

Interim Melamine and Analogues Safety/Risk Assessment Peer Review Report

June 7, 2007

Introduction and Summary of Comments

On May 24, 2007 FDA sent its Interim Melamine and Analogues Safety/Risk Assessment (S/RA)¹ to six experts and asked them to review it by May 31, 2007. The peer reviewers were:

Georges Benjamin, M.D.
Director, American Public Health Association

Samuel M. Cohen, M.D., Ph.D.
Professor and Chair, Pathology and Microbiology
Havlik-Wall Professor of Oncology
University of Nebraska Medical Center

Elaine M. Faustman, Ph.D.
Professor, University of Washington
School of Public Health and Community Medicine
Institute for Risk Analysis and Risk Communication

Lonnie King, D.V.M., M.P.A.
Director, National Center for Zoonotic, Vector-Borne and Enteric Diseases, CDC

Xavier Pi-Sunyer, M.D., M.P.H.
Director, Columbia University Obesity Research Center
St. Lukes Roosevelt Hospital Center

John Thomas, Ph.D., D.A.T.S., F.A.C.T
Professor, Indiana University School of Medicine
Department of Pharmacology and Toxicology

We greatly appreciate the peer reviewers' comments and suggestions, as well as their willingness to provide them on a very tight deadline. The reviewers' responses to the specific charge questions are provided below without attribution to the specific reviewer.

Overall, there was consensus from the peer reviewers that the conclusions of the S/RA were appropriate. In addition, recognizing the time-sensitive context in which the S/RA was developed and the time-sensitive need for the S/RA results, the peer reviewers

¹ Available at <http://www.cfsan.fda.gov/~lrd/fr070530.html>.

concluded that the methodology, data, assumptions, and exposure scenarios used were appropriate.

Several reviewers provided suggestions for FDA to consider in future revisions of the S/RA. These additional suggestions include the following:

- There may be other data sources from studies of similar compounds pertinent for consideration; for example, one reviewer suggested that we consider studies that were conducted to evaluate the toxicity and metabolism of “triazine” pesticide compounds.
- Multiple sources of exposure should be considered, as there could be an additive effect. For example, one reviewer suggested that there may be exposure to melamine and its analogues from plastic products.
- Although one reviewer stated that “there is no evidence of bioaccumulation,” other reviewers suggested that a scenario focused on possible chronic toxicity from longer duration exposure should be considered.
- There should be some consideration of possible formation of other (more toxic) compounds such as might be created during heating.

Finally, there was a consensus among the peer reviewers additional research is needed. The reviewers identified several areas for future investigation, including:

- Determine the concentration and crystallization of melamine compounds (MCs) in urine of different species, including possible co-crystallization (e.g., melamine and cyanuric acid) at lower concentrations due to hydrogen bonding effects between the MCs in enhancing crystallization and the effect of liquid and salt ingestion on the concentration of MCs in urine.
- Method development, such as more sensitive assays to detect low levels of MCs in tissues.
- Toxicological studies with different species, including the examination of co-toxicity from exposures to multiple MCs to determine whether there are other additive or synergistic effects.
- Studies to better understand melamine pharmacokinetics including the effects of dehydration, common medications (diuretics) and others that alter renal excretion.
- Determine whether heating MCs forms new compounds in foods with greater toxicity than the parent compounds.
- Conduct longer term studies to determine potential toxic effects, including liver, reproductive or endocrine dysfunction.
- Determine whether biomarkers can be identified for predicting renal failure secondary to exposure to MCs.

Several of these suggested research studies may provide more extensive and accurate data that could be used to reduce the uncertainties in future safety assessments of MCs. However, to some extent the long term testing that this suggested research would entail would need to be justified by evidence of chronic exposure, and we are unaware of such evidence at this point.

Peer Review Comments in Response to Charge Questions and FDA Response

Question 1. Does the S/RA report adequately identify the data and methodology used, and explain how data were identified and what criteria were used to determine the suitability of the data? Were these criteria adequate? Was the methodology appropriate?

Reviewer #1. I found that the report was adequate in both identifying the data used for the assessment and establishing the criteria and methodology for the S/RA. While these features were clear, only a single approach was used that was based on limited studies and data. There may be other data sources from studies of similar compounds that are pertinent for consideration and thus a broader approach to create the S/RA.

