-3)."

Reference
Lichtenstein AH. FATS AND OILS. In: Benjamin C, ed. Encyclopedia of Human Nutrition. Oxford: Elsevier 2005:177-86.

Revised
"Recent NHANES data reveal the main sources of SFA in the American diet are full-fat cheese, pizza and

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –**
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006
Page 5 of 6
Reviewer #1

desserts; other important sources include chicken dishes, cured meats, ribs and burgers (NCI 2010). The chief dietary sources of MUFA (C18:1) are desserts, meats, nuts, seeds, pizza, French fries and Mexican foods (NCI 2010). The major sources of n-6 PUFA consumed in the US are chicken dishes, desserts, salad dressings, chips, nuts, seeds and pizza (NCI 2010). The main sources of n-3 PUFA (C18:3n-3) are salad dressings, chicken dishes, desserts, pizza, bread, mayonnaise and pasta dishes; whereas, the main sources of long-chain n-3 PUFA (C20:5n-3 and C22:6n-3) are fish and fish mixed dishes (NCI 2010)."

References

U.S. National Institutes of Health. National Cancer Institute. Sources of Saturated Fat, Stearic Acid, & Cholesterol Raising Fat among the US Population, 2005–06. Risk Factor Monitoring and Methods Branch Web site. Applied Research Program. http://riskfactor.cancer.gov/diet/foodsources/sat_fat/.
Updated December 21, 2010. Accessed September 28, 2011.

U.S. National Institutes of Health. National Cancer Institute. Sources of Selected Fatty Acids among the US Population, 2005–06. Risk Factor Monitoring and Methods Branch Web site. Applied Research Program. http://riskfactor.cancer.gov/diet/foodsources/fatty_acids/.
Updated December 21, 2010. Accessed September 28, 2011.

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 6 of 6
Reviewer #1

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006

REPORT

July 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1.   Are the methods and analyses appropriate for the report objectives?
Yes (X) No ( )   Unsure ( )

Why?

This report lives up to its purpose to improve our understanding of the concentrations of biochemical indicators of the diet of the general population and of selected subpopulations. The comprehensive analyses of fatty acids, Isoflavones and Lignans reveal information on many compounds and metabolites of the major compounds. These compounds serve as biomarkers of dietary intake. The methods are consistent with previously used methods and with established analytical methodology protocols. The results of analyzing of fatty acids, Isoflavones and Lignans in subpopulations provide useful insights on racial and ethnic differences in these compounds and metabolites.

2.   Were the data analyzed in such a way to address appropriately the objectives of the report?
Yes (X) No ( )   Unsure ( )

Why?

Statistical analyses using geometric means are appropriate to address the objectives of the report. There is consistency from one set of analyses to the next. The statistical methods are conducted to reveal the results in an understandable fashion.

3. Are results presented and interpreted appropriately and completely?

Yes (X) No ( )   Unsure ( )

Why?

Results presented and interpreted nearly appropriately and completely. The one exception is with the discussion under the heading "Health Effects" of fatty acids. Concerns about maintaining the proper ratio of n-6 to n-3 fatty acids in the diet and the prevention of cardiovascular disease have pretty much been debunked. In his article: *The Omega-6/Omega-3 Ratio and Cardiovascular Disease Risk: Uses and Abuses,* Current Atherosclerosis Reports, 2006, 8:453-459, William Harris states that:

> "…the n-6/n-3 Fatty Acids ratio may be of value in interpreting biomarker data and in making nutritional recommendations. Although initially appealing, there are few human experimental and clinical trial data to support this view. This paper reviews a variety of studies that, in the aggregate, suggest that the ratio is, both on theoretical and evidential grounds, of little value. Metrics that includes the n-3 fatty acids alone, especially EPA and DHA, appear to hold the greatest promise."

Response: We reviewed the literature in this area and concur that high level interest in the n-6/n-3 ratio is almost exclusively limited to those working in agricultural or food science. However, a recent paper by Ramsden *et al.* (Ramsden CE, Hibbeln JR, Majchrzak SF, Davis JM. n-6 Fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. Brit J Nutr. 2010;104:1586-600) may revive interest in this discussion. It argues that by altering the n-6 to n-3 balance, diets high in n-6 PUFA tend to increase coronary heart disease risk. The impact of this paper, which stands in opposition to current guidelines, has yet to be determined and thus, it was not cited. We have removed the discussion about the ratio of n-6 to n-3 fatty acids from the "Health Effects" section.

