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

Reviewer Post-Meeting Comments

Submitted to:

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

Submitted by:

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Introduction

The Integrated Risk Information System (IRIS) is a U.S. Environmental Protection Agency (EPA) database containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances. IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process: hazard identification and dose-response evaluation. IRIS information includes a reference dose (RfD) for non-cancer health effects resulting from oral exposure, a reference concentration (RfC) for non-cancer health effects resulting from inhalation exposure, and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program within EPA's National Center for Environmental Assessment (NCEA) posted a draft Toxicological Review of ethylene glycol mono-butyl ether (EGBE) to the IRIS database in 1999. EPA's Office of Air Quality Planning and Standards (OAQPS) nominated EGBE for reassessment to support potential regulatory actions in response to a delisting petition under the Clean Air Act. Consequently, NCEA developed a draft revised Toxicological Review of EGBE.

In the fall of 2008, Eastern Research Group, Inc. (ERG), an EPA contractor, organized an independent peer review of EPA's *Draft Toxicological Review of Ethylene Glycol Mono-butyl Ether (EGBE)*. The review document contained a chronic inhalation RfC and a chronic oral RfD, but did not contain a quantitative cancer assessment. ERG identified seven nationally recognized experts (Appendix A) to conduct this review:

- David Jollow, Professor Emeritus, Medical University of South Carolina
- Michael Pereira, College of Medicine and Public Health, Ohio State University
- Andrew Salmon, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency
- Fletcher Hahn**,** Scientist Emeritus, Lovelace Respiratory Research Institute
- Rochelle Tyl**,** Life Sciences and Toxicology, RTI International
- D. Alan Warren**,** Environmental Health Sciences, University of South Carolina Beaufort
- Gregory Travlos, National Institute of Environmental Health Sciences

ERG provided the reviewers with a charge (page 3), which asked for their comments on the various aspects of the document. In addition to the review document, reviewers were also provided with:

- The 2005 EPA Report on "An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether" (EPA/600/R-04/123), which formed the basis, in part, of the external review draft IRIS assessment. The document includes external peer review comments and

EPA's disposition as an Appendix.

- A 1993 National Toxicology Program Technical Report on Toxicity Studies of Ethylene Glycol Ethers: 2-Methoxyethanol, 2-Ethoxyethanol, 2-Butoxyethanol (NIH Publication 93-3349).
- A 2000 National Toxicology Program Technical Report on the Toxicology and Carcinogenesis Studies of 2-Butoxyethanol (CAS No. 111-76-2) in F344/N Rats and B6C3F1 Mice (NIH Publication No. 00-3974).
- References.

Each reviewer also received complete copies of the written comments submitted during the public comment period, which they were asked to consider.

In the first stage of the review, the experts worked individually to prepare written pre-meeting comments, which were provided to all reviewers and EPA prior to a one-day peer review workshop. In the second stage, ERG convened the one-day workshop, on October 16, 2008, at EPA's facility in Research Triangle Park, North Carolina. The meeting was open to the public and attended by 16 observers and an ERG facilitator (Appendix B). Appendix C provides the workshop agenda. The meeting format included an opportunity for public comment. Four members of the public made oral comments. Reviewers were provided with the slides used by observers as they made their oral comments. After the meeting, reviewers revised their pre-meeting comments to reflect their views as they had evolved based on the workshop discussions. The reviewer final post-meeting comments are provided in this report. These comments reflect the individual opinions of the reviewers.

Peer Review Workshop for Toxicological Review of
Ethylene Glycol Monobutyl Ether (EGBE)

U.S. Environmental Protection Agency
Task Order No. 34
Contract No. EP-C-07-024

TECHNICAL CHARGE TO PEER REVIEW PANEL

Background

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of EGBE that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

An IRIS assessment for EGBE was posted to the database in 1999. The current draft health assessment includes a chronic Reference Dose (RfD) and Reference Concentration (RfC), and a carcinogenicity assessment. In 2005 EPA released "An Evaluation of the Human Carcinogenic Potential of Ethylene Glycol Butyl Ether" (EPA/600/R-04/123) (available at < http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=135268>). External peer review comments and EPA's disposition are available as an Appendix to the 2005 EPA document. This document formed the basis, in part, of the external review draft IRIS assessment.

Below is a set of charge questions that address scientific issues in the assessment of EGBE. Please provide detailed explanations for responses to the charge questions.

CHARGE QUESTIONS:

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of

uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. 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.

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

- A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are

sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).

- Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is

scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations.

Biographies

Fletcher Hahn, DVM, PhD

Scientist Emeritus
Lovelace Respiratory Research Institute

Dr. Fletcher Hahn received his BS in Biological Sciences and his DVM in Veterinary Medicine from Washington State University in 1964. He received his PhD in Comparative Pathology from the University of California-Davis in 1971. Prior to becoming a Scientist Emeritus for Lovelace Respiratory Research Institute in 2004, Dr. Hahn was a Senior Scientist conducting independent research on health effects of inhaled particles of environmental and occupational concern for the Institute. His primary interests are in the health effects of inhaled environmental contaminants, using exposed animals to determine possible effects in man. As a principal investigator and collaborator, he studied the morphologic changes and the pathogenesis of diseases resulting from materials inhaled by laboratory animals. The focus was on pulmonary inflammation, fibrosis, and neoplasia that result from inhaled chemical vapors, oxidant gases, wood and cigarette smoke, metallic particles, fibers, and radioactive materials. Extrapolation of results from animals to man is a recent focus as exemplified by a comparison of the pulmonary reactions of man and rats to inhaled dusts and the distribution of particles in the lungs of plutonium workers. Dr. Hahn has authored or co-authored over 270 open literature publications, primarily in these areas of interest. While working at the Inhalation Toxicology Research Institute, Dr. Hahn served as a pathologist on studies conducted using good laboratory practice (GLP) procedures. These included carcinogenicity bioassays of inhaled talc, nickel subsulfide, nickel oxide, and nickel sulfate. Also included were safety studies of laser diodes for treatment of benign prostatic hypertrophy, IL 12 combined with radiation for cancer therapy, inhaled hormones, inhaled polyacrylics, and inhaled gene therapy vectors. He also worked on pathology review panels in the area of inhalation pathology. Dr. Hahn also participated in training professionals in environmental and toxicologic pathology. For many years he was the on-site coordinator for a collaborative research training program conducted by the Lovelace Respiratory Research Institute in conjunction with the Department of Veterinary Pathobiology, Purdue University.

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

Dr. David John Jollow was appointed Professor of Pharmacology at the Medical University of South Carolina in Charleston, SC in 1974. He holds a PhD in Biochemistry from Monash University in Victoria, Australia and BSc and MSc degrees in Biochemistry from Sydney University, NSW, Australia. During his academic career, he has authored and co-authored over 150 books, articles, and abstracts. His research is focused on the mechanisms by which drugs and other environmental chemicals induce tissue-specific lesions in the liver and in red cells with particular attention to the contribution of chemically reactive intermediates, derived from such environmental chemicals, in the disease process. Dr. Jollow has served on many national committees, including as member and Chair of two National Institute of Health Study Sections (Toxicology and Environmental Health Sciences) and as member and Chair of the Safe Drinking Water Committee of the National Academy of Science. Editorial responsibilities include the Biological Reactive Intermediates series and several volumes of the Drinking Water and Health series of the National Academy of Sciences. On retirement in 2006, he was advanced to the rank of Professor Emeritus.

Michael Pereira, PhD

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

Dr. Michael Pereira received a BSc in Microbiology in 1967, and a PhD in Pharmacology and Toxicology in 1971 from Ohio State University. He then received a Damon Runyon Cancer Research Fellowship (1971–1973) to work at the National Institutes of Health (NIH). He is currently an Emeritus Professor of Medicine, Division Hematology and Oncology at Ohio State University. Dr. Pereira has over 40 years of experience in toxicology and carcinogenesis. This includes research performed as an employee of the US government (EPA), private industry (EHRT) and academia (New York University, Medical College of Ohio, and Ohio State University). As an employee of the US Environmental Protection Agency (EPA), he was responsible for the evaluation of the genotoxicity and carcinogenic of chemicals found in water, especially drinking water, and for the determination of their human health hazard. At EHRT, he was the Vice-President for Toxicology and Principal Investigator of numerous grant and contracts with the National Cancer Institute (NCI) and the National Institute for Occupational Safety and Health (NIOSH). He has continued in academia to be Principal Investigator of three grants from NCI, of two grants from EPA, one grant from National Institute of Environmental Health Sciences (NIEHS), and over 30 contracts with NCI. He was also the Principal Investigator of a contract with the City of Tampa to perform an evaluation of the toxicity of effluents from their wastewater reuse pilot plant. Dr. Pereira has over 230 publications in peer-reviewed journal that are related to toxicology and cancer. Since leaving EPA in 1986, he has reviewed numerous documents for the agency. Some of the other committees he has been a member of include the NCI Small Business Innovation Research (SBIR) Study Section, NCI Study Section for Program Project, NCI Specialized Program of Research Excellence (SPORE) in Lung-GU Cancer Review Committee (P50 applications), and NCI Chemo/Dietary Prevention Study Section.

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

Dr. Andrew Salmon is a Senior Toxicologist and Chief of the Air Toxicology and Risk Assessment Section at the Office of Environmental Health Hazard Assessment, which is part of the State of California's Environmental Protection Agency (Cal/EPA). As a research toxicologist in industry and academia, Dr. Salmon has worked on cancer mechanisms, metabolism and pharmacokinetics, inhalation toxicology, and safety evaluation for environmental and occupational exposures. His current activities include application of benchmark dose methodology and evaluation of special impacts on children's health in air toxics risk assessment. In addition to editing and contributing to numerous chemical-specific risk assessment documents and procedural guidance documents for the State of California, he is a contributing author on a number of papers published in journals such as *Preventive Medicine, Environmental Health Perspectives,* and the *Journal of Toxicology and Environmental Health*, and has made several presentations at meetings of the Society of Toxicology and Society for Risk Analysis. Dr. Salmon received his bachelor's and doctoral degrees in Biochemistry from Oxford University, U.K.

Gregory Travlos, DVM, DACVP

Veterinary Medical Officer, Clinical Pathologist
National Institute of Environmental Health Sciences

Dr. Gregory Travlos received his DVM in 1979 from the College of Veterinary Medicine at Iowa State University. He is currently the Veterinary Medical Officer at the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, NC, leads the Clinical Pathology Group within the Cellular and Molecular Pathology Branch, and is the Clinical Pathology Discipline Leader for the National Toxicology Program. Prior to joining NIEHS, he served as a Veterinary Clinical Pathologist and Manager of the Veterinary Division at MetPath, Inc. Dr. Travlos is a board certified Diplomate, American College of Veterinary Pathologists (clinical), and member of the American Society for Veterinary Clinical Pathology, American Association of Clinical Chemistry, and the Society of Toxicologic Pathology. Throughout his career, Dr. Travlos has published numerous peer-reviewed articles in leading biomedical journals such as *Fundamentals of Applied Toxicology, Toxicologic Pathology*, *Toxicology*, and *Toxicology and Applied Pharmacology,* as well as written several book chapters.

Rochelle Tyl, PhD

Senior Fellow, Toxicology
RTI International
Life Sciences and Toxicology

Dr. Rochelle W. Tyl has more than 40 years of experience in reproductive and developmental biology and toxicology. She is currently a Distinguished Research Fellow in Developmental and Reproductive Toxicology (DART) and Principal Investigator of DART studies in the Center for Life Sciences and Toxicology (LST) at RTI International (RTI). Dr. Tyl and her technical staff perform reproductive and developmental toxicity studies under the US Food and Drug Administration (FDA), the US Environmental Protection Agency (EPA) (the Toxic Substances Control Act [TSCA]; Federal Insecticide, Fungicide, and Rodenticide Act [FIFRA], and the Office of Prevention, Pesticides, and Toxic Substances [OPPTS]), the Organisation for Economic Co-operation and Development (OECD), and the International Conference on Harmonisation (ICH) testing guidelines and good laboratory practices (GLPs), as appropriate, and tailored studies for commercial clients and the government. She has served as a Diplomate of the American Board of Toxicology (DABT) since 1983, and served on the ABT Board from 2003 to 2007.

Dr. Tyl has served as Study Director for more than 30 multigeneration studies in rats and mice; for more than 150 developmental toxicity (Segment II) studies in rats, mice, and rabbits; for dominant lethal assays in rats and mice; for Chernoff–Kavlock assays in rats and mice; for modified and original OECD 421 and 422 reproductive/developmental toxicity screening assays; for FDA Segment I and III studies in rats and mice; and for developmental neurotoxicity studies. She has authored or co-authored more than 90 peer-reviewed articles, 18 book chapters, 10 reviews, more than 85 abstracts, and hundreds of study reports. She, along with her co-editor, is preparing a book on Reproductive Toxicology, Third Edition (for which she is also writing a chapter on FDA and ICH regulations).

Dr. Tyl has been an invited speaker in courses and symposia for universities and national and international organizations. She has participated, by invitation, in workgroups for the American Chemistry Council (formerly the Chemical Manufacturers Association [CMA]), EPA, the National Toxicology Program (NTP), the National Institute of Environmental Health Sciences (NIEHS), the FDA, the American Industrial Hygiene Association (AIHA), the National institute for Occupational Safety and Health (NIOSH), the International Life Sciences Institute (ILSI), and the Interagency Regulatory Liaison Group (IRLG). She was an invited member of the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC) for EPA and has represented the Agency in the OECD Test Guidelines program and at the OECD Conference on Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment. Dr. Tyl was a member of two National Academies' Expert Panels (Spacecraft Exposure Guidelines and Assessing Human Health Risks of Trichloroethylene) and on a number of ILSI/HESI expert panels. She is also an ad hoc reviewer for many journals. She was President of the Teratology Society for 2003–2004, and President of the Reproductive and Developmental Toxicology Specialty Section (RDTSS) for the Society of Toxicology for 2007–2008.

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

Since 2002, Dr. Alan Warren has served as Academic Program Director, Environmental Health Science, at the University of South Carolina Beaufort. In this capacity, he is responsible for the development of an undergraduate education program in Environmental Health Science, the conduct of field- and laboratory-based research in environmental and human health risk assessment, and providing instructional support in a variety of degree programs. Dr. Warren received his BS degree in Environmental Health from the University of Georgia (1985), an MPH degree from Yale University (1987), and a PhD in Toxicology from the University of Georgia (1995). He is a former Department of Defense Science and Engineering Graduate Fellow and National Research Council Research Associate in the Toxicology Division at Wright-Patterson Air Force Base. He was once employed as an industrial hygienist at the Georgia Tech Research Institute where he helped administer the State's Occupational Safety and Health Administration (OSHA) Consultation Program. He has also been a consulting toxicologist at TERRA, Inc. in Tallahassee, FL where he routinely evaluated the toxicology and theoretical risks associated with environmental chemicals and provided toxic tort litigation support to clients. His past research interests are varied but have focused on the pharmacokinetics and toxicity of a variety of solvents (e.g., JP-8 jet fuel, trichloroethylene, trichloroethane, perchloroethylene) and non-solvents alike (e.g., ammonium perchlorate, retinoic acid). At present, his research is largely focused on the fate of heavy metals and explosives constituents at small arms firing ranges and bombing ranges of the US Marine Corps. He has published on solvent toxicology in a variety of professional journals and recently co-authored the chapter entitled, "Toxic Effects of Solvents and Vapors" in the 7th edition of Casarett and Doull's Toxicology, The Basic Science of Poisons, which includes a section on glycol ethers.

Peer Reviewer Responses Organized by Charge Question

(G1) Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Fletcher Hahn

The draft Review is generally well presented, logical and clear. A strong point of the approach is the presentation of alternate models for calculation of various parameters and the liberal use of tables to present results.

David Jollow

This document is generally well written and laid out in a logical and concise manner. The scientific evidence for non-cancer and cancer end-points is presented clearly. However, there appears to be several areas where some additional discussion/modification may improve the document. These are:

i), The terms "lysis" and hemolysis" appear to be used interchangeably and seem to have led to the assumption that the hemolytic anemia seen *in vivo* after EGBE results from the direct lysis of damaged red cells within the vascular system. While this assumption is not unreasonable, the data available are not unequivocal and could conceivably lead to an under-estimation of the hemotoxic dose.

On the one hand, the data clearly indicate that incubation of rat red cells *in vitro* with high concentrations of BAA results in swelling of the red cells and direct lysis. However, *in vivo* data indicate that exposure to EGBE/BAA leads to an increase in the spleen/body weight ratio, implying a crucial role of the normal splenic sequestration process. The spleen removes whole cells (damaged or aged) by specific and highly selective receptor-mediated sequestration into resident macrophages. The liver (i.e, Kupffer cells) is key in the removal of broken cells and cell fragments. It should be appreciated that when a massive hemolytic event is occurring *in vivo*, the engorgement of the spleen may lead to failure of the resident macrophages to retain "sequested" red cells and hence result in spillage of damaged and partially-lysed red cells. In this situation, distinguishing between extra- and intra-vascular "lysis" becomes difficult when based on morphological changes (MCV etc) in the circulating red cells. The appearance of morphologically altered cells, even if the changes are dramatic and similar to those seen in *in vitro* experiments, does not indicate unequivocally that the hemotoxic event leading to drop in Hct is the spontaneous lysis of red cells throughout the circulation. (It is appreciated that since the splenic macrophages reside within the splenic vasculature, all hemolytic "events" are [strictly speaking] intravascular). However, the distinction is important in that it influences the selection of criteria for dose- and concentration-response relationships and for interspecies (rodent to human) extrapolation.

The analogous situation with the classical "hemolysin" phenylhydrazine (PHZ) illustrates this problem. PHZ has been studied for over 50 years and is well known that *ca* 2+g hr incubation of rat RBC with 2-10 mM PHZ results in morphological change (spherocytic echinocyte formation) and direct lysis. The EC_{50} for *in vitro* lysis is not precisely defined in the literature but may generally be considered to be in the 2-5 mM range. However, if rat RBCs are tagged with ^{51}Cr prior to the 2 hr PHZ exposure, then washed and returned to isologous rats, the exposed cells show a concentration-dependent decrease in their survival curves secondary to splenic sequestration (McMillan et al JPET 287:868-876, 1998) Under these conditions, the EC_{50} for the hemolytic activity of PHZ is ca 800 □M; at least a two to three fold decrease as compared with measurement of direct lysis. Direct cell lysis at 800 □M as measured in post incubation wash media was <1%.

Two issues seem to stand out from the lack of definition of the MOA. First, the kinetics of the toxic "hit" are undefined; specifically, if the toxicity of BAA towards the RBC is proportional to its AUC or its Cmax. AUC implies an accumulation of injury, whereas Cmax suggests a reversible association with a receptor or other protein. As discussed in section 5, the decision affects the selection of data for RfC etc. etc., and the use of PBPK modeling in rodent to human extrapolation. The observation that severity of the hematological effects does not progress in severity in the subchronic-to-chronic study (p75, line 2) does not allow distinction in that it is compatible with *both* a steady state of the mean age distribution of the red cells in treated rodents (i.e., a gradient of decreasing susceptibility of older-to-younger cells) and with the toxicokinetic-toxicodynamics of BBA interaction with a receptor. On the other hand, the observation that the hemotoxicity of EGBE is dependent on the mode of oral ingestion (page 78, line 10) is highly suggestive of the crucial role of concentration rather than AUC in that oral gavage may be expected to cause a more rapid ingestion and higher blood levels of EGBE than that provided by drinking water, spread over many hours of access. Higher peak levels of EGBE should give higher peak levels of BAA.

Of particular interest is the acute *vs* sub-chronic/chronic comparison. In the acute exposure situation, the % of affected red cells will be very high and splenic overload is unavoidable. The animals will appear to be experiencing a "spontaneous intravascular lysis". In more chronic dose situations, the initial dose(s) will remove the older red cells (consistent with the suggested red cell age-related susceptibility) leaving a younger mean-average age to the red cell population. On continued "steady-state" exposure to EGBE, one can expect that only a small fraction of the cell population will move into the age range that is susceptible to the "steady-state" BAA levels and that the amount of red cell mass removed per day will be very much less than that of the acute dose situation. During chronic doses studies, it is likely that the spleen would be able to cope with the "demand" for red cell sequestration. The feature central in the removal mechanism after single and initial doses of EGBE; viz, acute massive spillage of hemoglobin-iron with major involvement of the Kupffer cell population of the liver, may not be so important in the chronic exposure experiments. Of importance, the role of the spleen *vs* liver, with its associated risk of perturbation of normal iron turnover and storage mechanism (and hence hemosiderin deposition), may readily be resolved experimentally.

Second, the assumption of the crucial role of direct lysis may have deterred exploration of alternate hypotheses on the MOA. Of note is the observation of Ezov et al (Cardiovasc. Tox 2002) that EGBE causes disseminated thrombosis and infarction in Fischer 344 rats. This is suggestive of a role for loss of sidedness of the red cell membrane. The outer leaflet normally has a net positive charge due to its predominant presence of phosphatidyl choline. This is considered to be important in suppressing adhesion to the similarly positively charged membranes of epithelial cells lining the vascular. Ionic changes in the red cells induced by BAA may well activate the "scramblease" that promotes movement of phosphatidylserine from the inner to the outer leaflet with corresponding reduction of the normal repulsive behavior between the red cell surface and that of the vascular lining. If this mechanism is involved in BAA-induced hemotoxicity, it would provide a readily quantitatable index to establish the relative sensitivity of rodent *vs* human erythrocytes, with attendant implications for risk assessment.

Please replace the opening words of the 4.5 Synthesis/evaluation section of p 46; viz, "Intravascular hemolysis" with "Hemolytic anemia". Please modify the text here and

elsewhere to remove the suggestion that the toxicity of concern is "frank intravascular lysis" (e,g., p46 lines 30-32) with its attendant mechanistic implications. The requested modification is one of tone rather than major change in interpretation and does not preclude the use of available "lytic" data for comparison purposes provided that it is clear that we do not know the actual "toxic hit(s)" that lead to the premature removal of red cells from the circulation. Please identify the need for a firmer definition of the MOA for future research.

ii). The proposed MOA for the hemangiomosarcoma in male mouse liver resulting from chronic EGBE exposure is considered to be reasonable and is acceptable, even though the supporting data is far from overwhelming (e.g., lipid peroxidation is notoriously unreliable as an index of ROS activity). I have two concerns as to the use of hemosiderin as the POD for assessing risk of hemangiosarcoma:

a), the present data appears to rest on Prill Prussian blue positive cells in the liver. While morphometric analyses of this type provide important information, my experience has convinced me that they are inherently more susceptible to error as a quantitative index. Are liver samples from the test animals still available? If so, analysis of iron content might provide a more reliable index of "toxic load".

b), I have not found data in the document that describes the shape of the dose/response curve between amount of hemosiderin deposition with incidence of hemangiosarcoma. The discussion on page 56 is very general and that of Table 4-8 does not allow assessment of the shape of the D/R curve. From a biological perspective, it seems likely that this would be a "classical" sigmoidal relationship and should influence derivation of the BMD etc..

c), Inclusion of pentachloroanisole in Table 4-8 seems inappropriate and weakens the association between hemosiderin deposition and hemangiosarcoma.. This chemical is not hemolytic and hence hemosiderin-iron deposition is unlikely. The chemical (and especially its polycyclic analogs) is very likely to cause porphyria with deposition of porphyria-related pigments. This section would also be strengthened by inclusion of hemolytic compounds such as aniline, which induces iron overload and neoplasia in the spleen rather than the liver. In that the iron overload/ROS generation mechanism of initiation of neoplasia is of general interest for a variety of compounds, the need to understand the fundamental processes involved, including the basis of selectivity for target tissues, goes far beyond EGBE itself.

iii), Is there information on other parameters/aspects of the hemotoxicity such as levels of methemoglobin, haptoglobin, hemopexin? Has a Coombs test been done during chronic studies? It is appreciated that "free" hemoglobin in the circulation is rapidly converted to methemoglobin and that a distinction must be made between whole blood and intracellular RBCs levels of MetHb. Although unlikely, data and discussion should be included to rule out other well known causes of hemolytic events. As discussed below, data on haptoglobin and hemopexin levels and saturation during chronic exposure to EGBE may help in defining the relative importance of splenic *vs* liver macrophages in the premature removal of RBC.

iv), PBPK modeling of EGBE and BAA is not well illustrated in the document. Fig. 4-2 is not very informative. Is there a better figure to illustrate the metabolic relationships? The presentation in the document did not allow me to assess the reasonableness of the conclusions drawn.

v), The low renal clearance of BAA relative to GFR needs to be discussed in more depth. Is there data on plasma albumin binding of BAA and does it vary between rodents and humans? If the binding is less than ca 95%, it is unlikely to be the explanation of the restricted renal elimination . The alternate explanation of active *reuptake* by a carrier mechanism is amenable to study: is there relevant information in the literature? Resolution of this question is deemed crucial in understanding the factors that determine both the Cmax and AUC of BAA in the various species/sexes of rodents as well as the determination of HECs.

Minor problems/consideration:

a), The toxicokinetic discussion (section 3.2) is, perhaps of necessity, somewhat diffuse. I found myself going back and forth looking for specific parameters such as $T_{1/2}$ of EGBE and BAA in the several species/modes of administration/acute vs chronic etc. Is it possible to present a Table incorporating some of the relevant kinetic information?

b), Reversal of ADH and ALD in the text, e.g., p 11, lines 21/22.and p 44 lines 16-19. EGBE to BAL is catalyzed by alcohol dehydrogenase (ADH), and BAL to BAA by aldehyde dehydrogenase (ALD). Perhaps this confusion could be minimized by use of ALDH, a more common abbreviation for aldehyde dehydrogenase.

c), Table 4-2 title should include "and mice".

d), Table 4-2: the footnote explanation on incidence is unclear (especially the second sentence).

e), I know that it is a lost cause but p 20, line 3: nouns etc have gender, animals have sex! (regardless of the dictionary definitions!!)

f), Is there a discussion of the MTD of EGBE under the various experimental exposures? The comment of low survival in the male mice at 125 and 250 ppm "which may have been due to carcinogenic effects in the liver" (no autopsy??) seems a distracting lead-in to the next sentence; viz, "A high rate of hepatocellular carcinoma was found in these exposure groups". Please resolve.

Michael Pereira

The Review of the noncancer hazard is very logical, clear and easy to follow except for the justification of the use of hemisiderin as the critical effect. With respect to the cancer hazard, the Review places too much emphasis on non-statistically significant and very weak responses, although the overall conclusion that EGBE does not pose a carcinogenic hazard to human is correct.

There is also too much redundancy, speculation, extraneous information, and assumptions in the discussion of the cancer hazard.

Andrew Salmon

In general, the review is well laid out, logical and clear in its presentation of the evidence and of the Agency's interpretation of that evidence in deriving the RfC and RfD. Although I have some differences noted below, the document as a whole is thorough, objective and logically organized. A limitation of the approach presented, which should be addressed in the final version of the document, is the failure to adequately consider the

	respiratory system effects (hyaline degeneration of the olfactory epithelium) observed in the NTP long-term study as a possible endpoint for derivation of an RfC. This is an important alternative to the hematological effects and related sequelae, which is unlikely to show the large disparity in sensitivity between rodent and humans reported for the latter responses.
Gregory Travlos	Overall, the review of the literature was thorough and presentation of the available data was clear and objective.
Rochelle Tyl	In this reviewer's opinion, the Toxicological Review is logical and clear. The EPA has clearly and objectively represented and synthesized the extant scientific evidence for both the cancer and non-cancer hazard.
D. Alan Warren	Yes, the Toxicological Review is a well-written, logically-organized and objective interpretation of the state-of-the-science regarding EGBE. It accurately reflects the increased understanding of EGBE gained from targeted research studies conducted since posting of the 1999 IRIS assessment. It also effectively incorporates many of the suggestions made in external peer reviews of past position papers and technical reports. Particularly impressive is the length to which the Toxicological Review went to allow comparison of RfCs and RfDs derived using alternative "choices" to those ultimately selected (e.g., Figures 5-3 and 5-4). Doing so increases confidence in the newly-derived toxicity constants, particularly in their health conservatism.

(G2) Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

Fletcher Hahn

It was suggested during the review discussion that the incidence of hyaline degeneration of the olfactory epithelium of the nose of rats exposed in the two year NTP study be used to derive the point of departure for the RfC. The basis for the suggestion is the apparent similarity of the reactions of human volunteers and rats to inhalation of EGBE; nose and throat irritation in humans and accumulation of globules in the olfactory epithelium of rats. This is a reasonable suggestion, but it rests on shaky ground.