Reviewer #2. See response to Question 8.

Reviewer #3. The S/RA report clearly identifies the data and methodology used including the assumptions behind the methodology. Because of the general reported lack of clinical studies, a great deal of assumptions had to be made. While these may be found to be inexact in the future the authors make very conservative and reasonable assumptions to construct their methodology.

Reviewer #4. Extent of contamination—One of the interpretation difficulties inherent in an incident such as this is the representativeness of the analyzed samples. Knowledge about the sampling strategy used to analyze contaminated media can assist the reader in understanding the extent of the problem however such information was missing from this report. Obviously concern for source identification is important and hence samples were only identified by letter or number however in order for this reviewer to understand how these samples were chosen for analysis (i.e. random or were these chosen for most suspected to be contaminated; where these samples from multiple supplier sources or all from one supplier and thus representative of downstream variation.). Such information is needed in order for this reviewer to support the “conservative” characterization of the risk assessment that is proposed on page 3.

Risk assessment methodology—Standard approaches for using NOAEL based reference values, uncertainty factors and Margin of safety assessment techniques were employed. The calculation of the TDI was also representative of such established methodologies.

Exposure assessment—Based on this reviewers comments listed above I have concerns about whether the exposure assessment is based on appropriate estimates.

Reviewer #5. The S/RA adequately reports the data and methodology uses. It also explains how data were identified and what criteria were used to determine the suitability of the data. I believe that the criteria were adequate and the methodology was appropriate.

Reviewer #6. Yes.

FDA Response: The peer reviewers generally agreed that the report adequately identified the data and methodology used and that the data and methodology were appropriate. One reviewer requested additional information to better understand the sampling design of the analyzed feed and tissue samples. It is important to note that the samples were largely of an investigational nature and were obtained from facilities that were involved in the contamination incident; as such the reported samples were targeted to the incident and not "random" samples. We recognized in the S/RA that there was uncertainty associated with the sample design. However, we believe that this uncertainty was offset by the conservative nature of the assumptions in the S/RA. More information will be supplied in the S/RA to provide a better description of sampling methodology.

Question 2. Are uncertainties in the S/RA identified and characterized?

Reviewer #1. Further uncertainties may need to be considered in the assessment. There should be a factor of 10 used based on interspecies variability (identified in the interim S/RA) but also an additional factor of 10 should be considered based on variations in human sensitivities and exposure differences. Combining these premises would produce a lower uncertainty factor which would also be consistent with most standard RA practices.

Reviewer #2. See response to Question 8.

Reviewer # 3. The uncertainties concerning long term exposure could be strengthened. While Melamine compounds appear to be relatively inert, the complications of long term exposure remain unclear and there may be as yet unpredicted immunologic response secondary to some unknown trigger. I would simply strengthen these unknowns.

Reviewer #4. Please see comments for Question 1 responses. In addition, there are several additional issues that need to be addressed in this report. Please see my responses under question 7 for additional future data needs.

The report contains material in the appendices that does not appear to be fully addressed in main section of the report. For example, on page 9, Appendix 1, under the heading of Hazard and Toxicity both an LD₅₀ and phrase "Exp. Carcinogen" is listed. The text should provide additional references and discussion of this listing. On page 10, Appendix 1 for Cyanuric acid, there are listings under biological use and importance that hint at other biological activities that could be related to its hazard potential for example, its use as a herbicide and information that in an ester form it may be an anticonvulsant or hypnotic. Does FDA have any information on these properties or potential for these forms to be present under the current conditions? How potent are these materials? Is

there information available from the Pesticides office of EPA (in RED documents) for cyanuric acid as an herbicide? This same page also lists this agent as an eye and skin irritant. Where did this information come from? At what exposures do these effects occur?

Other missing information includes lack of toxicological information that this reviewer would believe is present in the toxicological documents reviewed for this assessment. Since, references were not cited it is difficult for this reviewer to double-check if this is true. For example, on page 2 of the report many NOAELs were given from various animal studies however minimal to no information was provided about complete numbers and dose levels tested in each study. At a minimum such information is useful for understanding if the strength of support for the NOAEL being a true NOAEL rather than just the lowest dose tested. Such information could influence the size of the uncertainty factor used in the later stages of the risk assessment.

Reviewer #5. The uncertainties with regard to this S/RA Assessment are identified and characterized.