4. Are the conclusions and recommendations appropriate and complete?

Yes (X) No ( )   Unsure ( )

Why?

Conclusions and recommendations are based on data interpreted in the context of authoritative reports including the 2010 Dietary guidelines for Americans and the IOM 2005 Report on Fatty Acids. In the section of Intake Recommendations for Fatty Acids, it would be preferable for the report to cite the recommendation from the Dietary Guidelines Advisory Committee Report and the 2010 DGA regarding recommended intakes of EPA and DHA rather that citing only the Am. Heart Association. The evidence-based 2010 DGA states that Moderate evidence shows that consumption of about 8 ounces/wk of a variety of seafood, which provide an average consumption of 250 mg/d of EPA and DHA is associated with reduced cardiac deaths among individuals with and without pre-existing cardiovascular disease.

Response: Based on the reviewer's recommendation, we revised the text as follows:

"Moderate evidence shows that consumption of about 8 oz of seafood per week, which provides an average of 250 mg per day of eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3), reduces cardiac deaths among persons with and without cardiovascular disease (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010). This amount of seafood is also associated with improved infant health outcomes such as visual and cognitive development, when consumed by pregnant or lactating women (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010)."

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –**
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006
Page 2 of 3
Reviewer #2

We retained the American Heart Association information and added ATP III guidance (Cleeman 2001) for those clinicians who rely on its recommendations:

"According to the American Heart Association, patients with coronary heart disease should be encouraged to increase their consumption of eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) to about 1 gram per day preferably from oily fish. The American Heart Association Dietary Guidelines recommend at least two servings of fish per week (particularly fatty fish) for patients without documented coronary heart disease. In addition, inclusion of vegetable oils (e.g., soybean, canola, walnut, flaxseed) and food sources (e.g., walnuts, flaxseeds) high in alpha-linolenic acid (C18:3n-3) is recommended in a healthy diet for the general population (*Kris-Etherton 2002*). The American Heart Association recommends 5-10% of energy from n-6 fatty acids (Harris 2009) while Adult Treatment Panel III recommends up to 10% of total calories may be consumed from polyunsaturated fats (Cleeman 2001)."

5.   Are there any overall comments on the data analysis and presentation?

Overall, the data look good. The analyses reveal a good bit of new information about population and subpopulations. The first-time NHANES data for 24 plasma fatty acids will be quite useful to the scientific community and researchers in interpreting these biomarkers in relationship to disease risks. Data on subpopulations will also prove to be useful. There were patterns of consistency with regard to some of the variables measured. Plasma concentrations of long-chain omega-3 fatty acids, EPA and DHA, showed racial and ethnic differences where Mexican Americans had lower levels compared to Non-Hispanic Blacks and Whites. The expansion of NHANES data to include fatty acids and Isoflavones will add a new dimension and enhance our understanding of biomarkers for various chronic disease risks.

6.   Select the appropriate category below:

(List recommended changes or reasons for not recommending)

A.   Recommend (X)

B.   Recommend with Required Changes ( )

C.   Not Recommended ( )

ADDITIONAL QUESTIONS:

1.   Are there any comments on NCEH/ATSDR's peer review process?

None

2.   Are there any other comments?

None

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –**
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006
Page 3 of 3
Reviewer #2

Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population

Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006

REPORT

July 2011

GUIDE TO REVIEWERS:

The objective of peer review conducted by the Office of Science is to ensure the highest quality of science for NCEH/ATSDR studies and results of research; therefore, your comments should be provided with this goal in mind. Unlike other peer review processes in which you may have participated, the questions to be addressed for NCEH/ATSDR are broadly based so that each reviewer may have a wide latitude in providing his/her comments. Any remarks you wish to make that have not been specifically covered by the General Questions section may be included under question # 2 in the Additional Questions section. Please note that your unaltered comments will be sent to the investigator for a response. You should receive a copy of the response to the peer review comments when they are available.

GENERAL QUESTIONS:

1.   **Are the methods and analyses appropriate for the report objectives?**
Yes (X) No ( ) Unsure (X)

Why?

Why often used I am unsure about the universal use of geometric means. I believe that this creates a level of complexity that will reduce the utility of this document. Transformations and back transformations are not as straightforward as it is assumed. As a result, it is my strong recommendation that the analyses for the next edition of this report be conducted in the untransformed units. By the same token, I suggest that the authors use only the arithmetic scale and not the log scale in plotting the data. Using the log scale without accompanying text will add more confusion than anything else.