Studies of six human volunteers exposed to butyl cellosolve (EGBE) indicated that eye, nose and throat irritation and headache were subjectively reported after exposure to 200 ppm for eight hours although there were no effects observed objectively (erythrocyte fragility, blood pressure, pulse rate). All involved agreed that 200 ppm was too high a concentration to breathe comfortably for eight hours. In addition, exposure to 100 ppm for eight hours (conducted days later) was judged to be nearly as uncomfortable as 200 ppm. The authors concluded that 100 ppm was appropriate hygienic standard for work room exposure (Carpenter et al 1957).

In the rats from the NTP study of inhaled EGBE (2000), the incidence of hyaline degeneration of the olfactory epithelium was significantly increased in all exposed groups of male and in females exposed to 62.5 or 125 ppm. Inflammation was present in the nose but at a much lower incidence and was not dose related. Hyaline degeneration is the accumulation of globules of homogeneous eosinophilic material in the cells of the olfactory epithelium of the nose as detected by microscopic examination. Similar changes were found in the mice, but the incidence was not significantly increased as in the rats. This finding is one seen on occasion in rodents exposed to vapors or gases (dimethylamine, Buckley el al 1985; pyridine Nikula et al 1995). It is also seen in aged rodents that are not exposed (St Clair 1992). The meaning of this finding is uncertain. It has been called an "adaptive" response (Buckley 1985) or an aging change (St Clair 1992). More recent studies have shown that the number and size of hyaline globules in the olfactory epithelium increase with increased and prolonged (32 week) exposure to cigarette smoke (Lewis et al 1994). The globules in smoke-exposed rats contain carboxylesterase (CE) and the amount of esterase in the globules increased with exposure. Although the CE is physically localized in the globules, the biochemistry of the localization is uncertain. Additional studies have shown that CE is an inducible enzyme in the olfactory mucosa. Rats exposed to pyridine at the threshold limit value concentration of 5 ppm, or at 444 ppm, 6 hr/day for 4 days showed the localization and amount of immunoreactive CE in olfactory mucosa (Nikula 1995). Quantitative densitometry showed a statistically significant, dose-related increase in the density of immunoreactive CE in olfactory mucosa of pyridine-exposed rats. These results indicate pyridine, and possibly other toxicants, can induce nasal CE, an enzyme not directly involved in the metabolism of those solvents, following low-dose, short-term inhalation exposure. The response is adaptive one. This same mechanism may have occurred in the rats exposed to EGBE.

Thus, in humans the reaction to 8 hr exposure to high concentrations of EGBE is irritation of the nose and throat, probably the result of mild inflammation. In rats, the reaction in the nose to many months exposure to a range of concentrations of EGBR is an adaptive

	response, not an inflammatory one.
David Jollow	As discussed above, I would like to see a more thorough characterization of BAA's MOA in damaging the rodent RBC, the fate of the damaged red cells (splenic sequestration or frank intravascular lysis), role of the liver Kupffer cells in removal of damaged red cells, the kinetics of the "toxic hit" (dependent on the concentration of BAA in a reversible fashion or the result of accumulation of injury over time[i.e., proportional to AUC of BAA rather than just concentration]). I would also like to see the hemosiderin deposition quantitated and chemically characterized by analytical techniques in addition to morphometric assessment by Prussian blue staining. Such characterization and quantitation would allow a more reliable and robust assessment of the shape of the D/R for the hemosiderin/neoplasia relationship.
Michael Pereira	There are no additional studies.
Andrew Salmon	None at this time.
Gregory Travlos	**a.**) Because there are pertinent hematology instrument methodology differences that result in the early EGBE-induced changes in mean cell volume (MCV) and hematocrit (Hct) being missed or misrepresented, I recommend the addition of the following reference: Ghanayem, B. I., Ward, S. M., Blair, P. C., and Matthews, H. B. (1990). Comparison of the hematological effects of 2-butoxyethanol using two types of hematology analyzers. *Toxicol. Appl. Pharmacol*. 106, 341-345. In this study, the authors demonstrated that a laser-based hematology analyzer could not determine the early EGBE-induced increases in Hct and MCV but these changes were detectable by impedence-based technology. Thus, instrument selection could substantially impact the performance/interpretation of the hematology evaluations as a pre-analytical source of variation. **b.**) Because hepatic iron (i.e., hemosiderin staining) was used as the critical effect for deriving the RfC and RfD, I suggest the review and possible addition of the following references: Smith, P. G. and Yeoh, G. C. (1996). Chronic iron overload in rats induces oval cells in the liver. *Am. J. Pathol*. 149(2): 389–398. Irving MG, Booth CJ, Devlin CM, Halliday JW, Powell LW. (1991). The effect of iron and ethanol on rat hepatocyte collagen synthesis. *Comp Biochem Physiol C.*100(3):583–590. Pietrangelo, A., Gualdi, R., Casalgrandi, G., Montosi, G., and Ventura, E. (1995). Molecular and cellular aspects of iron-induced hepatic cirrhosis in rodents. *J. Clin. Invest*. 95(4): 1824–1831.

Tsukamoto, H., Horne, W., Kamimura, S., Niemelä, O., Parkkila, S., Ylä-Herttuala, S., and Brittenham, G. M. (1995). Experimental liver cirrhosis induced by alcohol and iron. *J. Clin. Invest*. 96(1): 620–630.

Rothenberg, B. E., and Voland, J. R. (1996). beta2 knockout mice develop parenchymal iron overload: A putative role for class I genes of the major histocompatibility complex in iron metabolism. *Proc. Nat. Acad. Sci*. 93(4): 1529-1534.

Edwards, C. Q. (1999). Hemochromatosis. In Wintrobe's Clinical Hematology (G. R. Lee, J. Foerster, J. Lukens, F. Paraskevas, J. Greer, G. M. Rodgers, eds.). pp. 1056-1070. Williams & Wilkins, Baltimore, MD.

Britton, R.S. and Bacon, B.R., 1994. , Role of free radicals in liver diseases and hepatic fibrosis. *Hepatogastroenterology* **41**: 343–348.

Tector A. J., Olynyk J. K., Britton R. S., Janney C. G., O'Neill R., and Bacon B. R. (1995). Hepatic mitochondrial oxidative metabolism and lipid peroxidation in iron-loaded rats fed ethanol. *J. Lab. Clin. Med*. 126: 597-602.

Olynyk, J. K., Hall, P., Reed, W., Williams, P., Kerr, R., MacKinnon M. (1995). A long-term study of the interaction between iron and alcohol in an animal model of iron overload. *J. Hepatol*. 22:671-676.

Bomford, A., and Williams, R. (1976). Long term results of venesection therapy in idiopathic haemochromatosis. Quarterly Journal of Medicine 45, 611-23.

Niederau, C., Fischer, R., Sonnenberg, A., Stremmel, W., Trampisch, H. J., and Strohmeyer, G. (1985). Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N. Engl. J. Med*. 313(20):1256-1262.

Niederau, C., Fischer, R., Purschel, A., Stremmel, W., Haussinger, D., and Strohmeyer, G. (1996). Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *Gastroenterol.* 110(4):1107-1119.

Tiniakos, G. and Williams, R. (1988). Cirrhotic process, liver cell carcinoma and extrahepatic malignant tumors in idiopathic haemochromatosis. Study of 71 patients treated with venesection therapy. *Appl Pathol*. 6:128-138.

Deugnier YM, Guyader D, Crantock L, et al. (1993). Primary liver cancer in genetic hemochromatosis: A clinical, pathological, and pathogenetic study of 54 cases. *Gastroenterology*. 104:228-234.

Fellows, I. W., Stewart, M., Jeffcoate, W. J., Smith, P. G., and Toghil, P. J. (1988). Hepatocellular carcinoma in primary haemochromatosis in the absence of cirrhosis. *Gut*. 29(11): 1603–1606.

Rochelle Tyl

I am not aware of any extant additional studies which should be considered in the assessment of the cancer and non-cancer health effects of EGBE.

D. Alan Warren

While recognizing the Toxicological Review is not intended to be a complete treatise on EGBE's toxicity, the issue of thrombosis and infarction might warrant more than the single paragraph afforded it (p. 35). Granted, it is clearly not the most sensitive endpoint for dose-response assessment, but it is believed to occur secondary to intravascular hemolysis, has been examined at inhalation concentrations used in the subchronic and chronic NTP studies, and exhibits age-, gender- and species specificity. In addition, it informs the issue of intrahuman variability as a source of uncertainty, as patients with some hemolytic conditions are prone to thrombosis and infarction. Several published studies on the subject exist that are not referenced in the Toxicological Review (see a few listed below), among them that of Yoshizawa et al. (2005) that evaluated atrial thrombosis in NTP studies and identified EGBE as one of 13 compounds with increased incidences (20-100%) among high-dose groups.

Yoshizawa et al. (2005). Chemical-induced atrial thrombosis in NTP rodent studies. *Toxicol. Pathol.* 33(5):517-32.

Ramot et al. (April 2007). Age and dose sensitivities in the 2-butoxyethanol F344 rat model of hemolytic anemia and disseminated thrombosis. *Exp. Toxicol. Pathol.* 58(5):311-22.

Nyska et al., (1999): Disseminated thrombosis and bone infarction in female rats following inhalation exposure to 2-butoxyethanol. *Toxicol. Pathol.* 27(3):287-94.

On a related note, Udden and Patton (2005) is cited as the lone study in a very brief discussion of the mechanism of BAA-induced RBC hemolysis (see p. 45). These authors indicate that preliminary studies in their laboratory have shown the movement of phosphatidylserine from the inner to the outer leaflet of the lipid bilayer of rat RBCs incubated with BAA. This "externalization" of phosphatidylserine is associated with adhesion of RBCs to endothelial cells and the generation of thrombin, which is relevant given reports of disseminated thrombosis and infarction in EGBE-treated rats. Perhaps this information would be a reasonable addition to the existing mechanistic discussion, despite what appears to be only an abstract detailing the findings [Tamirisa et al., 2002. Annexin V binding and hemolysis of rat RBCs exposed to butoxyacetic acid, *Blood* 100:7b].

Lastly, for those readers not well informed on the role of Kupffer cells in hepatotoxicity and carcinogenicity, the following reference provides an outstanding overview: Roberts et al. (2007). Role of the Kuppfer cell in mediating hepatic toxicity and carcinogenesis. *Toxicol. Sci.* 96(1):2-15. The publication is an outgrowth of a symposium held at the Annual Meeting of the Society of Toxicology in 2006. The article suggests that Kupffer cell activation may initially be protective and become injurious only with continued and higher dose exposure. This obviously has potential dose-response implications for EGBE-induced liver cancer that should be taken into consideration.

(G3) Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Fletcher Hahn

Data to support the mode of action for mouse liver tumors should be strengthened.

A) The amount of iron in the liver should be measured. This would be a more precise indication of hepatic Fe than scoring of Fe-staining pigment. A dose response curve could be developed.

b) The doses required for triggering the initiation and promotion of cancer in liver cells by reactive oxidant species in both rodents and humans should be determined. (although I am unsure how to do this)

David Jollow

Specific research needs for risk assessment purposes include: definition of the red cell age susceptibility to BAA, definition of the MOA of the toxic insult(s) to the red cell with definition of dose metrics; contribution of loss of "sidedness" in the phospholipid composition of the outer leaflet of the red cell membrane to the premature loss of red cells from the circulation and the possibility that this may induce microvascular thombosis and infarction; role of the spleen *vs* the liver in the removal of damaged red cells and effects on haptoglobin and hemopexin levels and saturation during chronic exposure to EGBE; time and dose response relationships for hemosiderin deposition in the liver as a whole and in specific cell types; MOA and "dose"/response relationships for hemosiderin in hemoangiosarcoma development;

Specific experimental series include use of ^{51}Cr-tagging and/or fluorescent dye-tagging techniques to assess the immediate fate of the BAA-damaged red cells after *in vitro* incubations followed by reintroduction to isologous rodents (spleen vs liver vs generalized deposition throughout the vascular bed). Pretreatment of the rodents with clodronate liposomes may be used to determine the contribution of splenic vs liver macrophages. Flow cytometry studies with annexin binding will permit definition of the EC_{50} for phophatidylserine exposure after BAA, which may be directly compared with ^{51}Cr-EC_{50} for hemolytic activity, as determined above. As noted, this is of particular relevance for human health considerations interest in that microvascular infarction and thrombosis of the type proposed by Ezov et al., may occur as a consequence of occupational exposure to EGBE. The *in vitro* exposure/*in vivo* assessment of fate, type of approach allows concentration/hemolytic response definition and parallel assessment of biochemical change(s) in the red cell under directly comparable toxic conditions. Definition of the acute cellular changes can then allow specific probing of events during chronic exposure and perhaps lead to the recognition of key events that favor hemosiderin or other iron deposition in places that are crucial for localized ROS generation and toxicity.

Michael Pereira

General Question 3 discussed at the meeting.

Response:

A very important research need that would greatly increase the confidence of using hemisiderin as the critical effect is the determination of the dose and temporal relationship between EGBE induced hemisiderin and its induction of hemolysis.

Andrew Salmon

Although the effects of EGBE on animals, especially laboratory rodents, have been extensively studied, there is an intrinsic lack of information on the nature and dose response of effects seen in humans following moderate exposures. Laboratory studies of human erythrocytes, and case reports of human poisoning, are sufficient to demonstrate the humans are relatively insensitive to the hemolytic effects which are the critical effect for toxicity in rats, mice and rabbits. A few case reports and the original volunteer studies by Carpenter et al (1956) indicate other less severe responses, in particular sensory or respiratory irritation, but these are in general poorly characterized. Occupational studies have not been especially revealing so far, but there would appear to be a case for further studies in humans, especially studies using rigorous epidemiological methods and addressing mild effects which might not be a major concern in an occupational context but would be unacceptable in a community exposure situation.

Gregory Travlos

Since there is an overriding difference in erythrocyte sensitivity to EGBE-induced hemolysis between laboratory rodents (rats and mice) and humans, species differences in erythrocyte membrane physiology should be investigated and or reviewed.

Rochelle Tyl

It is clear (at least to me) that the missing "link" in the EPA Human Health Assessment of EGBE is long-term human inhalation (or oral) studies of EGBE. The short-term human volunteer studies of inhaled EGBE (and other chemicals of interest) were performed at Bushy Run Research Center by Dr. Carpenter and others at another time. It is highly unlikely that the IRB (Institutional Review Board for human studies) or IACUC (Institutional Animal Care and Use Committee for animal studies) committees would even consider human exposure studies of a known reproductive toxicant such as EGBE (or even anything else). It is important to note that in Carpenter's studies, humans (both men and women) experienced sensory respiratory irritation at both 100 and 190 ppm.

D. Alan Warren

Given the paucity of data on the mechanism(s) by which BAA induces RBC hemolysis, this would be an informative line of research, particularly if it addressed whether the mechanism(s) was conserved across species. Such research would also inform the issue of sensitive subpopulations, potentially impacting the uncertainty factor for intrahuman variability in RfC/RfD derivation. One outstanding question is whether Udden and Patton's laboratory pursued the mechanistic issue of phospholipid externalization after preliminarily reporting the externalization of phosphatidylserine by BAA in rat RBCs *in vitro.*

The experimental evidence supporting the hypothetical mechanism by which EGBE

produces hemangiosarcomas and hepatocellular carcinomas is discussed on p. 55. Based on this discussion, there are gains to be made by investigating the role of reactive oxygen species (ROS) in modulating gene expression specifically within endothelial cells and hepatocytes of male mice (and perhaps humans), the cell types that undergo neoplastic transformation. This line of research, coupled with *in vivo* studies designed to quantify the internal threshold doses that must be met to progress from one key precursor "event" in the mechanistic sequence to another (e.g., RBC hemolysis → hemosiderin deposition → ROS production or cytokine/growth factor release) would significantly enhance the EGBE database. In addition, determining the relative susceptibility of endothelial cells and hepatocytes to oxidative damage would be informative.

(G4) Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Fletcher Hahn

The key sources of uncertainty have been addressed fairly well. Table 5-15 is a helpful overview. The discussions of uncertainty in sections (5.1.3, 5.2.3) should be reviewed and expanded for clarity. Section 5.3 is much clearer.

David Jollow

With the exception of assumption of a direct lysis within the circulation as the MOA of BAA-induced hemotoxicity, I have no problems with the presentation in this section. The use of a BMD approach appears well justified based on the available biological information.

The selection of a UF of 1 for rodent to human extrapolation seems excessive and needs a more detailed specific justification. The available clinical "overdose", and epidemiology data (Section 4) suggests that humans are insensitive to EGBE-induced hemotoxicity. This insensitivity is supported by the comparative *in vitro* studies (although I am not comfortable with the "direct lysis" endpoint). Collectively, the clinical and experimental experience point to a relative resistance factor for humans of at least 10, suggesting that a UF significantly less than 1 would adequately safeguard humans. The UF of 10 for human variability also needs a more detailed justification. As noted (pp 86-87), human studies do not point to enhanced susceptibility in the elderly or in several red cell deficiencies. The rodent studies of Ghanayem et al (TAP 91:222-234, 1987) suggest toxicokinetic explanations rather than toxicodynamic effects. [An additional contribution not proposed by the authors is that the younger animals are rapidly growing and have a correspondingly rapid expansion of their red cell mass. The mean average age of their red cells is thus lower than that of the older animals. If (as suggested elsewhere) younger red cells are more resistant, the younger animals would of necessity appear more resistant to the hemotoxicity of EGBE].

The discussion on methods of analysis is outside my area of expertise.

Michael Pereira

The UF for variation in sensitivity within human populations (UF_H) should be 1 and not 10 and for interspecies variation (UF_A) should be 0.1 and not 1. Although the Document states numerous times that humans are much less sensitive to hemolytic effects of EGBE and that there would not be sensitive human populations, it still uses a UF_A equal to 1 and a UF_H equal to 10. These values are not consistent with the text. A UF_A equal to 0.1 and a UF_H equal to 1 would be much more consistent with the text of the Review.

Andrew Salmon

The discussion of uncertainty is thorough and lays out the assumptions used in the derivation of the RfC and RfD accurately and clearly as far as the issues addressed in the derivation are concerned. The consideration of alternative endpoints not related to the hematological effects in rodents has not been addressed.

Gregory Travlos

The identification and characterization of the sources of uncertainty were clearly identified and described. I may not agree with all the choices (for example, use of Kupffer cell hemosiderin accumulation versus erythron decreases (e.g., erythrocyte count) as the critical effect; the use of male data when females animals appeared to be more sensitive (to the erythron effects).

Rochelle Tyl

One of the finest, most clear and succinct summaries of the uncertainties in any risk assessment is found in Chapter 5 of this EGBE in Table 5-15 (p. 105). For each consideration, the table indicates the potential impact of its use (to increase or decrease the risk estimation), the decision made in the document, and the justification for the decision. This summary table, and the text in Chapters 5 and 6, more than adequately discuss the sources of uncertainty. The choices, assumptions, and the impact of the various uncertainties have been clearly, objectively, and transparently described, and the consequences evaluated. The biggest uncertainty is the animal to human extrapolation, whether the key events in the MOA proposed for rodents: forestomach accumulation of acidic HAA, leading to irritation, leading to cytotoxicity, leading to compensatory cell proliferation, leading to forestomach tumors (in female mice), **and** RBC hemolysis leading to hemosiderin accumulation in the Kupffer cells of the liver (in male rats and mice), leading to oxidative stress, leading to cell apoptosis and compensatory cell proliferation, leading to tumorigenesis (only in male mice), are relevant to humans in both a qualitative and quantitative sense, and whether these adverse effects are likely or not likely (rationales provided support that it is not likely) to occur in humans at the RfD or RfC. The impact of the uncertainty on the assessment has been clearly, transparently, and objectively described, and the values for the RfD and RfC under different aspects of uncertainty have been calculated and discussed. There was consensus at the meeting that the hemosiderin deposition, per se, is not toxic (in fact, it is likely protective), but it may be a useful biomarker. However, it is not really ever quantified. RBC count and/or hemolysis is continuous, quantifiable, directly related to MOA, and may be a better biomarker and/or POD.

D. Alan Warren

The discussion of uncertainty surrounding RfC/RfD derivation is a strong suit of the Toxicological Review. The discussion is comprehensive and "choices and assumptions" are transparently and objectively described and supported by an exemplary section 4. As mentioned in my response to general charge question no. 1, the Toxicological Review is particularly impressive in its extensive effort to qualitatively and quantitatively present the impact of alternative "choices" on the derivation of toxicity constants. To some extent, the presentation of uncertainty can be seen as evidence that the precautionary principle can remain intact while deriving a mechanistically driven set of toxicity constants.

As for section 6 (Major Conclusions in the Characterization of Hazard and Dose Response), it would be reasonable to conduct a margin of exposure-type analysis for EGBE in which maximum inhalation concentrations and oral daily doses encountered by humans are compared to the newly-derived RfC/RfD values. Such an analysis, in which Hazard Quotients and Hazard Indices are computed, was previously published in USEPA's proposed rule removing EGBE from the Hazardous Air Pollutants list (Federal Register, Vol.68, No. 225, November 21, 2003).

(A) Inhalation reference concentration (RfC) for EGBE

(A1) The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Fletcher Hahn

A.1.Response: The selection of the NTP (2000) 2 yr inhalation study as the principal study is scientifically justified. There are no reported studies of humans exposed for months or longer, the selection of an animal study is necessary. The NTP study has a number of positive attributes. It is the only chronic study available and has both hematology and histopathology endpoints. In addition, it has factors (more dose groups, large exposure groups, two species) that aid in more precise estimates of the RfC. In addition, NTP studies are carefully planned, executed, reviewed and reported adding to confidence in the data base. The study has not been described in much detail, but the original report is readily available through the NTP website.2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

David Jollow

The selection of the two year inhalation study by the NTP as the basis for the chronic inhalation RfC is scientifically justified. The study has been adequately described in the document. No additional/alternate studies are identified for consideration.

Michael Pereira

The NTP 2-year inhalation is very appropriate and justified as the basis for the chronic inhalation RfC.
The study for the most part is well and objectively described with the following points that need to be addressed and clarified:

a) Page 25, Line 7. Giving the survival for only the highest two treatment groups is meaningless without the value for the control group, unless it is zero. Please correct.
b) Page 28, Lines 20-25. The first sentence is not clear since it implies that there were neoplastic effects observed in female rats when there were not. The results of the 2-year bioassay in rats and mice should not be combined but rather reported separately. The first sentence of the paragraph should read "At the end…….no significant neoplastic effects were observed in male and female rats. However, a non-significant level of combined incidence of benign and malignant ……was observed, .ie., 3/50, 4/50, 1/49, and 8/49.
   Note: on Line 22 the and/or should be and.

c) Page 28, Line 26 and 27. Delete "may have been…….carcinogenic effects in the liver."
   Hepatocellular carcinomas are not lethal and since they were also found in a significant number of control mice (10/50) there should also have been death in this group. Since the number of mice supplying data in all treatment groups is reported as

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50, the actual mortality in the different groups should be given including when they occurred. As well as the reason why the mortality did not reduce the number of evaluated mice.

d) Page 28, Line 29. What statistical test was used to get a p-value <0.01? The data does not appear to be statistically significant.

e) Page 28, Line 30. Delete "However"

f) Page 29, Lines 1-4. This sentence is not true since female mice had the greatest EGBE reduction in body weight therefore a maximum tolerated does was used. It is more likely that the effect in male liver was not significant.

g) Page 29, Line 11 and Lines 12-15. Delete on Line 11 "While" and on Lines 12-15 from "the incidence of……hyperplasia." These tumors were increased in male mice.

Andrew Salmon

This study appears to be the best choice as the critical study for derivation of the RfC, being of sound design, adequate size and duration, and including exposure by the relevant route. NTP studies also benefit from thorough and objective analysis and reporting. The study description in the document is clear and accurate. There is a background issue with the selection of the test species in that since humans are clearly less sensitive than rodents to the critical effect in the NTP study (hemolysis and its various chronic sequelae) the choice of test species in this case could be applauded in that evidently the most sensitive test species has been selected, in accordance with the guidelines. On the other hand the choice of a rodent study could be criticized in that since humans are substantially less sensitive than rodents to the EGBE-induced hemolysis, they could be prone to other effects which are masked in the rodent study. If this is fact the case, the calculated RfC and RfD would be protective, but not predictive of the kind of toxic effects expected in humans if the RfC or RfD are exceeded: this might present a practical problem for risk management and mitigation. There does not seem to be any adequate alternative to the approach taken here unless more thorough epidemiological studies are eventually undertaken.

Gregory Travlos

Since I am not qualified to comment on all aspects of the chemical-specific charge questions, responses will be limited to questions, or parts of questions, that I feel my comments would be appropriate.

I believe the aforementioned study was adequately justified and appropriately represented.

Rochelle Tyl

The selection of the two-year inhalation bioassay in rats and mice by the National Toxicology Program (NTP, 2000) was appropriate and fully justified. It was clearly, fully, transparently, and objectively described in this document. I am unaware of any other study which should even be considered, let alone selected, as the principal study. The 91-day drinking water studies in rats and mice (with adjustments from subchronic to chronic) should at least be considered.

D. Alan Warren	The selection of NTP (2000) as the principal study has been scientifically justified, and transparently and objectively described in the Toxicological Review. No other studies, to my knowledge, are better suited as the basis for RfC derivation.

(A2) The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Fletcher Hahn

A.2. Response: The selection of hemosiderin staining in the liver is an appropriate critical event for liver tumors in male rats exposed to EGBE. The hemosiderin is probably not the form of iron that induces reactive oxidant species, but hemosiderin is most likely correlated with the body burden of iron. (*see earlier comments on studies to develop a dose response for iron effects in the liver and later comments on the need to use male mice as the species*)

David Jollow

The relationship between iron overload in tissues and increased incidence of fibrosis, cirrhosis, and tumors secondary to tissue iron overload is well documented for conditions such as hemochromatosis and after parental iron loading in β-thalassemia. Hemosiderin deposition may be considered to reflect the extent to which EGBE causes hemolytic episodes in excess of the normal red cell elimination capacity of the spleen and hence the extent to which redox-active iron is deposited in tissues. Whether hemosiderin itself is the source of reactive oxygen species (ROS) leading to hemangiosarcoma or whether it is a surrogate for redox-active iron deposited directly in the target cells is not clear, but does not weaken the use of hemosiderin as a critical effect for risk assessment purposes. The present use of the relationship is clearly relevant and reasonable. The criteria and rationale are adequately and objectively described.

Major deficiencies in the use of hemosiderin as the critical effect lie in its method of quantitation, lack of definition of its time-profile of accumulation, and of "dose"/response relationship(s) between hemosiderin levels and toxic events.
Alternate end points such as decrease in hematocrit (Hct) also have problems. Decrease in Hct could be used but it should be appreciated that Hct post EGBE exposure is the balance between enhanced loss of red cell mass and replacement by immature red cells etc., and by increased erythropoiesis. Thus the fall in Hct is a measure of both the toxic insult and the body's compensatory mechanisms, and not a direct simple assessment of the hematotoxicity of EGBE. Further, if the mechanism of removal of BAA-damaged red cells during chronic EGBE is splenic sequestration (i.e., post the relatively large decrease of the initial doses) rather than a "true" intravascular lysis, the fall in Hct may be a misleading indicator of the toxic potential of EGBE administration. Normal and "moderately" enhanced sequestration is associated with transferrin-mediated iron transport in a non-redox active form. Overflow protective mechanisms include plasma haptoglobin to sequester "free" hemoglobin and hemopexin to take up "free" heme". Deposition of redox-active iron in target tissues would be enhanced in situations where the transferrin/haptoglobin/hemopexin protective mechanisms are exceeded.

It seems reasonable to expect that at toxic EGBE dose levels, the decreased Hct of chronically-treated animals is associated with both maximal transferrin/haptoglobin/hemopexin sequestration of iron and its "excessive overflow" (by whatever mechanism). Thus the fall in Hct, per se, would be a measure of both normal and abnormal processes and not a direct estimate of "excessive overflow", and hence the

extent to which red cell iron turnover exceeds the body's capacity to sequester it away from deposition in ROS-generating form(s) in target tissues. If the neoplastic or other toxic potential of EGBE is, as postulated, secondary to redox-active iron deposition in tissues, the critical effect should reflect "excessive overflow" and not just total red cell loss (i.e., decreased Hct).

For risk assessment purposes, measurement of haptoglobin and hemopexin levels and saturation during chronic EGBE exposure and a more selective and analytical determination of the iron overload might resolve difficulties in selecting a critical effect.

Since the use of both hemosiderin and decreased Hct appear to have deficiencies as "critical effects", it is suggested that the utility of both parameters for POD purposes be discussed and illustrated in the review.