Reviewer #6. Yes.

FDA Response: One reviewer noted the need to provide more complete references for the S/RA. The reviewer also suggested including additional ancillary information. In general, we agree that more information could be provided and that the information should be properly cited and documented, and will take steps to further develop the references in this S/RA. Although adding references will enhance this document, we do not believe that providing such additional information would have changed our methodology, approach, or results.

One reviewer suggested an additional uncertainty factor should be considered “based on variations in human sensitivities and exposure differences.” At the current time the additional uncertainty factor would not be generally considered warranted. Human variability was considered in the selection of the total uncertainty factor of 100 that was used in the S/RA. Selection of the uncertainty factor of 100 was done according to typical safety assessment practices; the value was based on a factor of 10 for inter-species variability and a factor of 10 for intra-species sensitivity variation. As additional information becomes available through future research, the magnitude of the uncertainty factor can be revisited.

One reviewer questioned the information in Appendix I and whether further discussion in the text of the S/RA was needed on a listed LD₅₀ and a reference to “Experimental Carcinogen.” The same LD₅₀ reference was a part of and considered in Reference 2. The consideration of MCs as carcinogens was not considered relevant to short-term exposure to these compounds. There has been extensive testing of cyanuric acid and melamine; the results of this testing forms the basis for conclusions regarding the general systemic toxicity of these two compounds, as discussed in the S/RA at page 2. A table of studies contributing to the S/RA will be added to the S/RA. This table will list dose levels and durations of studies.

Question 3. Are there additional scientific/ technical studies available that were not considered?

Reviewer #1. There are a number of studies that have been conducted on other “triazine” compounds that are structurally closely related to melamine and its analogues that should be considered in the analysis and S/RA.

Reviewer #2. See response to Question 8.

Reviewer #3. I have done a brief review of the literature and have not [sic] found very little covering toxicity with melamine in humans. One article concerning inhalation of an aerosol including several chemicals one of which was melamine resulted in pulmonary edema. The causative compound was not felt to be melamine.

Reviewer #4. Since very few if any of the studies that were reported had unique references to specific studies this question was a bit difficult to answer directly. When RTECS [Registry of Toxic Effects of Chemical Substances] was reviewed there were additional references listed that should be specifically cited and discussed in this risk assessment document. For example the Tox and Applied Pharm article from 1984. Also for transparency I would have direct links from this document to these review sources. The RTECS site also lists mutation data and references and these should be referenced. In addition, IRAC [IARC, the International Agency for Research on Cancer] reviews should be cross-referenced. For background it may also be important to document that inhalation studies have been performed even though this route of exposure and toxicological findings are less relevant than oral studies they may at least provide some toxicological context. Note that both reproductive and liver function assessments were done following inhalation studies and these endpoints should be openly discussed for relevance. There is also a specific citation for eye irritation that provides limited exposure dose information. Please add this information.

Reviewer #5. To my knowledge, the available scientific/technical studies were referred to. I do not know of additional data that should have been included and was not.

Reviewer #6. Studies showing lack of DNA reactivity (non-genotoxicity). See International Agency for Research on Cancer (IARC) reference.

FDA Response: Before the S/RA is updated, we will review the studies identified by the peer reviewers to determine whether they are relevant to the data and assumptions used for this S/RA. We note that some of the studies identified by the reviewer concern occupational exposure and may investigate a route of exposure (e.g., inhalation, dermal) that is different from the route considered in this S/RA (oral) that may not be applicable to our analysis.

Question 4. Is the no observed adverse effect level (NOAEL) used the appropriate point of departure for calculating the margin of safety (MOS), or do data support the use of an alternative endpoint?

Reviewer #1. An alternative end point should be considered based on toxicity studies of atrazine and similar compounds; some have looked at occupational human exposures. These studies suggest that reproductive and developmental hazards and implications need to be considered in the final end point calculations. The animal study used was based on the carcinogenicity of calculi-forming pathways. This may or may not be applicable to the human situation.

Reviewer #2. See response to Question 8.

Reviewer #3. I think this (NOAEL) is the most appropriate point to use.

Reviewer #4. Based on the information provided in the assessment this review would concur with the chosen NOAEL and endpoint. However as discussed above, insufficient information about total dose-response curves for all toxicological studies was given that would allow for a full response to this question.

