Peer Review Workshop for EPA's Draft Toxicological Review of Ethylene Glycol Monobutyl Ether (EGBE)

Round 2 Comments on Revised Draft

Submitted to:

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

Submitted by:

Eastern Research Group
110 Hartwell Avenue
Lexington, MA 02421

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Reviewer Round 2 Comments

Fletcher Hahn, DVM, PhD
Scientist Emeritus
Lovelace Respiratory Research Institute

Comments on revised draft of Toxicological Review for EGBE

I have not substantive comments on the revised draft.

I particularly like the addition of Table 3-1 and the discussion of toxicokinetic parameters, the changes in Table 4-1 on Hematological data for 13 wks and the added discussion of the hemolytic mode of action and the hyaline degeneration.

Appendix C is particular helpful for increased transparency.

David Jollow, PhD
Professor Emeritus
Medical University of South Carolina
Department of Pharmacology

The document seems to me to be significantly improved by the incorporation of the suggestions of the several reviewers and by the general editing process that has taken place by the authors.

I still have a problem with the section of the discussion on the mechanism underlying the hemolytic anemia (Section 4.5, pp 53-54) in that it implies that we know and understand more than we do. Specifically, I feel that the suggestion that osmotic fragility *per se*, plays a role in the toxic sequence (page 54, line 6) is very misleading and needs to be either deleted or explained in greater detail.

Osmotic fragility is an *in vitro* test in which challenged red cells are subjected to hypo-osmolar media. While all red ells will lyse if the osmolarity is sufficiently low, a positive response is seen when affected red cells show a lesser capacity to resist hypo-osmolar conditions. The basis for this decreased ability to resist low tonicity is unknown, but presumably reflects loss of cellular homeostasis due to change in ionic components and/or gating and ionic transporters, and/or mechanisms controlling the structural elements of the cell.

Clearly, since hypo-osmolar conditions do not occur in vivo, red cells damaged by BAA *in vivo* are never subjected to this type of stress. Osmotic fragility *per se* with the attendant swelling and lysis seen *in vitro,* is not part of the toxic sequence that leads to the hemolytic anemia in the laboratory animals.

Equally clearly, the fundamental damage to the red cell may be the same *in vivo* and *in vitro.* However, this does not mean that the consequences of that damage must be the same. Swelling, if it occurs *in vivo*, does not preclude passage through the micro-circulation and does not necessitate fragmentation. Even severe morphological change such as the Heinz-body laden sphero-echinotic forms seen after phenylhydrazine treatment are not trapped in the micro-circulation but are selectively removed by the spleen, The contrast is the sickled RBC where morphological change correlates with impeded passage. We simply do not yet understand why the sickle cell adheres but the phenylhydrazine-damaged cell does not.

While the sequence implied in this section is attractive, it is not adequately supported by the available information. I would like to see a simple statement early in this section (e.g., the third sentence of Section

4.5, page 53, line 27) such as: ” The mechanisms underlying the hemolytic events are unknown.” This should be followed by a summary of the in vitro observations (as is presently done on lines 28-31) and the implications of Ezoz et al., etc re possible loss of sidedness of membrane phospholipids (as is down to p54, line 4). The comments re osmotic fragility and loss of deformability need to be deleted .

This should be followed by a statement such as: “The fate of the damaged red cells, whether direct lysis in the circulation or splenic sequestration, is unknown, It is likely that under low EGBE exposure conditions, splenic sequestration will predominate but at higher exposure rates, splenic spillage and/or frank intravascular lysis will occur. Heme transport mechanisms will be overloaded and iron containing fragments will accumulate in the phagocytic cells of the liver (Kuppfer cells) as hemosiderin.

ALSO

Page 54, lines 11-12. The sentence re association of PS externalization and apoptosis needs a qualifer “in nucleated cells”,

Page 57, lines 10-13. Please modify the reference to osmotic fragility. As discussed above. “osmotic fragility” cannot occur in an intact animal.

Michael Pereira, PhD
Professor, Division of Hematology and Oncology
College of Medicine and Public Health
Ohio State University

The single space text is from the EPA response followed by my comment in bold, 1.5 space.

