

Food and Drug Administration

Risk Assessment Arsenic in Apple Juice: Peer Review Report

December 17, 2012

Introduction and Summary of Contents:

Between December 2011 and early January 2012, the Food and Drug Administration (FDA) sent a draft document “An Assessment of Arsenic in Apple Juice,” to seven individuals who are expert in a range of scientific disciplines, identified below. These individuals were asked to peer review the document by providing individual, written comments in response to a specific set of questions and to provide other comments on the draft.

We greatly appreciate the peer reviewers’ comments and suggestions, as well as their willingness to provide them quickly. The peer reviewers are listed below along with their curriculum vitae. The charge questions for the peer review are provided thereafter, followed by the reviewers’ full responses to the specific charge questions, and other comments, without attribution to the specific reviewer. In the next section of this document, we respond to issues raised by the peer reviewers and explain why we agree or disagree with the comments and describe any actions we have taken in response. Finally, we list comments we received from the Centers for Disease Control and Prevention (CDC) and provide responses to those comments. The CDC comments were provided in response to an invitation by FDA to review the assessment outside of the individual peer review process.

The Peer Reviewers:

Supratim Choudhuri, Ph.D.

U.S. Food and Drug Administration (FDA)/Center for Food Safety and Applied Nutrition (CFSAN)

Office of Food Additive Safety (OFAS)/Division of Biotechnology and GRAS Notification Review (DBGNR)

College Park, MD 20740

Dr. Choudhuri received his Masters of Science and Doctorate at the University of Calcutta, India, with expertise in molecular toxicology. He currently serves as a toxicologist in DBGNR in CFSAN’s Office of Food Additive Safety. Within DBGNR, he is responsible for

toxicological evaluation and safety assessment of GRAS (Generally Recognized as Safe) notices, biotechnology notices, and food additive petitions. He also provides molecular biology, protein chemistry and immunology technical support in the biotechnology program. Dr. Choudhuri is an Adjunct Associate Professor in the Department of Pharmacology, Toxicology and Therapeutics at the University of Kansas Medical Center in Kansas City, KS. He has experience in the areas of molecular biology and genomics, biochemical and molecular toxicology, mechanisms of toxicity, and metabolism. He is a full member of the Society of Toxicology as well as a life member of the Zoological Society of Calcutta, and the D.N. Ganguly Academy of Biosphere, Calcutta, India. He has chaired sessions at the Society of Toxicology on Toxicogenomics; Role in Predictive Toxicology and Carcinogenicity, and Natural Products. He has published extensively on the topics of genomics and carcinogenesis and served as Editor for two special issues of *Toxicology Mechanisms and Methods* on Epigenetics and Toxicology and Genomics and Human Health.

Rebecca Prupurna Danam, Ph.D.

FDA/CFSAN
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Dr. Danam received a Bachelors of Science in Chemistry, Botany and Zoology from Osmania University, Hyderabad, India; a Masters of Science in Biochemistry from the University of Hyderabad and a Doctorate in Biochemistry from the University of Hyderabad, Hyderabad, India. She is currently a toxicologist in CFSAN's DBGNR providing toxicological safety evaluations for food ingredients to be considered as GRAS substances including two precedent setting GRAS notices, as well as providing scientific support for toxicological safety evaluations of botanicals for national and international enquiries. Her expertise is in the fields of epigenetic gene regulation, DNA repair, cancer, aging and certain neurological diseases. She has been appointed as a Temporary Adviser to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), World Health Organization. She has published and presented widely on topics related to her areas of expertise, including co-authoring a chapter on "Food ingredients are sometimes mixtures" in the book: Principles and Practices of Mixture Toxicology, (pg 477-490, 2010).

Penelope Ann Rice, Ph.D.

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OFAS/ Division of Food Contact Notification
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Dr. Rice received a Bachelors of Science in Physics from Clemson University, a Masters of Science in Physics from Florida State University, and a Doctorate of Science in Toxicology from the University of Rochester Medical School. She is currently a toxicologist in the Division of Food Contact Notification in CFSAN's Office of Food Additive Safety. She is an experienced toxicologist in research and technical review. She has published in the areas of immunology, neuropharmacology, and infectious disease. Dr. Rice has specialized experience in the review of pre-market safety applications for novel food ingredients, infant formulas, genetically-engineered crops, and food packaging materials. Before coming to FDA, Dr. Rice served as a Postdoctoral Research Assistant in the Department of Pulmonary and Critical Care Medicine at

the University of Maryland, where she conducted research in the field of immunology. She has also served as a Research Assistant at the University of Rochester in the Department of Environmental Medicine, where she conducted scientific research in the fields of immunology, signal transduction, and neurobiology.

Reeder Losch Sams II, Ph.D.

US Environmental Protection Agency (EPA)
National Center for Environmental Assessment (NCEA)
Hazardous Pollutant Assessment Group
Research Triangle Park, NC 27711

Dr. Sams received a Bachelors of Science in Pre-Veterinary Medicine from the College of Agriculture and Forestry, West Virginia University, Morgantown, WV, and a Doctorate of Science in Interdisciplinary Toxicology from the Medical Graduate School, University of Arkansas for Medical Sciences, Little Rock, AR. He is currently the Chief of the Hazardous Pollutant Assessment Group in EPA's National Center for Environmental Assessment, with responsibility for developing human health risk assessments and methodologies for use in human health risk assessment. His work in this area has included major human health risk assessments for acrylamide, methanol, and inorganic arsenic. He has also served as an Environmental Health Scientist for EPA's Integrated Risk Information System Staff where he helped develop human health risk assessments, with direct involvement in health risk assessments for arsenic, carbon tetrachloride, copper, methanol, platinum, and urea. He is also currently an Ad Hoc Graduate Faculty member in the Division of Environmental Sciences and Engineering at the University of North Carolina. Prior to his work at EPA, Dr. Sams served as a Research Chemist, Division of Biochemical Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR. He was an Expert Reviewer for the World Health Organization Technical Workshop for the Immunotoxicity Risk Assessment for Chemicals Guidelines in 2011 and co-chaired a Society of Toxicology workshop on Advances and Challenges for the Incorporation of Mode of Action in Human Health Risk Assessment.

John Jay Vandenberg, Ph.D.

U.S. Environmental Protection Agency,
National Center for Environmental Assessment (NCEA)
Office of Research and Development
Research Triangle Park, NC 27711

Dr. Vandenberg received a Bachelors of Science in Biology from the College of Wooster, Wooster, OH, and a Masters and Doctorate of Science in Biophysical Ecology from Duke University. He is currently the Director of the Research Triangle Park Division of the Environmental Protection Agency's NCEA. As such, he is responsible for directing preparation and communication of assessments used in environmental policy making including Integrated Science Assessments for the major air pollutants and Integrated Risk Information System assessments for hazardous air pollutants. Dr. Vandenberg represents EPA before the Clean Air Scientific Advisory Committee and other committees of EPA's Science Advisory Board, to the National Academy of Sciences. He is an Adjunct Professor at the Nicholas School of the Environment, Duke University, Durham, NC. Dr. Vandenberg previously served as the

Associate Director for Health in NCEA's Office of Research and Development where he was responsible for scientific leadership of EPA's comprehensive health risk assessment program; this program improves risk assessment methods and assessment products utilized by EPA regulatory programs, Regions, state and local agencies, industry and public health organizations. He has also served as the Acting Director of the Experimental Toxicology Division of EPA's Office of Research and Development where he had responsibility for scientific and managerial leadership of a comprehensive health research program encompassing pulmonary toxicology, immunotoxicology and pharmacokinetics focused on understanding and describing the fate, disposition and health consequences of chemicals in the body and ultimately developing quantitative models for extrapolation/prediction in the context of EPA's risk assessment activities. Dr. Vandenberg is an Elected Fellow of the Society for Risk Analysis and serves on numerous scientific advisory committees. He has published extensively on toxicology, risk assessment and health effects related to environmental contaminants.

Michael Phillip Waalkes, Ph.D.

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National Toxicology Program
Research Triangle Park, NC

Dr. Waalkes received a Bachelors of Science in Biology and Chemistry from Hope College, Holland, MI and a Doctorate of Science from the West Virginia University Department of Pharmacology and Toxicology. He was a Postdoctoral Fellow at the University of Kansas School of Medicine Department of Pharmacology, Toxicology and Therapeutics. He is currently the Branch Chief of the National Toxicology Program Laboratory and head of the Inorganic Toxicology Group, Division of the National Toxicology Program at NIEHS. Before coming to NIEHS, Dr. Waalkes was a Professor of Toxicology at the University of Maryland and served as the Chief of the Inorganic Carcinogenesis Section in the Laboratory of Comparative Carcinogenesis of the National Cancer Institute, Frederick Cancer Research and Development Center, Frederick MD. He is a former Editor-in-Chief of the journal *Toxicology and Applied Pharmacology* and is currently on the editorial boards for the *Journal of Toxicology and Environmental Health* and *Toxicology Mechanisms and Methods*. Dr. Waalkes is a full member of the Society of Toxicology, as well as the president of the Stem Cells Specialty Section. He has published and spoken widely on the toxicity of arsenic and other heavy metals.