Reviewer #5. I believe that the NOAEL used is the appropriate point of departure for calculating the margin of safety.

Reviewer #6. NOAEL is appropriate

FDA Response: In general, the peer reviewers agreed that the NOAEL chosen was appropriate. It would be challenging to analyze additional triazines for potential applicable toxicity with regard to expected effects of the MCs. The rather extensive testing performed with melamine and cyanuric acid to date do not reveal effects relating to the other toxicological endpoints mentioned by the outside reviewers at exposures that are relevant to those potentially received by humans in this instance. The reviewers for this S/RA felt that the most relevant toxicological data would be provided by that of melamine and cyanuric acid. Other substances e.g., atrazine, do demonstrate additional toxicity compared to MCs; however, the central nervous system toxicity manifested by atrazine may not be applicable to the MCs due to atrazine's less polar nature. As noted, we will look again at the literature on triazine pesticides and consider the possibility that other biological endpoints should be considered in future assessments.

Question 5. Were scientific assumptions that are not strictly linked to the data explained and appropriate?

Reviewer #1. The S/RA as currently being considered is quite narrow in its interpretation and data consideration without the additional consideration of pertinent studies involving atrazine and similar chemical compounds.

Reviewer #2. See response to Question 8.

Reviewer #3. The real challenge is extracting [extrapolating] rodent data to humans (I understand the studies are mostly in this species). However, since the event was precipitated by feline illness I would suggest you discuss what you do know about the pathophysiology of this exposure in cats and then transition to people.

Reviewer #4. No, for example the discussion on carcinogenic potential was not fully explained or sufficiently documented. One of the other reviewers of this assessment is Dr. Sam Cohen and his research on rodent tumors with saccharin is very relevant for this study however none of his mechanistic research is mentioned or summarized for relevance. Also, in order to support a non-genotoxic mechanism for cancer, the available mutation data and references would need to be cited. Some of this was listed in RTECS but not fully described or referenced in this document.

Reviewer #5. A certain number of assumptions had to be made in carrying out this assessment. The assumptions made seem reasonable and sound to me.

Reviewer #6. Yes.

FDA Response: With one exception, the peer reviewers agreed that the assumptions were explained and appropriate. The additional suggested considerations will be evaluated when the interim S/RA is updated. As stated previously, the potential chronic toxicity and carcinogenicity of the MCs were not evaluated in this S/RA due to the temporary nature of exposure to these substances (see FDA Response to research suggestions by the outside reviewers and FDA Response to Question #4). One reviewer suggested including a discussion of the mechanism of action. There are no data found in the literature on the mechanism of action specific for melamine. We do not agree that including data on the mechanism of action for the formation of bladder tumors from ingestion of high doses of saccharin is useful for the melamine S/RA because it is not known whether the molecular events which trigger bladder tumor formation from ingestion of high levels of saccharin would be the same as those for MCs; however, Dr. Cohen was one of our reviewers and we will follow up on this suggestion with him. Finally, thus far, little or no experimental data have been found where the cat was used as the subject of testing.

Question 6. Are the scenarios addressed representative and comprehensive, considering the public health risk evaluated?

Reviewer #1. The three scenarios are quite useful but may not be completely representative or comprehensive. There may actually be worse case scenarios that should be considered in addressing potential public health risk. There might be a greater additive effect if multiple sources of exposure are considered. Another factor that should be considered is human consumption of pet food which does occur in some human populations. The final issue is the need to consider possible background exposures to melamine and its analogues likely through plastic products. One scenario should also focus on a longer duration of exposures and the potential outcome of chronicity.

Reviewer #2. See response to Question 8.

Reviewer #3. The three scenarios are appropriate and cover the scope of risk categories. They are at the macro-level and define the gross risk of consumption. The scenarios appear to assume no bioaccumulation over time (This is of course adjusted some by the calculation for Tolerable Daily Intake but may need to be readjusted if substantial bioaccumulation is found to occur in future studies).

Reviewer #4. Please see my earlier comments regarding representativeness of the exposure media assessment and how this would impact uncertainty in the exposure scenarios. As a reviewer of this document, I would need to understand more about the rationale to use 50 ppb for the concentration of melamine in all edible tissues. Because of the lack of information about representativeness of the measurements of the tissues and feed, this reviewer could not determine the “conservative” nature of this assumption as is stated on page 3, second paragraph.