Response: We thank the reviewer for the suggestions. The "Data Presented for each Biochemical Indicator" section in the Introduction provides information on why geometric means were selected over arithmetic means for most biochemical indicators. With mostly right-skewed data, the geometric mean is the best estimate of central tendency and is commonly used to describe the center of the population distribution.

"The majority of the biochemical indicators reviewed in this report, with the exception of vitamin C and body iron stores, have a long upper tail (skewed right). For these biochemical indicators, a geometric mean provides a better estimate of central tendency because it is less influenced by high values than is the arithmetic mean. However, the arithmetic mean is presented for vitamin C and body iron stores as the distributions for these biochemical indicators were reasonably symmetric."

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 1 of 6
Reviewer #3

The "Data Analysis" section in the Introduction provides information on how geometric means and corresponding confidence intervals were calculated:

"Geometric means were calculated by taking the log of each concentration, calculating the mean of those log values, then taking the antilog of that mean (the calculation can be done by use of any log base, such as 10 or e). The confidence interval uses the standard error and mean on the log scale and the appropriate critical value from the t-distribution to calculate upper and lower confidence limits on the log scale. The confidence intervals of geometric means in this report are based on taking the antilog of those upper and lower confidence intervals."

We re-assessed the distribution for each indicator through the estimation of the box-cox transformation parameter and using a combination of histograms and normal probability plots, and confirmed that the use of the geometric mean is appropriate to describe the center of the population distribution for the majority of the biochemical indicators with the exception of vitamin C and body iron. These were the only two indicators that were not sufficiently right skewed to make a log transformation necessary. Therefore, we have presented arithmetic means for these two analytes.

With regards to the use of a log scale to plot the data: this technique is commonly used when an axis spans several orders of magnitude. We only used this for two highlight figures in the entire report: one showing geometric mean levels of 24 fatty acids and other showing geometric mean levels of 5 isoflavones and lignans.

2.   Were the data analyzed in such a way to address appropriately the objectives of the report?
Yes ( ) No ( ) Unsure (X)

Why?

Please see response to #1.

Response: Please see response to #1.

3.   Are results presented and interpreted appropriately and completely?
Yes (X) No ( )   Unsure ( )

Why?

4.   Are the conclusions and recommendations appropriate and complete?
Yes ( ) No ( ) Unsure (X)

Why? Too much of a focus on omega-3 and omega-6 fatty acids and not enough focus on saturated fatty acids.

Response: By way of background, the original focus of the CDC Nutritional Biomarkers Laboratory for this study was to measure absolute concentrations of polyunsaturated fatty acids (PUFA) with emphasis on those for which there are Adequate Intake recommendations (linoleic and *alpha*-linolenic acid) and those providing heart health benefits (fish-derived EPA and DHA). A review of the literature showed that using older technology (flame ionization detection), the analytical precision for absolute concentrations was not very good (CV 15-17% for major fatty acids) and this apparently influenced researchers to present individual fatty acid results in percent-of-total units which were significantly more reproducible (CV <3% for all major fatty acids). We were certain that analytical precision could be improved, using mass spectrometry

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –**
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006
Page 2 of 6
Reviewer #3

together with isotopically-labeled internal standards, to allow satisfactory determination of absolute concentration reference values for the U.S. population. High quality concentration data have not been available as an investigational tool, so we planned to address this gap and provide the most accurate and precise fatty acid concentration data possible. These data could prove to be highly useful, as fatty acids are major components of complex lipids; perhaps knowing individual fatty acid concentrations would add value to cholesterol and triglyceride measurements. Considering that advice about dietary intake is often given in terms of avoiding, minimizing or maximizing intake of different classes of fatty acids, it seems that plasma fatty acid concentrations should be more useful as a measure of dietary compliance than aggregate lipid concentrations (cholesteryl esters and triglycerides) which are composed of many different types of fatty acids. As the project evolved, however, meetings with lipid experts convinced us that in addition to providing concentration data we needed to measure as many fatty acids as was practical to report in the more commonly used percent-of-total units. Otherwise, experts believed that workers in the field will be at a loss as to how to compare the NHANES data with their own.