Tong Zhou, M.S., Ph.D., DABT

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Office of New Animal Drug Evaluation
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Dr. Zhou received a Bachelors of Science in Biology from Peking University, People's Republic of China, a Masters of Science in Biology/ Environmental Sciences from the University of Maryland, and a Doctorate of Science in Biology/Environmental Toxicology from Rutgers University. He currently serves as a toxicology reviewer in the Office of New Animal Drug Evaluation in FDA's Center for Veterinary Medicine (CVM). He is an experienced senior toxicologist with extensive expertise in human food safety risk assessment for new animal drugs and feed ingredients, involving both qualitative and quantitative toxicological risk assessment,

including carcinogenic risk assessment. He is currently the CVM representative for the FDA-NIEHS Toxicology Study Selection and Review Committee (TSSRC) and the U.S. representative in the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Safety Expert Working Group. Dr. Zhou is a Diplomat of the American Board of Toxicology and a member of both the Society of Toxicology and the Society of Risk Analysis. He has made oral presentations on topics related to risk assessment and human food safety assessment at meetings of the Society of Toxicology, the Society of Risk Analysis, and the American Academy of Veterinary Pharmacology and Therapeutics. Dr. Zhou participated in post-doctoral research at EPA's Toxicology Training Program, Curriculum in Toxicology, University of North Carolina-Chapel Hill, where he conducted *in vivo* perinatal developmental studies on endocrine disrupting chemicals, such as polybrominated diphenyl ethers. He also served a post-doctoral fellowship at the Department of Biological Sciences, Rutgers University, where he conducted research on evaluation of environmental contaminants on fish thyroid hormone levels.

List of Acronyms and Abbreviations

ADME, Adsorption, Distribution, Metabolism and Excretion
AIC, Akaike Information Criterion
ATSDR, Agency for Toxic Substances and Disease Registry
CDC, Centers for Disease Control and Prevention
CFSAN, Center for Food Safety and Applied Nutrition
CFSII, Continuing Survey of Food Intakes by Individuals
CSF, Cancer Slope Factor
CVM, Center for Veterinary Medicine
DMA^V, dimethylarsinic acid
DR, Dose/Response
EFSA, European Food Safety Authority
EPA, Environmental Protection Agency
FAO, Food and Agriculture Organization of the United Nations
FDA, Food and Drug Administration
GRAS, Generally Recognized as Safe
iAs, inorganic Arsenic
IARC, International Agency for Research on Cancer
IRIS, Integrated Risk Information System
JECFA, Joint FAO/WHO Expert Committee on Food Additives
LOC, Level of Concern
LOQ, Level of Quantification
MMA^V, monomethylarsonic acid
MOA, Modes of Action
NCEA, National Center for Environmental Assessment
NHANES, National Health Examination and Nutrition Survey
NIEHS, National Institute of Environmental Health Sciences
NIH, National Institutes of Health
NRC, National Research Council
OFAS, Office of Food Additive Safety
PK, pharmacokinetics
SE, Standard Error
TEP, Toxic Elements Program
TSSRC, Toxicology Study Selection and Review Committee
USEPA, United States Environmental Protection Agency
VICH, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
WHO, World Health Organization

The Charge to the Peer Reviewers:

Each expert peer reviewer was provided with a written “charge” concerning the document, as follows:

FDA has generated a draft risk assessment for arsenic in apple juice, in anticipation of preparing a guidance document to establish levels (limits) for inorganic arsenic in apple juice. The peer review should provide input on the reasonableness of judgments made in the assessment from the scientific evidence. The results should a determination by each peer reviewer as to the reasonableness of: (a) the assumptions made and the hypotheses used, (b) the methodology used, (c) the quality and relevance of the data and information, and (d) whether the conclusions reached are supported.

Charge Questions:

1. Is the document logical and clear?
2. Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?
3. Is use of average consumption data from CDC’s National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?
4. Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?
5. Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?
6. Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?
7. The dose/response (DR) function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?

The Peer Reviewer Comments (provided in random order and without attribution):

Peer Reviewer Number 1:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** Not really. The document was rather wordy and confusing, particularly when compared with the JECFA drinking water assessment for arsenic. Some parts, such as the discussion of the evidence underpinning the conclusion that iAs was carcinogenic in humans, needed a lot more detail. Moreover, the selection of the endpoint of carcinogenicity for the focus of the RA needs to be explained in the context of the other toxic effects observed with chronic intake and/or intake during development. What is the evidence that carcinogenicity being the most sensitive, relevant endpoint for the two exposure scenarios being modeled?
2. ***Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?*** No, the explanation of the criteria used, which appear to be the same criteria as used in JECFA, 2011, need to be explained in more detail. The JECFA document, for instance, makes clear that there were other epidemiology studies reporting an association between As exposure and cancer, but that these studies measured arsenic (As) exposure via toenail As concentrations, which were considered unreliable indicators of daily dietary exposure. As that document makes clear, only the 2 Chen et al studies met JECFA's criteria of clear association between cancer and As exposure, clear quantification of daily As intake (via drinking water), and a DR-range that allowed reliable extrapolation to low doses. However, it should be noted that these 2 papers are really one study done on a single population cohort. As such, the confidence in the risk assessments derived from these two papers, as opposed to an RA derived from data from multiple studies, must be qualified accordingly in the RA document. The criteria by which the Chen study was considered pivotal should also be spelled out in more detail in the RA document.
3. ***Is use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** Yes.
4. ***Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?*** At OFAS, we normally use the 50th and 90th-percentile consumption values for the general population. As the RA document states that the risk associated with children's exposure via data on juice consumption at 0-6 years old was also modeled, this seems like an adequate approach.
5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** Yes, as people don't usually drink apple juice

concentrate, although it seems inconsistent that the concentrate would have a lower concentration than the ready-to-drink juice.

6. ***Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?*** It appears to be the most conservative approach, given the limitations of the analytical equipment used. Moreover, as the majority of iAs is rapidly converted in vivo to organic forms, even estimating risk from total iAs may be overly conservative.
7. ***The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** As the exact mechanism of action of iAs in causing cancer is unknown and there are no suitable animal models with which to explore the MOA for this process, it should be assumed that the same MOA for carcinogenesis would be present in children as adults. The draft RA made no mention of an PK data available for iAs in humans, but it is possible that less efficient methylation capacity in extremely young children could shift the DR-curve to the left for that age group. The draft RA made no mention of in vitro studies with isolated hepatocytes identifying the Phase I enzymes responsible for the methylation. Identification of those enzymes, combined with data on the induction of those enzymes during postnatal development, could allow for comparison of the detoxification response during early childhood with that of adults and improve the accuracy of the RA with respect to childhood cancer risk. Alternatively, OFAS uses an additional factor of 10 in the calculation of risk for infant exposure to account for the possibility of increased sensitivity to the carcinogen during the period of concern. This approach may also be used here.

Peer Reviewer Number 2:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** Yes, the document is logical and clear, but could still be improved. This document draws significantly (and justifiably so) from the JECFA 2011 document, which has been referenced. Nevertheless, this document is CFSAN's document on arsenic risk assessment; hence it should be a stand-alone and complete document. Consequently, a little more discussion on various studies on arsenic, particularly various epidemiological studies reporting on the same or similar endpoints, would be very useful. This will help explain the rationale for using Chen et al.'s studies as the pivotal studies.
2. ***Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were***

these criteria adequate? This point has been addressed already in relation to Question 1 above. To reiterate, a justification for using the studies by Chen et al. should emphasize two aspects: (1) the strength of Chen et al.'s studies, and (2) the flaws of other epidemiological studies reporting on the carcinogenesis endpoints. The second aspect is missing from this document. A brief discussion (before discussing Chen et al.'s studies) why other similar epidemiological studies were not considered, can be used to reinforce why Chen et al.'s studies were chosen as the pivotal studies for this risk assessment document. Such a discussion would be helpful for the completeness of this document.

3. ***Is use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** Yes
4. ***Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?*** Yes but could be improved. No justification has been provided why the 3X (and not 2X or 4X) has been considered.

In OFAS, we use the 50th and 90th percentile consumption and we mostly deal with non-linear dose-response function derived from toxicological studies. In such a situation, the data on the 50th and 90th percentile consumption add significantly to the safety assessment.

Although this document is about risk assessment, an explanation will still be helpful. In a linear dose-response function the disease rates from 3X exposure is predictably 3-times the average exposure; but in non-linear dose-response function such predictability is missing. Therefore, for this document, a rationale for using the 3X exposure should be added. For children, this could be easily fixed by citing published literature (For example: Dennison, B.A. 1996. *J. American College of Nutrition* 15(5 Suppl): 4S-11S.). Other sources of citation may include data reported by juice manufacturers on the excess consumption of fruit juice. Also, standard practice may be cited.

5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** Yes, the use of the finished product with higher arsenic levels makes the risk assessment more conservative; hence more useful.

It is pertinent to mention in this context that in OFAS, we consider whether the consumer exposure to similar (or same) compounds could be additive or substitutional. The consumption of apple juice could be regarded as substitutional for water, that is, a consumer of apple juice is expected not to drink simultaneously the same fluid volume of water to quench thirst or for hydration. Therefore, if the limit of arsenic in water is 10 ppb, the arsenic level in apple juice should not exceed 10 ppb. [In real life, it could be further improved based on good apple growing practice, and further purification of water at the plant for making juice. This will be a risk management decision].

6. ***Is it reasonable to base risk estimation on the basis of total Arsenic, even though in doing so, total Arsenic includes some varying amounts of organic Arsenic?*** Yes. Since arsenic in water is mostly in inorganic form, using total arsenic data is expected to yield a reasonably accurate risk estimate of inorganic arsenic in apple juice.

Additionally, using total water arsenic data as a surrogate for inorganic arsenic will make it a conservative risk assessment; yet it will not create a regulatory burden for the industry to achieve the lower inorganic arsenic level in apple juice.

7. ***The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** Yes, but may need an explanation for children. From the perspective of dose-response function alone, a linear dose-response usually assumes zero risk only in the event of zero exposure. Any exposure will have some risk of adverse effects. Therefore, this is the most conservative approach, particularly when carcinogenesis is used as the endpoint.