Michael Pereira

Hemosederin is not a critical adverse effect but rather might be a biomarker for exposure. The adverse effect is hemolysis. The quantitative if any relationship between hemosederin and hemolysis is not established. In fact the discussion of the two effects indicates that they do not correlate, especially the fact that hmosederin increased with time while the hemolytic indices did not. This would suggest that the rate of accumulation of iron (hemolysis) does not change (increase) with time but rather the extent of hemosiderin staining does increase. Hence, hemosiderin staining could be present after two years of exposure without any significant adverse clinical effect of hemolysis; there could be a slight subclinical increase in hemolysis without any significant deleterious health effect. This should be discussed in full at the meeting.

Furthermore as discussed at the meeting in RTP, the hyaline degeneration of olfactory epithelium in both sexes of rats is a significant effect of EGBE and should be further discussed (Page 25, Lines14-18). This additional discussion should include a comparison of the resulting RfC using olfactory degeneration to the RfC resulting from hemolysis or for that matter hemisiderin.

Andrew Salmon

If one is going to be concerned about hemolysis and its various chronic sequelae as an endpoint in defining the RfC, then the choice of hemosiderin staining is an excellent indicator. The document clearly identifies this as a recognized consequence of chronic hemolysis, and a precursor of both cancer and non-cancer lesions in the rodent liver. The mechanistic basis of these assumptions is laid out in convincing detail. Moreover the quality of data in the NTP (2000) study appears to be good, although one might have hoped for a continuous measure of hemosiderin accumulation rather than a quantal (present/absent) evaluation. It also has the advantage, as a systemic endpoint, of being susceptible to route-to-route comparisons in support of the RfD as well as the RfC, without concern for possible portal-of-entry effects.

On the other hand, there are also suitable direct measures of the hemolytic impact of EGBE (hematocrit, MCV etc) which are equally sensitive and could also be used as a critical endpoint in deriving the RfC: in fact this was the approach used in the previous (1999) toxicological review for EGBE. Since all the available indicators have some limitations in measurement and/or biological interpretation, it would be preferable to fully evaluate all the possible hemolysis-related endpoints rather than to concentrate most of the effort on hemosiderin deposition. Since these different endpoints appear to indicate

similar points of departure for the RfC derivation, the overall synthesis of all these data would strengthen the confidence in the value derived.

However, choice of any endpoint related to the hemolytic effect of EGBE is problematic in the context of identifying an RfC or RfD since there is evidence that humans are considerably (50 -150 fold?) less sensitive than rodents. Indeed the whole basis of the argument that the liver tumors observed in rodents are not relevant to human health risk is based on the finding that hemolytic doses are unlikely to be achieved in humans by inhalation, and are not always even reached following accidental or suicidal poisoning by the oral route. It is therefore rather confusing to nevertheless use this endpoint as the critical effect for deriving a health protective level for non-cancer effects. This can perhaps be justified pragmatically on the grounds that the levels so derived, with the conservative assumption of equivalent toxicity between species, is health protective against the possibility of either cancer of non-cancer effects in humans. However, it seems that this particular issue has not been explored fully in the document: in particular any other possible endpoints besides those based on erythrocyte fragility, hemolysis and related markers such as hemosiderin are dismissed without any detailed quantitative analysis being presented.

One effect which should be considered is the hyaline degeneration of the olfactory epithelium observed in both male and female rats by NTP (2000). This has the advantage of being apparently unrelated to the hematological effects, and also parallels the finding of respiratory irritation in human studies by Carpenter et al. (1956) and in some case studies. On the other hand this is clearly, as described by NTP (2000), a relatively mild effect, but it is not obvious why this was summarily dismissed as “adaptive”. It is at the mild end of a spectrum of responses seen following chronic exposure of rodents to a number of inhaled chemicals with irritant effects: in the case of more severe irritants such as reactive aldehydes the response progresses to necrosis, hyperplasia and/or metaplasia. As such it can reasonably be regarded as an adverse effect. In this case the incidence shows a clear dose-response with increasing EGBE concentration. This is often done by means of individual scores, which can be used as a pseudo-continuous variable in benchmark analysis. Although there are some factors needing to be considered with regard to time- and interspecies extrapolations for this type of portal-of entry effect, a simple benchmark dose analysis using applied concentration as the dose metric suggests that a point of departure based on this endpoint would not be enormously different from that based on the hemosiderin response (see attached supplementary material showing output of such a benchmark analysis). It is unfortunate that the only data readily available are quantal incidences: it is often much more informative to provide severity information as well.

Gregory Travlos

I am not sure increased hemosiderin staining in Kupffer cells have been clearly established as a critical effect. The Nyska et al. (2004) retrospective report demonstrated an apparently strong association ($p < 0.001$) between hemangiosarcoma and hemosiderin accumulation, but the number of studies was small (6 studies) and two of the studies (33% of the study set) had hemosiderin increases but no hemangiosarcoma development.

While hemosiderin and ferritin are considered nontoxic storage forms of iron, iron released from storage sites may react with hydrogen peroxide to form reactive oxygen species (ROS) (Edwards, 1999). Iron-catalyzed ROS injury has been demonstrated in liver (Britton and Bacon, 1994; Tector et. al., 1995; Olynyk et. al., 1995). It has been

reported that humans that are hemochromatosis homozygotes, and who have liver cirrhosis, develop hepatocellular carcinoma, but not hepatoma, in approximately 10 to 30% of the affected patients (Niederau et. al., 1985, 1996; Tiniakos and Williams, 1988; Deugnier et. al., 1993). Hepatocellular carcinoma is about 200-fold more common in hemochromatosis patients as in unaffected individuals (Niederau et. al., 1985). There are a few patient reports of hepatoma in cases where cirrhosis was not present (Fellows et. al., 1988). To my knowledge, hemangiosarcomas have not been related to iron overload in humans (or other species). As noted in the report (section 4.7), the iron accumulation in hemachromatosis patients occurs in liver parenchymal cells, and to a lesser extent, macrophages (e.g., Kupffer cells); with iron overload in rats, iron accumulation also appears to preferentially affect hepatocytes with Kupffer cells affected to a lesser extent (Smith and Yeoh, 1996).

As noted in the report (section 4.7), humans that develop hepatocellular carcinomas, as a consequence of hemochromatosis, usually demonstrate liver cirrhosis, thus, reflecting the chronic nature of the disease. In laboratory rodents, iron overload demonstrates a similar fibrotic process. For example, dietary iron overload in rats results in increased collagen gene expression, activating collagen production within lipocytes and leads to hepatic fibrosis (Irving et. al, 1991; Pietrangelo et. al, 1995; Tsukamoto et. al, 1995). Smith and Yeoh (1996), reported that iron overloading of the liver, as a means of inducing liver damage over an extended period, promoted oval cell proliferation. Rats fed a 2% carbonyl-iron-supplemented diet for 3 or 6 months demonstrated extensive periportal iron deposits in hepatocytes and some Kupffer cells; iron deposition was less pronounced pericentrally. Small oval-like cells, morphologically and immunocytochemically similar to CDE-derived oval cells, were identified and quantified. Oval cells first emerged periportally and subsequently in small tracts or foci nearer central regions and stained positively for alpha-fetoprotein, pi-class glutathione S-transferase, and the embryonic form of pyruvate kinase. They contained very few iron deposits and were classified as iron free. The major difference between CDE- and iron-overload-derived oval cells was that the latter were negative for transferrin. They concluded that cellular changes occurring in iron-overloaded rat liver are similar to those observed in rats placed on a hepatocarcinogenic diet and in rats chronically exposed to alcohol.

A β_2 microglobulin knockout mouse model of hemochromatosis has been reported (Rothenberg and Voland, 1996). The authors tested the hypothesis that animals lacking the β-analogous promoter gene would experience upregulated iron absorption and iron overload. They demonstrated age-dependent, increased hepatic iron in the β_2 knockout mice and some mice developed sinusoidal fibrosis, hyperglycemia and hepatoma (hemangiosarcomas were not observed).

Thus, it appears iron overload in rats and mice result in some level of hepatic fibrosis (as has been reported for humans). For the studies used in this report, there was no increase in hepatic fibrosis reported. Thus, the clinical significance of the Kupffer cell hemosiderin used as the “critical effect” in this report could be questioned. It is clear, however, that hemolysis is a direct toxic effect of the EGBE metabolite, butoxyacetic acid (BAA). It would seem that the hemolysis would be the critical effect and that the accumulation of hemosiderin in Kupffer cells would be simply a secondary response to the increased erythrocyte turnover.

Rochelle Tyl

The EPA selected hemosiderin staining (intracellular accumulation of iron from

hemolysis) in the liver of male rats (which interestingly do not develop liver tumors from EGBE exposure) as the critical effect, because it is considered by EPA as a precursor to an adverse effect (hepatocellular tumors in male mice). The EPA has made a strong case for use of this "critical effect" since it is necessary (but not sufficient) for the subsequent tumor formation, and for the use of the rat (rather than the mouse) because the hemosiderin staining occurs at a lower EGBE dose in male rats than in male mice. The use of RBC count or hemolysis might be a better approach, and the females appeared more sensitive to hemosiderin deposition than did the males. The criteria, justification, and rationale for this effect in the more sensitive species and sex have been thoroughly, clearly, transparently, and objectively presented in the document. It is this reviewer's considered opinion that it is a defensible best choice for the critical effect and resulted in the lowest, most protective calculated RfD and RfC.

D. Alan Warren

The selection of hemosiderin staining in the liver of male rats as the critical effect, as opposed to one of several hematological parameters, has been scientifically justified. The basis for its selection is transparently and objectively described and includes the following: 1) its sensitivity to EGBE (a LOAEL of 31 ppm was identified in rats of both sexes, although Table 5-2 does not indicate a statistically significant difference from the control incidence at this concentration; indeed, the NTP Technical report indicates that hemosiderin deposition was increased relative to chamber controls at 62.5 and 125 ppm in the 2-year study); 2) its clear progression, as indicated by dramatic changes in incidence with continuing exposure, coupled with the lack of progression of hematological endpoints; 3) uncertainties surrounding what changes in hematological endpoints actually represent (toxicity vs. compensation), the mechanisms behind the changes, and their frequent lack of adherence to the fundamental tenet of dose-response (all of which preclude an informed decision as to which hematological endpoint is most appropriate); and 4) the recognition that all of the hematological endpoints discussed are considered precursors to hemosiderin deposition, a clear pathological finding with experimental evidence linking it to neoplastic transformation. The selection of hemosiderin deposition as the critical endpoint is further justified, in a regulatory sense, by the fact that it represents the lower end of the RfC range when compared to other potential endpoints (see Figure 5-3). It also reflects an acknowledgement of hemosiderin deposition as a key mechanistic step or unifying mechanism behind liver tumor formation independent of exposure route.

While I can support hemosiderin deposition as the critical effect, it is not without significant reservation. While the incidence of this effect is increased in male and female rats at 2 years and 31 ppm, the increase is not statistically significant (Table 5-2). By contrast, RBC count is significantly decreased at 31 ppm in male and female rats at 14 weeks (prior to any increase in hemosiderin deposition; NTP Technical Report, Table 3) and in female rats at 3 and 6 months (NTP Technical Report, Table 9). Thus, an argument might be made for RBC count as the critical effect on the basis of sensitivity alone. However, as shown in Table 5-3 of the Review, its use as such does not result in a more health conservative RfC. In addition, hemosiderin deposition is treated as a quantal response for risk assessment purposes, when in reality it is not an all or none proposition. This is particularly noteworthy given that severity scores for hemosiderin deposition were reported in the subchronic drinking water study, but not in the 2-year inhalation study. Furthermore, the severity of hemosiderin deposition was “minimal” regardless of oral dose.

(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.

Fletcher Hahn

A.3.Response: As modeling is not my area of expertise, I can not comment on the modeling approaches for determining the POD.

The benchmark response (10% extra risk of hemosiderin staining in the liver) is appropriate. However, the selection of male rats upon which to base the POD does not seem appropriate. Why shouldn't the species (male mice) that has been shown to have the critical effect correlated with the adverse (tumor) effect be used? This would be the logical choice from the scientific stand point. Male rats show the bench mark effect at the lowest exposure concentration and their choice will obviously result in a "more protective" RfC. My thought is that such choices would be more transparent if they were made at when the uncertainty factors are chosen.

David Jollow

The crucial role of hemotoxicity leading to iron overload of hepatic tissue is well justified indicating that a non-linear relationship exists between EGBE exposure and hemangiosarcoma incidence. The BMD modeling of hemosiderin staining is acceptable as the POD.

The methodological approaches for application of the POD are well presented. Their adequacy is outside my area of expertise

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Michael Pereira

The BMD modeling and the determine POD is appropriate and objectively applied. However, male rats were used instead of female rats. Since female rats are more sensitive and were used in the previous IRIS assessment, why were male rats used in the document?

Also, the document states that a NOAEL was not determined for EGBE in either male or female rats. However with respect to female and male rats, in the 14-week study a NOAEL for hemosiderin and RBC count was observated at 31ppm and in the 2-year bioassay, a NOAEL of 31ppm was also observed for hemosiderin.

Andrew Salmon

There is now an extensive literature demonstrating the superiority of BMD modeling over the more "traditional" NOAEL/LOAEL approach. This observation is sometimes qualified by adding restrictions such as "providing the data are adequate". It is worth pointing out that, providing the BMD procedure can be run at all, it is especially the preferred approach when data are inadequate, since it takes all the available data into account with appropriate statistical weighting. In those few cases where a BMD model cannot be used (free-standing NOAELS, all 100% responses, only one data point etc.) it may sometimes be possible to derive a number which can be described as a NOAEL or

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LOAEL, but the relationship of this number to the actual dose-response for that receptor, effect and compound is remote, if not downright mythical.

a. The application of BMD modeling in this report is clearly and completely reported, and follows the recommendations for application of this approach given by U.S. EPA and others.

b. This is the one point where I disagree with the choices made by the analysts for the EGBE document. The original guidelines for BMD analysis recommended that the $BMDL_{10}$ (i.e. lower 95 % confidence limit on the dose producing 10% response) be used by default as the POD. However, extensive experience with this techniques by various analysts (including myself, and others working for California and U.S. risk assessment groups) has shown that a better default choice for POD in analysis of quantal data in animal toxicity studies is the $BMDL_{05}$ (i.e. using a 5% response rate). This value is generally found to most resemble the NOAEL in well-designed and well-conducted studies, whereas a $BMDL_{10}$ typically approximates a LOAEL. It is therefore more appropriate to use the standard uncertainty factors (UFs) (UF_H, UF_A but not UF_L) with the $BMDL_{05}$.

This is of course a default recommendation and the analyst may choose a different response rate to set the POD depending on their analysis of the data. In the case of this EGBE report it appears that the 10% response rate was simply selected as a default, there being no particular attempt to justify its selection in the context of the specific data being analyzed.

c. The analysts also report derivation of PODs using the NOAEL/LOAEL methodology, but correctly point out that the BMD approach is superior. These are the two generally recognized ways of analyzing this type of data.

Gregory Travlos

Discounting the hemosiderin accumulation, since the female animals demonstrated the most sensitivity to the hematological effects, it would seem the use of the female gender would have been more appropriate?

Rochelle Tyl

Benchmark Dose (BMD) modeling was applied to the incidence data for hemosiderin staining in the rat liver in the NTP study, to derive the Point of Departure (POD) for the calculation of the RfC. It is this reviewer's considered opinion that BMD modeling is the best approach for determining the POD and was appropriately conducted, and clearly, transparently and objectively described in terms of its use, its results, and the consequences of the outcome. The benchmark response (BMR) selected for use in the POD, 10% excess risk of hemosiderin deposition (staining) in the liver (necessary but not sufficient), was justified (as necessary, but not sufficient for the downstream liver tumor outcome in male mice, but present at lower EGBE doses in the male rat). It was also clearly, transparently, and objectively described. I am not aware of any better alternative approaches for the determination of the POD, and I concur with EPA's choice of the BMD modeling.

D. Alan Warren

The data set to which BMD modeling was applied for determination of the POD is suitable for such an analysis. In this particular case, BMD modeling does represent the

best approach, as the BMCL is informed by response information from all dose groups compared to the LOAEL/NOAEL that is constrained to one of the experimental doses. The BMD modeling is objectively described and its conduct is transparent. Based on the Toxicological Review's narrative, Tables 5-6 and 5-7, and review of model parameters and output (Appendix B-8 to B-10), the modeling appears to have been correctly conducted, with any one of three potential model choices sufficing. In addition, the dose metric selected (i.e., AUC BAA) is appropriate, as the critical effect is more than likely a function of cumulative exposure rather than peak concentration. Comparison of the LOAEL/NOAEL and BMD/PBPK approaches was informative, with the comparable results increasing confidence in use of the latter. In addition, BMD modeling using an alternative endpoint (changes in RBC counts among rats of both sexes) and dose metric (Cmax BAA at 3 months) served to inform the debate over the most appropriate critical effect.

(A4) PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

Fletcher Hahn

A.4.Response: As modeling is not my area of expertise, I can not comment on the scientific justification of the PBPK modeling.

David Jollow

PBPK modeling for extrapolation from experimental animals to humans is well accepted as the preferred approach to assess tissue target dose of a toxicant. The models appear to have been appropriately utilized Difficulties noted by other reviewers in the selection of models and parameters need to be resolved and scientifically supported.

As noted above, an improved illustration and explication of the PBPK model for rodents and human would enhance the document.

Michael Pereira

The PBPK modeling is appropriate.

Andrew Salmon

The PBPK modeling approach used is typical of the current approach to this type of problem, and appears to have been well executed and described. It takes a fairly standard approach using measured values for key parameters where these are available. Although an extensive sensitivity analysis for the selected parameter values was not presented here, the fact that this analysis relies largely on published and peer-reviewed models is an advantage. While more could have been done in this document to validate the model structure and parameter values used, this is on the whole a reasonable way of addressing the extrapolation of the POD from test animals to humans, and certainly an improvement on the use of default uncertainty factors which is the obvious alternative. The analysis described is similar in general terms to the PBPK analysis presented in the previous (1999) toxicological review; however the conclusions drawn in the version are somewhat different with respect to the extrapolation of dose metrics from rats to humans. Without wishing to question the validity of the new analysis, it would be helpful to identify and explain the differences between this and the previous version.

It needs to be pointed out that if my earlier suggestion to consider the hyaline degeneration of olfactory epithelium endpoint, a "portal of entry" effect rather than a systemic effect like the hemosiderin deposition and hemolytic effects, were to be adopted, a different PBPK approach would be required. There are a number of models available in the literature which address the issues of deposition in various parts of the upper respiratory tract, for compounds with a range of properties (water solubility being a particularly important factor), including values appropriate for EGBE. These should be considered in any such analysis, particularly in preference to the earlier default RGDR calculation which has not proved to be as reliable as one might have hoped, relative to specific PBPK deposition and metabolism models incorporating chemical-specific information.

Gregory Travlos	*No comment provided.*
Rochelle Tyl	PBPK modeling, to extrapolate the POD from rats to humans, is the model of choice to perform species-to-species extrapolation when the appropriate data are available in both species and the assumptions are explicitly presented and discussed. The model was scientifically justified and fully, transparently, and objectively described. We have toxicokinetic data from the rodent and human (a rarity!), and they were appropriately represented and applied. The model assumptions, parameter values, and selection of dose metrics were also clearly presented and scientifically supported. It would have been useful to compare and contrast the discussion in this document with that in the 1998 document.
D. Alan Warren	Based on Table 5-3, Summary of PBPK models, it appears the model of Corley et al. (1994, 1997) is the only one capable of extrapolating between rats and humans. Corley et al.'s model is essentially a coupling of two models, one describing the disposition of EGBE and the other BAA. The model includes the introduction of EGBE via IV infusion, inhalation, ingestion and dermal absorption; the distribution of EGBE to the liver; the metabolism of EGBE to BAA solely in the liver following Michaelis-Menten kinetics; protein binding of BAA in the blood; and distribution of BAA to tissues, including the kidney where it is excreted via an active transport process in the urine. It is appropriate for the experimentally validated model of Corley et al. to be exercised to convert the BMCL for the RfC (based on AUC BAA in rats, for example) to a human equivalent concentration (HEC) or oral human equivalent dose (HED). Footnote 8 on p. 78 indicates that a review of PBPK models was conducted prior to their use in the 1999 EGBE Toxicological Review. Furthermore, the text on p. 78 notes that established EPA methods and procedures were used to review, select and apply the chosen PBPK models. It can therefore be assumed that any errors in model structure, parameterization or application would have been remedied prior to its use in the current context. My review of the basic components of the Corley et al. model, including the parameters listed in Appendix A, Table A-1, found nothing to suggest otherwise.

(A5) Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

- **A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., 101/2).**

- **Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).**

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

Fletcher Hahn

A.5. Response: *(These should be scientifically sound, not policy decisions.)*

UF_H – A default of 10 is conservative and accounts for the unknown response of infants and young.
UF_A – The factor of 1, reduced from the default of 10, is appropriate because there is a good database on the pharmacokinetic and pharmacodynamics. Four research papers have presented PBPK modeling of EGBE. Four additional research papers using in vitro and in vivo approaches have shown that humans are less sensitive than rodents by a large factor (40-150) does account for the striking difference in concentrations required for hemolysis in humans compared with rodent.

- The PBPK modeling does appear to be justified. At least four research articles have presented such models for EGBE in humans, rats and mice. The draft text is not clear on the definition of PK-UF.

- The PD-UF of 1 is justified. However, I do think that the striking difference in the sensitivity to EGBE hemolysis between humans and rodents should be taken into account. The draft text is not clear on the definition of PD-UF either.

David Jollow

As discussed previously, the UF values of 1 for rodent to human and of 10 for human variability need greater justification. I appreciate that there is a lack of human data in chronic exposure situations and that this may necessitate larger UFs. Of importance, if the

selection of a UF of 1 for rodent to human is based on an EPA policy decision to cover unknown uncertainties rather than on the available *in vitro* data and acute clinical observations, this should be clearly stated. Clearly, the absence of chronic exposure data for human is of concern and needs to be addressed. Please expand.

Michael Pereira

The UF for variation in sensitivity within human populations (UF_H) should be 1 and not 10 and for interspecies variation (UF_A) should be 0.1 and not 1. As state in the document mice, rats and other species are much more sensitive than humans to hemolysis by EGBE. Therefore, scientific evidence strongly indicate a UF_A of 1/30 (Page 59, Line 5: For example, in vitro study of RBCs indicated that humans are more than 150-fold more resistant to EGBE than rats). However, a UF_A of 0.1 would be acceptable.

Similar studies of RBCs from human children and elderly individuals were no more sensitive to EGBE than those from adults. Hence, an UF_H of 1 not 10 is recommended.

Andrew Salmon

- It is reasonable to replace the PK-UF component of UF_A with an appropriate PBPK model which includes a data-based extrapolation between the test species and humans. In this case the use of this model instead of an uncertainty factor (UF_A – PK) greater than 1 is justified and adequately defended in the document.

- In so far as this uncertainty factor is applied to the protection of humans from the hemolytic effect and its chronic sequelae, this is a reasonable choice from the point of public health protection: indeed, it could be considered conservative although this is not an argument for departing from this suggestion. The actual data in fact indicated a considerably lower sensitivity of humans to the hemolytic effect, so based on the data alone a UF_A of 0.1 or even 0.03 could be justified. However, this has various risks including the possibility of alternative endpoints becoming critical for humans, as noted below. The decision not to use UF_A values less than 1 is therefore a policy decision (with a valid basis and justification) and should be so characterized in the document. The issues relating to possible susceptible human subpopulations have been addressed in the document, and there do not appear to be any identifiable susceptibilities which undermine the proposal of a PD-UF_A of 1 for this class of effect.

  There is no particular reason to suppose that this assumption is health protective for other endpoints: there is no quantitative basis for such an extrapolation unless other plausible endpoints are actually evaluated quantitatively.

- If the hyaline degeneration of olfactory epithelium endpoint were to be addressed quantitative, it would be desirable to use an appropriate deposition related PBPK model rather than a default PK-UF_A. Since this is a portal-of-entry effect which probably does not depend on metabolism or distribution, a reduced PK-UF_H might also be justified. Interspecies extrapolation of the toxicodynamic effects in this case would need to be considered carefully by the analyst: there would not be an automatic presumption of greater human sensitivity to such an effect (particularly since this is a mild endpoint), but on the other hand the possibility of exacerbation or induction of asthma needs to be considered as a human variability factor for respiratory irritants, especially where children are exposed.

Gregory Travlos

No comment provided.

Rochelle Tyl

Comments on the selection of all of the uncertainty factors (Ufs) applied to the POD for the derivation of the chronic RfC (predominantly from Chapter 5.1.3, pages 86 ff).

- An UF(H) value of 10 was applied to account for the variability in sensitivity within the human population. This factor would cover potentially susceptible subpopulations (individuals with enhanced metabolism, decreased excretion of BAA, and/or individuals whose RBC membranes are more susceptible to lysis). However, it should be noted that in vitro assessments of RBC lysis from newborn, or elderly patients and patients with genetic blood dyscrasias, are <u>not</u> more sensitive to the hemolytic effects of EGBE. Perhaps a UF of 10 is too high (3?). In animal studies, older animals are more sensitive (perhaps due to older animals having older and therefore more vulnerable RBCs) than neonates, and females are more sensitive than males. Developmental toxicity studies in rodents do not indicate increased susceptibility in fetuses and/or neonates. However, there are no long-term human exposure studies in normal or predisposed individuals which drove the decision to use an UF(H) of 10. These arguments were clearly, transparently explained and scientifically supported.

- A PK-UF of 1 was selected to cover potential differences in pharmacokinetic parameters between the animal models and humans. Since there are good PK data in both animals and humans, an HEC (Human Exposure Concentration) was calculated using known animal blood levels and PBPK modeling data. It appears that the human is much less sensitive to the hemolytic effects of EGBE; therefore, a PK-UF of 1 was considered sufficient. However, humans <u>are</u> sensitive to respiratory irritation. These arguments were clear and transparent and scientifically supported.

- A PD-UF of 1 was selected to cover potential differences in pharmacodynamic parameters between the animal models and humans. The PD-UF of 1 was selected based on clear evidence that the human is much less sensitive to EGBE concentrations that cause hemolytic effects in human red blood cells than are the animals. The chosen PD-UF is sufficiently conservative; a value less than 1 is not as protective, since we have not evaluated all sensitive subpopulations of humans, long-term human exposures, humans of different ages, or humans with all blood dyscrasias; at the meeting, we were told that as a matter of policy, UF of less than 1 are not used. There is one concern by this reviewer: The parameters assessed in the in vitro human blood tests with EGBE, etc., included MCV (mean corpuscular volume), i.e., the size of the red blood cell (RBC) (page 74, lines 7-8 described the in vivo animal studies; page 71, lines 14-17 described the human in vitro blood studies). MCV is a sensitive parameter in vivo, since animals with reduced RBC counts exhibit larger MCVs, from release into the circulatory system of younger, larger, more immature RBCs from the bone marrow (and rarely from extramedullary hematopoiesis in the spleen or liver). This release cannot occur in vitro using just blood samples. It is likely that the authors meant that there was RBC membrane deformation and/or RBC swelling (and bursting) due to induced membrane fragility. This distinction must be explicitly made. The UF is appropriate and protective, and

was presented clearly and transparently, with strong scientific justification.

- A UF to account for extrapolation from subchronic to chronic exposure was not needed since the RfC was derived from a chronic study (page 87, lines 19-20). The suggestion to use the 91-day rat drinking water study would necessitate a UF.

- A UF to account for extrapolation from LOAEL to NOAEL was also not needed since this factor was one of the considerations in selecting the BMR of 10% increase in hemosiderin staining for BMD modeling. This 10% increase was considered "a biologically significant change" (page 87, lines 21-24).

D. Alan Warren

As mentioned in the response to general charge question no. 4, the discussion of uncertainty surrounding RfC derivation is a strong suit of the Toxicological Review. In my opinion, all of the uncertainty factors (UF) applied to the POD for RfC derivation are valued appropriately, scientifically justified, and transparently and objectively described. This includes the overall UF for interspecies extrapolation of 1 and both of its component parts (i.e., interspecies differences in toxicokinetics and toxicodynamics). The use of the experimentally validated PBPK model of Corley et al. for animal to human extrapolation obviates the need for any interspecies UF for toxicokinetic differences. As for an UF to account for toxicodynamic differences, any uncertainty stemming from the lack of chronic, human exposure data is offset by the decreased susceptibility of human RBCs to hemolysis as demonstrated *in vitro* and in acute poisoning incidents.

Despite my comments above, I believe that some consideration should be given to reducing the intraspecies UF from 10 to 3 given the outcome of studies conducted with RBCs from what were suspected to be potentially sensitive human subpopulations. At the same time, however, I would consider an increase in the database UF from 1 to 3 given USEPA's medium-to-high confidence in the RfC (and RfD) assessment and in the database that supports it. Such a confidence level is seemingly inconsistent with a database UF of 1. If both changes mentioned above were made, the overall UF applied to RfC (and RfD) derivation would obviously remain unchanged from that currently proposed.