Returning to this report in which data are presented in absolute concentration units (µmol/L), very few published data are available against which to compare our results, thus we are limited in our ability to make contrasts. This report is not the right platform in which to probe how to interpret the data; at very least additional information about intake is required. We plan to write a paper in which we will provide advice about how to compute percent of total fatty acids using multiple imputation of missing concentration data. In that paper we will provide tables of percent-of-total data for the 24 fatty acids and describe how NHANES fatty acid data compare with selected data from larger studies. We also plan to write a paper about the relationship between absolute concentrations and percentage of total fatty acids compared with intake (diet and supplements) at which time we can delve into the issue of plasma fatty acid concentrations in those Americans who follow expert dietary guidance, such as limiting intake of saturated fat, compared with those who do not.

Finally, our emphasis on PUFA seems justified based on data in the literature showing that in comparison with other classes of fatty acids, plasma PUFA are more strongly correlated with intake. Because saturated (SFA) and monounsaturated (MUFA) fatty acids can be synthesized *de novo* they are reported to be less strongly correlated with dietary intake.

5. **Are there any overall comments on the data analysis and presentation?**

Please see comments made under #1. In addition, I have the following comments.

My comments concern primarily the chapter on Plasma Fatty Acids and most specifically the introduction to the chapter. The introduction to the chapter on fatty acids is very well written. There can be no criticism of the English composition – it is superb.

However, a great deal of space in the "Health Effects" section - too much, much too much - is devoted to essential fatty acids and to **Eicosapentaenoic Acid** (EPA or also icosapentaenoic acid) and Docosahexaenoic acid (DHA) both of which are referred to as omega-3 fatty acids. That is the focus is on everything except what is central to diet and CHD risk – saturated fatty acids. First of all, if Americans have a nutritional deficiency problem it is not essential fatty acid deficiency. Second, I would like to suggest that as far as CVD risk and prevention is concerned more focus should be given to saturated fatty acids and most especially to the two fatty acids in the diet responsible for raising serum cholesterol levels, i.e. Myristic and Palmitic Acids.

There is a wonderful review article on the effects of diet on serum lipids and lipoproteins. It is a bit old but it is still very useful and informative. It is: Grundy SM and Denke MA. Dietary Influences on Serum Lipids and Lipoproteins. J Lipid Research 1990;31:1149-1172. As the authors discuss, the central tenant of "Diet Heart" is and remains the lowering of saturated fatty acid and dietary cholesterol intake. Omega-3 fatty acids, I believe, are a side issue.

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –**
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006
Page 3 of 6
Reviewer #3

Reading the "Introduction" of this chapter one comes away with the impression that essential fatty acids and omega-3 fatty acids have supplanted saturated fatty acids in importance to CHD prevention. The shorter chain saturated fatty acids, as shown long ago by Keys et al and Hegstead et al, are the ones which raised blood levels of total cholesterol. As mentioned above they are the C14 (Myristic) and C16 (Palmitic) saturated fatty acids.

In the "Health Effects" section there is also an attempt to describe the differential cholesterol raising effects of saturated, PUFA and MUFA. The best way to describe their effects is to present the equations for predicting effects of diet on serum total cholesterol. The two oldest and most recognized are those developed by Keys et al and Hegsted et al (Please see Table 1 Grudy and Denke):

TABLE 1. Equations for predicting effects of diet on total serum cholesterol

Equation of Keys et al. (21)
$$\Delta \text{ Cholesterol (mg/dl)} = 2.7\Delta S - 1.35\Delta P + 1.5\Delta C^{1/2} \text{ mg/1000 cal-day}$$

Equation of Hegsted et al. (22)
$$\Delta \text{ Cholesterol (mg/dl)} = 2.16\Delta S - 1.65\Delta P + 0.068\Delta C_{mg/day}$$

Where S = saturated fatty acids (% of total calories); P = poly-unsaturated fatty acids (% of total calories); C = dietary cholesterol.

Both groups show that the short chain saturated fatty acids (c12-c16) raise total cholesterol levels while Stearic acid (c18) does not. MUFA were not included in their equations because they felt MUFA were neutral in their impact on serum total cholesterol levels.

More recently, articles by Mensink et al and Yu et al have conducted meta analyses to examine the predictive capabilities of individual fatty acids (attached). Based on their analyses it seems as though the cholesterol lowering effect of PUFA may be smaller than originally thought and as proposed in the equations from Keys and Hegsted. In addition, there is some data in the more recent papers to suggest that MUFA may indeed lower serum total cholesterol levels.