However, arsenic-induced carcinogenesis usually takes significantly higher exposure for a longer period of time. Referring to Chen et al.'s studies this document states, "*In total, 8086 subjects aged 40 years and older were recruited into the study, with an average of 11.5 years of follow-up*". Thus, questions may be raised as to what is the likelihood of children 0-6 years developing cancer from arsenic exposure? Is carcinogenesis the most appropriate/sensitive endpoint of arsenic exposure in children? Can there be a better marker of arsenic exposure and arsenic-induced adverse effects in children 0-6 years? An explanation will significantly improve the document.

Peer Reviewer Number 3:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** It is logical but lacks pointers that clearly connect the information described and in some portions needed more detail. It would have been helpful to clearly state the reasons for the lack of good animal models for carcinogenicity of inorganic arsenic and why they were excluded for risk assessment. It would have been better to compare other studies and highlight the robustness of the chosen pivotal study. In some instances, it would have been more evident, for example, as to why smoking as a variable was adjusted in Chen's study, if the studies that report an increased risk of bladder cancer in smokers but not in nonsmokers, exposed to relatively low concentrations of arsenic in drinking water were described earlier. With respect to the mechanism of action, the animal and in vitro studies that report the biochemical and cytotoxic effects at low doses and concentrations that are potentially attainable in human tissues following ingestion of drinking water need to be included. The necessity for the use of 8 different dose response models for risk assessment and the model that finally provided the best fit should be justified.

2. ***Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?*** No. In view of the extensive availability of epidemiological studies linking arsenic in drinking water to increased risk of skin, urinary and lung cancer and skin lesions, the various criteria used to identify the data and its suitability for the risk assessment is not adequately described. The criteria used for the selection of a single prospective cohort study conducted by Chen et al. (2010 a, b) as the basis for risk assessment and the dose-response modeling should be explained in more detail. The strength of the data from Chen's study should be enunciated clearly to justify its use for quantitative risk assessment. It is not sufficient to refer to the JECFA monographs for additional information regarding the study selection criteria, although they are discussed clearly in that document. The rationale for choosing carcinogenicity, (urinary tract and lung cancer as opposed to skin cancer) as the most appropriate end point rather than other adverse effects associated with long term arsenic exposure needs to be explained. Further, it is reasonable to justify the exclusion of other epidemiological studies that examined the bladder and lung cancer as well as large population studies from West Bengal, Bangladesh, and Inner Mongolia that examined the dose-response relationships between arsenic intake via drinking water and skin lesions. The limitations of the study design, or the methodologies utilized, the confounding factors, the uncertainties or the assumptions made in those studies that do not allow them to be suitable for the cancer risk assessment need to be discussed.
3. ***Is use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** Yes.
4. ***Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?*** Yes. Typically the mean and the 90th or the 95th (European countries) percentile exposure estimates vary by a factor of 2 or 3. The use of three times the average is reasonable and would provide a conservative estimate.
5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** Yes. It is most appropriate to estimate the exposure assessment based on the finished consumer product, which would then take into account the contribution of water which is the diluent typically used, that may be contaminated with high or low or zero levels of arsenic.
6. ***Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?*** Yes. In the context of juices, it would be reasonable to base risk estimation on total arsenic, because the principal form of arsenic in drinking water is inorganic. Since inorganic arsenic is rapidly metabolized to organic forms, estimating risk based on total arsenic content would be a conservative approach. A risk assessment based on inorganic arsenic will be more straightforward and a highly conservative approach. However, given the lack of validated analytical methods for extraction and measurement of speciation, it would be

more challenging to obtain more consistent, and accurate data. The need for speciation data needs to be encouraged. In the context of estimating dietary arsenic exposure, considering total arsenic would lead to an overestimation of health risk as it is shown that in foods, especially in seafoods, arsenic is present in organic forms that are less toxic.

7. ***The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** In children, the impact of arsenic exposure on cancer endpoint may not be of serious concern as it depends on the lifetime exposure and the level of exposure. Based on available data, it is not clear whether children differ from adults with regard to their sensitivity to the carcinogenic effects of arsenic in drinking water. However, there is some evidence suggesting that children methylate arsenic less efficiently and there are some differences in the inherent methylation patterns between children and adults. The differences in the pharmacokinetics of ingested arsenic and other factors such as nutritional status, socio-economic status would also contribute to the effects of arsenic toxicity. Therefore, the possibility of a differential susceptibility to arsenic exposure cannot be ignored. It is not known whether childhood exposure to arsenic would lead to an increased risk of cancer later in life.

It may be more useful and relevant to consider other non-carcinogenic biological effects such as neurobehavioural, pulmonary or cardiovascular effects as endpoints in children for risk assessment. Few studies in school aged children indicate a possible adverse effect on the neuro-cognitive development showing lower verbal comprehension scores. The dose response relationship and the critical times of exposure for these effects need to be investigated.

II. SPECIFIC OBSERVATIONS

A number of specific edits/comments on the draft risk assessment document were offered to correct typos, improve clarity, point out inconsistencies in nomenclature and font size, and make recommendations for repositioning text within the document.

Peer Reviewer Number 4:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** Overall the assessment presents a logical stepwise progression to assess the potential risk of health effects due to consumption of apple juice over a lifetime. The assessment would benefit from referencing or including citations in the text to provide support and context throughout the assessment. The science basis for conclusions in the assessment would also benefit from providing the corresponding citations (see response to Charge Question 2). Many of the tables and figures in the document could be improved by a greater level of detail in figure legends and footnotes. Lastly, on page 10, lines 1-16 made a comparison with the current assessment (FDA 2011) and assessments conducted by the EPA. These types of comparisons would be most appropriately made with the EPA 2001 assessment, EPA 1988, and the NRC 2001.

The most relevant and up to date comparisons of the cancer potency of inorganic arsenic in drinking water would be the NRC 2001. The NRC 2001 report was the basis for the draft EPA 2010 assessment

(http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=219111). The draft EPA 2010 assessment was a pre-dissemination copy for review purposes only, it should not be cited or quoted. A cancer slope factor or cancer potency estimate can be calculated from the NRC 2001 report as follows:

The cancer risk estimates presented in Table 1 for consumption of drinking water with specified arsenic concentrations provide information that is scientifically equivalent to estimates of CSFs. The NRC's (2001) recommended risk models provide estimates that consumption of drinking water containing 10 µg/L arsenic is associated with the site specific cancer risks below. Note that the same CSF values, other than small differences due to rounding error, would be obtained starting with any of the water concentrations presented in the NRC (2001).

Table 1. Maximum likelihood estimates of excess lifetime risk (incidence per 10,000 people) of lung cancer and bladder cancer for US populations

Arsenic concentration (µg /L)	Bladder		Lung	
	Male	Female	Male	Female
10	23	12	14	18

Source: NRC (2001)

The equivalent CSFs can be calculated as follows:

- Using the exposure factors for US populations applied in NRC (2001), consumption of 10 µg/L arsenic in drinking water results in a daily exposure of $(10 \mu\text{g/L}) \times (1 \text{ L/d}) \times (1 \text{ mg}/1,000 \mu\text{g}) \times (1/70 \text{ kg}) = 0.000143 \text{ mg/kg-day}$ of inorganic arsenic. As the NRC risk estimates are linear (proportional to dose) for these exposures, equivalent CSF values come from the equation:
- Risk = CSF (per mg/kg-d) \times dose (mg/kg-d)
- As an example, applying this equation to bladder cancers in females:
- $12 \times 10^{-4} = \text{CSF} \times 0.000143 \text{ mg/kg-d}$, or CSF = 8.4 per mg/kg-day

Thus the CSF estimates resulting from Table 1 are shown below in Table 2.

Table 2. Arsenic oral CSFs (per mg/kg-d) for lung cancer and bladder cancer in US populations

Bladder		Lung	
Male	Female	Male	Female
16	8	10	13

As these are maximum likelihood estimates, it is appropriate to add risks across the two sites resulting in combined CSFs for lung and bladder cancer of 21 and 26 per mg/kg-day in females and males respectively.

[NRC](http://www.nap.edu/openbook.php?record_id=10194&page=R1). (National Research Council). (2001). Arsenic in drinking water: 2001 Update. Washington, DC: Author. Retrieved from http://www.nap.edu/openbook.php?record_id=10194&page=R1.

[U.S. EPA](http://www.epa.gov). (U.S. Environmental Protection Agency). (2001). National primary drinking water regulations; Arsenic and clarifications to compliance and new source contaminants monitoring. Fed. Reg., 66, 6975-7022.

[U.S. EPA](http://www.epa.gov). (U.S. Environmental Protection Agency). (1988). Integrated Risk Information System (IRIS) Summary. Washington, DC: National Center for Environmental Assessment. Retrieved from <http://www.epa.gov/NCEA/iris/subst/0278.htm>.

2. ***Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?*** Even though many of the conclusions presented in this assessment may be reasonable, the basis for these conclusions is not transparently communicated in such a way that the reader of the document can evaluate the data and reach the same or different conclusion. The conclusions would be appropriately supported by providing an increased synthesis of the available science (with references), supporting analyses (conducted by FDA or WHO 2011) and the criteria by which the significant conclusions in the assessment were determined. Examples of this include:
 - a. Page 6, line 27-29 derives a qualitative conclusion regarding the dose-response for cancer versus noncancer effects which states that carcinogenic effects occur at lower doses than non cancer effects. This conclusion should be supported by the available science and some type of comparative analysis.
 - b. Page 6, lines 33-36 presents the selection of principal studies (Chen et al., 2010a,b) with which to conduct a dose-response analysis for estimating the potency of arsenic in drinking water or to derive a cancer potency estimate. The rationale and basis for this conclusion cites WHO 2011 as providing the supporting information for this decision. At a minimum this assessment should provide a rationale (even if it is excerpted from WHO 2011) within the document to enable the reader of the FDA assessment to evaluate and to determine whether or not they agree with the conclusion.
 - c. It is not clear if the dose-response analysis evaluates the combined risk of lung and bladder cancer (page7-8) from the Chen et al., (2010 a,b) studies. However Table 7 does specify combined risk for the tumor types. This needs to be clarified in the text.
3. ***Is the use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** The use of the NHANES data seems to be a reasonable starting point. A sensitivity analysis using several exposure / consumption rates which may include a 95% percentile level would be useful to characterize the uncertainty for this model assumption.