Note that the text does not specifically state how the contaminant level in the feed (Table A-3, Source numbers 1-19) related in any way to the tissue levels reported in Table A-4. Also, this reviewer noted that on page 3, second paragraph the text states that the “analogue levels were generally no greater than melamine levels” however Table A-2 lists multiple examples where this statement was not true. This reviewer probably did not disagree with the over all statement but feels that more specific responses and statements are needed rather than just saying “generally” state the specific number of time the analogues exceeded the levels of melamine.

Reviewer #5. I think that the three scenarios presented are representative and comprehensive, considering the public health risk evaluated.

Reviewer #6. Yes.

FDA Response: The additional suggested scenarios will be taken into consideration when the interim S/RA is updated. The pharmacokinetic data on melamine and cyanuric acid indicate that both of these substances are absorbed readily, distributed to the total body water and excreted relatively quickly (half lives of 2-3 hours). There is no evidence of bioaccumulation of these two substances at the doses tested. One reviewer assumes that the FDA's S/RA used 50 ppb as the level of contamination by MCs; however, the actual level of contamination was assumed to be 100 ppb. (See S/RA at p. 3.) Thus, the degree of conservatism with regard to assumed exposure was even greater than this reviewer suggested. When the S/RA is updated we will have a more complete analysis of samples for the compilation of putative exposures and their variation.

Question 7. What are the prioritized research needs in relation to melamine and melamine related compounds, including the research that is needed to reduce the uncertainty associated with the S/RA?

Reviewer #1. There is clearly a need to develop and implement several research studies to help fill in knowledge gaps. These studies are listed in priority order from my perspective: 1. Identify complete structural activity to make sure that we are comparing the various compounds correctly; 2. Take contaminated food stuffs and identify exact chemical compounds that might result after heating the foods and then evaluate if the new compounds have greater toxicity; 3. Conduct longer term studies to determine other potential toxic effects including liver and reproductive or endocrine dysfunction; 4. Determine the possible additive and/or synergistic effect of melamine and its analogues that might result from co-exposures.

Reviewer #2. See response to Question 8.

Reviewer #3. The most obvious one is to look at other organs like gut, spleen, liver etc. for sub clinical accumulation or effects. Also, to better understand the effects of dehydration, common medications (diuretics and others that alter renal excretion of melamine pharmacokinetics).

Reviewer #4. This reviewer is supportive of the research needs listed in the document but feels that additional needs should include information on kinetics especially as this is directly related to the clearance issues. Determining whether these compounds breakdown internally or form esters internally would also be important to confirm. The importance of determining toxicity under co-exposure conditions is important. The report contains only a single line regarding reproductive and developmental toxicity and this would need to be verified or available data more thoroughly described. Much more exposure information about contaminate sources is needed.

Reviewer #5. Information on the toxicity of melamine compounds and on the levels of melamine compounds in edible tissues would be very useful. It would be important to have more accurate analytical methods to detect low levels of melamine compounds in tissues. The crystals that have been found in kidneys should be characterized. Basic toxicological studies should be done in several species. The toxicity studies should include examination of co-toxicity when there is exposure to more than one than one melamine compound. Is there synergism or just additivity? Could biomarkers be identified that would raise an alert about renal failure secondary to melamine compounds?

Reviewer #6. Concentration and precipitation in urine of different species. Synergistic effects of the 4 chemicals in enhancing crystallization. More sensitive assay. Effect of liquid and salt ingestion of concentration of MC's in urine.

FDA Response: Thank you for the suggested research needs.

Question 8. Do the S/RA results adequately support the conclusion (i.e., the low risk to human health from consumption of fish, chicken, poultry and pork inadvertently fed melamine contaminated feed)?

Reviewer #1. While the statement of a low risk to human health is the likely conclusion, I would hope that a true worst case scenario be constructed and considered based on exposures mentioned in question # 6. While the half-life of the compounds is relatively short, there is still a lingering question about what happens to melamine and analogues after cooking and/or heating. Are new and more toxic compounds created as they combine with resins? If this is true, then the current S/RA would seem to miss the most potentially relevant exposures. It seems that this question needs to be addressed before a final conclusion is considered.