(A6) Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

Fletcher Hahn

The database for EGBE is fairly solid and a database UF of 1 is appropriate. The toxicokinetics of EGBE has been studied in both humans and animals. Absorption rates by dermal inhalation and oral exposure have been determined. Studies in humans and rodents have determined the pathways of EGBE metabolism and determined the metabolite responsible for hemolytic toxicity. Factors that influence metabolism, such as age dose and inhibitors have been studied.

David Jollow

As discussed above, I am not comfortable with the rationale for selecting a UF of 1 for rodent to human extrapolation and 10 for human variability. As noted, it is appreciated that the lack of human chronic exposure needs to be incorporated into the selection of UFs. A more detailed justification is requested with clear separation between the policy and scientific basis of the decisions.

Michael Pereira

This uncertainty factor of 1 is appropriate.

Andrew Salmon

There appear to be adequate data on frequently deficient types of data such as developmental and reproductive effects to justify the use of a UF_D of 1. Application of database deficiency factors is usually used to address missing toxicological data. It is an unnecessary confusion to include uncertainties about the toxicokinetic data in this factor: these should properly be reflected in the separate uncertainty factors to which they relate, i.e. UF_A PK and UF_H PK. These issues are adequately addressed in the document, apart from the necessity to consider endpoints unrelated to the hematological effect.

Gregory Travlos

No comment provided.

Rochelle Tyl

Specific comments on the UF for the database. A UF(D) of 1 was selected for the database, since there were adequate animal studies of appropriate sizes, doses, routes, durations, ages from prenatal to older than 2-years of age, and with sufficient range of endpoints to provide a robust animal database. There are limited human studies under short-term exposure conditions (with additional studies unlikely) and no human studies of long-term exposures(again, not likely to occur) (page 87, lines 25-28). Perhaps a UF(D) of 3 is more defensible. This UF(D) is clearly, cogently, and transparently explained and well supported scientifically.

D. Alan Warren

A database UF of 3 or 10 is generally used when extrapolating from valid results in experimental animals when data are "incomplete." It is intended to account for what some believe is the inability of any single study to adequately address all possible adverse outcomes. As indicated in the response to charge question no. 5 above, I can support a database UF of 1, particularly as an UF for intrahuman variability of 10 exist to account for the outstanding questions surrounding potentially sensitive subpopulations. The data base UF of 1 is scientifically justified, and its basis transparently and objectively described in the Toxicological Review. The Toxicological Review expresses medium-to-high confidence in the RfC (and RfD) assessment and in the database that supports it (which is seemingly inconsistent with a database UF of 1). I am somewhat more optimistic and believe the bioassays detailed in NTP (2000), coupled with existing studies of hemato- and hepatotoxicity, are sufficient to identify the critical effect and develop a high confidence estimate of its sub-threshold concentration or dose. USEPA indicates that its confidence in the database in not high since the potential for effects in humans from repeat, long-term exposures has not been investigated. USEPA does acknowledge, however, that the existing data suggest long-term exposures in humans would be no more adverse, and likely less so, than long-term rat exposures. As opportunities to examine humans after repeated, long-term exposures are rare, if they exist at all, USEPA's reservations over the completeness of the database are not likely to be resolved.

Regarding the body of literature specific to the hemato- and hepatotoxicity of EGBE, in my opinion it is sufficiently robust to formulate with a high degree of confidence a hypothetical, threshold-dependent mechanism beginning with EGBE's metabolic activation to a hemolytic metabolite and ending with neoplastic transformation of two liver cell types. This high degree of confidence should not be misconstrued as approaching absolute certainty. The toxicokinetics of EGBE have been well investigated in terms of their species-, dose-, age- and route-dependency, and have been used to develop and validate predictive PBPK models for rats, mice and humans. Internal doses of BAA at which hemolysis is seen in rodents have been measured and provide a basis for model predictions of the exposure circumstances, if any, under which such an effect might be seen in humans. As for the role of toxicokinetics in determining the database UF, I suppose the qualitative similarity in kinetics across species (and the ability for interspecies extrapolation via a PBPK model) makes the numerous rodent studies relative to the prediction of human risk, barring toxicodynamic differences that suggest otherwise. This increases the utility of the database for human health risk assessment and lessens the concern for the lack of chronic human data.

Despite my comments above, I will reiterate a portion of my response to charge question no. 5, as it is applicable here as well. I believe that some consideration should be given to increasing the database UF from 1 to 3 given USEPA's medium-to-high confidence in the RfC (and RfD) assessment and in the database that supports it. Such a confidence level is seemingly inconsistent with a database UF of 1. However, should this be done, I would advocate for a reduction in the intraspecies UF from 10 to 3 given the outcome of studies conducted with RBCs from what were suspected to be potentially sensitive human subpopulations. If both changes mentioned above were made, the overall UF applied to RfC (and RfD) derivation would obviously remain unchanged from that currently proposed.

(B) Oral reference dose (RfD) for EGBE

(B1) A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

Fletcher Hahn

Rationale for not developing an RfD based on the 91 day drinking water study needs to be clarified. Exactly what are the deficiencies? In the rest of the document analyses using more than one approach were used, which is a strength. Why not use multiple approaches here?

David Jollow

Available date suggests that the decrease in Hct represents a steady state situation without progression from sub-chronic to chronic exposure. The rationale for not using the 91-day drinking water study described in the document needs a more detailed discussion and justification.

Michael Pereira

The document does not justify why the 91-day drinking water should not be used to calculate chronic RfD, since this study was previously used to calculate one. No further toxicity, including any toxicity at a lower dose level was observed in the 2-year inhalation study beyond those observed at earlier times. Thus 91-day exposure is sufficient for determination of the chronic RfD. Using the previous calculation an of the BMD50 HED of 5.1 mg/kg-day and a total uncertainty factor of 0.1, an RfD of 51 mg/kg-day is recommended value.

The document should describe in detail the procedure used in the 1998 document and in the present document using the 91-day drinking water study. The description should highlight where the two approaches using the drinking water study differ, give the explanation the differences and justified any difference in the present document.

It is recognized that there is no chronic drinking water study. Hence a database uncertainty (UF_S) of 10 would be reasonable.

Andrew Salmon

The rationale for this approach is reasonably laid out and defended in the document. However, it does seem unnecessarily severe to eliminate the 90-day drinking water study (NTP, 1993) entirely from consideration. It does have the important advantage of using the relevant route of exposure, although the duration falls short of a full chronic exposure. There are standard ways of allowing for this limitation as noted below in the discussion of UFs. With these adjustments the conclusions based on this study appear essentially supportive of the derivation using the NTP inhalation study with route-to-route extrapolation. Apart from this, there do not appear to be any additional studies which would modify the conclusion presented in the document.

Gregory Travlos

Oral data does exist and was used previously (USEPA, 1999). But the present report suggests that because the data is limited the inhalation data set is more appropriate, even

in light of the lack of forestomach hyperplasia in the drinking water studies (NTP, 1993) compared to the observed forestomach lesions observed in the inhalation studies (NTP, 2000)?

Rochelle Tyl

A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. The rationale provided (Chapter 5, Section 5.2, page 89 ff) for not deriving a chronic oral RfD, i.e., no chronic (or subchronic) oral human studies, and no chronic oral animal studies, is presented clearly and transparently and is obvious. Hemolysis is not viewed as progressive (it does not get worse), but it is accumulative (as the animals and their RBCs age). The two 91-day drinking water EGBE studies in rats and mice (NTP, 1993) are available. Use of the PBPK model for EGBE could allow their use. This would require a UF from subchronic to chronic if the effect is genuinely accumulative.

D. Alan Warren

The rationale for performing a route-to-route extrapolation from inhalation data to derive an RfD is transparent and objectively described. The Toxicological Review notes that the oral database for EGBE is quite limited relative to that of inhalation, with no chronic oral studies in any species. It also accurately points out that the hematological effects considered precursors to hemosiderin deposition are consistent between the oral and inhalation exposure routes. Nonetheless, it is noteworthy that RBC count was significantly reduced and MCV significantly elevated in female rats at the lowest dose administered in the 13-week drinking water study of NTP (1993). Furthermore, 13 weeks was sufficient to generate a dose-response for minimal to mild Kuppfer cell pigmentation (hemosiderin deposition) among female rats of 0/10 (control), 0/10 (69 mg/kg-day), 2/10 (129 mg/kg-day), 10/10 (281 mg/kg-day), 10/10 (367 mg/kg-day) and 10/10 (452 mg/kg-day).

As the hematological effects of EGBE/BAA are not progressive, the subchronic nature of the NTP (1993) study should not be a major consideration when determining the value of these endpoints for RfD derivation. It was therefore appropriate that the Toxicological Review would apply the BMD/PBPK approach to hematological data for RfD derivation. Unfortunately, the Toxicological Review failed to do likewise for the hemosiderin deposition data in female rats from the same study. Granted, the Corley et al. PBPK model is based on male rat kinetic data and use of the NTP (1993) study may necessitate an UF for subchronic to chronic exposure duration, but these considerations did not preclude the application of BMD/PBPK methodology to RBC count data. It is therefore suggested that the same methodology be applied to the hemosiderin deposition data using AUC BAA as the dose metric. Doing so would further inform the decision of whether route-to-route extrapolation is preferred to the use of subchronic data, albeit data from the opposite sex to that which served as the basis for RfC derivation. At present, I am not opposed to the use of the route-to-route extrapolation for RfD derivation. Nor am I convinced, however, that the subchronic data have no utility in this regard. This is only reinforced by the demonstration that BMDL(HEDs) based on subchronic RBC counts and route-to-route extrapolation are comparable (3 vs. 1.4 mg/kg-day).

(B2) A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

Fletcher Hahn	Modeling is not my area of expertise.
David Jollow	If the chronic inhalation study is to be used to determine the RfD, PBPK modeling is essential. However, as discussed above in General Comments, there is a lack of adequate definition of the MOA by which BAA damages red cells. There is at present no way to decide whether the AUC or the concentration (perhaps above a MEC) of BAA is the appropriate dose metric. Since both absorption and elimination are first order processes, the Cmax of EGBE (and hence of BAA) will be more dependent on the absorption rate constant than is the AUC. The AUC may vary little between oral and inhalation exposure, whereas the Cmax may be very different. Without definition of the MOA and hence of the appropriate dose metric, avoidance of extrapolation seems desirable. If extrapolation is to be done, the PBPK modeling approach is essential. As noted, it is felt that the PBPK modeling is not adequately described for the non-PBPK specialists reading this document.
Michael Pereira	The extrapolation is correct, however this extrapolation is not required since there is an adequate oral (drinking water) study.
Andrew Salmon	This seems a reasonable an appropriate approach to route-to-route extrapolation, and the data and model used appear to be adequate and correctly applied. In the case of the RfD the concern about consideration of additional endpoints in the respiratory system does not apply. Portal of entry effects have been adequately addressed in the separate discussion of forestomach irritation and carcinogenesis, so the choice to base the RfD on route-to-route extrapolation of the hemosiderin effect in the NTP inhalation study appears to be a proper health-protective decision. However, the concern for basing the standard on an effect which will probably never be seen in humans remains.
Gregory Travlos	*No comment provided.*
Rochelle Tyl	A route-to-route extrapolation was done to derive the chronic RfD, using the chronic inhalation study in rats and PBPK modeling. The Human Equivalency Concentration (HEC) was based on "continuous" oral exposure to EGBE in the drinking water, that would yield the same AUC (area under the curve) for the metabolite BAA in arterial

	blood over 3 months as that estimated for the rat following external inhalation exposure to EGBE at the proposed POD (i.e., BMCL10). In this reviewer's opinion, using PBPK modeling for the route-to-route extrapolation to obtain an RfD was a very smart idea in the absence of adequate oral animal data or adequate human dose-response data. The rationale was well described. The extrapolation was correctly performed (to the best of my knowledge) and objectively and transparently documented and interpreted.
D. Alan Warren	The PBPK model is thought to be adequate for the conduct of route-to-route extrapolation in the derivation of the RfD, despite it being a potential source of uncertainty. The extrapolation appears to have been correctly performed and is transparently documented. Please refer to my response to the charge question immediately above for reservations about accepting the route-to-route extrapolation outright in lieu of using data from the subchronic study of NTP (1993).

(B3) Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

Fletcher Hahn	Since the UF of 1 is appropriate for determining the RfC it should be appropriate for the RfD.
David Jollow	As with the previous question, the rationale for selection of UF factors needs more explication
Michael Pereira	The database uncertainty factor of 1 is appropriate.
Andrew Salmon	I repeat my earlier comment in respect of the RfC: "Application of database deficiency factors is usually used to address missing toxicological data. It is an unnecessary confusion to include uncertainties about the toxicokinetic data in this factor: these should properly be reflected in the separate uncertainty factors to which they relate, i.e. UF_A PK and UF_H PK." In view of all the uncertainties considered in the RfD derivation, the choice of values for UF_A, UF_H and UF_D in the document seems reasonable and are adequately defended for the case presented. If in the final version of the document a derivation from the 90-day drinking water study (NTP, 1993) is also included, a UF_C of 10 would be indicated as standard practice to allow for the extrapolation from a 90-day study to a full lifetime exposure.
Gregory Travlos	*No comment provided.*
Rochelle Tyl	A UF for the database in the RfD derivation was selected as 1. The use of measured internal dose in rats (more sensitive than mice to the deposition of hemosiderin in the liver) and a human PBPK model were appropriate, given the data available, to derive the RfD. The selection of the UF (RfD) of 1 was clearly, transparently, and objectively described and scientifically justified.
D. Alan Warren	In the Toxicological Review, the descriptions of UFs applied to RfC and RfD derivations are essentially the same. This might be expected, as the RfD reflects the inhalation database for EGBE more so than the oral database given the means by which it was derived (i.e., inhalation to oral extrapolation). Accordingly, most of my comments on the

database UF applied in RfC derivation are applicable here. As for the uncertainty potentially introduced by route-to-route extrapolation, the similarity between HEDs derived using this method (1.4 mg/kg-day), the NOAEL/LOAEL method (7.6 mg/kg-day) and back-calculation from a rat BMDL (3 mg/kg-day), suggest that it is not of sufficient magnitude to disqualify the model's use in this regard.

(C) Carcinogenicity of EGBE

(C1) Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

Fletcher Hahn

I concur that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations; however, the "expected concentrations" should be noted. The description is appropriate because studies in mice (NTP 2000) have show slight increase in liver tumors and mild increases in forestomach tumors. The human studies are limited in number and are focused on non-cancer effects. None of the results have suggested that tumors are associated with human exposures to EGBE. However, such a determination is exceedingly difficult to make from studying exposed humans and the absence of finding tumors does not definitely indicate that EGBE is not carcinogenic. In the NTP study (2000) female mice developed squamous cell papillomas and carcinomas of the forestomach after prolonged exposure (> 582 d). These tumors were preceded by ulcers of the forestomach. Subsequent studies have shown that EBGE is bound by food stored in the fore stomach leading to a prolonged, relatively high local exposure of the mucosa. The incidence of these tumors was relatively low (2-12%) and showed a response to increasing dose. It is unlikely, however, that this mode of action is operative in humans.

Male mice developed a significantly increased incidence of hepatocellular carcinomas and hemangiosarcomas of the liver in the highest exposure groups. In vitro and in vivo studies were used to develop the mode of action based on an indirect mechanism of hemolysis, uptake of hemosiderin by hepatic phagocytes, and resultant production of reactive oxidant species. The MOA is plausible; however, it has not been shown that the same events occur in humans at potential exposure concentrations.

The weight of evidence has been adequately described.

David Jollow

The conclusion that EGBE is not likely to be carcinogenic for humans at expected exposures is well justified and the rationale adequately presented.

Michael Pereira

Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans*. The phase "at expected exposure concentrations" could be deleted (Page 51, Lines 2 and 30-31). This is because EGBE was not carcinogenic in rats, was only very weakly carcinogenic in mice and appears to have a MOA at the RfD and RfC values that is unlikely to be carcinogenic in humans.

Should the document include the phase "at expected exposure concentrations," then it should also include the reported levels of EGBE exposure, i.e., the range of EGBE concentrations found in occupational exposure, in drinking, ground, and surface water, and in the air. This information could be added to Section 2 (Chemical, Physical and Exposure Information). This exposure information is important for the Reader to be

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confident that EGBE is not likely to be carcinogenic to humans at **expected exposure concentrations**.

Alternatively the document could state: "EGBE is not likely to be carcinogenic to humans at **the calculated RfC and RfD values presented in this document**. This is the statement I recommend since it is what I believe the EPA wants to convey. That is that the calculated RfC and RfD would protect humans not only from the toxicity of EGBE but also from any possible carcinogenic activity.

Page 53, Lines 6-9 should be deleted or changed to "The NTP (2000)…….did not demonstrate carcinogenic activity for EGBE in male or female rats."

Andrew Salmon

The document identifies proposed mechanisms of action for the observed tumor endpoints in rodents, and carefully reviews the plausibility of these mechanisms at each step of the proposed explanations. The overall conclusions reached for both liver and forestomach tumors are carefully explained, showing that although such effects are not impossible in humans it is very unlikely that they would be observed in real human exposure situations involving inhalation exposure or chronic oral exposure. The weight of evidence characterization has been carefully developed and well described.

Gregory Travlos

No comment provided.

Rochelle Tyl

Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment, the Agency concluded that EGBE is not likely to be carcinogenic in humans at expected exposure concentrations. The effects of EGBE on hemosiderin deposition in the liver and forestomach irritation may both have qualitative relevance to humans. However, the exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC/RfD and well above the concentrations necessary to cause these effects in mice (the more sensitive species) (page 110, lines 25-29). Chapter 4.5 (Synthesis and Evaluation of Major Noncancer Effects and Mode of Action: Oral and Inhalation) and Chapter 4.6 (Evaluation of Carcinogenicity) provide detailed rationales for the weight of evidence for cancer (and noncancer) endpoints and excellent scientific justification for the evaluations. The weight of evidence "descriptor" has been sufficiently, transparently, and objectively described and scientifically justified.

D. Alan Warren

Yes, the scientific justification for describing EGBE as "not likely to be carcinogenic to humans" at expected exposure concentrations is sufficiently, transparently and objectively described in the Toxicological Review. In other words, the descriptor is appropriately presented in the context of a weight-of-evidence narrative. As pointed out in the *Guidelines for Carcinogen Risk Assessment*, the descriptor is appropriate when the available data, as in this case, are considered robust for deciding that there is not a basis for human hazard concern. Use of the descriptor obviously does not depend upon the absence of positive bioassay data or cancer mechanism(s) that are likely operable only in experimental animals. Rather, the descriptor can be applied to chemicals such as EGBE that are clearly animal carcinogens by at least one mechanism that, in theory, might be

operable in humans at extreme doses rarely, if ever, encountered. Use of the descriptor under these circumstances, however, is contingent on the unlikelihood that human doses above the threshold for precursor effects essential to tumor formation would ever be realized. In other words, such a description as the one applied to EGBE and qualified by exposure concentration or dose is typically reserved for carcinogens for which sufficient evidence of a non-linear mechanism exist. Such is the case for EGBE. The descriptor applied to EGBE is further supported by 1) the chemical's general lack of mutagenicity and clastogenicity; 2) a PBPK exercise demonstrating that vapor pressure limitations preclude inhalation exposures sufficient to achieve hemolytic blood levels of BAA in humans; 3) the experimental demonstration of the relative insensitivity of humans to RBC hemolysis, an essential precursor to liver tumor formation; 4) a BMD/PBPK analysis confirming that the RfC and RfD derived on the basis of hemosiderin deposition were also protective against forestomach hyperplasia and tumors; and 5) the likelihood that high doses of EGBE in humans would result in metabolic acidosis before hemolysis, which would require treatment and likely result in discontinuation of the exposure scenario.

Based on the above discussion, it is not anticipated that the descriptor applied to EGBE will be the subject of debate. Nonetheless, as suggested in my response to general charge question no. 4, it would be reasonable to add a margin of exposure-type analysis for EGBE to the Toxicological Review in which maximum inhalation concentrations and oral daily doses encountered by humans were compared to the newly-derived RfC/RfD values.

(C2) EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

Fletcher Hahn

The MOA for male mouse liver cancer following EGBE is a solid proposal generally supported by scientific data. The initial steps, metabolism of EGBE to BAA and the swelling and hemolysis of RBCs by BAA have been established by several studies to occur in humans, rats and mice, as well as other animals species. Excess hemoglobin resulting from hemolysis taken up by phagocytic cells of the liver and spleen and stored as hemosiderin has also been reported in humans, and in other mammalian species. The subsequent steps are less well supported. Oxidative damage and increased synthesis of endothelial and hepatocyte DNA are proposed to be initiated by generation of reactive oxygen species from hemoglobin derived iron in Kupffer or other cells in the liver or by production of cytokines/growth factors by the Kupfer cells that suppress apoptosis and promote cell proliferation, or both. These actions have been shown with *in vitro* studies but have not been shown to occur *in vivo* and at cellular doses that occur in exposed animals or humans. In addition the reactive oxidative species also are purported to damage DNA (which has been shown *in vivo* with comet assays in one study), modulate liver cell gene expression (extrapolated from work in mammalian cell lines). ROS have been shown to stimulate cell proliferation and inhibit apoptosis. Increased DNA synthesis in endothelial cells and hepatocyte has been shown in mice, but not rats, exposed to EBGE. The initiation and promotion of liver cells by ROS generated by EBGE is less well supported by laboratory studies. The MOA could be strengthened by relating the doses required to trigger the steps involving oxidative damage to what is required to initiate similar damage in human cells.

David Jollow

The proposed MOA is reasonable and supported by available data. The reasoning is sound and transparently and objectively described.

Michael Pereira

The discussion of the Mode of Action for the apparent carcinogenic activity of EGBE should be greatly decreased since:

a) it give too much credence to activity that is not statistical significant,
b) EGBE is very unlikely to be a human carcinogen, and
c) contains too much speculation.

Page 54, Line 13-16: Change to "it is possible thqat events leading to oxidative stress could contribute to the development of hemangiosarcomas and hepatocellular carcinomas in male mice. Note: HGBE does not cause transformation.

Page 54, Line 31-32 and Page 55, Line 1-2: Delete these points of 5-8. Because:

a) There is no evidence for HGBE causing oxidative damage to DNA.
b) Alteration in gene expression is a meaningless point since it is obvious that to have increased DNA synthesis and cell proliferation there most be alterations in gene expression. This is an important point only should alteration in genes specific for

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the activity of HGBE are identified.

c) Point (7) was already stated in Point (4b)

d) There is no evidence that HGBE promotes initiation of hepatocycte, no less endothelial cells. HGBE is non-genotoxic.

Page 55, Line 26-29. Delete since too speculative and most likely not involve in HGBE carcinogenic activity.

Page 55, Lines 31-36. This possible MOA of ROS induced increase in cell proliferation is consistent with the lack of genotoxicity of HGBE (see Section 4.4.4).

Page 55, Line 33 and though out the document delete "spontaneous" in describing tumors and neoplasms for which you might not know the cause. These are not spontaneous but rather the result of heredity, hormones, such as estrogen, oxidative damage, etc.

Page 56, Line 1. Delete "hepatic hemosiderin buildup". The cause of the oxidative damage is hemolysis.

Page 56, Line 10-31 and Table 4-8. Delete these lines and the table. The critical dose-response relationships that should be discussed are those of Siesky et al. (2002) and of hemolysis in the NTP studies.

Page 57, Section 4.6.3.1.3 should be deleted since it is not relevant or adds anything to the understanding of the activity of HGBE. The relevant points in these paragraphs such as the discussion of Kamendulis et al. (1999) and Siesky et al. (2002) has already been given.

Is it appropriate to use a non-peer review reference like Kamendulis? If so it should be identified as a report and not a publication, especially since it was written in 1999, but not let published.

Page 59, Lines 10-22. Delete this paragraph since it is redundant and already discussed in Section 4.

Page 59, Lines 23-34 and Page 59, Lines 1 and 2. This paragraph is not relevant since HGBE is not genotoxic. A two sentence paragraph is all that is needed. One sentence stating the possible of a genotoxic MOA for HGBE, followed by a second sentence stating that this is not appropriate for HGBE since it is not genotoxic.

Andrew Salmon

There is now a considerable literature examining the mode of causation of liver tumors, especially hemangiosarcomas, associated with hemosiderin (iron) deposition from various causes. It appears that the relatively low incidences of these tumors reported for EGBE and other agents acting via this mechanism are consistently associated with oxidative damage induced by the excess iron deposits, and the interaction of these chemical deposits with cellular metabolism in cells (such as the Kupfer cell) where oxidative metabolism is naturally active. This mechanism is contrasted with the different causation of hemangiosarcomas and other liver tumors by chemicals (such as vinyl chloride) which give rise to reactive and directly DNA-damaging metabolites. The lack of genotoxicity of EGBE in standard assays provides supportive (although not definitive) evidence reinforcing the contrast with those clearly genotoxic liver carcinogens. The document

describes this analysis and carefully reviews its plausibility. The general conclusion is that this mechanism is well supported by the available data.

Gregory Travlos

No comment provided.

Rochelle Tyl

Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The EPA has proposed a MOA (mode of action) for male mouse liver cancer (the more sensitive species and sex). The sequence of events:

- EGBE metabolism to BAA
- Hemolysis of RBCs from BAA in the blood
- Hemosiderin deposition in the liver from bioaccumulation of iron from lysed RBCs
- Oxidative damage to hepatic cells
- Compensatory proliferation, leading to
- Tumor induction Chapter 4.6.3.1 (derived from Step Event, page 54)

The hypothesized MOA for the liver tumors following EGBE treatment involves exposure to high doses for prolonged periods of time. Each step in the proposed process has been confirmed in humans (first steps) and in animal models (last steps)(Chapter 4.6 ff). EGBE is not a genotoxic carcinogen, again supported by animal evidence. This analysis and proposed MOA are both scientifically sound, transparently and objectively described and fully supported by the data available. An analysis of the NTP database on chemicals which produce hemosiderin deposition in the liver in subchronic or chronic exposures (and which were carcinogens), indicated a highly statistically significant association, p less than 0.001, between the studies exhibiting deposition of hemosiderin (6) and those studies with liver carcinogenesis (hemangiosarcoma and hepatocarcinoma), Table 4-8 (page 57). Why is there no hemosiderin deposition in the spleen? In addition, tumor induction from initiated cells is speculation. No other viable MOAs have been identified that explain the existing laboratory animal and human observations (Chapter 4.6.3.3, page 67, lines 29-30). However, the hemosiderin deposition dose response is steeper in females (in all females, 10/10, in the top 3 doses) versus male mice (only 7/10 in the top dose). Again, hemosiderin deposition is a measure of exposure, <u>not</u> effect.

D. Alan Warren

The analysis regarding the MOA for liver hemangiosarcoma and hepatocellular carcinoma is scientifically sound, and transparently and objectively described in the Toxicological Review. In particular, the stepwise progression from metabolic activation of EGBE to neoplasm formation (pp. 54-55) is a nice way of bringing disparate data sources together in the form of a single MOA, that while hypothetical, nonetheless enjoys considerable experimental support. In addition, the specific experimental support for each of the nine “steps” in the hypothesized MOA is discussed in the text, independent of the section on biological plausibility. This impressive compilation of supportive studies alone is sufficient to increase confidence and decrease uncertainty in the MOA.

Confidence in the MOA is further increased by a discussion of other chemicals that, like EGBE, increase the incidence of both liver tumors and hemosiderin deposition among male mice. Knowledge of the hypothesized MOA is more than sufficient to select an appropriate dose metric (AUC BAA), critical effect (hemosiderin deposition) and low-dose extrapolation method (BMD modeling with back-extrapolation via a PBPK model to a human equivalent concentration), with the latter being the source of uncertainty with the greatest potential impact on EGBE's RfC. Lastly, the Toxicological Review contains a statement to the effect that no other viable MOAs have been identified to explain the hemato- and hepatotoxicological observations among laboratory animals and humans following EGBE exposure.

Despite the above statement, I remain somewhat hesitant to fully embrace hemosiderin deposition as the critical effect given the hypothetical nature of the MOA. Might reactive oxygen species be generated without Kupffer cell involvement? If so, hemosiderin deposition within Kupffer cells might be more of a biomarker of exposure rather than effect, and given the "minimal" severity of the deposition regardless of dose, not a good one at that. Selection of RBC hemolysis as the critical effect, while not making the hypothesized MOA any less viable, would avoid having to place more confidence in the hypothesized MOA than might arguably be justified.