4. ***Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?*** The document would be strengthened by providing the rationale, an analysis, and corresponding references that provides support for this assumption. As a reader of the assessment, I found myself asking “Does three times the NHANES average apple juice consumption equal the 95% percentile for consumption?” Walking through a real-life exposure scenario may be a useful addition to the assessment. Along the lines of the following:

For a 10 kg infant (~20lbs), the consumption at three times the NHANES average would be approximately 93 ml (~ 3 ounces) of apple juice per day. From a parent’s perspective this may seem somewhat low. From personal observations some infants consume significantly more apple juice on a regular basis. I am not suggesting that this should be the example, but a more definitive analysis would be beneficial for the assessment.

5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** This assumption is reasonable, but again characterization of the uncertainty surrounding this assumption should be presented as an analysis in the assessment. Essentially all water sources in the United States contain some level of arsenic with the average being approximately ~3 ppb (USEPA, 2000). Data should be utilized to inform this assumption and its impact on the final level of arsenic in reconstituted apple juice.

Another variable that should be evaluated in the assessment is the range of the arsenic concentration observed in apple juice samples (Tables 4 & 5). A real-world scenario may include brand loyalty by consumers. If specific brands of apple juice consistently represent the upper range (e.g., 47 ppb as reported in Table 4) of the tested samples there may exist populations that consistently consume higher levels of arsenic in apple juice. USEPA (2000) Arsenic occurrence in public drinking water supplies. EPA 8105-R-00-023.

6. ***Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?*** Yes, this is a reasonable assumption and the worst-case scenario would overestimate the potential risk due to inorganic arsenic contained within apple juice.
7. ***The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** The assumption regarding the potential increased susceptibility for children due to exposure to inorganic arsenic through apple juice consumption should be explored more fully within the assessment. In utero and early-life exposure to inorganic arsenic and the resulting health effects is a rapidly expanding area of research. As a starting point, the following references may useful to characterize the current scientific evidence:

Noncancer effects:

- Dauphiné, D. C.; Ferreccio, C.; Guntur, S.; Yuan, Y.; Hammond, S. K.; Balmes, J., . . . Steinmaus, C. Lung function in adults following in utero and childhood exposure to arsenic in drinking water: preliminary findings. *Int Arch Occup Environ Health*. <http://dx.doi.org/10.1007/s00420-010-0591-6>.
- Smith, A. H.; Marshall, G.; Yuan, Y.; Ferreccio, C.; Liaw, J.; von Ehrenstein, O., . . . Selvin, S. (2006) Increased mortality from lung cancer and bronchiectasis in young adults following exposure to arsenic in utero and in early childhood. *Environ Health Perspect* 114: 1293-1296. <http://dx.doi.org/10.1289/ehp.8832>.
- von Ehrenstein, O. S.; Poddar, S.; Yuan, Y.; Mazumder, D. G.; Eskenazi, B.; Basu, A., . . . Smith, A. H. (2007) Children's intellectual function in relation to arsenic exposure. *Epidemiology* 18: 44-51. <Go to ISI>://WOS:000242836700010.
- Wang, S.-X.; Wang, Z.-H.; Cheng, X.-T.; Li, J.; Sang, Z.-P.; Zhang, X.-D., . . . Wang, Z.-Q. (2007) Arsenic and fluoride exposure in drinking water: Children's IQ and growth in Shanyin county, Shanxi province, China. *Environ Health Perspect* 115: 643-647. <Go to ISI>://WOS:000245412800049.
- Wasserman, G. A.; Liu, X. H.; Parvez, F.; Ahsan, H.; Factor-Litvak, P.; van Geen, A., . . . Graziano, J. H. (2004) Water arsenic exposure and children's intellectual function in Araihaazar, Bangladesh. *Environ Health Perspect* 112: 1329-1333. <Go to ISI>://WOS:000224547000035.
- Wasserman, G. A.; Liu, X.; Parvez, F.; Ahsan, H.; Factor-Litvak, P.; Kline, J., . . . Graziano, J. H. (2007) Water arsenic exposure and intellectual function in 6-year-old children in Araihaazar, Bangladesh. *Environ Health Perspect* 115: 285-289. <Go to ISI>://WOS:000243946800040.
- Wasserman, G. A.; Liu, X. H.; Parvez, F.; Factor-Litvak, P.; Ahsan, H.; Levy, D., . . . Graziano, J. H. (2011) Arsenic and manganese exposure and children's intellectual function. *Neurotoxicology* 32: 450-457. <Go to ISI>://WOS:000291762600011.

Carcinogenic effects:

- Liaw, J.; Marshall, G.; Yuan, Y.; Ferreccio, C.; Steinmaus, C.; Smith, A. H. (2008) Increased childhood liver cancer mortality and arsenic in drinking water in northern Chile. *Cancer Epidemiology Biomarkers & Prevention* 17: 1982-1987. <Go to ISI>://WOS:000258800800021.
- Marshall, G.; Ferreccio, C.; Yuan, Y.; Bates, M. N.; Steinmaus, C.; Selvin, S., . . . Smith, A. H. (2007) Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. *J Natl Cancer Inst* 99: 920-928. <http://dx.doi.org/10.1093/jnci/djm004>.
- Smith, A. H.; Marshall, G.; Yuan, Y.; Ferreccio, C.; Liaw, J.; von Ehrenstein, O., . . . Selvin, S. (2006) Increased mortality from lung cancer and bronchiectasis in young adults following exposure to arsenic in utero and in early childhood. *Environ Health Perspect* 114: 1293-1296. <http://dx.doi.org/10.1289/ehp.8832>.

- Tokar, E. J.; Diwan, S. A.; Waalkes, M. P. (2010) Arsenic Exposure Transforms Human Epithelial Stem/Progenitor Cells into a Cancer Stem-like Phenotype. *Environ Health Perspect* 118: 108-115. <Go to ISI>://WOS:000273292800033.
- Tokar, E. J.; Diwan, B. A.; Ward, J. M.; Delker, D. A.; Waalkes, M. P. (2011) Carcinogenic Effects of "Whole-Life" Exposure to Inorganic Arsenic in CD1 Mice. *Toxicol Sci* 119: 73-83. <Go to ISI>://WOS:000285845400007.

Peer Reviewer Number 5:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** Overall, the document is logical and clear. To enhance the clarity of the document, the reviewer recommends that: (1) the objective of the risk assessment be clearly stated in the introduction section (the introduction as it stands now only contains discussions on occurrence of arsenic in the environment and food); (2) explicitly explain why the assessment was focused on inorganic arsenic (inorganic vs. organic arsenic toxicity) and why the cancer endpoint of toxicity was chosen for the risk assessment (a sensitive toxicity endpoint); (3) add subheadings for the hazard assessment, such as mechanisms of toxicity, animal studies and human epidemiology studies; (4) add notes or legends for figures and tables, for example, adding figure legends (or notes as presented in 2011 JECFA Monographs) for Figures 1&2; and (5) add more detail information on dose-response modeling and risk estimates (see Specific Comments below).
2. ***Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?*** In general, there were sufficient information and explanations presented describing the data selection and the criteria used regarding data for cancer dose response assessment and data for exposure assessment (apple juice consumption and arsenic concentrations in apple juice). For the purpose of this assessment of arsenic in apple juice as a contaminant, the criteria were generally reasonable. One suggestion is to consider combining datasets from northeast and southwest Taiwan to see how the dose-response relationship will be modified. In addition, questions remain as to how the relative sensitivity in carcinogenic response to arsenic exposures between Taiwanese and US populations is addressed so that the arsenic-related cancer risks in the Taiwanese population can be extrapolated to US populations.
3. ***Is use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** It is unclear how the average consumption was calculated. Table 3 shows that children aged 6 or less have much higher apple juice consumption than adults. Was the weighted average used in calculating the population average?
4. ***Is the use of three times the NHANES average a reasonable estimation for the high end apple juice consumption?*** Assuming that the consumption data are normally

distributed, the use of three times of the average may reflect the 95th-percentile of the high end apple juice consumption. Given that there is nothing known about the distribution of apple juice consumption, it seems this is one of the reasonable ways to estimate the high end consumption. It may be helpful to also include the 95th percentile for comparison purpose.

5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** If the purpose is to set a single standard/limit of arsenic for all juice and concentrate, the reviewer would agree that exposure assessment based on single strength juice rather than concentrate would be conservative, given that in comparison to single strength juice, concentrate appears to contain lower concentrations of total arsenic and assuming that concentrate would also contain lower concentrations of inorganic arsenic.
6. ***Is it reasonable to base risk estimate on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?*** Yes. Because inorganic arsenic is more toxic than organic arsenic and it is the inorganic form of arsenic which gives rise to the carcinogenic concern, the risk estimate based on the total arsenic would be conservative in protecting public health.
7. ***The dose/response function does not assume greater or lesser response to dose, i.e., susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** The endpoint of toxicological concern is carcinogenicity, which usually is associated with long-term exposure, and the dose-response relationship for carcinogenic effects is based on human epidemiology studies. Studies showed a significant dose-response relationship between cancer mortality and increased years of exposure to arsenic (Chen et al., 1986). However, in the absence of data on whether and the extend of childhood exposures to arsenic may result in increased cancer risk later in life, the reviewer agrees that based on the available data that it is reasonable not to assume greater or less response to dose for children. In addition, the reviewer notes that the 2010 draft EPA document did not apply age-dependent adjustment factor to the cancer slope factor and combine with early-life exposure estimates when estimating cancer risks from exposure to inorganic arsenic because a mutagenic mode-of-action for carcinogenesis of inorganic arsenic has not been established.