Reviewer #2. I have reviewed the document entitled, “Interim Melamine and Analogues Safety/Risk Assessment”, dated May 24, 2007. The various assumptions used in developing the three (3) Scenarios, coupled with the analytical results, indicate that melamine and its analogues should pose no undue risks to human health. The Scenarios appear to be plausible. While additional investigations may be warranted, there are no overt adverse effects caused by these compounds that should concern humans who might have inadvertently ingested melamine-contaminated animal food/feed.

It is noteworthy that melamine was synthesized over 150 years ago, and that it has enjoyed a wide variety of commercial uses for several decades.

Melamine is minimally toxic, and has an LD-50 in experimental animals that is similar to that of common table salt or sodium chloride. There is no evidence of bioaccumulation.

Reviewer #3. I believe the S/RA adequately supports the conclusions for short and moderate term exposure. Longer term exposure (years) should be cautionary until further studies are done.

Reviewer #4. This reviewer would support that the fact that the interim risk assessment suggests that the risk is low for humans following acute exposure scenarios presented. Questions that need to be determined include the above points regarding lack of completeness regarding the extent of contamination—for example only livestock, pet and fish food contamination issues were discussed. Do we really know and can ensure that no contamination of human food stuffs were directly contaminated? In addition, because of the uncertainty of the linkage of exposure data and effects in livestock, it appears that only one of the studies was directly linked (U of California Davis study) hence significant uncertainty exists yet no additional factors for uncertainty are included in the margin of safety calculations. If an additional factor of at least 10 were included the same general risk/safety conclusion could be made but the MOS values would be more modest and in this reviewer's assessment more reflective of the situation at the current state of knowledge.

Reviewer #5. The S/RA adequately supports the conclusion that there has been low risk to human health from consumption of fish, chicken, poultry, and pork inadvertently fed melamine contaminated food, given the amounts that were ingested.

Reviewer #6. Yes.

FDA Response: We will take the suggestion to consider the toxicity of other potential toxic compounds and implications of chronic exposure into account when the interim S/RA is updated. We agree that a separate S/RA to evaluate the risk from exposure to other human food items would be useful, but note that we are unaware of evidence of direct contamination of human food items. Scenario 3 was meant to address in part the uncertainty of whether all potentially contaminated human food items had been identified when the S/RA was conducted.

Question 9. Do you have any additional comments that would assist FDA in refining the S/RA?

Reviewer #1. My comments and perspectives have already been stated.

Reviewer #2. See response to Question 8.

Reviewer #3. No.

Reviewer #4. This reviewer felt that a conference call or face to face meeting with the reviewers could have readily resolved some of the issues of this risk assessment and could have facilitated a quicker more comprehensive response.

Reviewer #5. No. I feel the S/RA is comprehensive, reasonable, and evidence-based.

Reviewer #6. Reference to IARC (IARC Monogr. Eval. Carcinogen. Risks Human, 73:329-338, 1999) and RSI (Crit. Rev. Toxicol., 33:591-654, 2003) (sponsored by US-EPA and Health Canada) reviews.

FDA Response: We thank the reviewer for providing the two additional references for our consideration. One reviewer suggested that instead of a letter peer review, that a panel review could have facilitated the speed of the responses. We believe that considering the urgency of the situation, that the letter review was appropriate. In addition, we note that the June 14, 2007 public meeting of the Science Board to the FDA will provide an additional opportunity for scientific review of the S/RA.

Question 10. Do the information and data in Appendices II and III support the underlying assumptions used in the interim assessment?

Reviewer #1. Yes, the assumptions are supported by data available and used; the issue remains, should other data sets be considered for this S/RA? I believe that they should be considered. I am aware of the need to make decisions with data that are available and there is certainly a paucity of information. The S/RA was restricted by this reality. Yet at this point, further work needs to be done to shift this report from an interim status to a final report and S/RA. My comments are made to be constructive and useful with an understanding of the difficulties involved in this situational and the need for timeliness of the analysis.

Reviewer #2. See response to Question 8.

Reviewer #3. Yes they do.

Reviewer #4. See the specific comments above.

Reviewer #5. I believe that the information and data in Appendices II and III support the underlying assumptions used in the interim statement.

Reviewer #6. Yes.

FDA Response: The peer reviewers concurred that the data in the appendices support the underlying assumptions. No additional response is needed.