(C3) EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

Fletcher Hahn

The proposed MOA for forestomach tumors is a plausible model backed by reasonable scientific evidence. The initial steps of deposition, retention and metabolism of EGBE in the forestomach are well documented. The prolonged retention is much longer than what conventional wisdom might predict. The next step of irritation with a compensatory proliferative response in the gastric epithelium is also well documented. This step is consistent with the current thinking that inflammation can be an initiation/promotion factor in carcinogenesis (Mantovani 2008)

David Jollow

The MOA proposed for forestomach tumors appears adequately supported by the available data. The reasoning is sound and transparently and objectively described.

Michael Pereira

Again, the discussion of the Mode of Action for the apparent carcinogenic activity of EGBE should be greatly decreased since:

a) it give too much credence to activity that is not statistical significant,
b) EGBE is very unlikely to be a human carcinogen, and
c) contains too much speculation.

Page 62, Line 23 & 24. Step (5) should be deleted since it is redundant with Step (4).
Page 62, Line 25 & 26. Step (6) is probably not true and in any case too speculative. There is no evidence for clonal growth; it is more likely a field effect. Also, there is no evidence for spontaneously initiated cells. High level of cell proliferation could lead to genetic and epigenetic alterations that enhance the occurrence of tumors. I would recommend deleting this step and adding to the end of Step (4) "that enhance the occurrence of tumors.

Page 65, Line 4. Change induce to "increase the incidence of"

Page 65, Line17-35. This paragraph is redundant with what was previously stated for HGBE and its metabolites and just should be deleted.

Page 66, Line 28 to Page 67, Line 10. This paragraph is not relevant since HGBE is not genotoxic. A two sentence paragraph is all that is needed. One sentence stating the possible of a genotoxic MOA for HGBE, followed by a second sentence stating that this is not appropriate for HGBE since it is not genotoxic.

Page 67, Lines 11-16. This paragraph does not belong in this section and should be deleted.

Page 68, Line 14. Delete "at expected environmental concentrations.

Andrew Salmon

The issue of relevance of rodent forestomach tumors and their relevance to possible human stomach (or esophageal) cancer is a contentious issue which has been extensively debated with regard to a wide range of different chemicals found to induce such tumors by various exposure routes. The conclusion in the specific case of EGBE appears to be based on considerations of plausible exposure routes and levels, and the specific functional and anatomical properties of the mouse forestomach. The document provides a thorough description of the various factors taken into account in the overall conclusion presented. Some of the arguments used to discount the relevance of tumors at this site in rodents appear rather speculative: it is not clear to what extent actual data support the presumed route of exposure and accumulation in the forestomach (grooming and swallowing of inhaled material, adsorption onto retained food, etc.). However, on balance it would seem reasonable to conclude that the proposed mechanism of action is plausible for this particular case, especially as it is very hard to identify a mechanism of action involving genotoxic effects at this site. However, it should not be presumed that this particular case provides a significant precedent for discounting the relevance to human cancer risk of tumors induced by other chemicals at this site.

Gregory Travlos

No comment provided.

Rochelle Tyl

EPA has proposed a MOA for female mouse forestomach tumors (the most sensitive species and sex). The sequence of events is:

- Deposition of EGBE/metabolite BAA in the stomach and forestomach (humans do not have a forestomach) via consumption or reingestion of EGBE-laden mucus, salivary excretions and fur material
- Retention of EGBE/BAA in food particles of the forestomach long after being cleared from other organs
- Metabolism of EGBE to BAL, which is rapidly metabolized to BAA systemically and in the forestomach
- Irritation of target cells by BAA leading to hyperplasia and ulceration
- Continued injury by BAA and degeneration leading to high cell proliferation and turnover, leading to
- Clonal growth of spontaneously initiated forestomach cells (estrogen-dependent event, speculation…?) (Chapter 4.6.3.2, Step Event, p. 62).

This analysis and MOA are both scientifically sound and transparently and objectively described. The first two steps have been demonstrated in animal studies. Step 3 requires ALD and ADH, which have been evaluated in the stomachs and forestomachs of mice. These enzymes have been shown to be heavily localized in the stratified squamous epithelim of the mouse and rat forestomach (while their distribution in the rodent and human stomach is more diffuse) (page 63, lines 16-22). Human stomach tissues, with less amounts and diffuse distribution of these deyhdrogenase enzymes, would be less capable of accumulating and localizing BAA than rat/mouse tissues, and would less likely be exposed to the irritating effects of BAA (Chapter 4.6.3.2, line 37, p. 63; and lines 1-5, p. 64). The process in rodents is well described and confirmed. The MOA also explains

why humans would not be expected to exhibit the full MOA and therefore the tumors in the (human) stomach. Interestingly, no hyperplasia or tumors were observed in the inhalation studies of EGBE in rats (NTP, 2000) or in the drinking water studies of mice (NTP, 1993), supporting the requirement for all of the steps above to occur prior to tumor formation (Chapter 4.6.3.2.1, page 64, lines 25-27). Again, the use of the 91-day drinking water studies in rats and mice is strongly suggested. One panelist suggested that it is "unwise to dismiss forestomach tumors out of hand." The discussion in the document was considered by many to be too speculative. The lack of use of historical control data on incidence and severity of the tumors by the initial authors and by the reviewers is regrettable. We don't know enough biologically; what is critical: dose, dose rate, accumulative dose (i.e., exposure duration, timing (specific vulnerable life stage(s)?

D. Alan Warren

The analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Similar to that for the liver, the Toxicological Review presents the MOA for forestomach tumors as a stepwise progression from deposition and metabolic activation of EGBE in the forestomach to the promotion of initiated forestomach cells via a proliferative response to cell injury (p. 62). Again, this is a nice way of bringing disparate data sources together in the form of a single MOA, that while hypothetical, nonetheless enjoys considerable experimental support. The experimental support for each of the six "steps" in the hypothesized MOA is discussed in the text, independent of a section on biological plausibility. This impressive compilation of supportive studies alone is sufficient to increase confidence and decrease uncertainty in the MOA. Confidence in the MOA is further increased by acknowledgment that several other chemicals, like EGBE, are capable of inducing forestomach hyperplasia after inhalation exposure. Knowledge of the hypothesized MOA is more than sufficient to select an appropriate dose metric (Cmax of blood BAA), critical effect (epithelial hyperplasia of the forestomach) and low-dose extrapolation method (BMD modeling with back-extrapolation via a PBPK model to a human equivalent oral dose and air concentration). Extensive uncertainty surrounding the relevance of the MOA in mice for humans persists and is due to the absence of a forestomach in humans and differences in enzyme distribution and kinetics between the glandular and forestomach tissues of the two species. The Toxicological Review has, however, effectively eliminated any concern for forestomach tumors created by opting for a critical effect related to the liver, as a BMD/PBPK analysis demonstrated RfC and RfD values for EGBE are protective against forestomach hyperplasia. Lastly, the Toxicological Review contains a statement to the effect that no other viable MOAs have been identified to explain the toxicity of EGBE to the forestomach.

(C4) EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

Fletcher Hahn

I do not recommend development a MOA for pheochromocytomas in female rats exposed to EGBE. The uncertainty in differentiating hyperplasia from tumor, the marginal dose response, the lack of significantly increased incidence and the lack of tumors in other organs of the body are valid reasons for giving scant weight to this observation. In addition, the significance of this tumor for human carcinogenesis is questionable. Typically, male F344 rats are the ones to show increased incidence of pheochromocytomas in chronic bioassays (Nyska 1999). The increased incidence in NTP chronic bioassays has been associated with severe nephropathy (possibly by disruption of calcium metabolism) and with space-occupying lung lesions (Ozaki 2002). In addition, multiple disparate factors can affect the incidence including xenobiotic agents, dietary factors, factors from pituitary tumors (common in F344 rats) and stimulation of the autonomic nervous system. In most cases the exogenous factors do not cause DNA damage, but may affect the response by indirect mechanisms. These factors complicate any extrapolation of the MOA to humans.

David Jollow

The reasoning is sound and transparently and objectively described. No alternate analysis is warranted.

Michael Pereira

The incidence of pheochromocytomas of the adrenal medulla in female rats was not statistically significant and therefore should not be given any weight in the qualitative or quantitative assessment of EGBE cancer potential. If anything the document puts to much emphasis on this non-significant observation.

Andrew Salmon

Tumors at this site are not a particularly unusual observation in rat bioassays, and there have been a number of discussions among NTP scientists and pathology consultants as to whether these tumors should be considered dose related and/or indicative of human cancer risk. Whereas there appear to be a number of instances where these tumors are dose-related and apparently caused by exposure to a carcinogen, in this particular case it is hard to disagree with, or significantly expand upon, the NTP's conclusion that the association of these tumors with EGBE exposure in this study is "equivocal". In view of this it is reasonable for the document not to place extensive reliance on this particular endpoint. Additional defense of NTP's conclusion could be undertaken in this document in support of the conclusion not to weight this endpoint significantly. In terms of how a risk estimate might e prepared to illustrate the effect of considering this endpoint, it might be interesting to develop a default (linear extrapolation) potency estimate from rodent tumors at this site. This would require the important caveat that such an estimate has a

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low level of reliability both as regards the relation to dose in the rodents, and its extrapolation to humans. There really do not appear to be any quantitative data available to support any other type of risk estimation procedure.

Gregory Travlos

No comment provided.

Rochelle Tyl

EPA has not proposed an MOA for female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as indicating "equivocal" evidence of carcinogenic activity. The pathology report for that study expressed concern whether the observed tumors met the established criteria used for the diagnosis of these tumors. Therefore, this tumor was not given "significant weight" in the qualitative or quantitative assessment of the carcinogenic potential of EGBE. This reviewer concurs with the EPA's (and NTP) concerns and conclusions. Examination of the NTP final report on 2-butoxyethanol (Appendix B, Table B1, Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of 2-butoxyethanol) indicates that in the adrenal medulla, the incidence of malignant pheochromocytoma was 0 (0%) at control, 0 at 31.2 ppm (low), 0 at 62.5 ppm (mid), and 1 (2%) at 125 ppm (high). The incidence of benign pheochromocytoma was 3 (6%) in controls, 4 (8%) at 31.2 ppm (low), 1 (2%) at 62.5 ppm (mid), and 6 (12%) at 125 ppm (high). A third entry was for benign pheochromocytoma, bilateral, reported only at 125 ppm (high concentration) in 1 (2%).

The report states (p. 58, left column) that: "The incidences of benign or malignant pheochromocytoma (combined) occurred with a positive trend in females; however, the incidence in females exposed to 125 ppm was not significantly increased relative to the chamber controls (Tables 10 and B3), but exceeded the range for historical controls from 2-year inhalation studies (Tables 10 and B4). One pheochromocytoma in the 125 ppm female group was malignant and another, while benign, was bilateral (Tables 10 and B1). The incidence of medullary hyperplasia was slightly, although not significantly, greater in females in the 125 ppm group than in the chamber controls (Tables 10 and B5). The primary criterion used to distinguish pheochromocytoma from medullary hyperplasia was the presence of mild to moderate compression of the adjacent tissue. Most of the pheochromocytomas were small and not substantially larger than the more severe grade of adrenal medullary hyperplasia." At best this is an uncertain, equivocal finding.

The analysis regarding the female rat pheochromocytomas is scientifically sound (and concurs with the concerns of the NTP Pathology Working Group for the 2-year inhalation bioassay of EGBE). I could not find a detailed discussion in the Toxicological Review of this tumor type. This tumor type (and incidence and severity) in the female rat is not considered to be adequate to support alternative analyses of qualitative and quantitative cancer risks to humans. Approaches to take to see if such analyses are warranted would include (but are <u>not</u> recommended) are:

- Search to see if these tumor types have occurred in other NTP bioassays and, if so, in what chemicals, by what routes, at what dose/concentration levels
- Search to see what the background incidence of this tumor type is in female rats
- Search to see what the background incidence of this tumor is in people (males and

females)

- Determine whether there is a MOA for this tumor type in animal studies (I don't know of any), and see if the human is at risk based on MOA

My guess is that at the exposure levels to which humans are exposed, even if there were a MOA, human exposures would be below the effects level(s).

D. Alan Warren

Page 53 of the Toxicological Review (which is part of the overall weight of evidence summary for EGBE's carcinogenicity) contains a one paragraph justification as to why pheochromocytomas in female rats were not significantly weighted in the assessment of EGBE's cancer potential. The paragraph is an accurate reflection of the concerns expressed in NTP's Technical Report. Though concise, it provides a scientifically sound, transparent and objective basis for USEPA's dismissal of the pheochromocytoma data. After all, there were no increased incidences among males; the incidence even at the highest exposure concentration (16%) barely exceeded the highest incidence observed in any one historical inhalation (13%) or non-inhalation control group (14%); while a trend for combined benign and malignant tumor incidence was seen among females, it was not strictly concentration dependent, nor were there any statistically significant pairwise comparisons; and, the time to first tumor incidence was not inversely related to concentration. Therefore, NTP's characterization of the incidences of pheochromocytoma as equivocal findings not clearly related to EGBE exposure is justified, as is USEPA's decision to exclude them from consideration in quantitatively assessing EGBE's hazard potential.

(C5) Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations.

Fletcher Hahn

The nonlinear threshold approach is certainly warranted in this evaluation based on the lack of mutagenicity of EGBE and the modes of action for induction of liver tumors (initial hemolysis leading to iron deposition in the liver and production of ROS) and forestomach tumors (local irritation, inflammation and hyperplasic response) The section describing this (p103 l. 1-6) could be strengthened by showing or referring to a non linear dose response curve for some hematologic parameter (e.g. RBC count or Hb).

Although the linear dose response needs to be mentioned, the discussion in 5.4.1.1 is a distraction for me, particularly Table 5-16. Although there are several disclaimers that the table is for illustrative purposes, I think the casual reader may get the wrong impression, based on a linear dose response, that this compound may be a likely cancer agent.

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	St Clair MGB and Morgan KT. Changes in the upper respiratory tract in Pathobiology of the Aging Rat Mohr U. Dungworth DL. Capen CC, eds. pp 111-128 ILSI Press 1992
David Jollow	The non-linear approach is appropriate and scientifically sound. The alternate linear analysis is felt to be inappropriate.
Michael Pereira	The choice of the nonlinear threshold approach is scientifically sound and objectively described. However, I recommend that a quantitative assessment of the carcinogenic potential of EGBE not be included in the document. Instead the document should state that "The evaluation of EGBE for carcinogenic activity indicates that it is not likely to be a human carcinogen and since there is not evidence to suggest otherwise, a quantitative assessment of the carcinogenic potential of EGBE was not done." To perform such an assessment would result in the false suggestion that EGBE represents a carcinogenic hazard to humans.
Andrew Salmon	Reasonably plausible mechanistic arguments have been presented in support of the interpretation that EGBE carcinogenesis in the rodent (particularly with regard to the liver tumors) proceeds by a mechanism which involves a practical threshold. The arguments do not necessarily support an absolute threshold where the risk is actually zero below a certain critical dose, but they do at least support the concept that the risk would be very low indeed until such a dose level was reached. It is therefore reasonable to propose the threshold approach to risk estimation for this compound, and also to argue that provided the threshold dose for hemosiderin deposition (used as a criterion for the noncancer RfC and RfD derivations) is not exceeded the human cancer risk is negligible. However it is an important part of the uncertainty analysis to present the consequences of alternative choices for the dose response model, so presentation of the linear alternative is useful in illustrating what the risk estimates would be if the threshold assumption were in fact incorrect. Part of the necessary decision logic in rejecting alternative hypotheses requires consideration of how severe the consequences would be if a particular choice was wrong: obviously the risk assessor needs to be very confident in rejecting a particular mechanistic analysis if the consequences of that analysis are severe, although perhaps less plausible than more reassuring alternatives.
Gregory Travlos	*No comment provided.*
Rochelle Tyl	The choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE is the correct one. A number of the initiating and subsequent steps (each necessary but not sufficient in itself) are nonlinear with thresholds below which the adverse effect does not occur. This is true for the hepatocarcinomas in male mice and for the forestomach tumors in female mice. In fact, the strongest evidence for little or no risk to humans for these tumors (not counting that we do not have a forestomach) is that our exposures are below the determined RfC and RfD, and at these lower doses/concentration, the sequential progression of necessary adverse events does not happen. I concur completely that the EPA has provided clear, transparent objectives

and cogent arguments, with strong scientific justification that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations.

D. Alan Warren

Whether applying it to data on hematological factors such as RBC count, hemosiderin deposition or forestomach hyperplasia, I support the choice of the non-linear threshold approach employed in the Toxicological Review. The approach appears to be scientifically sound, and transparently and objectively described. It is clearly applicable based on the hypothesized MOAs of EGBE within the two target tissues in which tumors were clearly elevated. As a result, key "steps" in the MOA that include critical effects and all downstream events (including cancer) are unlikely to occur at or below the RfC or RfD. I favor retaining the analysis in section 5.4.1 as a means of reinforcing the importance of reducing uncertainties and strengthening the database to the point where a mechanistically-driven assessment of hazard potential is possible. Its retention could be further justified in those cases where a minority of genotoxicity data or structural analogy suggests the possibility of low-dose linearity and direct interaction with DNA. I do not, however, support its presentation as an alternative to the threshold approach clearly warranted in the case of EGBE.

Additional Comments

Fletcher Hahn

No comment provided.

David Jollow

No comment provided.

Michael Pereira

1. **General and Major Comments:** extremely well written and is very easy to follow the rationale and procedure used for the hazard and dose-response assessment of EGBE, including the calculation of the RfC and RfD. The document also includes a very comprehensive and complete review of the literature pertaining to EGBE.
2. The use of hemosiderin as the critical adverse effect needs to be better justified in the document. Rather the effect of EGBE on hemosiderin staining would appear to be more suitable as a biomarker for exposure to EGBE.
3. The UF for variation in sensitivity within human populations (UF_H) should be 1 and not 10 and for interspecies variation (UF_A) should be 0.1 and not 1.
4. The discussion of the Mode of Action for the apparent carcinogenic activity of EGBE could be greatly decrease since:
   a) it give too much credence to activity that is not statistical significant,
   b) EGBE is not likely to be a human carcinogen, and
   c) Contains too much speculation.

Specific Comments:

1. Page 1, Line 15: change to: exposure (<10% of lifetime)…. Reason: The lower limit of duration is critical and not the upper limit of a lifetime.

2. Page 3, Line 15: delete "it is anticipated that"
3. Page 8, Line 9: change to: "were more than two orders of magnitude"…. Reason: 46 is more than 100 times greater than 0.29.
4. Page 11, Lines 5 and 6. Give the maximum $t_{1/2}$ value for rats and mice. Reason: As written there sentence does not indicate that the value was different for the two species. For example: both species could have a $t_{1/2}$ value of 4.
5. Page 15, Lines 21-23. The sentence starting with "By" does not make sense. Significantly increased relative to what? Mean value of what values? Should it be: " was reported to be as high as 39% of the total….."?
6. P16, Line 19. The use of plural for target is not clear. Do you mean to say: "the putative cancer target organ" or "the putative cancer target cells."
7. Page 17, Line 28: Change lesions to alterations.
8. Page 18, Table 4-1. In the footnotes define "§". Mean ±SE?
9. Page 19, Table 4-2. The title is wrong; lacks mice and organ weights. It would be best to separate into two tables; one for rats and one for mice. The title for the rat table should include "histopathological alterations" and not "lesions". The title of the mouse table should include "body and kidney weight …….in female mice." Also, give the body weight before the organ weight. Also, include the actual kidney weights, even though they were not different among the treatment groups.
10. Page 20, Lines 20-22. Move to Section 4.2.2 since this is a cancer study and not a toxicity study, even though for only 120 days. Line 22. Change "induce increases in tumors" to' induce an increase in tumors", since as written there could have been an increase in tumors in the different sites.
11. Page 27, Line 5 change "anda" to "and."
12. Page 27, Table 4-6. Please check that 48.7 ±1.9 (Male mice, 62.5 ppm, 12 months) is significantly different than the Control, 47.9 ± 0.4

Andrew Salmon

See page 78 for Supporting material: Benchmark dose analysis of data on hyaline degeneration in the olfactory epithelium of rats (NTP, 2000)

Gregory Travlos

The document is well written and the literature review thorough and logically presented. As mentioned in my pre-meeting comments, it appeared to me that the hemolytic effect (decreased erythron) and not the kupffer cell hemosiderin accumulation was the critical effect. And, while I will not totally discount the proposed potential mechanism for development of the hemangiosarcomas, the use of hemochromatosis in humans as a major part of the justification for the mechanism does not seem to be totally appropriate (the severity of the iron overload is different, the location [i.e., cell populations] of the iron accumulation within the liver is different, and the tumors associated with iron overload—in humans and rodents—is different). If the hemolytic effect was the critical effect, then there was appropriate oral exposure data (that has already been used in a previous report, EPA, 1999) for determination of the RfD.

I have one comment to add after the workshop. From the discussions it became evident that due to the species (rat v. human) differences in sensitivity of erythrocytes to the toxic metabolite (BAA), the assigned UF(A) of 1 appeared to be excessively high and not based on the data presented in the text. This suggested the UF(A) was selected for reasons other than science-based. Since, I do not understand all the issues (including policy) involved in such selections, it would be appropriate to justify the UF(A) selection in the text rather

than have lingering questions regarding its selection rationale.

Otherwise, my post-meeting comments are the same as my pre-meeting comments, presented below.

Editorial Comments:

Page 52, line 16: The historical control information reported on this line "(6.4-3.5%; range 2-13%)" appears to be incorrect.

Page 71, line 32: The female information (NTP, 1993) represented as an "increased urea nitrogen creatine" is incorrect. Firstly it should be presented as an increased urea nitrogen and creatinine concentrations. Creatine was not analyzed. Secondly, the males also had increased urea nitrogen concentrations. Thus, the idea that the changes in these markers of renal injury indicate supportive information that the females were more sensitive to EGBE administration is probably overstating/overinterpreting the findings.

Page 99, line 6: There is no section "5.1.2.4".

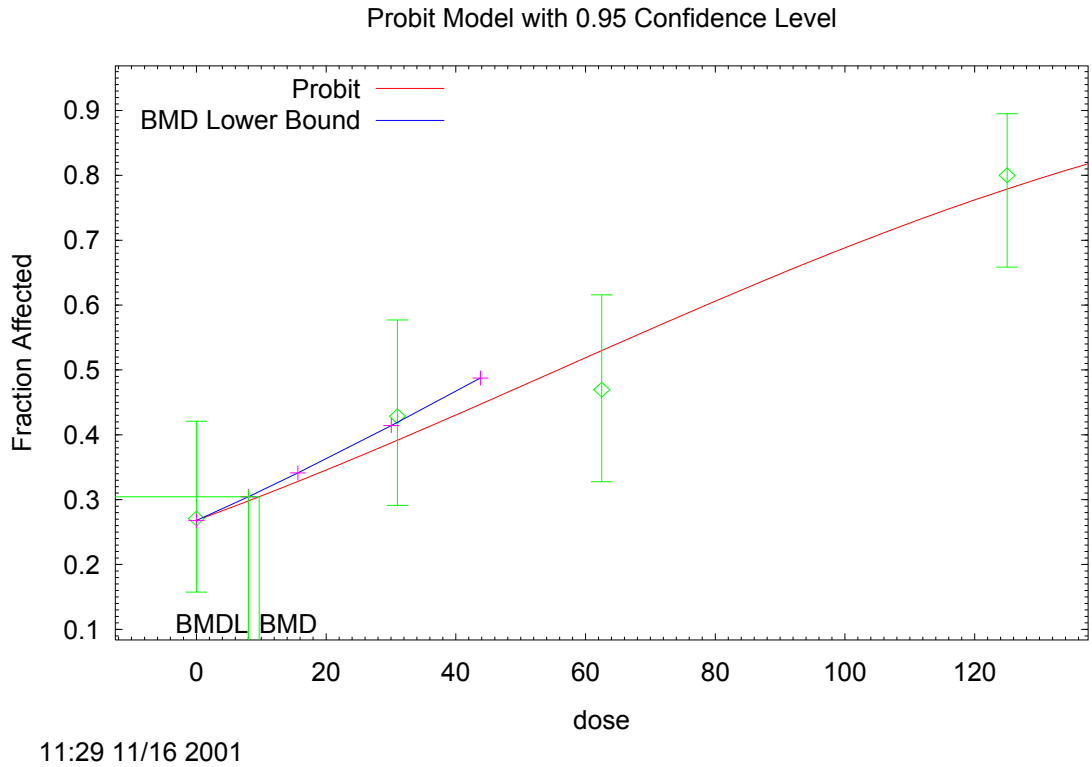
Rochelle Tyl

No comment provided.

D. Alan Warren

No comment provided.

Andrew Salmon
Supporting material: Benchmark dose analysis of data on hyaline degeneration in the olfactory epithelium of rats (NTP, 2000)
Male rats – hyaline degeneration of the olfactory epithelium

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        Gnuplot Plotting File:  D:\BMDS\DATA\EGBEMALERATNTP.plt
                                                Fri Nov 16 11:29:02 2001
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = COLUMN3
   Independent variable = COLUMN1
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
                  Default Initial (and Specified) Parameter Values
```

```
background =            0   Specified
 intercept =    -0.615103
     slope =    0.0111337
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.77
slope	-0.77	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.619211	0.14673
slope	0.0111431	0.00211812

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-120.391			
Fitted model	-120.954	1.12625	2	0.5694
Reduced model	-135.847	30.9112	3	<.0001

AIC: 245.908

Goodness of Fit

Dose(ppm)	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2679	12.859	13	48	0.04606
31.0000	0.3921	19.214	21	49	0.5225
62.5000	0.5308	26.008	23	49	-0.8611
125.0000	0.7804	39.022	40	50	0.3342

Chi-square = 1.13 DF = 2 P-value = 0.5688

Benchmark Dose Computation

Specified effect = 0.05

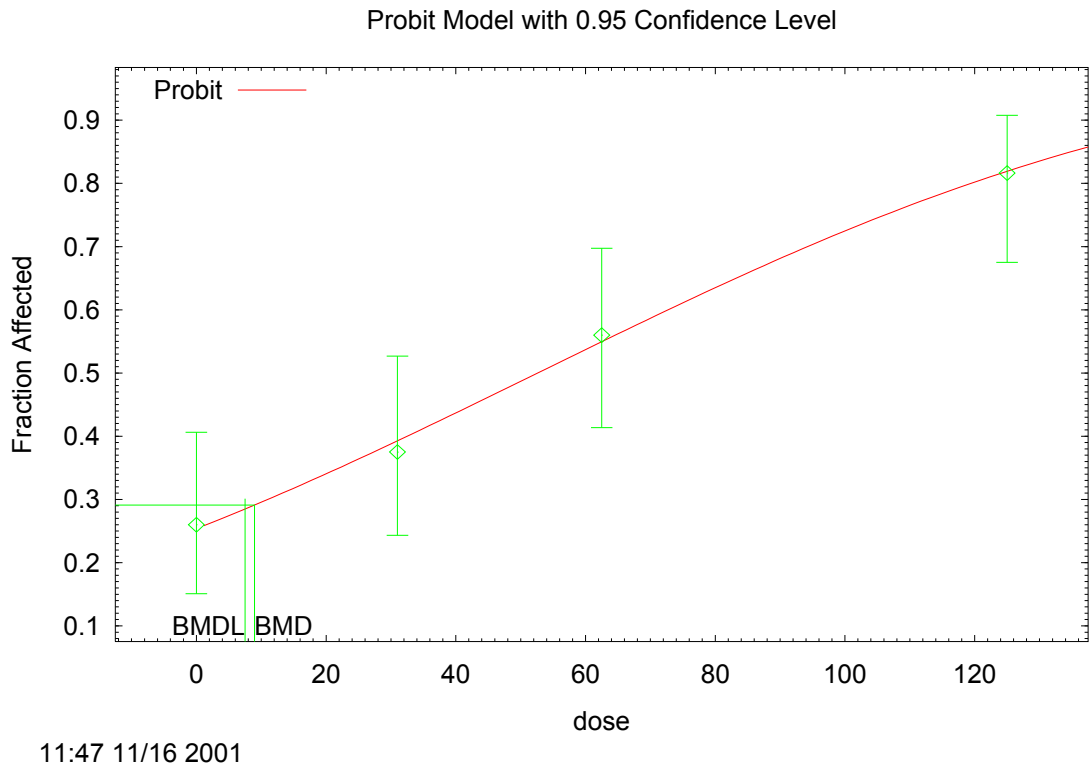
Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.66459

BMDL = 8.01404 ppm = 38.7 mg/m^3

Female rats – hyaline degeneration of the olfactory epithelium

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        Gnuplot Plotting File:  D:\BMDS\DATA\EGBEFEMALERATNTP.plt
                                                Fri Nov 16 11:47:55 2001
 ====================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = COLUMN3
   Independent variable = COLUMN1
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =     -0.65546
                          slope =    0.0123976
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.76
slope	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.66314	0.146919
slope	0.0125616	0.00216835

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-118.073			
Fitted model	-118.122	0.0975191	2	0.9524
Reduced model	-136.547	36.9482	3	<.0001

AIC: 240.244

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2536	12.681	13	50	0.1037
31.0000	0.3921	18.823	18	48	-0.2433
62.5000	0.5485	27.427	28	50	0.1629
125.0000	0.8178	40.073	40	49	-0.02694

Chi-square = 0.10 DF = 2 P-value = 0.9526

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 8.95568

BMDL = 7.4974 ppm = 36.2 mg/m^3

Mean of BMDL for M & F rats = 7.75572 ppm or 37.46 mg/m^3

Individual Reviewer Post-Meeting Comments

Fletcher Hahn

IRIS Toxicological Review of Ethylene Glycol Monobutyl Ether (EGBE)
Fletcher Hahn Post-meeting Comments

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

The draft Review is generally well presented, logical and clear. A strong point of the approach is the presentation of alternate models for calculation of various parameters and the liberal use of tables to present results.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

It was suggested during the review discussion that the incidence of hyaline degeneration of the olfactory epithelium of the nose of rats exposed in the two year NTP study be used to derive the point of departure for the RfC. The basis for the suggestion is the apparent similarity of the reactions of human volunteers and rats to inhalation of EGBE; nose and throat irritation in humans and accumulation of globules in the olfactory epithelium of rats. This is a reasonable suggestion, but it rests on shaky ground.