II. SPECIFIC OBSERVATIONS

Data for Cancer Dose Response Assessment

The urinary and lung cancer data from northwest Taiwan (Chen et al., 2010a&b) were selected for dose-response modeling, which is consistent with the dose-response data selected in 2011 JECFA Monographs. Though recognizing the strength of these recent studies (a prospective cohort study with a relatively large population, individual data available, inclusion of relatively low concentrations of inorganic arsenic in drinking water), the reviewer notes that in a recent

review article, Gibb et al (2001) concluded that “*the data from southwest Taiwan continue to provide the best basis for the quantitative risk assessment of lung and bladder cancer from ingested arsenic*” and individual data from Chen et al. (2010 a&b) “*should provide an excellent database on which to improve the assessment.*” The EPA IRIS draft carcinogenic risk assessment for inorganic arsenic (2010) is based on the southwest Taiwan data (Chen et al., 1988&1992; Wu et al., 1989). In order to address carcinogenic concerns for approved animal drug residues in edible tissues of food-producing animals, CVM conducted a carcinogenic risk assessment in 2010 using the same dataset and the dose-response modeling approach as EPA IRIS’s; in addition, as CVM considers a meta-analysis on combined epidemiological data from different regions may be a stronger approach, CVM has been working on the meta-analysis since then. The reviewer suggests also consider combining datasets from northeast and southwest Taiwan to evaluate how the shape of the dose-response curve would be modified.

Dose-Response Modeling

It was indicated in the document that eight different models were used to model the data shown in Table 2 and cohort incidences were adjusted. It is unclear as to (1) what data were used in the modeling; Figures 1&2 have frequency as y-axis and the data presented do not match the cohort incidence (converted to percentage) shown in Table 2; and (2) which model(s) provides the best fit. The reviewer suggests providing the modeling results in an Appendix.

The reviewer recommends including a discussion on appropriateness of using bootstrap analysis to address uncertainties associated with the dose-response relationships and the assumptions made in using this analysis. Will the Taiwanese samples represent unbiased samples for humans? How will the variability in carcinogenic response between populations (US vs. Taiwanese) be addressed in order to extrapolate the arsenic-related cancer risks in the Taiwanese population to US populations?

The fitted dose-response curves go beyond the observed range of the dose; the curves intersect at the zero dose, suggesting a non-linear low dose extrapolation that is questionable for a chemical without an established mode-of-action for carcinogenesis. As it is stated in the document that studies were selected in order to avoid extrapolation below the observed range in the dose modeling, the reviewer recommends presenting the fitted dose response curve within the observed range of the data.

Peer Reviewer Number 6:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** The 12/20/11 draft is organized in a logical fashion, with a brief introduction followed appropriately by a discussion of ADME, a hazard evaluation including discussion of proposed modes of action and evidence of hazard from animal and epidemiological studies, a review of arsenic toxicity data for children, a review of data for dose-response assessment and modeling of selected data, a comparison to other assessments by USEPA, an exposure assessment specifically focused on apple

juice, a summary of risk estimates, and a summary conclusion paragraph and a list of 7 references.

The draft is logical and generally reads well, but this reviewer has concerns that the very few references to the primary literature reduce the clarity of the information presented in the draft. That is, throughout the draft a number of statements of fact are made that, while presumably correct, are not supported through citations to the source of the information. For example, there are only two references to the primary literature (Chen et al, 2010a and 2010b), and the other 5 references included in the draft are to ATSDR, EPA, EFSA, IARC or WHO authoritative but secondary documents. This approach to referencing makes the draft relatively easy to read for the informed public and others, but it may leave the scientific community wanting much more specificity regarding the source of information presented in the draft.

The draft would also benefit from inclusion, either as part of the introduction or as a separate section, of a statement regarding the purpose and scope of the document. Inorganic arsenic causes health outcomes other than cancer, and the FDA draft document should indicate why these other health outcomes are not considered in the assessment.

A comparison to other assessments may be useful, and reference can be made to the following published EPA and National Research Council documents: (1) the 2001 EPA arsenic assessment; (2) the 1988 IRIS assessment for arsenic; (3) the 2001 National Research Council report on arsenic; (4) the 2007 EPA Science Advisory Board peer review report of a 2005 EPA draft assessment; and (6) to the 2011 EPA Science Advisory Board peer review report on a 2010 draft EPA assessment document. Reference can be made for informational purposes only and to provide context for the 2007 and 2010 EPA SAB reports, and not as a primary source of scientific information, to the EPA 2005 and 2010 draft IRIS assessments, respectively, that were released for public comment and peer review. The section of the FDA draft report that reviews other dose-response assessments can refer to the aforementioned documents and should be corrected so that there is no reference to a 2011 draft IRIS cancer assessment for inorganic arsenic.

- 2. Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?** The information and explanations given to describe how the data were identified and criteria were used to determine the suitability of the data are sufficient. The pivotal study used as the basis for the risk estimates is a prospective cohort study in north-eastern Taiwan for urinary cancer (Chen et al, 2010a) and lung cancer (Chen et al., 2010b). The draft clearly states that “pivotal” studies were identified from epidemiological studies associated with inorganic arsenic exposure and those adverse effects with the greatest strength of evidence for a causal association, citing a recent WHO (2010) report as providing additional discussion of study selection. A review of the WHO (2010) report demonstrates that the report reviews a number of potential studies and clearly describes the criteria applied to select pivotal studies i.e., “studies were preferred that included documentation of relatively high concentrations of inorganic arsenic in drinking-water (e.g. >300 µg/l) and also relatively low

concentrations (e.g. <100 µg/l) in order to avoid extrapolation below the observed range in the dose–response modelling. Pivotal studies were identified from epidemiological studies reporting a positive association with inorganic arsenic exposure and those adverse effects with the greatest strength of evidence for a causal association, as described in the following section.” (WHO, 2010, Section 8.1.2)

Further, the approach described by WHO (2010) for modeling dose-response evidently is part of the basis for the approach employed in the FDA draft report. The WHO report includes discussion of recommendations made by EPA’s Science Advisory Board, in 2007, for evaluation of parameters used in modeling the epidemiological study data, and the FDA draft report follows the WHO approach closely. Therefore, the FDA draft report is consistent with the approach used by WHO (2010) and with the fuller documentation found in the WHO (2010) report.

There are some limitations to the WHO and thus FDA approach that merit discussion. First, neither the WHO or FDA report quantitatively evaluates published alternative studies to determine if the dose-response estimates from other populations, such as from south-western Taiwan, are comparable. In addition, sensitivity analyses of some alternative modeling assumptions (e.g., the level of arsenic in non-water exposures such as from food) as recommended by EPA’s Science Advisory Board (2007) are described in the WHO (2010) report. A rationale and description of such analyses is not included in the FDA draft. Reference is made to a draft EPA assessment where alternative model parameters were evaluated, though notably these EPA analyses were applied to a different study population from Taiwan than evaluated by FDA. Sensitivity analyses were provided in the WHO report in terms of analysis of alternative benchmark dose models (e.g., logistic, probit), which FDA extended through a bootstrap analysis to represent multiple uncertainties. The FDA bootstrap analyses focused on the likely most important model parameters (arsenic intake from drinking water and from food) though the description of the modeling approach is very limited and difficult to fully evaluate. More complete sensitivity analyses (e.g., of alternative studies, alternative model parameters) would be useful in interpreting the FDA modeling and risk estimation results, though such work can take considerable time and resources and may require access to the original study data, which were not available to the FDA. Therefore, while the pivotal study selected by WHO and FDA (Chen et al, 2010a, 2010b) is reasonable, the lack of a criterion to evaluate alternative studies to gain insights on the robustness of the selection of the ‘pivotal’ study and accompanying aspects of dose-response modeling is a limitation.

To improve clarity and transparency, it is recommended that the FDA report more fully reference the WHO (2010) report as the basis for the dose-response modeling approach and modeling assumptions and parameters where applicable, and to distinguish where in the dose-response modeling the FDA made alternative decisions to the WHO approach or applied alternative approaches (e.g., bootstrap analyses) or developed model parameters or information not included in the WHO report.