A3. Benchmark dose (BMD) modeling was applied to incidence data for hemosiderin staining in male rat liver to derive the point of departure (POD) for the RfC. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response (BMR) selected for use in deriving the POD (i.e., 10% extra risk of hemosiderin staining in the liver) been scientifically justified, and transparently and objectively described? Please identify and provide the rationale for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Comments:
All reviewers agreed that BMD modeling was the best approach for determining the POD. However, alternatives were suggested for the choice of species and gender. One reviewer commented that male mice should have been used since they have shown correlation between the critical effect and the adverse effect (tumor). Two other reviewers commented that female rats should have been used since they have been shown to be more sensitive to the hemolytic effects and were used in the previous IRIS assessment. In addition, one reviewer commented on the choice of 10% response. This reviewer felt that a 5% response rate is more scientifically supported approximating the NOAEL.

Response:
Based on the majority of comments agreeing with the choice of gender and endpoint to model, no substantial changes to the *Toxicological Review* are indicated. Rationale for the selection of the species and gender are discussed in Section 5.3.9. Briefly, the male rat was chosen based on the NTP 2000 report showing male rats were the most sensitive species and gender with respect to the critical effect (hemosiderin deposition). In addition, the modeling of the data for male rats provided a much better fit than the modeling for the female data. As for the selection of the response rate, 10% was chosen based on EPA guidance on BMD modeling of qualitative data and that 10% was within the range of experimental responses and appropriate for the power of the study. Additional justification has been added regarding the fact that this tumor response may not have been observed in the rats because the rats were exposed to lower doses and for a shorter portion of their average lifespan than the mice.

My Comments:

Section 5.3.9 does not discuss any rationale but rather states that male rats are more sensitive. How can male rats be more sensitive if the 31 ppm is a LOAEL in female rats but a NOAEL in male rats? As stated in the text on page 23 "The LOAEL for hematological alterations was 31 ppm for female rats and 62.5 ppm for male rats. The 31 ppm exposure level was considered a NOAEL for male rats." Hemosiderin Incidence at 62.5 ppm in females was 10/10 but 0/10 in males.

A5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC.

Comments:
Several reviewers commented that the choice of uncertainty factor values was not justified by the available science.
a. Two reviewers felt that the UF_A should be less than 1.

Response:

The current UF_A value in the *Toxicological Review* is 1. The UF_A accounts for a reduction from 3 for the toxicokinetic differences between animals and humans through the use of a PBPK model for extrapolation of doses. The toxicodynamic portion, likewise was reduced from 3 to 1. The use of a 1 for the toxicodyanamic portion of the UF_A represents the lowest reduction described by the current guidance (USEPA, 1994). The toxicological effect in question being the deposition of hemosiderin is a key event of the MOA for the development of liver hepatomas in mice. To implement a further reduction in the UF_A (i.e., fractional) would logically indicate a preponderance of data are available to fully describe this key event (toxicodynamics) in both animals and humans to the extent of describing why humans are less sensitive to the hemolytic effects leading to hemosiderin deposition. This is not the case, although in vitro data (Udden and Patton, 2005; Udden, 2002 and 2000; Ghanayem, 1989) do suggest humans are less sensitive than rodents to the hemolytic effects of EGBE. Likewise, the few human studies (Haufroid et al., 1997; Carpenter et al., 1956) indicate the same finding. However, these studies (Udden and Patton, 2005; Udden, 2002 and 2000; Haufroid et al., 1997; Ghanayem, 1989; Carpenter et al., 1956) do not characterize hemosiderin deposition, the key event for the MOA.

The current UF_A value in the *Toxicological Review* is 1 and provides an adequate margin of safety based on the limited studies available as well as the unknown effect of EGBE on the cellular events leading to hemolysis in human populations. In addition, little is known of the long term or repeated exposure responses in humans to EGBE.

Lastly, part of the definition of an uncertainty factor is that it is represented by a number that is generally an order of magnitude (i.e., 10). Implementation of uncertainty factors is described by dividing the point of departure by a factor of 1, 3, or 10 for each of the defined UFs to calculate a human health toxicity value. The use of a fractional UF would represent a deviation from the current guidance and is beyond the scope of this *Toxicological Review*.

My Comment:

I still believe the UF_A should be less than 1. EPA response to it not being less than 1 is that the EPA never considers humans to be less sensitive than animals as stated in their last three sentences. Otherwise a fractional UF would be allowed under the current guidance. There is very good evidence presented in the document that humans are less sensitive than the animals and thus scientific support for a value less than 1. However, there is no evidence for a sensitive human subpopulation let a hypothetical subpopulation of humans is proposed with a UF_H of 10. How can the EPA use a UF_A of greater than when scientific evidence indicates it is less than 1, while using a UF_H of without any scientific justification?