Studies of six human volunteers exposed to butyl cellosolve (EGBE) indicated that eye, nose and throat irritation and headache were subjectively reported after exposure to 200 ppm for eight hours although there were no effects observed objectively (erythrocyte fragility, blood pressure, pulse rate). All involved agreed that 200 ppm was too high a concentration to breathe comfortably for eight hours. In addition, exposure to 100 ppm for eight hours (conducted days later) was judged to be nearly as uncomfortable as 200 ppm. The authors concluded that 100 ppm was appropriate hygienic standard for work room exposure (Carpenter et al 1957).

In the rats from the NTP study of inhaled EGBE (2000), the incidence of hyaline degeneration of the olfactory epithelium was significantly increased in all exposed groups of male and in females exposed to 62.5 or 125 ppm. Inflammation was present in the nose but at a much lower incidence and was not dose related. Hyaline degeneration is the accumulation of globules of

homogeneous eosinophilic material in the cells of the olfactory epithelium of the nose as detected by microscopic examination. Similar changes were found in the mice, but the incidence was not significantly increased as in the rats. This finding is one seen on occasion in rodents exposed to vapors or gases (dimethylamine, Buckley el al 1985; pyridine Nikula et al 1995). It is also seen in aged rodents that are not exposed (St Clair 1992). The meaning of this finding is uncertain. It has been called an "adaptive" response (Buckley 1985) or an aging change (St Clair 1992). More recent studies have shown that the number and size of hyaline globules in the olfactory epithelium increase with increased and prolonged (32 week) exposure to cigarette smoke (Lewis et al 1994). The globules in smoke-exposed rats contain carboxylesterase (CE) and the amount of esterase in the globules increased with exposure. Although the CE is physically localized in the globules, the biochemistry of the localization is uncertain. Additional studies have shown that CE is an inducible enzyme in the olfactory mucosa. Rats exposed to pyridine at the threshold limit value concentration of 5 ppm, or at 444 ppm, 6 hr/day for 4 days showed the localization and amount of immunoreactive CE in olfactory mucosa (Nikula 1995). Quantitative densitometry showed a statistically significant, dose-related increase in the density of immunoreactive CE in olfactory mucosa of pyridine-exposed rats. These results indicate pyridine, and possibly other toxicants, can induce nasal CE, an enzyme not directly involved in the metabolism of those solvents, following low-dose, short-term inhalation exposure. The response is adaptive one. This same mechanism may have occurred in the rats exposed to EGBE.

Thus, in humans the reaction to 8 hr exposure to high concentrations of EGBE is irritation of the nose and throat, probably the result of mild inflammation. In rats, the reaction in the nose to many months exposure to a range of concentrations of EGBR is an adaptive response, not an inflammatory one.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Data to support the mode of action for mouse liver tumors should be strengthened.

A) The amount of iron in the liver should be measured. This would be a more precise indication of hepatic Fe than scoring of Fe-staining pigment. A dose response curve could be developed.

b) The doses required for triggering the initiation and promotion of cancer in liver cells by reactive oxidant species in both rodents and humans should be determined. (although I am unsure how to do this)

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

The key sources of uncertainty have been addressed fairly well. Table 5-15 is a helpful overview. The discussions of uncertainty in sections (5.1.3, 5.2.3) should be reviewed and expanded for clarity. Section 5.3 is much clearer.

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

A.1.Response: The selection of the NTP (2000) 2 yr inhalation study as the principal study is scientifically justified. There are no reported studies of humans exposed for months or longer, the selection of an animal study is necessary. The NTP study has a number of positive attributes. It is the only chronic study available and has both hematology and histopathology endpoints. In addition, it has factors (more dose groups, large exposure groups, two species) that aid in more precise estimates of the RfC. In addition, NTP studies are carefully planned, executed, reviewed and reported adding to confidence in the data base. The study has not been described in much detail, but the original report is readily available through the NTP website.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

A.2. Response: The selection of hemosiderin staining in the liver is an appropriate critical event for liver tumors in male rats exposed to EGBE. The hemosiderin is probably not the form of iron that induces reactive oxidant species, but hemosiderin is most likely correlated with the body burden of iron. (*see earlier comments on studies to develop a dose response for iron effects in the liver and later comments on the need to use male mice as the species*)

3. 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.

A.3.Response: As modeling is not my area of expertise, I can not comment on the modeling approaches for determining the POD.

The benchmark response (10% extra risk of hemosiderin staining in the liver) is appropriate. However, the selection of male rats upon which to base the POD does not seem appropriate. Why shouldn't the species (male mice) that has been shown to have the critical effect correlated with the adverse (tumor) effect be used? This would be the logical choice from the scientific stand point. Male rats show the bench mark effect at the lowest exposure concentration and their choice will obviously result in a "more protective" RfC. My thought is that such choices would be more transparent if they were made at when the uncertainty factors are chosen.

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

A.4.Response: As modeling is not my area of expertise, I can not comment on the scientific justification of the PBPK modeling.

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

A.5. Response: *(These should be scientifically sound, not policy decisions.)*

UF_H – A default of 10 is conservative and accounts for the unknown response of infants and young.

UF_A – The factor of 1, reduced from the default of 10, is appropriate because there is a good database on the pharmacokinetic and pharmacodynamics. Four research papers have presented PBPK modeling of EGBE. Four additional research papers using in vitro and in vivo approaches have shown that humans are less sensitive than rodents by a large factor (40-150) does account for the striking difference in concentrations required for hemolysis in humans compared with rodent.

- A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).

  Response: The PBPK modeling does appear to be justified. At least four research articles have presented such models for EGBE in humans, rats and mice. The draft text is not clear on the definition of PK-UF.

Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

Response: The PD-UF of 1 is justified. However, I do think that the striking difference in the sensitivity to EGBE hemolysis between humans and rodents should be taken into account. The draft text is not clear on the definition of PD-UF either

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

A.6. Response: The database for EGBE is fairly solid and a database UF of 1 is appropriate. The toxicokinetics of EGBE has been studied in both humans and animals. Absorption rates by dermal inhalation and oral exposure have been determined. Studies in humans and rodents have determined the pathways of EGBE metabolism and determined the metabolite responsible for hemolytic toxicity. Factors that influence metabolism, such as age dose and inhibitors have been studied.

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

B.1.Response: Rationale for not developing an RfD based on the 91 day drinking water study needs to be clarified. Exactly what are the deficiencies? In the rest of the document analyses using more than one approach were used, which is a strength. Why not use multiple approaches here?

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

B.2.Response: Modeling is not my area of expertise.

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

B.3.Response: Since the UF of 1 is appropriate for determining the RfC it should be appropriate for the RfD.

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

C.1. Response: I concur that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations; however, the "expected concentrations" should be noted. The description is appropriate because studies in mice (NTP 2000) have show slight increase in liver tumors and mild increases in forestomach tumors. The human studies are limited in number and are focused on non-cancer effects. None of the results have suggested that tumors are associated with human exposures to EGBE. However, such a determination is exceedingly difficult to make from studying exposed humans and the absence of finding tumors does not definitely indicate that EGBE is not carcinogenic. In the NTP study (2000) female mice developed squamous cell papillomas and carcinomas of the forestomach after prolonged exposure (> 582 d). These tumors were preceded by ulcers of the forestomach. Subsequent studies have shown that EBGE is bound by food stored in the fore stomach leading to a prolonged, relatively high local exposure of the mucosa. The incidence of these tumors was relatively low (2-12%) and showed a response to increasing dose. It is unlikely, however, that this mode of action is operative in humans.

Male mice developed a significantly increased incidence of hepatocellular carcinomas and hemangiosarcomas of the liver in the highest exposure groups. In vitro and in vivo studies were used to develop the mode of action based on an indirect mechanism of hemolysis, uptake of hemosiderin by hepatic phagocytes, and resultant production of reactive oxidant species. The MOA is plausible; however, it has not been shown that the same events occur in humans at potential exposure concentrations.
The weight of evidence has been adequately described.

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

C.2. Response: The MOA for male mouse liver cancer following EGBE is a solid proposal generally supported by scientific data. The initial steps, metabolism of EGBE to BAA and the swelling and hemolysis of RBCs by BAA have been established by several studies to occur in humans, rats and mice, as well as other animals species. Excess hemoglobin resulting from hemolysis taken up by phagocytic cells of the liver and spleen and stored as hemosiderin has also been reported in humans, and in other mammalian species. The subsequent steps are less well supported. Oxidative damage and increased synthesis of endothelial and hepatocyte DNA are proposed to be initiated by generation of reactive oxygen species from hemoglobin derived iron in Kupffer or other cells in the liver or by production of cytokines/growth factors by the Kupfer cells that suppress apoptosis and promote cell proliferation, or both. These actions have been shown with *in vitro* studies but have not been shown to occur *in vivo* and at cellular doses that occur in exposed animals or humans. In addition the reactive oxidative species also are purported to damage DNA (which has been shown *in vivo* with comet assays in one study), modulate liver cell gene expression (extrapolated from work in mammalian cell lines). ROS have been shown to stimulate cell proliferation and inhibit apoptosis. Increased DNA synthesis in endothelial cells and hepatocyte has been shown in mice, but not rats, exposed to EBGE. The initiation and promotion of liver cells by ROS generated by EBGE is less well supported by laboratory studies. The MOA could be strengthened by relating the doses required to trigger the steps involving oxidative damage to what is required to initiate similar damage in human cells.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

C.3. Response: The proposed MOA for forestomach tumors is a plausible model backed by reasonable scientific evidence. The initial steps of deposition, retention and metabolism of EGBE in the forestomach are well documented. The prolonged retention is much longer than what conventional wisdom might predict. The next step of irritation with a compensatory proliferative response in the gastric epithelium is also well documented. This step is consistent with the current thinking that inflammation can be an initiation/promotion factor in carcinogenesis (Mantovani 2008)

4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

C.4. Response: I do not recommend development a MOA for pheochromocytomas in female rats exposed to EGBE. The uncertainty in differentiating hyperplasia from tumor, the marginal dose response, the lack of significantly increased incidence and the lack of tumors in other organs of the body are valid reasons for giving scant weight to this observation. In addition, the significance of this tumor for human carcinogenesis is questionable. Typically, male F344 rats are the ones to show increased incidence of pheochromocytomas in chronic bioassays (Nyska 1999). The increased incidence in NTP chronic bioassays has been associated with severe nephropathy (possibly by disruption of calcium metabolism) and with space-occupying lung lesions (Ozaki 2002). In addition, multiple disparate factors can affect the incidence including xenobiotic agents, dietary factors, factors from pituitary tumors (common in F344 rats) and stimulation of the autonomic nervous system. In most cases the exogenous factors do not cause DNA damage, but may affect the response by indirect mechanisms. These factors complicate any extrapolation of the MOA to humans.

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations.

C.5. Response: The nonlinear threshold approach is certainly warranted in this evaluation based on the lack of mutagenicity of EGBE and the modes of action for induction of liver tumors (initial hemolysis leading to iron deposition in the liver and production of ROS) and forestomach tumors (local irritation, inflammation and hyperplasic response) The section describing this (p103 l. 1-6) could be strengthened by showing or referring to a non linear dose response curve for some hematologic parameter (e.g. RBC count or Hb).

Although the linear dose response needs to be mentioned, the discussion in 5.4.1.1 is a distraction for me, particularly Table 5-16. Although there are several disclaimers that the table is for illustrative purposes, I think the casual reader may get the wrong impression, based on a linear dose response, that this compound may be a likely cancer agent.

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David Jollow

David Jollow Post-meeting Comments
IRIS Toxicological Review of Ethylene Glycol Monobutyl Ether (EGBE)

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

This document is generally well written and laid out in a logical and concise manner. The scientific evidence for non-cancer and cancer end-points is presented clearly. However, there appears to be several areas where some additional discussion/modification may improve the document. These are:

i), The terms "lysis" and hemolysis" appear to be used interchangeably and seem to have led to the assumption that the hemolytic anemia seen *in vivo* after EGBE results from the direct lysis of damaged red cells within the vascular system. While this assumption is not unreasonable, the data available are not unequivocal and could conceivably lead to an under-estimation of the hemotoxic dose.

On the one hand, the data clearly indicate that incubation of rat red cells *in vitro* with high concentrations of BAA results in swelling of the red cells and direct lysis. However, *in vivo* data indicate that exposure to EGBE/BAA leads to an increase in the spleen/body weight ratio, implying a crucial role of the normal splenic sequestration process. The spleen removes whole cells (damaged or aged) by specific and highly selective receptor-mediated sequestration into resident macrophages. The liver (i.e, Kupffer cells) is key in the removal of broken cells and cell fragments. It should be appreciated that when a massive hemolytic event is occurring *in vivo*, the engorgement of the spleen may lead to failure of the resident macrophages to retain "sequested" red cells and hence result in spillage of damaged and partially-lysed red cells. In this situation, distinguishing between extra- and intra-vascular "lysis" becomes difficult when based on morphological changes (MCV etc) in the circulating red cells. The appearance of morphologically altered cells, even if the changes are dramatic and similar to those seen in *in vitro* experiments, does not indicate unequivocally that the hemotoxic event leading to drop in

Hct is the spontaneous lysis of red cells throughout the circulation. (It is appreciated that since the splenic macrophages reside within the splenic vasculature, all hemolytic "events" are [strictly speaking] intravascular). However, the distinction is important in that it influences the selection of criteria for dose- and concentration-response relationships and for interspecies (rodent to human) extrapolation.

The analogous situation with the classical "hemolysin" phenylhydrazine (PHZ) illustrates this problem. PHZ has been studied for over 50 years and is well known that *ca* 2+g hr incubation of rat RBC with 2-10 mM PHZ results in morphological change (spherocytic echinocyte formation) and direct lysis. The EC_{50} for *in vitro* lysis is not precisely defined in the literature but may generally be considered to be in the 2-5 mM range. However, if rat RBCs are tagged with ^{51}Cr prior to the 2 hr PHZ exposure, then washed and returned to isologous rats, the exposed cells show a concentration-dependent decrease in their survival curves secondary to splenic sequestration (McMillan et al JPET 287:868-876, 1998) Under these conditions, the EC_{50} for the hemolytic activity of PHZ is ca 800 □M; at least a two to three fold decrease as compared with measurement of direct lysis. Direct cell lysis at 800 □M as measured in post incubation wash media was <1%.

Two issues seem to stand out from the lack of definition of the MOA. First, the kinetics of the toxic "hit" are undefined; specifically, if the toxicity of BAA towards the RBC is proportional to its AUC or its Cmax. AUC implies an accumulation of injury, whereas Cmax suggests a reversible association with a receptor or other protein. As discussed in section 5, the decision affects the selection of data for RfC etc. etc., and the use of PBPK modeling in rodent to human extrapolation. The observation that severity of the hematological effects does not progress in severity in the subchronic-to-chronic study (p75, line 2) does not allow distinction in that it is compatible with *both* a steady state of the mean age distribution of the red cells in treated rodents (i.e., a gradient of decreasing susceptibility of older-to-younger cells) and with the toxicokinetic-toxicodynamics of BBA interaction with a receptor. On the other hand, the observation that the hemotoxicity of EGBE is dependent on the mode of oral ingestion (page 78, line 10) is highly suggestive of the crucial role of concentration rather than AUC in that oral gavage may be expected to cause a more rapid ingestion and higher blood levels of EGBE than that provided by

drinking water, spread over many hours of access. Higher peak levels of EGBE should give higher peak levels of BAA.

Of particular interest is the acute *vs* sub-chronic/chronic comparison. In the acute exposure situation, the % of affected red cells will be very high and splenic overload is unavoidable. The animals will appear to be experiencing a "spontaneous intravascular lysis". In more chronic dose situations, the initial dose(s) will remove the older red cells (consistent with the suggested red cell age-related susceptibility) leaving a younger mean-average age to the red cell population. On continued "steady-state" exposure to EGBE, one can expect that only a small fraction of the cell population will move into the age range that is susceptible to the "steady-state" BAA levels and that the amount of red cell mass removed per day will be very much less than that of the acute dose situation. During chronic doses studies, it is likely that the spleen would be able to cope with the "demand" for red cell sequestration. The feature central in the removal mechanism after single and initial doses of EGBE; viz, acute massive spillage of hemoglobin-iron with major involvement of the Kupffer cell population of the liver, may not be so important in the chronic exposure experiments. Of importance, the role of the spleen *vs* liver, with its associated risk of perturbation of normal iron turnover and storage mechanism (and hence hemosiderin deposition), may readily be resolved experimentally.

Second, the assumption of the crucial role of direct lysis may have deterred exploration of alternate hypotheses on the MOA. Of note is the observation of Ezov et al (Cardiovasc. Tox 2002) that EGBE causes disseminated thrombosis and infarction in Fischer 344 rats. This is suggestive of a role for loss of sidedness of the red cell membrane. The outer leaflet normally has a net positive charge due to its predominant presence of phosphatidyl choline. This is considered to be important in suppressing adhesion to the similarly positively charged membranes of epithelial cells lining the vascular. Ionic changes in the red cells induced by BAA may well activate the "scramblease" that promotes movement of phosphatidylserine from the inner to the outer leaflet with corresponding reduction of the normal repulsive behavior between the red cell surface and that of the vascular lining. If this mechanism is involved in BAA-induced hemotoxicity, it would provide a readily quantitatable index to establish the relative sensitivity of rodent *vs* human erythrocytes, with attendant implications for risk assessment.

Please replace the opening words of the 4.5 Synthesis/evaluation section of p 46; viz, "Intravascular hemolysis" with "Hemolytic anemia". Please modify the text here and elsewhere to remove the suggestion that the toxicity of concern is "frank intravascular lysis" (e,g., p46 lines 30-32) with its attendant mechanistic implications. The requested modification is one of tone rather than major change in interpretation and does not preclude the use of available "lytic" data for comparison purposes provided that it is clear that we do not know the actual "toxic hit(s)" that lead to the premature removal of red cells from the circulation. Please identify the need for a firmer definition of the MOA for future research.

ii). The proposed MOA for the hemangiomosarcoma in male mouse liver resulting from chronic EGBE exposure is considered to be reasonable and is acceptable, even though the supporting data is far from overwhelming (e.g., lipid peroxidation is notoriously unreliable as an index of ROS activity). I have two concerns as to the use of hemosiderin as the POD for assessing risk of hemangiosarcoma:

a), the present data appears to rest on Prill Prussian blue positive cells in the liver. While morphometric analyses of this type provide important information, my experience has convinced me that they are inherently more susceptible to error as a quantitative index. Are liver samples from the test animals still available? If so, analysis of iron content might provide a more reliable index of "toxic load".

b), I have not found data in the document that describes the shape of the dose/response curve between amount of hemosiderin deposition with incidence of hemangiosarcoma. The discussion on page 56 is very general and that of Table 4-8 does not allow assessment of the shape of the D/R curve. From a biological perspective, it seems likely that this would be a "classical" sigmoidal relationship and should influence derivation of the BMD etc..

c), Inclusion of pentachloroanisole in Table 4-8 seems inappropriate and weakens the association between hemosiderin deposition and hemangiosarcoma.. This chemical is not hemolytic and hence hemosiderin-iron deposition is unlikely. The chemical (and especially its polycyclic analogs) is very likely to cause porphyria with deposition of porphyria-related pigments. This section would also be strengthened by inclusion of hemolytic compounds such as aniline, which induces iron overload and neoplasia in the spleen rather than the liver. In that the iron overload/ROS generation mechanism of initiation of neoplasia is of general interest for a

variety of compounds, the need to understand the fundamental processes involved, including the basis of selectivity for target tissues, goes far beyond EGBE itself.

iii), Is there information on other parameters/aspects of the hemotoxicity such as levels of methemoglobin, haptoglobin, hemopexin? Has a Coombs test been done during chronic studies? It is appreciated that "free" hemoglobin in the circulation is rapidly converted to methemoglobin and that a distinction must be made between whole blood and intracellular RBCs levels of MetHb. Although unlikely, data and discussion should be included to rule out other well known causes of hemolytic events. As discussed below, data on haptoglobin and hemopexin levels and saturation during chronic exposure to EGBE may help in defining the relative importance of splenic *vs* liver macrophages in the premature removal of RBC.

iv), PBPK modeling of EGBE and BAA is not well illustrated in the document. Fig. 4-2 is not very informative. Is there a better figure to illustrate the metabolic relationships? The presentation in the document did not allow me to assess the reasonableness of the conclusions drawn.

v), The low renal clearance of BAA relative to GFR needs to be discussed in more depth. Is there data on plasma albumin binding of BAA and does it vary between rodents and humans? If the binding is less than ca 95%, it is unlikely to be the explanation of the restricted renal elimination . The alternate explanation of active *reuptake* by a carrier mechanism is amenable to study: is there relevant information in the literature? Resolution of this question is deemed crucial in understanding the factors that determine both the Cmax and AUC of BAA in the various species/sexes of rodents as well as the determination of HECs.

Minor problems/consideration:

a), The toxicokinetic discussion (section 3.2) is, perhaps of necessity, somewhat diffuse. I found myself going back and forth looking for specific parameters such as $T_{1/2}$ of EGBE and BAA in the several species/modes of administration/acute vs chronic etc. Is it possible to present a Table incorporating some of the relevant kinetic information?

b), Reversal of ADH and ALD in the text, e.g., p 11, lines 21/22.and p 44 lines 16-19. EGBE to BAL is catalyzed by alcohol dehydrogenase (ADH), and BAL to BAA by aldehyde dehydrogenase (ALD). Perhaps this confusion could be minimized by use of ALDH, a more common abbreviation for aldehyde dehydrogenase.

c), Table 4-2 title should include "and mice".

d), Table 4-2: the footnote explanation on incidence is unclear (especially the second sentence).

e), I know that it is a lost cause but p 20, line 3: nouns etc have gender, animals have sex! (regardless of the dictionary definitions!!)

f), Is there a discussion of the MTD of EGBE under the various experimental exposures? The comment of low survival in the male mice at 125 and 250 ppm "which may have been due to carcinogenic effects in the liver" (no autopsy??) seems a distracting lead-in to the next sentence; viz, "A high rate of hepatocellular carcinoma was found in these exposure groups". Please resolve.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

As discussed above, I would like to see a more thorough characterization of BAA's MOA in damaging the rodent RBC, the fate of the damaged red cells (splenic sequestration or frank intravascular lysis), role of the liver Kupffer cells in removal of damaged red cells, the kinetics of the "toxic hit" (dependent on the concentration of BAA in a reversible fashion or the result of accumulation of injury over time[i.e., proportional to AUC of BAA rather than just concentration]). I would also like to see the hemosiderin deposition quantitated and chemically characterized by analytical techniques in addition to morphometric assessment by Prussian blue staining. Such characterization and quantitation would allow a more reliable and robust assessment of the shape of the D/R for the hemosiderin/neoplasia relationship.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Specific research needs for risk assessment purposes include: definition of the red cell age susceptibility to BAA, definition of the MOA of the toxic insult(s) to the red cell with definition of dose metrics; contribution of loss of "sidedness" in the phospholipid composition of the outer leaflet of the red cell membrane to the premature loss of red cells from the circulation and the possibility that this may induce microvascular thombosis and infarction; role of the spleen *vs* the liver in the removal of damaged red cells and effects on haptoglobin and hemopexin levels and saturation during chronic exposure to EGBE; time and dose response relationships for hemosiderin deposition in the liver as a whole and in specific cell types; MOA and "dose"/response relationships for hemosiderin in hemoangiosarcoma development;

Specific experimental series include use of ^{51}Cr-tagging and/or fluorescent dye-tagging techniques to assess the immediate fate of the BAA-damaged red cells after *in vitro* incubations followed by reintroduction to isologous rodents (spleen vs liver vs generalized deposition throughout the vascular bed). Pretreatment of the rodents with clodronate liposomes may be used to determine the contribution of splenic vs liver macrophages. Flow cytometry studies with annexin binding will permit definition of the EC_{50} for phophatidylserine exposure after BAA, which may be directly compared with ^{51}Cr-EC_{50} for hemolytic activity, as determined above. As noted, this is of particular relevance for human health considerations interest in that microvascular infarction and thrombosis of the type proposed by Ezov et al., may occur as a consequence of occupational exposure to EGBE. The *in vitro* exposure/*in vivo* assessment of fate, type of approach allows concentration/hemolytic response definition and parallel assessment of biochemical change(s) in the red cell under directly comparable toxic conditions. Definition of the acute cellular changes can then allow specific probing of events during chronic exposure and perhaps lead to the recognition of key events that favor hemosiderin or other iron deposition in places that are crucial for localized ROS generation and toxicity.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

With the exception of assumption of a direct lysis within the circulation as the MOA of BAA-induced hemotoxicity, I have no problems with the presentation in this section. The use of a BMD approach appears well justified based on the available biological information.

The selection of a UF of 1 for rodent to human extrapolation seems excessive and needs a more detailed specific justification. The available clinical "overdose", and epidemiology data (Section 4) suggests that humans are insensitive to EGBE-induced hemotoxicity. This insensitivity is supported by the comparative *in vitro* studies (although I am not comfortable with the "direct lysis" endpoint). Collectively, the clinical and experimental experience point to a relative resistance factor for humans of at least 10, suggesting that a UF significantly less than 1 would adequately safeguard humans. The UF of 10 for human variability also needs a more detailed justification. As noted (pp 86-87), human studies do not point to enhanced susceptibility in the elderly or in several red cell deficiencies. The rodent studies of Ghanayem et al (TAP 91:222-234, 1987) suggest toxicokinetic explanations rather than toxicodynamic effects. [An additional contribution not proposed by the authors is that the younger animals are rapidly growing and have a correspondingly rapid expansion of their red cell mass. The mean average age of their red cells is thus lower than that of the older animals. If (as suggested elsewhere) younger red cells are more resistant, the younger animals would of necessity appear more resistant to the hemotoxicity of EGBE].

The discussion on methods of analysis is outside my area of expertise.

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

The selection of the two year inhalation study by the NTP as the basis for the chronic inhalation RfC is scientifically justified. The study has been adequately described in the document. No additional/alternate studies are identified for consideration.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

The relationship between iron overload in tissues and increased incidence of fibrosis, cirrhosis, and tumors secondary to tissue iron overload is well documented for conditions such as hemochromatosis and after parental iron loading in β-thalassemia. Hemosiderin deposition may be considered to reflect the extent to which EGBE causes hemolytic episodes in excess of the normal red cell elimination capacity of the spleen and hence the extent to which redox-active iron is deposited in tissues. Whether hemosiderin itself is the source of reactive oxygen species (ROS) leading to hemangiosarcoma or whether it is a surrogate for redox-active iron deposited directly in the target cells is not clear, but does not weaken the use of hemosiderin as a critical effect for risk assessment purposes. The present use of the relationship is clearly relevant and reasonable. The criteria and rationale are adequately and objectively described.

Major deficiencies in the use of hemosiderin as the critical effect lie in its method of quantitation, lack of definition of its time-profile of accumulation, and of "dose"/response relationship(s) between hemosiderin levels and toxic events.