3. ***Is use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** The FDA draft report indicates an average consumption of apple juice as 3.1 g/kg-day for children aged 0-6 and refers to NHANES as the source of these data, though a citation is not included in the draft making it difficult to be certain of the source. I searched the NHANES summary data for juice consumption at the following website (http://wwwn.cdc.gov/nchs/nhanes/bibliography/key_statistics.aspx) under Diet and Nutritional Health Status/Fruits, Vegetables and Grains and found that for ages 1-3 the mean total daily fruit intake in 1 cup equivalents (128 g/cup) is 1.5 cups (equaling 192 g). Assuming this fruit consumption is via apple juice for a child assumed to weigh 10 kg (an approximate mean weight for a 1 year old, see Table 8-1 in EPA's Exposure Factors Handbook (2011) <http://www.epa.gov/ncea/efh/pdfs/efh-chapter08.pdf>) then this would suggest about 19 g/kg-day. In contrast, EPA's Child-Specific Exposure Factors Handbook (2008) (<http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199243>) evaluates USDA CFSII survey data and indicates (Chapter 9) a mean consumption of apples (as fruit, mixtures or juice) of 2.32 g/kg-day (SE = 0.13) for birth to 1 year old, 1.79 g/kg-day (SE = 0.09) for 1-2 year olds and 1.64 g/kg-day (SE = 0.05) for 3 to 5 year olds. The average consumption data for apple juice used in the FDA draft may be reasonable, but it is recommended that further analysis and documentation be conducted to support use of this assumption in the exposure assessment.
4. ***Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?*** The document would be strengthened by providing a rationale, an analysis, or a reference that provides support for this assumption. As noted above, the standard error for mean consumption of apples (as fruit, mixtures or juice) from EPA's Child-Specific Exposure Factors Handbook (2008) provides means and standard errors that could be used to estimate a confidence level for high end apple juice consumption. For example, assuming a normal distribution and using the data referred to in response to Charge Question 3 from the EPA's Child-Specific Exposure Factors Handbook, the standard error for birth to 1 year old of 0.13 and mean of 2.32 g/kg-day indicates a 95% upper confidence interval level of $(2.32 + 1.96*0.13) = 2.6$ g/kg-day.
5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** The draft FDA report evaluates the evidence of arsenic contamination in finished and concentrated apple juice products. It would appear the available data provide an opportunity to evaluate alternative assumptions in the exposure assessment. While it seems reasonable to base the exposure assessment on the finished juice product, since some consumers may prefer or only be exposed to this product, it would seem possible to evaluate other approaches as well.
6. ***Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?*** The varying amount of inorganic versus organic arsenic in apple juice (and via other exposures) is an area of uncertainty in the assessment due to differences in the potential hazard and dose-

response for carcinogenicity of the various forms of arsenic. The draft FDA report states (page 12):

“Since the arsenic concentrations in most of the [apple juice survey] sample were not speciated, and the concentrations of organic species were low (< 1 ppb) in those that were, total arsenic is reported.”

Based on this information, it seems reasonable to base the FDA assessment on total arsenic consumed in apple juice, though this may overstate the potential risks from these exposures if the forms of arsenic vary. I am not aware of other information that may inform this assumption.

7. ***The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** This is an important question and a National Research Council report (http://dels.nas.edu/resources/static-assets/materials-based-on-reports/special-products/inutero_final_April2011.pdf) summarizes a recent (2010) workshop that included a presentation titled “The Influence of Early Exposure to Arsenic in Later-Life Outcomes.” In this session, Dr. Michael Waalkes of the National Institute of Environmental Health Sciences summarized recent research on mechanisms of arsenic-induced cancer using an animal model of fetal exposure. Using a transplacental model, Waalkes found mice exposed in utero to arsenic via maternal drinking water and those exposed postnatally were more likely than controls to develop tumors in many of the same sites as those shown in studies of humans. An animal model indicated it was more likely tumors would occur that were more severe and at a lower dose in offspring exposed whole-life to arsenic (pre-conception through adulthood exposure) than in utero alone. These results suggest that early life exposure (including before birth) may result in greater risks than exposures occurring just in utero.

Considering the Chen et al study (2010a, 2010b), it is not known if whole-life exposures occurred, but it may be reasonable to assume that with a relatively stable population, as is believed to be the case in rural Taiwan, the population was exposed via drinking water for their entire life. This consideration of assumed exposure history would suggest the FDA assumption is reasonable.

In addition, the US EPA *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA/630/R-03/003F) (2005) recommends application of an age dependent adjustment factor in risk assessment in cases where a carcinogen is acting by a mutagenic mode of action. To this reviewer’s knowledge neither EPA nor any other authoritative body has concluded that the evidence is sufficient to conclude that inorganic arsenic is acting by a mutagenic mode of action. This would suggest the FDA assumption is reasonable.

Importantly, the intriguing results from the research by Waalkes, which were not available at the time of the development of the EPA *Supplemental Guidance*, raise

concern about early life exposures to arsenic and suggest caution in drawing conclusions regarding the potential risks from childhood exposure to arsenic via apple juice.

Peer Reviewer Number 7:

I. RESPONSE TO CHARGE QUESTIONS

1. ***Is the document logical and clear?*** The document is logical and clear.
2. ***Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?*** Information and explanation are sufficient to describe how the data were identified and deemed suitable. The criteria use for suitability are adequate and in line with other similar endeavors by other groups.
3. ***Is use of average consumption data from CDC's National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?*** The data from NHANES is a more than reasonable estimation for lifetime apple juice consumption.
4. ***Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?*** A three fold multiplier seems adequate for high end estimates of consumption.
5. ***Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?*** The conservative basing of exposure assessment on single strength juice add validity to the conclusions.
6. ***Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?*** Again, the conservative approach of assuming total arsenic is the basis of risk estimation even though it may include some element of organo-arsenicals is appropriate.
7. ***The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?*** There are emerging data that clearly indicate the early life stage may be a time of higher sensitivity to arsenic for carcinogenesis, neurotoxicity, etc. The document largely missed these publications. The three fold factor used may account for this sensitivity, and the available human data on early life sensitivity probably do not lend themselves to this sort of analyses.

Other comments: Also IARC volume 100 that reviews arsenic and arsenic compounds in now available which is not included as a key reference. It discussed additional studies and also some

of the emerging data on early life sensitivity. The work emerging from the Smith group at Berkeley is key in this regard.

FDA Response to the Peer Review Comments:

The following are FDA's responses to the peer reviewer comments organized by original charge question. Where more than one peer reviewer raised the same or similar issue, the comments were synthesized into a single item for response.

Is the document logical and clear?

1. The objective of the risk assessment needs to be clearly stated in the introduction section.

FDA Response: We agree. An executive summary has been added that now begins by identifying the purposes and scope of the assessment, as both a stand-alone document that estimates risk and as a tool to inform risk management decision making. Given the public interest in this subject, we have attempted to draft this introduction in non-technical terms.

2. A comparison to other assessments may be useful. Comparisons would be most appropriately made with the EPA 2001 assessment, EPA1988, and NRC 2001. The most relevant and up to date comparisons of the cancer potency of inorganic arsenic in drinking water would be the NRC 2001.

FDA Response: We agree and have added a section devoted to the assessment used by the Environmental Protection Agency (EPA) as a basis for its 2001 drinking water regulation. This section includes a quantitative comparison between the EPA 2001 assessment and the FDA assessment. That comparison essentially examines the similarities between the dose-response functions. The EPA 1988 assessment involves a different health endpoint at higher doses (skin cancer), so it was not regarded as relevant for comparison purposes. A comparison was not made to the NRC 2011 assessment because it was not used in the development of the drinking water standard. A discussion of the issues underlying the development of dose response models from the Taiwanese data has been added.

3. It would have been helpful to clearly state the reasons for the lack of good animal models for carcinogenicity of inorganic arsenic and why they were excluded for risk assessment.

FDA Response: We agree and have added text to explain why animal models were not used. In short, animal data are not typically incorporated into the dose-response modeling in quantitative risk assessments when there are adequate human data available to develop a dose-response function, as was the case in this situation.

4. Explain why the assessment was focused on inorganic arsenic (organic vs. inorganic arsenic toxicity).

FDA Response: We agree that this explanation is important. The first paragraph of the executive summary now addresses this point, and an in-depth discussion later in the text addresses toxicological and exposure considerations (i.e., inorganic arsenic is more toxic, and most of the arsenic in apple juice is inorganic).

5. Selection of the endpoint of carcinogenicity for the focus of the risk assessment needs to be explained in the context of the other toxic effects observed with chronic intake and/or intake during development.

FDA Response: We agree and have added text on this point. In short, FDA has already established a “Level of Concern” (LOC) of 23 parts per billion for certain non-cancer endpoints in children. That LOC is based on a Reference Dose (RfD) of 23 parts per billion developed by the EPA for skin lesions and certain cardiovascular effects in children. As is the case in the development of an RfD, the most sensitive non-cancer endpoints were chosen for its basis. Unlike other endpoints, cancer is a health hazard that is generally assumed to occur at very low doses. In any case, the risk assessments for arsenic have focused on cancer endpoints, i.e., the EPA risk assessment for drinking water; the JECFA assessment for juice that formed the basis for the FDA assessment; and now our assessment.

6. The assessment would benefit from referencing or including citations in the text to provide support and context throughout the assessment. A number of statements of fact are made that, while presumably correct, are not supported through citations to the source of the information.

FDA Response: We agree and have added additional references. In a number of places in the text the statements derive primarily from one reference source. Also, given the extensive literature on arsenic toxicity, we have not attempted to provide primary references for all statements, and rely on other reviews throughout much of the text.

7. IARC volume 100 that reviews arsenic and arsenic compounds is now available which is not included as a key reference. It discussed additional studies and also some of the emerging data on early life sensitivity. The work emerging from the Smith group at Berkeley is key in this regard.

FDA Response: We appreciate this information and agree that it should be used as a key reference. We now use it. In addition, a section was added that discusses early lifetime exposure with a different literature review (Tokar et al., 2011). This section also includes several citations from the Smith group, and the risk assessment model was modified in consideration of these studies to place more emphasis on early exposure.

8. Add subheadings for the hazard assessment, such as mechanisms of toxicity, animal studies and human epidemiology studies; add notes or legends for figures and tables.

FDA Response: We agree that subheadings would be helpful and these have been added.

9. To improve clarity and transparency, it is recommended that the FDA report more fully reference the World Health Organization (WHO 2011) report as the basis for the dose-response modeling approach and modeling assumptions and parameters where applicable, and to distinguish where in the dose-response modeling the FDA made alternative decisions to the WHO approach or applied alternative approaches (e.g., bootstrap analyses) or developed model parameters or information not included in the WHO report.

FDA Response: We agree and have added text summarizing the rationale for selection of the Chen reports for dose-response modeling in WHO (2011). That report is extensive, however, and readers are also referred to it for additional discussion.

Were sufficient information and explanations given to describe how the data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate?