Response: In response to the reviewer's concern that we are effectively misleading the reader in that we stress the positive relationship between PUFA and CVD and gloss over the negative relationship between SFA and CVD, we reviewed the literature more carefully, including those papers provided by the reviewer. With the understanding that the Health Effects paragraphs are relatively short throughout the report, the following text seems to summarize the state of knowledge with regard to the effects of SFA, MUFA and PUFA on CVD:

"**Health Effects.** The most common MUFA is oleic acid (C18:1n-9); although humans can synthesize this fatty acid, it is obtained largely through the diet. Evidence suggests that replacing dietary carbohydrates with MUFA decreases LDL-cholesterol concentration, but there is little evidence that MUFA are associated with coronary heart disease (Astrup 2011). Currently, intense debate surrounds the question whether reduction in dietary SFA reduces risk of cardiovascular disease (Zelman 2011). Restricting fat intake and replacing SFA with other nutrients has revealed added complexity in the diet-heart issue. According to evidence from human studies, replacing SFA with PUFA lowers coronary heart disease risk, whereas replacing SFA with carbohydrate either has no benefit (Micha 2010) or may be harmful or beneficial depending upon the quality of the carbohydrate (Jakobsen 2009). Carbohydrates either rapidly or slowly increase blood glucose; highly refined carbohydrates are amongst the former, and higher intake of these is associated with greater risk for development of diabetes. Replacing SFA with highly refined carbohydrates and added sugars may be increasing heart disease risk through promotion of obesity and diabetes (Hu 2010)."

Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU, *et al*. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? Am J Clin Nutr. 2011;93:684–688.
Hu FB. Are refined carbohydrates worse than saturated fat? Am J Clin Nutr 2010;91:1541-2.

Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, *et al*. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr. 2009;89:1425–1432.

Micha R, Mozaffarian D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a Fresh look at the evidence. Lipids. 2010;45:893–905.

Zelman K. The great fat debate: A closer look at the controversy - Questioning the validity of age-old dietary guidance. J Am Diet Assoc. 2011;111(5):655–658.

Hopefully, the chapter seems more balanced even though additional attention has not been given to certain SFA (e.g., palmitic, myristic). Again, there are essentially no data available with which to compare our concentrations; to understand if absolute concentrations are meaningful, we need to analyze their relationships to serum cholesterol and other lipid fractions, as well as to dietary intake.

Finally, there is notably no reference given to the National Cholesterol Education Program's Adult Treatment Panel 3 (ATP 3) and to the dietary recommendations in ATP3 (attached). The complete version is an excellent discussion of the impact of diet on serum lipids. It provides guidelines for dietary intakes of fatty acids and cholesterol. The recommendations in ATP3 are the ones physicians follow - not Dietary Guidelines for Americans or the 2005 IOM Report - and it is therefore useful to cite them.

Response: Thank you for the reminder about citing the ATP III recommendations (Cleeman 2001). We have added these citations to the report as follows:

"Lowering dietary intake of SFA to no more than 10% of caloric intake and replacing them with MUFA and PUFA is recommended to reduce the risk of cardiovascular disease; moreover, lowering the percentage of calories derived from SFA to 7% of calories, can further reduce risk of cardiovascular disease (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010; Cleeman 2001). The National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) recommends that MUFA not exceed 20% of calories (Cleeman 2001)." …

"The American Heart Association recommends 5-10% of energy from n-6 fatty acids (Harris 2009) while Adult Treatment Panel III recommends up to 10% of total calories may be consumed from polyunsaturated fats (Cleeman 2001)."

Cleeman JI, Grundy SM, Becker D, Clark LT, Cooper RS, Denke MA, *et al*. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA. 2001;285(19):2486–2497

6.   **Select the appropriate category below:**

(List recommended changes or reasons for not recommending)

Recommend (X):
I made a number of suggested changes to the text for the author's consideration. However, I will leave it up to them as to include or not include them.

A. Recommend with Required Changes ( )

B. Not Recommended ( )

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 5 of 6
Reviewer #3

1.   **Are there any comments on NCEH/ATSDR's peer review process?**

None.

2.   **Are there any other comments?**

None.

Title: **Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population –
Data for plasma fatty acids for NHANES 2003-2004 and urine phytoestrogens for NHANES 2005-2006**
Page 6 of 6
Reviewer #3