Andrew Salmon, PhD
Senior Toxicologist and Chief
Air Toxicology and Risk Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Satisfactory unless otherwise noted below.:

Charge question 1 was generally addressed. The additional PBPK data are helpful

Charge question 2. Most points were adequately addressed, but the response to the comment under 2e relating to hyaline degeneration was an addition of derisory proportions which did not in any way address the point of the original comment: it appears merely as a half-hearted attempt to justify the Agency's determination to ignore the issue. This histological observation may perfectly well be regarded as a precursor lesion and might be useful at least for comparison with other possible endpoints. It was not suggested that this would be a suitable critical endpoint in isolation, but it may be important as a comparison, since it is the only reported effect of inhalation which is probably NOT related to the hematological effects underlying the other endpoints examined.

Question A3: The discussion in the document is now actually more convincing than the response to this comment. One of the points of the comment was that the "EPA guidance" referred to briefly is somewhat inconsistent with the actual data and judgments appearing in various specific assessments by U.S. EPA and others.

Question A5: The additional discussion of UFs is helpful.

Other additions and clarifications noted in the responses are helpful to the final document and substantially address the comments received.

A few minor problems in the actual text of the review:

Page 5 line 28: Blood from rats, mice, and rabbits <u>were</u> (replacing "was") more rapidly …
Still wrong grammatically – subject and verb must agree either singular or plural. Could say "Bloods from … were …" or perhaps a more natural style would be "Blood samples from …"

Page numbers have disappeared after page 24.

Page 44 *et seq.* (according to MS word): section 4.3. Reproductive and Developmental Studies: Oral and Inhalation. Repeatedly in this section the word "adverse" was replaced either with "significant" or "biologically significant".

It's not clear why one term was used in some cases and the other elsewhere. Usually the term "significant" without qualification means statistically significant. If in some cases the observation described was statistically significant but not biologically significant this needs to be noted explicitly and the conclusion of lack of biological significance needs to be explained and justified.

Page 48, line 10: should surely read "… noted thereafter, or at any time in offspring." or something to that effect.

Page 75, line 10: "As shown in **Error! Reference source not found.** Figure 4-1,". Field codes messed up here? Is this Figure 4-21? Several other figure and table references in the document look a little odd. Some tables flow across page breaks, and some headings are separated from their body paragraphs, figures etc. A cross-reference on Page 128, line 10 is actually missing.

Gregory Travlos, DVM, DACVP
Veterinary Medical Officer, Clinical Pathologist
National Institute of Environmental Health Sciences

This reviewer did not conduct a round 2 review on the revised draft assessment.

Rochelle Tyl, PhD
Senior Fellow, Toxicology
RTI International
Life Sciences and Toxicology

I have no further comments on the EGBE Toxicological Review. Thank you for the opportunity to assist EPA.

D. Alan Warren, MPH, PhD
Academic Program Director, Environmental Health Sciences
University of South Carolina Beaufort

Based on my review of pre-meeting comments, personal notes taken during the peer review workshop, and the redline version of the EGBE Draft Toxicological Review (with emphasis on Appendix A, Summary of External Peer Review and Public Comments and Disposition), it appears that U.S. EPA has carefully considered the review panel's recommendations and modified the Toxicological Review accordingly. That the principal study, critical effect, and methodologies used for RfC and RfD derivation remained intact after external review is testament to the high quality of the original draft. I do wish to make one trivial point, however. The latest version of the Review states, in reference to the database UF of 1, the following: *One reviewer commented that the value should be higher, which would make it consistent with the USEPA's confidence level of medium to high* (see pp. A-11 and A-12). In actuality, I wasn't necessarily in disagreement with the UF of 1, but simply felt as though it was inconsistent with the Agency's expression of medium-to-high confidence in the RfC assessment and in the database that supports it. Lastly, the Review does contain a few errors in punctuation, such as the subject-verb disagreement in the following sentence (p. 5, line 28): Blood from rats, mice, and rabbits were more rapidly hemolyzed than blood from humans, monkeys, dogs, or guinea pigs when incubated in vitro at 37.5°C with a saline solution of 0.1% of the sodium salt of BAA