1. Criteria used for selecting which studies to incorporate into the dose-response modeling need to be explained in more detail. The confidence in risk assessment derived from the Chen study, as opposed to a risk assessment derived from data from multiple studies, must be qualified accordingly in the RA [risk assessment] document. A justification for using the studies by Chen et al. should emphasize two aspects: (1) the strength of the Chen, et al, studies and (2) the flaws of other epidemiological studies reporting on the carcinogenesis endpoints.

FDA Response: We agree and have added discussion concerning the selection of the Chen et al. (2010) papers as the basis for the assessment. In summary, Chen et al. is superior for our purposes because it is a longer term study and it is prospective. The other studies are all of shorter duration and not all of them are prospective. The other studies could still be useful, however, in a meta-analysis to characterize the dose-response relationship, but this would be difficult to do well without individual subject data that are not currently available. It is our understanding that EPA is currently in the process of obtaining it.

2. Qualitative conclusions regarding the dose-response for cancer vs non-cancer effects, which state that carcinogenic effects occur at lower doses than non-cancer effects, should be supported by the available science and some type of comparative analysis.

FDA Response: We agree but are limited by current data. See the response to Comment #5 under the previous header (“Is the document logical and clear?”).

3. The risk assessment needs to clarify whether or not the dose-response analysis evaluates the combined risk of lung and bladder cancer from the Chen et al., (2010 a, b) studies.

FDA Response: We address this point in the second paragraph under the header “Estimated Risks.” In summary, the dose-response analysis evaluated the combined risk by modeling them separately and then adding them together to estimate combined rates.

4. Consider combining datasets from northeast and southwest Taiwan to see how the dose-response relationship will be modified.

FDA Response: We agree that this would be useful, but are unable to do so at this time without individual subject data. Such data have not been made publicly available. This is a common situation in the conduct of quantitative assessments involving results from human studies.

5. How is the relative sensitivity in carcinogenic response to arsenic exposures between Taiwanese and US populations addressed so that the arsenic-related cancer risks in the Taiwanese population can be extrapolated to U.S. populations?

FDA Response: This is a good question; however, because there are no comparable results from the United States, we are compelled to assume that persons in the United States are equally susceptible. We address this assumption in a discussion of the many assumptions that go into modeling the Taiwanese studies.

Is use of average consumption data from CDC’s National Health Examination and Nutrition Survey (NHANES) a reasonable basis for estimating lifetime consumption of apple juice?

1. A sensitivity analysis using several exposure / consumption rates which may include a 95% percentile level would be useful to characterize the uncertainty for this model assumption.

FDA Response: We agree that a sensitivity analysis would be desirable. The problem is that the NHANES survey is for two days at a particular age in each person. Therefore, we have no idea what a chronic lifetime 95 percentile might be. The result for 3 times the average is, in effect, a sensitivity analysis with a rate of consumption that is higher than average. We make this point in text under the header “Consumption of Apple Juice” and at the bottom of Table 5, “Apple Juice Consumption Estimates.

2. It is unclear how the average consumption was calculated. Table 3 shows that children aged 6 or less have much higher apple juice consumption than adults. Was the weighted average used in calculating the population average?

FDA Response: A weighted average was used only for calculating risk attributable to childhood exposure. (A weight of 7 is addressed in the first “bullet” under the header “Estimated Risks.”) Exposure from ages 0-50 years did not need weighting because they were based on actual averages. The same was true for lifetime exposure. These three categories of exposure are addressed in Table 9.

3. The average consumption data for apple juice used in the FDA draft may be reasonable, but it is recommended that further analysis and documentation be conducted to support use of this assumption in the exposure assessment.

FDA Response: We agree and have added text to clarify this point. In short, the use of average consumption is a standard approach for cancer risk assessment because it is based on the general assumption that the dose-response function for cancer is linear. All the alternative models used in the current analysis are linear at low doses. We acknowledge in the assessment that this approach only yields per capita disease rates and does not characterize the risks for persons consuming apple juice at a rate that is higher than average. In order to characterize risks in populations with higher rates of consumption, we also estimate risks for a hypothetical population with a consumption rate that is three times the per capita average.

Is the use of three times the NHANES average a reasonable estimation for high end apple juice consumption (see Table 3)?

1. No justification has been provided why the 3X (and not 2X or 4X) has been considered.

FDA Response: The risk estimate at 3 times average exposure is presented as a sensitivity analysis to characterize the risk to a population consuming apple juice at rates that are well above average. It was selected somewhat arbitrarily since we do not know what above average consumption typically is. In any event, the reader can easily calculate risk at some other level, e.g., two or four times average exposure. Because the dose-response function is linear, risk at 3 times average is roughly 3 times higher than average risk.

Likewise, two times average exposure would produce risk that is roughly twice that at average risk while four times average exposure would produce risk that is roughly four times that at average exposure. This point is addressed in the text immediately after Table 11.

2. Sense of a rationale for using the 3X exposure could be easily fixed by citing published literature (For example: Dennison, B.A. 1996. *J. American College of Nutrition* 15(5 Suppl): 4S-11S.). Other sources of citation may include data reported by juice manufacturers on the excess consumption of fruit juice.

FDA Response: We appreciate being pointed to these cites in the published literature; unfortunately they refer to short-term consumption estimates, not lifetime exposure, so they are of limited value in this assessment. Regarding manufacturers' data on excess consumption, we would expect manufacturers to know how much they sell, but not know the extent to which individuals might consume above average, especially over a lifetime. Nonetheless, we would welcome any data that manufacturers' have on that subject. In the absence of such data we have no choice but to provide hypothetical examples of risk at higher than average consumption, such as at 3 times higher than average.

Does it make sense to base exposure assessment on single strength juice (i.e., finished product) rather than concentrate, given that concentrate appears to have lower arsenic levels when single strength is calculated?

1. This assumption is reasonable, but again characterization of the uncertainty surrounding this assumption should be presented as an analysis in the assessment.

FDA Response: To the best of our knowledge, all apple juice is consumed as single strength. For that reason, it is no longer clear to us whether the question we have asked on this point is germane. If we receive information from the public that there are uses for concentrate other than to make single strength juice, we would reconsider whether such practices would have a bearing on risk to the point where further analysis would be needed. We do note, however, that the assessment does contain an analysis that includes values derived from concentrates, but these values are only for total arsenic and not for inorganic arsenic, so the analysis is of limited value.

2. Another variable that should be evaluated in the assessment is the range of the arsenic concentration observed in apple juice samples (Tables 4 & 5). A real-world scenario may include brand loyalty by consumers.

FDA Response: We agree in the sense that some brands might contain more arsenic than others and some individuals might only drink apple juice from those brands. As a practical matter, however, the amount of sampling of individual brands in order to be confident that we can differentiate arsenic levels from brand to brand would be significantly greater than what has been conducted to date. Fortunately, the fact that our speciated sampling indicates that juice with more than 10 ppb inorganic arsenic is likely to be uncommon regardless of brand indicates that our inability to differentiate one brand from another is not likely to mean that we are missing a major undetected risk.

3. Consider whether the consumer exposure to similar (or same) compounds could be additive or substitutional. The consumption of apple juice could be regarded as substitutional for water, that is, a consumer of apple juice is expected not to drink simultaneously the same fluid volume of water to quench thirst or for hydration.

FDA response: This suggestion would be useful if the exposure assessment characterized the total arsenic burden from all sources, including water and all types of juice in addition to apple juice. At some point this could well be an appropriate subject of a quantitative assessment and we intend to consider it. This assessment could be regarded as an early step in such a direction that is designed to inform us about the risk solely from apple juice.

Is it reasonable to base risk estimation on the basis of total arsenic, even though in doing so, total arsenic includes some varying amounts of organic arsenic?

1. In the context of estimating dietary arsenic exposure, considering total arsenic would lead to an overestimation of health risk as it is shown that in foods, especially in seafoods, arsenic is present in organic forms that are less toxic.

FDA Response: We agree and the risk assessment now looks specifically at inorganic arsenic in apple juice.

2. As the majority of inorganic arsenic is rapidly converted in vivo to organic forms, even estimating risk from total inorganic arsenic may be overly conservative.

FDA Response: The dose-response model is based on the external dose of inorganic arsenic before it becomes converted in any way in the body. Consequently, conversions from inorganic to organic would not be expected to affect the estimate of risk.

The dose/response function does not assume greater or lesser response to dose, i.e. susceptibility, on the part of children. Is that a reasonable assumption, based on the available data?

1. The draft risk assessment made no mention of in vitro studies with isolated hepatocytes identifying the Phase I enzymes responsible for the methylation. Identification of those enzymes, combined with data on the induction of those enzymes during postnatal development, could allow for comparison of the detoxification response during early childhood with that of adults and improve the accuracy of the assessment with respect to childhood cancer risk. Alternatively, an additional factor of 10 in the calculation of risk for infant exposure to account for the possibility of increased sensitivity to the carcinogen during the period of concern could be used here.

FDA Response: We have added a section in the assessment on childhood susceptibility. There is evidence for and against enhanced childhood susceptibility. The evidence both ways is inconclusive to the point where we could just as easily assume less sensitivity as greater sensitivity. For that reason we do not assume either way. An additional factor of 10 in the modeling would be based on an assumption of greater sensitivity without knowing the extent of it. This is an area in which more research would be useful.

2. Is carcinogenesis the most appropriate/sensitive endpoint of arsenic exposure in children? Can there be a better marker of arsenic exposure and arsenic-induced adverse effects in children 0-6 years? An explanation will significantly improve the document.

FDA Response: We have added a discussion on this point. It is always possible that a better marker could be developed but it simply has not happened yet. We refer the reader to response number 5 under the header “Is the document logical and clear.”

3. The possibility of a differential susceptibility to arsenic exposure cannot be ignored. It is not known whether childhood exposure to arsenic would lead to an increased risk of cancer later in life. It may be more useful and relevant to consider other non-carcinogenic biological effects such as neurobehavioural, pulmonary or cardiovascular effects as endpoints in children for risk assessment.

FDA Response: We refer the reader to response number 5 under the header “Is the document logical and clear?”

4. In the absence of data on whether and the extent of childhood exposures to arsenic may result in increased cancer risk later in life, the reviewer agrees that based on the available data that it is reasonable not to assume greater or less response to dose for children.

FDA Response: Although the assessment does not assume that children are more sensitive, it does recognize that exposures earlier in life are more likely to result in earlier effects. This recognition derives from a study in northern Chile that documented progressively increasing rates of lung and bladder cancer over a period of approximately 25 years following an episodic exposure to arsenic in drinking water.

5. There are emerging data that clearly indicate the early life stage may be a time of higher sensitivity to arsenic for carcinogenesis, neurotoxicity, etc. The document largely missed these publications. The three fold factor used may account for this sensitivity, and the available human data on early life sensitivity probably do not lend themselves to this sort of analyses.

FDA Response: While there is evidence that exposure during childhood can increase the rate of cancer later in life, we did not find any support for the notion that exposure relative to body weight is more harmful in children. However, we did find evidence that exposures earlier in life are more apt to influence the development of cancer, and we therefore changed the period of exposure from lifetime to 0-50 years. Since childhood constitutes a greater proportion of this time period, the present risk assessment places greater weight on the exposure of children. We have added references to several papers and reviews, including the new IARC report that published after the draft risk assessment was submitted for peer review.

**COMMENTS FROM THE NATIONAL CENTER FOR ENVIRONMENTAL HEALTH
(NCEH) AND THE AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY
(ATSDR) IN THE CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC)
AND FDA RESPONSES**

NCEH/ATSDR has reviewed the draft document “An Assessment of Arsenic in Apple Juice, by the Chemical Hazards Assessment Team, Center for Food Safety and Applied Nutrition, US Food and Drug Administration.

We agree with the use of 9.3 g/kg-d as an estimate of children’s consumption of apple juice. We agree with using the Chen 2010 a & b studies as your basis for the dose-response modeling. We point out that these studies are different from, but do not contradict, the studies used by EPA in its draft assessment (i.e., Wu 1989 and Chen 1992).

We suggest adding additional details to the document. In particular, the cancer slope factor (CSF) and unit risk derived from modeling, calculations that are the basis for Tables 7-9, calculations using EPA’s draft CSF, and specific references to the points in the Assessment paper (an example is on Page 2, lines 26-27, “Small amounts of MMA(V) and DMA (V) are also found...”. The document should include a discussion as to why the FDA potency estimate is considerably lower than EPA’s draft potency estimate and a justification for prorating the estimated life-time cancer risk to childhood exposure.

FDA Response: We do not mention the EPA draft because it is a draft and because the EPA website requests that it not be cited or quoted. However, we have added text that discusses the many issues that must be addressed when estimating cancer risk from epidemiological studies.

More specific comments:

1. On page 3 lines 17-18, arsenic in hair and nails is NOT a reliable biomarker for exposure assessment due to contamination issues and analytical issues (ATSDR 1999 expert panel report).

FDA Response: We have added the missing “not.” Thank you for calling this to our attention.

2. Update the human data on page 3, lines 26-28 with the NHANES data from NHANES 2003-2004 paper by Caldwell et al in J Expos Sci Environ Epi 2009;19:59. This provides recent data on total and speciated results in the US population, with % of each.

FDA Response: We have included a reference to this paper and have specifically recognized the finding of high levels of arsenobetaine in individuals with high urinary arsenic concentrations.

3. On page 6, lines 30-32, is a bold statement that is not referenced. We suspect the intent is to say "there is no evidence that children have specific health effects (or toxicity) that are distinct from what may be seen in adults”.

FDA Response: That sentence has been rewritten in a new section on Arsenic Toxicity in Children.

4. Tables 1 and 2. FDA used the “central estimate” of the arsenic concentrations for Chen et al. 2010a and 2010b as 5, 30, 75, 200, and 450 ug/l. These values were also used by the World Health Organization Evaluation (WHO 2011). However, Chen et al. 2010a used the median doses of 0.95, 25.47, 74.87, 139.23, and 535.56 ug/l. Why do these point estimates differ and how were they derived?

FDA Response: The well water concentrations that were used to derive the dose-response function in the risk assessment are based on the median values of the well water concentrations used to define each group. These were also used by the Joint FAO/WHO Expert Committee on Food Additives. We agree that using the median values or average values given in the Chen urinary tract paper and Chiou et al. (2001; *Am J Epidemiol* 153:411), respectively would be preferable. Consequently, we have rerun the dose-response analysis with the average values in Chiou et al. (2001) and have found the resulting risk estimates to be slightly reduced compared to those in the report (median of 7.7 cases per million rather than 8.0 cases per million). Because the results are so close, we have not modified the assessment.

5. Data in Table 1 and 2 are used to model the dose response analysis (page 7 lines 31-32 and lines 36-37) and the dose-response curve is graphically shown on Figures 1 and 2. These figures show a dose-response graph relating dose versus “Frequency” as a percentage.

- a. What is the percent frequency? How is it calculated? Should this be labeled differently?

FDA Response: These are population disease frequencies expressed as a percentage.

- b. What was the 2-3 year “adjustment factor” used for this variable and how was it calculated?

FDA Response: The derivation of the factor is described in greater detail in the last paragraph of the modeling methodology section.

- c. Can the “frequency” variable be listed in Tables 1 and 2?

FDA Response: The column labeled “cohort incidence” has the frequencies estimated with 11.5 years of follow-up.

6. Dose Response Modeling

- a. Page 10 lines 11– Microsoft Excel solver – although a very reasonably effective and popular spreadsheet, some authors have challenged MS Excel’s statistical performance. See <http://www.practicalstats.com/xlsstats/excelstats.html>, particularly comments related to Solver.

FDA Response: We agree that using Solver can be a bit tricky, and it is often necessary to try different initial estimates in order to get a reasonable fit. However, because the models appear to fit the data we believe that the Solver estimates are reasonable approximations.

- b. Page 10 lines 12-19. The bootstrap procedure comments:

- i. Can you list the number of bootstrap samples that were generated?
FDA Response: Yes, it is listed (300 bootstraps)
 - ii. Page 10 lines 13-17. Can you identify the uncertainties used in the models? Page 10 lines 17-18. Was a single model bootstrapped or for each bootstrap, was the best fitting model selected of the eight candidate models?
FDA Response: Yes, the uncertainties are stated in the dose-response modeling section.
 - iii. Figures 1 and 2 – Please define the “lower bound” and “upper bound” of the simulation for the point estimates of the dose/response.
FDA Response: Yes, we have added the 5th and 95th to define the lower and upper bound everywhere necessary for purposes of clarification.
 - iv. Please describe which of the eight candidate models was used for the final bootstrap model.
FDA Response: Table 3 now provides the frequency of model usage in the bootstrap analysis.
 - v. Should the diagnostic statistics such as chi-squared goodness of fit and **Akaike Information Criterion (AIC)** for the different models be included in your report?
FDA Response: We appreciate the suggestion but it would not be feasible to do so since there are eight goodness-of-fit estimates for each of the 300 bootstraps.
7. Table 4- Total Arsenic Concentration Results from By Survey and Year - Check title of table – missing Toxic Elements Program(?)
FDA Response: We have clarified that TEP refers to the Toxic Elements Program.
8. According to the FDA web site the laboratory method for arsenic in apple juice , the level of **quantification** is 1.0 and the level of **detection** is 0.1 (ppb). The term “limit of detection” should be changed throughout the text to be “limit of quantification” (see Table 4, note 2; Table 5, note 2; and Table 6, note 1).
FDA Response: We agree and have made the change.
9. Non detection treatment. Note 2 in Table 5 states that *FDA used 0.5 for calculating average values when the reported level was below 1 or below the level of detection.*
- a. Does this mean that the level of quantification (LOQ) varies?
FDA Response: Some levels were below 1 because they were calculated from levels made from concentrate. This had nothing to do with the LOQ.
 - i. If the LOQ is always 1.0 ppb this statement should read: *FDA used 0.5 for calculating average values when the reported level was below 1.0, the level of quantification.*
FDA Response: No, see the answer to “a,” above.
 - ii. If the LOQ did vary, FDA should have used x, where $x=0.5(\text{LOQ})$ to adjust for missing values.

FDA Response: No, see the answer to “a,” above.

- b. Thirty percent of the observations are not quantifiable. FDA should determine if the estimated average As concentrations vary significantly based upon different ways of adjusting for missing values. If the data are sensitive to the type of adjustment for missing data, FDA should select the method that predicts the highest As average concentration.

FDA Response: Since all of the missing values are at the low end of the distribution, the missing values have minimal impact on estimated average concentrations and no impact on the incremental risk estimates at higher concentrations

10. On Table 5, note #1, “assumes no additional arsenic is added...”. This should be defined and the lab consulted to see if they used arsenic “free” lab purified water.

FDA Response: The water was added by the manufacturer and not at the laboratory. We have clarified the text on that point.

11. Estimated Risks

- a. Table 7-9. Please define the intervals given in the tables that appear next to the point estimates. Are these 95 percent confidence bounds?

FDA Response: Yes; we have clarified the text on this point.

- b. We suggest changing the 2nd column heading to Cancer risk per million persons (average intake) and the 3rd column to Cancer risk per million persons (3x average intake).

FDA Response: We have adjusted the heading to read “Disease Rate Per Million.”