Alternate end points such as decrease in hematocrit (Hct) also have problems. Decrease in Hct could be used but it should be appreciated that Hct post EGBE exposure is the balance between enhanced loss of red cell mass and replacement by immature red cells etc., and by increased erythropoiesis. Thus the fall in Hct is a measure of both the toxic insult and the body's compensatory mechanisms, and not a direct simple assessment of the hematotoxicity of EGBE. Further, if the mechanism of removal of BAA-damaged red cells during chronic EGBE is splenic sequestration (i.e., post the relatively large decrease of the initial doses) rather than a "true" intravascular lysis, the fall in Hct may be a misleading indicator of the toxic potential of EGBE administration. Normal and "moderately" enhanced sequestration is associated with transferrin-mediated iron transport in a non-redox active form. Overflow protective mechanisms include plasma haptoglobin to sequester "free" hemoglobin and hemopexin to take up "free" heme". Deposition of redox-active iron in target tissues would be enhanced in situations where the transferrin/haptoglobin/hemopexin protective mechanisms are exceeded.

It seems reasonable to expect that at toxic EGBE dose levels, the decreased Hct of chronically-treated animals is associated with both maximal transferrin/haptoglobin/hemopexin sequestration of iron and its "excessive overflow" (by whatever mechanism). Thus the fall in Hct, per se, would be a measure of both normal and abnormal processes and not a direct estimate of "excessive overflow", and hence the extent to which red cell iron turnover exceeds the body's capacity to sequester it away from deposition in ROS-generating form(s) in target tissues. If the neoplastic or other toxic potential of EGBE is, as postulated, secondary to redox-active iron deposition in tissues, the critical effect should reflect "excessive overflow" and not just total red cell loss (i.e., decreased Hct).

For risk assessment purposes, measurement of haptoglobin and hemopexin levels and saturation during chronic EGBE exposure and a more selective and analytical determination of the iron overload might resolve difficulties in selecting a critical effect.

Since the use of both hemosiderin and decreased Hct appear to have deficiencies as "critical effects", it is suggested that the utility of both parameters for POD purposes be discussed and illustrated in the review.

3. 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.

The crucial role of hemotoxicity leading to iron overload of hepatic tissue is well justified indicating that a non-linear relationship exists between EGBE exposure and hemangiosarcoma incidence. The BMD modeling of hemosiderin staining is acceptable as the POD.

The methodological approaches for application of the POD are well presented. Their adequacy is outside my area of expertise,

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

PBPK modeling for extrapolation from experimental animals to humans is well accepted as the preferred approach to assess tissue target dose of a toxicant. The models appear to have been appropriately utilized Difficulties noted by other reviewers in the selection of models and parameters need to be resolved and scientifically supported.

As noted above, an improved illustration and explication of the PBPK model for rodents and human would enhance the document.

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

- A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).
- Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

  Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

As discussed above, the UF values of 1 for rodent to human and of 10 for human variability need greater justification. I appreciate that there is a lack of human data in chronic exposure situations and that this may necessitate larger UFs. Of importance, if the selection of a UF of 1 for rodent to human is based on an EPA policy decision to cover unknown uncertainties rather than on the available *in vitro* data and acute clinical observations, this should be clearly stated. Clearly, the

absence of chronic exposure data for human is of concern and needs to be addressed. Please expand.

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

As discussed above, I am not comfortable with the rationale for selecting a UF of 1 for rodent to human extrapolation and 10 for human variability. As noted, it is appreciated that the lack of human chronic exposure needs to be incorporated into the selection of UFs. A more detailed justification is requested with clear separation between the policy and scientific basis of the decisions.

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify rand provide the rationale for their use.

Available date suggests that the decrease in Hct represents a steady state situation without progression from sub-chronic to chronic exposure. The rationale for not using the 91-day drinking water study described in the document needs a more detailed discussion and justification.

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

If the chronic inhalation study is to be used to determine the RfD, PBPK modeling is essential. However, as discussed above in General Comments, there is a lack of adequate definition of the MOA by which BAA damages red cells. There is at present no way to decide whether the AUC or the concentration (perhaps above a MEC) of BAA is the appropriate dose metric. Since both absorption and elimination are first order processes, the Cmax of EGBE (and hence of BAA) will be more dependent on the absorption rate constant than is the AUC. The AUC may vary little between oral and inhalation exposure, whereas the Cmax may be very different. Without definition of the MOA and hence of the appropriate dose metric, avoidance of extrapolation seems desirable.

If extrapolation is to be done, the PBPK modeling approach is essential. As noted, it is felt that the PBPK modeling is not adequately described for the non-PBPK specialists reading this document.

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

As above, the rationale for selection of UF factors needs more explication

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

The conclusion that EGBE is not likely to be carcinogenic for humans at expected exposures is well justified and the rationale adequately presented.

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The proposed MOA is reasonable and supported by available data. The reasoning is sound and transparently and objectively described.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The MOA proposed for forestomach tumors appears adequately supported by the available data. The reasoning is sound and transparently and objectively described.

4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

The reasoning is sound and transparently and objectively described. No alternate analysis is warranted.

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations.

The non-linear approach is appropriate and scientifically sound. The alternate linear analysis is felt to be inappropriate.

Michael Pereira

Review of Toxicological Review of Ethylene Glycol Mono-Butyl Ether
Michael A. Pereira Post-meeting Comments

1. **General and Major Comments:** extremely well written and is very easy to follow the rationale and procedure used for the hazard and dose-response assessment of EGBE, including the calculation of the RfC and RfD. The document also includes a very comprehensive and complete review of the literature pertaining to EGBE.
2. The use of hemosiderin as the critical adverse effect needs to be better justified in the document. Rather the effect of EGBE on hemosiderin staining would appear to be more suitable as a biomarker for exposure to EGBE.
3. The UF for variation in sensitivity within human populations (UF_H) should be 1 and not 10 and for interspecies variation (UF_A) should be 0.1 and not 1.
4. The discussion of the Mode of Action for the apparent carcinogenic activity of EGBE could be greatly decrease since:
   d) it give too much credence to activity that is not statistical significant,
   e) EGBE is not likely to be a human carcinogen, and
   f) Contains too much speculation.

Specific Comments:

1. Page 1, Line 15: change to: exposure (<10% of lifetime)…. Reason: The lower limit of duration is critical and not the upper limit of a lifetime.
2. Page 3, Line 15: delete "it is anticipated that"
3. Page 8, Line 9: change to: "were more than two orders of magnitude"…. Reason: 46 is more than 100 times greater than 0.29.
4. Page 11, Lines 5 and 6. Give the maximum $t_{1/2}$ value for rats and mice. Reason: As written there sentence does not indicate that the value was different for the two species. For example: both species could have a $t_{1/2}$ value of 4.
5. Page 15, Lines 21-23. The sentence starting with "By" does not make sense. Significantly increased relative to what? Mean value of what values? Should it be: " was reported to be as high as 39% of the total….."?
6. P16, Line 19. The use of plural for target is not clear. Do you mean to say: "the putative cancer target organ" or "the putative cancer target cells."

7. Page 17, Line 28: Change lesions to alterations.
8. Page 18, Table 4-1. In the footnotes define "§". Mean ±SE?
9. Page 19, Table 4-2. The title is wrong; lacks mice and organ weights. It would be best to separate into two tables; one for rats and one for mice. The title for the rat table should include "histopathological alterations" and not "lesions". The title of the mouse table should include "body and kidney weight …….in female mice." Also, give the body weight before the organ weight. Also, include the actual kidney weights, even though they were not different among the treatment groups.
10. Page 20, Lines 20-22. Move to Section 4.2.2 since this is a cancer study and not a toxicity study, even though for only 120 days. Line 22. Change "induce increases in tumors" to' induce an increase in tumors", since as written there could have been an increase in tumors in the different sites.
11. Page 27, Line 5 change "anda" to "and."
12. Page 27, Table 4-6. Please check that 48.7 ±1.9 (Male mice, 62.5 ppm, 12 months) is significantly different than the Control, 47.9 ± 0.4

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Response:
The Review of the noncancer hazard is very logical, clear and easy to follow except for the justification of the use of hemisiderin as the critical effect. With respect to the cancer hazard, the Review places too much emphasis on non-statistically significant and very weak responses, although the overall conclusion that EGBE does not pose a carcinogenic hazard to human is correct.

There is also too much redundancy, speculation, extraneous information, and assumptions in the discussion of the cancer hazard.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

Response:

There are no additional studies.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Response:
No further research is needed, nor would any likely increase confidence.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Response:

The UF for variation in sensitivity within human populations (UF_H) should be 1 and not 10 and for interspecies variation (UF_A) should be 0.1 and not 1. Although the Document states numerous times that humans are much less sensitive to hemolytic effects of EGBE and that there would not be sensitive human populations, it still uses a UF_A equal to 1 and a UF_H equal to 10. These values are not consistent with the text. A UF_A equal to 0.1 and a UF_H equal to 1 would be much more consistent with the text of the Review.

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Response:
The NTP 2-year inhalation is very appropriate and justified as the basis for the chronic inhalation RfC.

The study for the most part is well and objectively described with the following points that need to be addressed and clarified:

a) Page 25, Line 7. Giving the survival for only the highest two treatment groups is meaningless without the value for the control group, unless it is zero. Please correct.
b) Page 28, Lines 20-25. The first sentence is not clear since it implies that there were neoplastic effects observed in female rats when there were not. The results of the 2-year bioassay in rats and mice should not be combined but rather reported separately. The first sentence of the paragraph should read "At the end…….no significant neoplastic effects were observed in male and female rats. However, a non-significant level of combined incidence of benign and malignant ……was observed, .ie., 3/50, 4/50, 1/49, and 8/49.

Note: on Line 22 the and/or should be and.

c) Page 28, Line 26 and 27. Delete "may have been…….carcinogenic effects in the liver." Hepatocellular carcinomas are not lethal and since they were also found in a significant number of control mice (10/50) there should also have been death in this group. Since the number of mice supplying data in all treatment groups is reported as 50, the actual mortality in the different groups should be given including when they occurred. As well as the reason why the mortality did not reduce the number of evaluated mice.

d) Page 28, Line 29. What statistical test was used to get a p-value <0.01? The data does not appear to be statistically significant.

e) Page 28, Line 30. Delete "However"

f) Page 29, Lines 1-4. This sentence is not true since female mice had the greatest EGBE reduction in body weight therefore a maximum tolerated does was used. It is more likely that the effect in male liver was not significant.

g) Page 29, Line 11 and Lines 12-15. Delete on Line 11 "While" and on Lines 12-15 from "the incidence of……hyperplasia." These tumors were increased in male mice.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Response:

Hemosederin is not a critical adverse effect but rather might be a biomarker for exposure. The adverse effect is hemolysis. The quantitative if any relationship between hemosederin and hemolysis is not established. In fact the discussion of the two effects indicates that they do not correlate, especially the fact that hmosederin increased with time while the hemolytic indices did not. This would suggest that the rate of accumulation of iron (hemolysis) does not change (increase) with time but rather the extent of hemosiderin staining does increase. Hence, hemosiderin staining could be present after two years of exposure without any significant adverse clinical effect of hemolysis; there could be a slight subclinical increase in hemolysis without any significant deleterious health effect. This should be discussed in full at the meeting.

Furthermore as discussed at the meeting in RTP, the hyaline degeneration of olfactory epithelium in both sexes of rats is a significant effect of EGBE and should be further discussed (Page 25, Lines14-18). This additional discussion should include a comparison of the resulting RfC using olfactory degeneration to the RfC resulting from hemolysis or for that matter hemisiderin.

3. 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.

Response:

The BMD modeling and the determine POD is appropriate and objectively applied. However, male rats were used instead of female rats. Since female rats are more sensitive and were used in the previous IRIS assessment, why were male rats used in the document?

Also, the document states that a NOAEL was not determined for EGBE in either male or female rats. However with respect to female and male rats, in the 14-week study a NOAEL for hemosiderin and RBC count was observated at 31ppm and in the 2-year bioassay, a NOAEL of 31ppm was also observed for hemosiderin.

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

Response:

The PBPK modeling is appropriate.

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

- A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).
- Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs),

including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

Response:

The UF for variation in sensitivity within human populations (UF_H) should be 1 and not 10 and for interspecies variation (UF_A) should be 0.1 and not 1. As state in the document mice, rats and other species are much more sensitive than humans to hemolysis by EGBE. Therefore, scientific evidence strongly indicate a UF_A of 1/30 (Page 59, Line 5: For example, in vitro study of RBCs indicated that humans are more than 150-fold more resistant to EGBE than rats). However, a UF_A of 0.1 would be acceptable.

Similar studies of RBCs from human children and elderly individuals were no more sensitive to EGBE than those from adults. Hence, an UF_H of 1 not 10 is recommended.

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

Response:

This uncertainty factor of 1 is appropriate.

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

Response:

The document does not justify why the 91-day drinking water should not be used to calculate chronic RfD, since this study was previously used to calculate one. No further toxicity, including any toxicity at a lower dose level was observed in the 2-year inhalation study beyond those observed at earlier times. Thus 91-day exposure is sufficient for determination of the chronic RfD. Using the previous calculation an of the BMD50 HED of 5.1 mg/kg-day and a total uncertainty factor of 0.1, an RfD of 51 mg/kg-day is recommended value.

The document should describe in detail the procedure used in the 1998 document and in the present document using the 91-day drinking water study. The description should highlight where the two approaches using the drinking water study differ, give the explanation the differences and justified any difference in the present document.

It is recognized that there is no chronic drinking water study. Hence a database uncertainty (UF_S) of 10 would be reasonable.

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

Response:

The extrapolation is correct, however this extrapolation is not required since there is an adequate oral (drinking water) study.

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the

database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

Response:

The database uncertainty factor of 1 is appropriate.

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

Response:

Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/ background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans.* The phase "at expected exposure concentrations" could be deleted (Page 51, Lines 2 and 30-31). This is because EGBE was not carcinogenic in rats, was only very weakly carcinogenic in mice and appears to have a MOA at the RfD and RfC values that is unlikely to be carcinogenic in humans.

Should the document include the phase "at expected exposure concentrations," then it should also include the reported levels of EGBE exposure, i.e., the range of EGBE concentrations found in occupational exposure, in drinking, ground, and surface water, and in the air. This information could be added to Section 2 (Chemical, Physical and Exposure Information). This exposure information is important for the Reader to be confident that EGBE is not likely to be carcinogenic to humans at **expected exposure concentrations**.

Alternatively the document could state: "EGBE is not likely to be carcinogenic to humans at **the calculated RfC and RfD values presented in this document**. This is the statement I recommend since it is what I believe the EPA wants to convey. That is that the calculated RfC

and RfD would protect humans not only from the toxicity of EGBE but also from any possible carcinogenic activity.

Page 53, Lines 6-9 should be deleted or changed to "The NTP (2000)…….did not demonstrate carcinogenic activity for EGBE in male or female rats."

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

Response:

The discussion of the Mode of Action for the apparent carcinogenic activity of EGBE should be greatly decreased since:

a) it give too much credence to activity that is not statistical significant,

b) EGBE is very unlikely to be a human carcinogen, and

c) contains too much speculation.

Page 54, Line 13-16: Change to "it is possible thqat events leading to oxidative stress could contribute to the development of hemangiosarcomas and hepatocellular carcinomas in male mice. Note: HGBE does not cause transformation.

Page 54, Line 31-32 and Page 55, Line 1-2: Delete these points of 5-8. Because:

a) There is no evidence for HGBE causing oxidative damage to DNA.

b) Alteration in gene expression is a meaningless point since it is obvious that to have increased DNA synthesis and cell proliferation there most be alterations in gene expression. This is an important point only should alteration in genes specific for the activity of HGBE are identified.

c) Point (7) was already stated in Point (4b)

d) There is no evidence that HGBE promotes initiation of hepatocycte, no less endothelial cells. HGBE is non-genotoxic.

Page 55, Line 26-29. Delete since too speculative and most likely not involve in HGBE carcinogenic activity.

Page 55, Lines 31-36. This possible MOA of ROS induced increase in cell proliferation is consistent with the lack of genotoxicity of HGBE (see Section 4.4.4).

Page 55, Line 33 and though out the document delete "spontaneous" in describing tumors and neoplasms for which you might not know the cause. These are not spontaneous but rather the result of heredity, hormones, such as estrogen, oxidative damage, etc.

Page 56, Line 1. Delete "hepatic hemosiderin buildup". The cause of the oxidative damage is hemolysis.

Page 56, Line 10-31 and Table 4-8. Delete these lines and the table. The critical dose-response relationships that should be discussed are those of Siesky et al. (2002) and of hemolysis in the NTP studies.

Page 57, Section 4.6.3.1.3 should be deleted since it is not relevant or adds anything to the understanding of the activity of HGBE. The relevant points in these paragraphs such as the discussion of Kamendulis et al. (1999) and Siesky et al. (2002) has already been given.

Is it appropriate to use a non-peer review reference like Kamendulis? If so it should be identified as a report and not a publication, especially since it was written in 1999, but not let published.

Page 59, Lines 10-22. Delete this paragraph since it is redundant and already discussed in Section 4.

Page 59, Lines 23-34 and Page 59, Lines 1 and 2. This paragraph is not relevant since HGBE is not genotoxic. A two sentence paragraph is all that is needed. One sentence stating the possible of a genotoxic MOA for HGBE, followed by a second sentence stating that this is not appropriate for HGBE since it is not genotoxic.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

Response:

Again, the discussion of the Mode of Action for the apparent carcinogenic activity of EGBE should be greatly decreased since:

a) it give too much credence to activity that is not statistical significant,

b) EGBE is very unlikely to be a human carcinogen, and

c) contains too much speculation.

Page 62, Line 23 & 24. Step (5) should be deleted since it is redundant with Step (4).

Page 62, Line 25 & 26. Step (6) is probably not true and in any case too speculative. There is no evidence for clonal growth; it is more likely a field effect. Also, there is no evidence for spontaneously initiated cells. High level of cell proliferation could lead to genetic and epigenetic alterations that enhance the occurrence of tumors. I would recommend deleting this step and adding to the end of Step (4) "that enhance the occurrence of tumors.

Page 65, Line 4. Change induce to "increase the incidence of"

Page 65, Line17-35. This paragraph is redundant with what was previously stated for HGBE and its metabolites and just should be deleted.

Page 66, Line 28 to Page 67, Line 10. This paragraph is not relevant since HGBE is not genotoxic. A two sentence paragraph is all that is needed. One sentence stating the possible of

a genotoxic MOA for HGBE, followed by a second sentence stating that this is not appropriate for HGBE since it is not genotoxic.

Page 67, Lines 11-16. This paragraph does not belong in this section and should be deleted.

Page 68, Line 14. Delete "at expected environmental concentrations.

4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

Response:

The incidence of pheochromocytomas of the adrenal medulla in female rats was not statistically significant and therefore should not be given any weight in the qualitative or quantitative assessment of EGBE cancer potential. If anything the document puts to much emphasis on this non-significant observation.

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations.

Response:

The choice of the nonlinear threshold approach is scientifically sound and objectively described. However, I recommend that a quantitative assessment of the carcinogenic potential of EGBE not be included in the document. Instead the document should state that "The evaluation of EGBE

for carcinogenic activity indicates that it is not likely to be a human carcinogen and since there is not evidence to suggest otherwise, a quantitative assessment of the carcinogenic potential of EGBE was not done."
To perform such an assessment would result in the false suggestion that EGBE represents a carcinogenic hazard to humans.

General Question 3 discussed at the meeting.

Response:
A very important research need that would greatly increase the confidence of using hemisiderin as the critical effect is the determination of the dose and temporal relationship between EGBE induced hemisiderin and its induction of hemolysis.

Andrew Salmon

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

In general, the review is well laid out, logical and clear in its presentation of the evidence and of the Agency's interpretation of that evidence in deriving the RfC and RfD. Although I have some differences noted below, the document as a whole is thorough, objective and logically organized. A limitation of the approach presented, which should be addressed in the final version of the document, is the failure to adequately consider the respiratory system effects (hyaline degeneration of the olfactory epithelium) observed in the NTP long-term study as a possible endpoint for derivation of an RfC. This is an important alternative to the hematological effects and related sequelae, which is unlikely to show the large disparity in sensitivity between rodent and humans reported for the latter responses.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

None at this time.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Although the effects of EGBE on animals, especially laboratory rodents, have been extensively studied, there is an intrinsic lack of information on the nature and dose response of effects seen in humans following moderate exposures. Laboratory studies of human erythrocytes, and case reports of human poisoning, are sufficient to demonstrate the humans are relatively insensitive to the hemolytic effects which are the critical effect for toxicity in rats, mice and rabbits. A few case reports and the original volunteer studies by Carpenter et al (1956) indicate other less severe responses, in particular sensory or respiratory irritation, but these are in general poorly characterized. Occupational studies have not been especially revealing so far, but there would appear to be a case for further studies in humans, especially studies using rigorous

epidemiological methods and addressing mild effects which might not be a major concern in an occupational context but would be unacceptable in a community exposure situation.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

The discussion of uncertainty is thorough and lays out the assumptions used in the derivation of the RfC and RfD accurately and clearly as far as the issues addressed in the derivation are concerned. The consideration of alternative endpoints not related to the hematological effects in rodents has not been addressed.

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. *The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.*

This study appears to be the best choice as the critical study for derivation of the RfC, being of sound design, adequate size and duration, and including exposure by the relevant route. NTP studies also benefit from thorough and objective analysis and reporting. The study description in the document is clear and accurate. There is a background issue with the selection of the test species in that since humans are clearly less sensitive than rodents to the critical effect in the NTP study (hemolysis and its various chronic sequelae) the choice of test species in this case could be applauded in that evidently the most sensitive test species has been selected, in accordance with the guidelines. On the other hand the choice of a rodent study could be criticized in that since humans are substantially less sensitive than rodents to the EGBE-induced hemolysis, they could be prone to other effects which are masked in the rodent study. If this is

fact the case, the calculated RfC and RfD would be protective, but not predictive of the kind of toxic effects expected in humans if the RfC or RfD are exceeded: this might present a practical problem for risk management and mitigation. There does not seem to be any adequate alternative to the approach taken here unless more thorough epidemiological studies are eventually undertaken.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

If one is going to be concerned about hemolysis and its various chronic sequelae as an endpoint in defining the RfC, then the choice of hemosiderin staining is an excellent indicator. The document clearly identifies this as a recognized consequence of chronic hemolysis, and a precursor of both cancer and non-cancer lesions in the rodent liver. The mechanistic basis of these assumptions is laid out in convincing detail. Moreover the quality of data in the NTP (2000) study appears to be good, although one might have hoped for a continuous measure of hemosiderin accumulation rather than a quantal (present/absent) evaluation. It also has the advantage, as a systemic endpoint, of being susceptible to route-to-route comparisons in support of the RfD as well as the RfC, without concern for possible portal-of-entry effects.

On the other hand, there are also suitable direct measures of the hemolytic impact of EGBE (hematocrit, MCV etc) which are equally sensitive and could also be used as a critical endpoint in deriving the RfC: in fact this was the approach used in the previous (1999) toxicological review for EGBE. Since all the available indicators have some limitations in measurement and/or biological interpretation, it would be preferable to fully evaluate all the possible hemolysis-related endpoints rather than to concentrate most of the effort on hemosiderin deposition. Since these different endpoints appear to indicate similar points of departure for the RfC derivation, the overall synthesis of all these data would strengthen the confidence in the value derived.

However, choice of any endpoint related to the hemolytic effect of EGBE is problematic in the context of identifying an RfC or RfD since there is evidence that humans are considerably (50 -150 fold?) less sensitive than rodents. Indeed the whole basis of the argument that the liver tumors observed in rodents are not relevant to human health risk is based on the finding that hemolytic doses are unlikely to be achieved in humans by inhalation, and are not always even reached following accidental or suicidal poisoning by the oral route. It is therefore rather confusing to nevertheless use this endpoint as the critical effect for deriving a health protective level for non-cancer effects. This can perhaps be justified pragmatically on the grounds that the levels so derived, with the conservative assumption of equivalent toxicity between species, is health protective against the possibility of either cancer of non-cancer effects in humans. However, it seems that this particular issue has not been explored fully in the document: in particular any other possible endpoints besides those based on erythrocyte fragility, hemolysis and related markers such as hemosiderin are dismissed without any detailed quantitative analysis being presented.

One effect which should be considered is the hyaline degeneration of the olfactory epithelium observed in both male and female rats by NTP (2000). This has the advantage of being apparently unrelated to the hematological effects, and also parallels the finding of respiratory irritation in human studies by Carpenter et al. (1956) and in some case studies. On the other hand this is clearly, as described by NTP (2000), a relatively mild effect, but it is not obvious why this was summarily dismissed as “adaptive”. It is at the mild end of a spectrum of responses seen following chronic exposure of rodents to a number of inhaled chemicals with irritant effects: in the case of more severe irritants such as reactive aldehydes the response progresses to necrosis, hyperplasia and/or metaplasia. As such it can reasonably be regarded as an adverse effect. In this case the incidence shows a clear dose-response with increasing EGBE concentration. This is often done by means of individual scores, which can be used as a pseudo-continuous variable in benchmark analysis. Although there are some factors needing to be considered with regard to time- and interspecies extrapolations for this type of portal-of entry effect, a simple benchmark dose analysis using applied concentration as the dose metric suggests that a point of departure based on this endpoint would not be enormously different from that based on the hemosiderin response (see attached supplementary material showing output of such

a benchmark analysis). It is unfortunate that the only data readily available are quantal incidences: it is often much more informative to provide severity information as well.

3. *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.*

There is now an extensive literature demonstrating the superiority of BMD modeling over the more "traditional" NOAEL/LOAEL approach. This observation is sometimes qualified by adding restrictions such as "providing the data are adequate". It is worth pointing out that, providing the BMD procedure can be run at all, it is especially the preferred approach when data are inadequate, since it takes all the available data into account with appropriate statistical weighting. In those few cases where a BMD model cannot be used (free-standing NOAELS, all 100% responses, only one data point etc.) it may sometimes be possible to derive a number which can be described as a NOAEL or LOAEL, but the relationship of this number to the actual dose-response for that receptor, effect and compound is remote, if not downright mythical.

a. Has the BMD modeling been appropriately conducted and objectively and transparently described?

The application of BMD modeling in this report is clearly and completely reported, and follows the recommendations for application of this approach given by U.S. EPA and others.

b. 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?

This is the one point where I disagree with the choices made by the analysts for the EGBE document. The original guidelines for BMD analysis recommended that the $BMDL_{10}$ (i.e. lower 95 % confidence limit on the dose producing 10% response) be used by default as the POD. However, extensive experience with this techniques by various analysts (including myself, and others working for California and U.S. risk assessment groups) has shown that a better default

choice for POD in analysis of quantal data in animal toxicity studies is the $BMDL_{05}$ (i.e. using a 5% response rate). This value is generally found to most resemble the NOAEL in well-designed and well-conducted studies, whereas a $BMDL_{10}$ typically approximates a LOAEL. It is therefore more appropriate to use the standard uncertainty factors (UFs) (UF_H, UF_A but not UF_L) with the $BMDL_{05}$.

This is of course a default recommendation and the analyst may choose a different response rate to set the POD depending on their analysis of the data. In the case of this EGBE report it appears that the 10% response rate was simply selected as a default, there being no particular attempt to justify its selection in the context of the specific data being analyzed.

c. 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.

The analysts also report derivation of PODs using the NOAEL/LOAEL methodology, but correctly point out that the BMD approach is superior. These are the two generally recognized ways of analyzing this type of data.

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

The PBPK modeling approach used is typical of the current approach to this type of problem, and appears to have been well executed and described. It takes a fairly standard approach using measured values for key parameters where these are available. Although an extensive sensitivity analysis for the selected parameter values was not presented here, the fact that this analysis relies largely on published and peer-reviewed models is an advantage. While more could have been done in this document to validate the model structure and parameter values used, this is on the whole a reasonable way of addressing the extrapolation of the POD from test animals to humans, and certainly an improvement on the use of default uncertainty factors which is the obvious

alternative. The analysis described is similar in general terms to the PBPK analysis presented in the previous (1999) toxicological review; however the conclusions drawn in the version are somewhat different with respect to the extrapolation of dose metrics from rats to humans. Without wishing to question the validity of the new analysis, it would be helpful to identify and explain the differences between this and the previous version.

It needs to be pointed out that if my earlier suggestion to consider the hyaline degeneration of olfactory epithelium endpoint, a "portal of entry" effect rather than a systemic effect like the hemosiderin deposition and hemolytic effects, were to be adopted, a different PBPK approach would be required. There are a number of models available in the literature which address the issues of deposition in various parts of the upper respiratory tract, for compounds with a range of properties (water solubility being a particularly important factor), including values appropriate for EGBE. These should be considered in any such analysis, particularly in preference to the earlier default RGDR calculation which has not proved to be as reliable as one might have hoped, relative to specific PBPK deposition and metabolism models incorporating chemical-specific information.

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

    - A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).

It is reasonable to replace the PK-UF component of UF_A with an appropriate PBPK model which includes a data-based extrapolation between the test species and humans. In this case the use of this model instead of an uncertainty factor (UF_A – PK) greater than 1 is justified and adequately defended in the document.

- Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

In so far as this uncertainty factor is applied to the protection of humans from the hemolytic effect and its chronic sequelae, this is a reasonable choice from the point of public health protection: indeed, it could be considered conservative although this is not an argument for departing from this suggestion. The actual data in fact indicated a considerably lower sensitivity of humans to the hemolytic effect, so based on the data alone a UF_A of 0.1 or even 0.03 could be justified. However, this has various risks including the possibility of alternative endpoints becoming critical for humans, as noted below. The decision not to use UF_A values less than 1 is therefore a policy decision (with a valid basis and justification) and should be so characterized in the document. The issues relating to possible susceptible human subpopulations have been addressed in the document, and there do not appear to be any identifiable susceptibilities which undermine the proposal of a PD-UF_A of 1 for this class of effect.

There is no particular reason to suppose that this assumption is health protective for other endpoints: there is no quantitative basis for such an extrapolation unless other plausible endpoints are actually evaluated quantitatively.

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

If the hyaline degeneration of olfactory epithelium endpoint were to be addressed quantitative, it would be desirable to use an appropriate deposition related PBPK model rather than a default PK-UF_A. Since this is a portal-of-entry effect which probably does not depend on metabolism or distribution, a reduced PK-UF_H might also be justified. Interspecies extrapolation of the toxicodynamic effects in this case would need to be considered carefully by the analyst: there

would not be an automatic presumption of greater human sensitivity to such an effect (particularly since this is a mild endpoint), but on the other hand the possibility of exacerbation or induction of asthma needs to be considered as a human variability factor for respiratory irritants, especially where children are exposed.

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

There appear to be adequate data on frequently deficient types of data such as developmental and reproductive effects to justify the use of a UF_D of 1. Application of database deficiency factors is usually used to address missing toxicological data. It is an unnecessary confusion to include uncertainties about the toxicokinetic data in this factor: these should properly be reflected in the separate uncertainty factors to which they relate, i.e. UF_A PK and UF_H PK. These issues are adequately addressed in the document, apart from the necessity to consider endpoints unrelated to the hematological effect.

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

The rationale for this approach is reasonably laid out and defended in the document. However, it does seem unnecessarily severe to eliminate the 90-day drinking water study (NTP, 1993) entirely from consideration. It does have the important advantage of using the relevant route of exposure, although the duration falls short of a full chronic exposure. There are standard ways of allowing for this limitation as noted below in the discussion of UFs. With these adjustments the conclusions based on this study appear essentially supportive of the derivation using the NTP

inhalation study with route-to-route extrapolation. Apart from this, there do not appear to be any additional studies which would modify the conclusion presented in the document.

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

This seems a reasonable an appropriate approach to route-to-route extrapolation, and the data and model used appear to be adequate and correctly applied. In the case of the RfD the concern about consideration of additional endpoints in the respiratory system does not apply. Portal of entry effects have been adequately addressed in the separate discussion of forestomach irritation and carcinogenesis, so the choice to base the RfD on route-to-route extrapolation of the hemosiderin effect in the NTP inhalation study appears to be a proper health-protective decision. However, the concern for basing the standard on an effect which will probably never be seen in humans remains.

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

I repeat my earlier comment in respect of the RfC:

"Application of database deficiency factors is usually used to address missing toxicological data. It is an unnecessary confusion to include uncertainties about the toxicokinetic data in this factor:

these should properly be reflected in the separate uncertainty factors to which they relate, i.e. UF_A PK and UF_H PK."

In view of all the uncertainties considered in the RfD derivation, the choice of values for UF_A, UF_H and UF_D in the document seems reasonable and are adequately defended for the case presented. If in the final version of the document a derivation from the 90-day drinking water study (NTP, 1993) is also included, a UF_C of 10 would be indicated as standard practice to allow for the extrapolation from a 90-day study to a full lifetime exposure..

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

The document identifies proposed mechanisms of action for the observed tumor endpoints in rodents, and carefully reviews the plausibility of these mechanisms at each step of the proposed explanations. The overall conclusions reached for both liver and forestomach tumors are carefully explained, showing that although such effects are not impossible in humans it is very unlikely that they would be observed in real human exposure situations involving inhalation exposure or chronic oral exposure. The weight of evidence characterization has been carefully developed and well described.

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

There is now a considerable literature examining the mode of causation of liver tumors, especially hemangiosarcomas, associated with hemosiderin (iron) deposition from various

causes. It appears that the relatively low incidences of these tumors reported for EGBE and other agents acting via this mechanism are consistently associated with oxidative damage induced by the excess iron deposits, and the interaction of these chemical deposits with cellular metabolism in cells (such as the Kupfer cell) where oxidative metabolism is naturally active. This mechanism is contrasted with the different causation of hemangiosarcomas and other liver tumors by chemicals (such as vinyl chloride) which give rise to reactive and directly DNA-damaging metabolites. The lack of genotoxicity of EGBE in standard assays provides supportive (although not definitive) evidence reinforcing the contrast with those clearly genotoxic liver carcinogens. The document describes this analysis and carefully reviews its plausibility. The general conclusion is that this mechanism is well supported by the available data.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The issue of relevance of rodent forestomach tumors and their relevance to possible human stomach (or esophageal) cancer is a contentious issue which has been extensively debated with regard to a wide range of different chemicals found to induce such tumors by various exposure routes. The conclusion in the specific case of EGBE appears to be based on considerations of plausible exposure routes and levels, and the specific functional and anatomical properties of the mouse forestomach. The document provides a thorough description of the various factors taken into account in the overall conclusion presented. Some of the arguments used to discount the relevance of tumors at this site in rodents appear rather speculative: it is not clear to what extent actual data support the presumed route of exposure and accumulation in the forestomach (grooming and swallowing of inhaled material, adsorption onto retained food, etc.). However, on balance it would seem reasonable to conclude that the proposed mechanism of action is plausible for this particular case, especially as it is very hard to identify a mechanism of action involving genotoxic effects at this site. However, it should not be presumed that this particular

case provides a significant precedent for discounting the relevance to human cancer risk of tumors induced by other chemicals at this site.

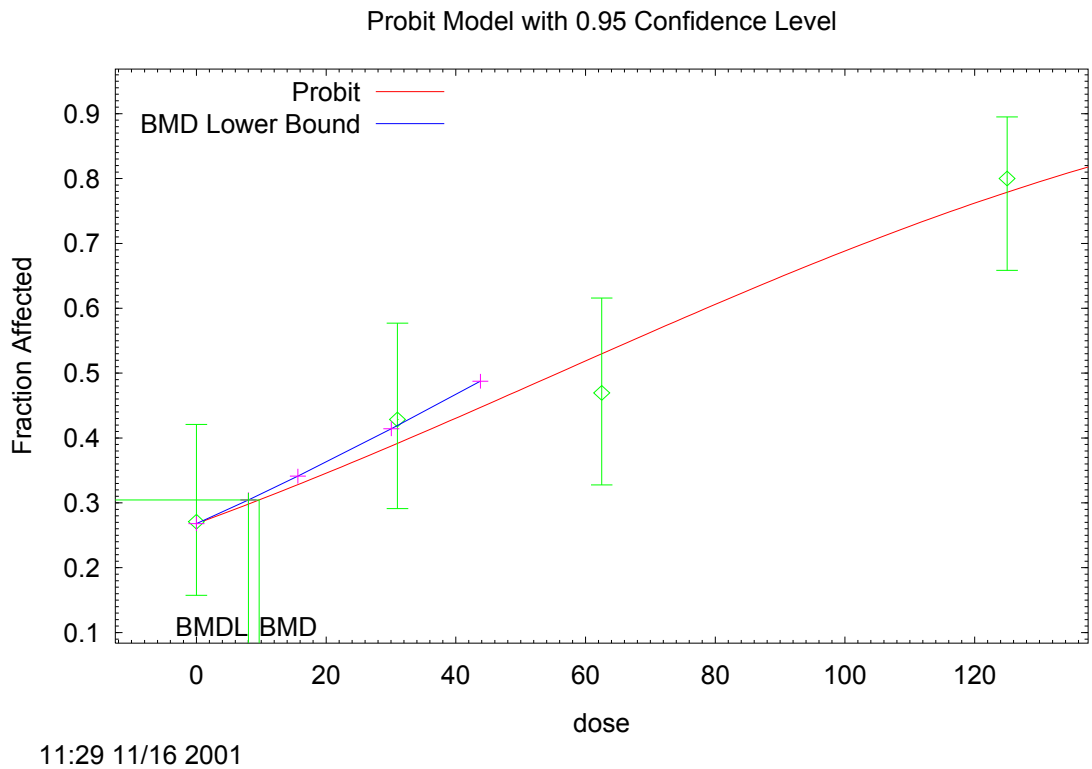
4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

Tumors at this site are not a particularly unusual observation in rat bioassays, and there have been a number of discussions among NTP scientists and pathology consultants as to whether these tumors should be considered dose related and/or indicative of human cancer risk. Whereas there appear to be a number of instances where these tumors are dose-related and apparently caused by exposure to a carcinogen, in this particular case it is hard to disagree with, or significantly expand upon, the NTP's conclusion that the association of these tumors with EGBE exposure in this study is "equivocal". In view of this it is reasonable for the document not to place extensive reliance on this particular endpoint. Additional defense of NTP's conclusion could be undertaken in this document in support of the conclusion not to weight this endpoint significantly. In terms of how a risk estimate might e prepared to illustrate the effect of considering this endpoint, it might be interesting to develop a default (linear extrapolation) potency estimate from rodent tumors at this site. This would require the important caveat that such an estimate has a low level of reliability both as regards the relation to dose in the rodents, and its extrapolation to humans. There really do not appear to be any quantitative data available to support any other type of risk estimation procedure.

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations.

Reasonably plausible mechanistic arguments have been presented in support of the interpretation that EGBE carcinogenesis in the rodent (particularly with regard to the liver tumors) proceeds by a mechanism which involves a practical threshold. The arguments do not necessarily support an absolute threshold where the risk is actually zero below a certain critical dose, but they do at least support the concept that the risk would be very low indeed until such a dose level was reached. It is therefore reasonable to propose the threshold approach to risk estimation for this compound, and also to argue that provided the threshold dose for hemosiderin deposition (used as a criterion for the noncancer RfC and RfD derivations) is not exceeded the human cancer risk is negligible. However it is an important part of the uncertainty analysis to present the consequences of alternative choices for the dose response model, so presentation of the linear alternative is useful in illustrating what the risk estimates would be if the threshold assumption were in fact incorrect. Part of the necessary decision logic in rejecting alternative hypotheses requires consideration of how severe the consequences would be if a particular choice was wrong: obviously the risk assessor needs to be very confident in rejecting a particular mechanistic analysis if the consequences of that analysis are severe, although perhaps less plausible than more reassuring alternatives.

Supporting material: Benchmark dose analysis of data on hyaline degeneration in the olfactory epithelium of rats (NTP, 2000)
Male rats – hyaline degeneration of the olfactory epithelium




```
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        Gnuplot Plotting File:  D:\BMDS\DATA\EGBEMALERATNTP.plt
                                          Fri Nov 16 11:29:02 2001
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = COLUMN3
   Independent variable = COLUMN1
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008
                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =    -0.615103
```

slope = 0.0111337

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.77
slope	-0.77	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.619211	0.14673
slope	0.0111431	0.00211812

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-120.391			
Fitted model	-120.954	1.12625	2	0.5694
Reduced model	-135.847	30.9112	3	<.0001

AIC: 245.908

Goodness of Fit

Dose(ppm)	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2679	12.859	13	48	0.04606
31.0000	0.3921	19.214	21	49	0.5225
62.5000	0.5308	26.008	23	49	-0.8611
125.0000	0.7804	39.022	40	50	0.3342

Chi-square = 1.13 DF = 2 P-value = 0.5688

Benchmark Dose Computation

Specified effect = 0.05

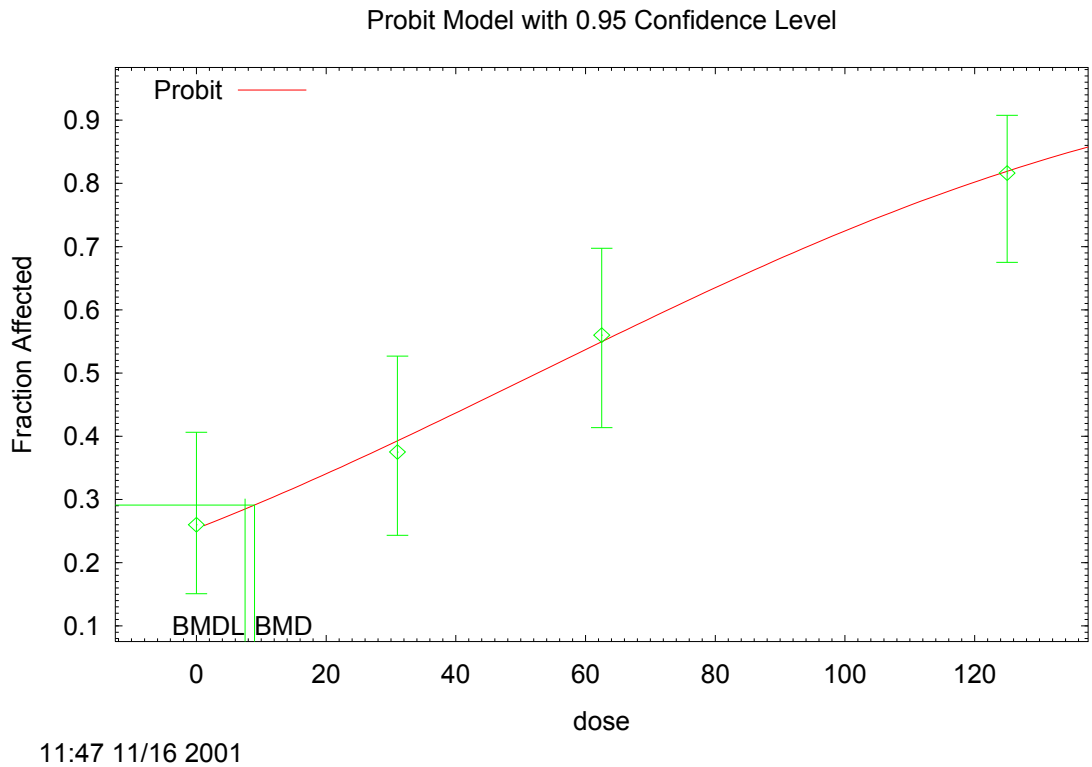
Risk Type = Extra risk

Confidence level = 0.95

BMD = 9.66459

BMDL = 8.01404 ppm = 38.7 mg/m^3

Female rats – hyaline degeneration of the olfactory epithelium

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        Gnuplot Plotting File:  D:\BMDS\DATA\EGBEFEMALERATNTP.plt
                                                Fri Nov 16 11:47:55 2001
 ====================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = CumNorm(Intercept+Slope*Dose),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = COLUMN3
   Independent variable = COLUMN1
   Slope parameter is not restricted

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                  Default Initial (and Specified) Parameter Values
                     background =            0   Specified
                      intercept =     -0.65546
                          slope =    0.0123976
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept	slope
intercept	1	-0.76
slope	-0.76	1

Parameter Estimates

Variable	Estimate	Std. Err.
intercept	-0.66314	0.146919
slope	0.0125616	0.00216835

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-118.073			
Fitted model	-118.122	0.0975191	2	0.9524
Reduced model	-136.547	36.9482	3	<.0001

AIC: 240.244

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2536	12.681	13	50	0.1037
31.0000	0.3921	18.823	18	48	-0.2433
62.5000	0.5485	27.427	28	50	0.1629
125.0000	0.8178	40.073	40	49	-0.02694

Chi-square = 0.10 DF = 2 P-value = 0.9526

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 8.95568

BMDL = 7.4974 ppm = 36.2 mg/m^3

Mean of BMDL for M & F rats = 7.75572 ppm or 37.46 mg/m^3

Gregory Travlos

External Peer Review for the
IRIS Toxicological Review of Ethylene Glycol Monobutyl Ether (EGBE)
November 3, 2008
Environmental Protection Agency
Research Triangle Park, NC

Reviewer: Dr. Gregory Travlos

Post-Meeting Comments

The document is well written and the literature review thorough and logically presented. As mentioned in my pre-meeting comments, it appeared to me that the hemolytic effect (decreased erythron) and not the kupffer cell hemosiderin accumulation was the critical effect. And, while I will not totally discount the proposed potential mechanism for development of the hemangiosarcomas, the use of hemochromatosis in humans as a major part of the justification for the mechanism does not seem to be totally appropriate (the severity of the iron overload is different, the location [i.e., cell populations] of the iron accumulation within the liver is different, and the tumors associated with iron overload—in humans and rodents—is different). If the hemolytic effect was the critical effect, then there was appropriate oral exposure data (that has already been used in a previous report, EPA, 1999) for determination of the RfD.

I have one comment to add after the workshop. From the discussions it became evident that due to the species (rat v. human) differences in sensitivity of erythrocytes to the toxic metabolite (BAA), the assigned UF(A) of 1 appeared to be excessively high and not based on the data presented in the text. This suggested the UF(A) was selected for reasons other than science-based. Since, I do not understand all the issues (including policy) involved in such selections, it would be appropriate to justify the UF(A) selection in the text rather than have lingering questions regarding its selection rationale.

Otherwise, my post-meeting comments are the same as my pre-meeting comments, presented below.

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Overall, the review of the literature was thorough and presentation of the available data was clear and objective.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

a.) Because there are pertinent hematology instrument methodology differences that result in the early EGBE-induced changes in mean cell volume (MCV) and hematocrit (Hct) being missed or misrepresented, I recommend the addition of the following reference:

Ghanayem, B. I., Ward, S. M., Blair, P. C., and Matthews, H. B. (1990). Comparison of the hematological effects of 2-butoxyethanol using two types of hematology analyzers. *Toxicol. Appl. Pharmacol*. 106, 341-345.

In this study, the authors demonstrated that a laser-based hematology analyzer could not determine the early EGBE-induced increases in Hct and MCV but these changes were detectable by impedence-based technology. Thus, instrument selection could substantially impact the performance/interpretation of the hematology evaluations as a pre-analytical source of variation.

b.) Because hepatic iron (i.e., hemosiderin staining) was used as the critical effect for deriving the RfC and RfD, I suggest the review and possible addition of the following references:

Smith, P. G. and Yeoh, G. C. (1996). Chronic iron overload in rats induces oval cells in the liver. *Am. J. Pathol*. 149(2): 389–398.

Irving MG, Booth CJ, Devlin CM, Halliday JW, Powell LW. (1991). The effect of iron and ethanol on rat hepatocyte collagen synthesis. *Comp Biochem Physiol C.*100(3):583–590.

Pietrangelo, A., Gualdi, R., Casalgrandi, G., Montosi, G., and Ventura, E. (1995). Molecular and cellular aspects of iron-induced hepatic cirrhosis in rodents. *J. Clin. Invest*. 95(4): 1824–1831.

Tsukamoto, H., Horne, W., Kamimura, S., Niemelä, O., Parkkila, S., Ylä-Herttuala, S., and Brittenham, G. M. (1995). Experimental liver cirrhosis induced by alcohol and iron. *J. Clin. Invest*. 96(1): 620–630.

Rothenberg, B. E., and Voland, J. R. (1996). beta2 knockout mice develop parenchymal iron overload: A putative role for class I genes of the major histocompatibility complex in iron metabolism. *Proc. Nat. Acad. Sci*. 93(4): 1529-1534.

Edwards, C. Q. (1999). Hemochromatosis. In Wintrobe's Clinical Hematology (G. R. Lee, J. Foerster, J. Lukens, F. Paraskevas, J. Greer, G. M. Rodgers, eds.). pp. 1056-1070. Williams & Wilkins, Baltimore, MD.

Britton, R.S. and Bacon, B.R., 1994. , Role of free radicals in liver diseases and hepatic fibrosis. *Hepatogastroenterology* **41**: 343–348.

Tector A. J., Olynyk J. K., Britton R. S., Janney C. G., O'Neill R., and Bacon B. R. (1995). Hepatic mitochondrial oxidative metabolism and lipid peroxidation in iron-loaded rats fed ethanol. *J. Lab. Clin. Med*. 126: 597-602.

Olynyk, J. K., Hall, P., Reed, W., Williams, P., Kerr, R., MacKinnon M. (1995). A long-term study of the interaction between iron and alcohol in an animal model of iron overload. *J. Hepatol*. 22:671-676.

Bomford, A., and Williams, R. (1976). Long term results of venesection therapy in idiopathic haemochromatosis. Quarterly Journal of Medicine 45, 611-23.

Niederau, C., Fischer, R., Sonnenberg, A., Stremmel, W., Trampisch, H. J., and Strohmeyer, G. (1985). Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N. Engl. J. Med*. 313(20):1256-1262.

Niederau, C., Fischer, R., Purschel, A., Stremmel, W., Haussinger, D., and Strohmeyer, G. (1996). Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *Gastroenterol.* 110(4):1107-1119.

Tiniakos, G. and Williams, R. (1988). Cirrhotic process, liver cell carcinoma and extrahepatic malignant tumors in idiopathic haemochromatosis. Study of 71 patients treated with venesection therapy. *Appl Pathol*. 6:128-138.

Deugnier YM, Guyader D, Crantock L, et al. (1993). Primary liver cancer in genetic hemochromatosis: A clinical, pathological, and pathogenetic study of 54 cases. *Gastroenterology*. 104:228-234.

Fellows, I. W., Stewart, M., Jeffcoate, W. J., Smith, P. G., and Toghil, P. J. (1988). Hepatocellular carcinoma in primary haemochromatosis in the absence of cirrhosis. *Gut*. 29(11): 1603–1606.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Since there is an overriding difference in erythrocyte sensitivity to EGBE-induced hemolysis between laboratory rodents (rats and mice) and humans, species differences in erythrocyte membrane physiology should be investigated and or reviewed.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

The identification and characterization of the sources of uncertainty were clearly identified and described. I may not agree with all the choices (for example, use of Kupffer cell hemosiderin accumulation versus erythron decreases (e.g., erythrocyte count) as the critical effect; the use of male data when females animals appeared to be more sensitive (to the erythron effects).

Chemical-Specific Charge Questions:

Since I am not qualified to comment on all aspects of the chemical-specific charge questions, responses will be limited to questions, or parts of questions, that I feel my comments would be appropriate.

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document?

I believe the aforementioned study was adequately justified and appropriately represented.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

I am not sure increased hemosiderin staining in Kupffer cells have been clearly established as a critical effect. The Nyska et al. (2004) retrospective report demonstrated an apparently strong association ($p < 0.001$) between hemangiosarcoma and hemosiderin accumulation, but the number of studies was small (6 studies) and two of the studies (33% of the study set) had hemosiderin increases but no hemangiosarcoma development.

While hemosiderin and ferritin are considered nontoxic storage forms of iron, iron released from storage sites may react with hydrogen peroxide to form reactive oxygen species (ROS) (Edwards, 1999). Iron-catalyzed ROS injury has been demonstrated in liver (Britton and Bacon, 1994; Tector et. al., 1995; Olynyk et. al., 1995). It has been reported that humans that are hemochromatosis homozygotes, and who have liver cirrhosis, develop hepatocellular carcinoma, but not hepatoma, in approximately 10 to 30% of the affected patients (Niederau et. al., 1985, 1996; Tiniakos and Williams, 1988; Deugnier et. al., 1993). Hepatocellular carcinoma is about 200-fold more common in hemochromatosis patients as in unaffected individuals (Niederau et. al., 1985). There are a few patient reports of hepatoma in cases where cirrhosis was not present (Fellows et. al., 1988). To my knowledge, hemangiosarcomas have not been related to iron overload in humans (or other species). As noted in the report (section 4.7), the iron accumulation in hemachromatosis patients occurs in liver parenchymal cells, and to a lesser extent, macrophages (e.g., Kupffer cells); with iron overload in rats, iron accumulation also appears to preferentially affect hepatocytes with Kupffer cells affected to a lesser extent (Smith and Yeoh, 1996).

As noted in the report (section 4.7), humans that develop hepatocellular carcinomas, as a consequence of hemochromatosis, usually demonstrate liver cirrhosis, thus, reflecting the chronic nature of the disease. In laboratory rodents, iron overload demonstrates a similar fibrotic process. For example, dietary iron overload in rats results in increased collagen gene expression, activating collagen production within lipocytes and leads to hepatic fibrosis (Irving et. al, 1991; Pietrangelo et. al, 1995; Tsukamoto et. al, 1995). Smith and Yeoh (1996), reported that iron overloading of the liver, as a means of inducing liver damage over an extended period, promoted oval cell proliferation. Rats fed a 2% carbonyl-iron-supplemented diet for 3 or 6 months demonstrated extensive periportal iron deposits in hepatocytes and some Kupffer cells; iron

deposition was less pronounced pericentrally. Small oval-like cells, morphologically and immunocytochemically similar to CDE-derived oval cells, were identified and quantified. Oval cells first emerged periportally and subsequently in small tracts or foci nearer central regions and stained positively for alpha-fetoprotein, pi-class glutathione S-transferase, and the embryonic form of pyruvate kinase. They contained very few iron deposits and were classified as iron free. The major difference between CDE- and iron-overload-derived oval cells was that the latter were negative for transferrin. They concluded that cellular changes occurring in iron-overloaded rat liver are similar to those observed in rats placed on a hepatocarcinogenic diet and in rats chronically exposed to alcohol.

A β_2 microglobulin knockout mouse model of hemochromatosis has been reported (Rothenberg and Voland, 1996). The authors tested the hypothesis that animals lacking the β-analogous promoter gene would experience upregulated iron absorption and iron overload. They demonstrated age-dependent, increased hepatic iron in the β_2 knockout mice and some mice developed sinusoidal fibrosis, hyperglycemia and hepatoma (hemangiosarcomas were not observed).

Thus, it appears iron overload in rats and mice result in some level of hepatic fibrosis (as has been reported for humans). For the studies used in this report, there was no increase in hepatic fibrosis reported. Thus, the clinical significance of the Kupffer cell hemosiderin used as the "critical effect" in this report could be questioned. It is clear, however, that hemolysis is a direct toxic effect of the EGBE metabolite, butoxyacetic acid (BAA). It would seem that the hemolysis would be the critical effect and that the accumulation of hemosiderin in Kupffer cells would be simply a secondary response to the increased erythrocyte turnover.

3. 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.

Discounting the hemosiderin accumulation, since the female animals demonstrated the most sensitivity to the hematological effects, it would seem the use of the female gender would have been more appropriate?

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

Oral data does exist and was used previously (USEPA, 1999). But the present report suggests that because the data is limited the inhalation data set is more appropriate, even in light of the lack of forestomach hyperplasia in the drinking water studies (NTP, 1993) compared to the observed forestomach lesions observed in the inhalation studies (NTP, 2000)?

(C) Editorial Comments

Page 52, line 16: The historical control information reported on this line "(6.4-3.5%; range 2-13%)" appears to be incorrect.

Page 71, line 32: The female information (NTP, 1993) represented as an "increased urea nitrogen creatine" is incorrect. Firstly it should be presented as an increased urea nitrogen and creatinine concentrations. Creatine was not analyzed. Secondly, the males also had increased urea nitrogen concentrations. Thus, the idea that the changes in these markers of renal injury indicate supportive information that the females were more sensitive to EGBE administration is probably overstating/overinterpreting the findings.

Page 99, line 6: There is no section "5.1.2.4".

Rochelle Tyl

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Toxicological Review of Ethylene Glycol Monobutyl Ether (EGBE)
Rochelle Tyl Post-meeting Comments

General Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

   In this reviewer's opinion, the Toxicological Review is logical and clear. The EPA has clearly and objectively represented and synthesized the extant scientific evidence for both the cancer and non-cancer hazard.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

   I am not aware of any extant additional studies which should be considered in the assessment of the cancer and non-cancer health effects of EGBE.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

   It is clear (at least to me) that the missing "link" in the EPA Human Health Assessment of EGBE is long-term human inhalation (or oral) studies of EGBE. The short-term human volunteer studies of inhaled EGBE (and other chemicals of interest) were performed at Bushy Run Research Center by Dr. Carpenter and others at another time. It is highly unlikely that the IRB (Institutional Review Board for human studies) or IACUC (Institutional Animal Care and Use Committee for animal studies) committees would even consider human exposure studies of a known reproductive toxicant such as EGBE (or even anything else). It is important to note that in Carpenter's studies, humans (both men and women) experienced sensory respiratory irritation at both 100 and 190 ppm.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

One of the finest, most clear and succinct summaries of the uncertainties in any risk assessment is found in Chapter 5 of this EGBE in Table 5-15 (p. 105). For each consideration, the table indicates the potential impact of its use (to increase or decrease the risk estimation), the decision made in the document, and the justification for the decision. This summary table, and the text in Chapters 5 and 6, more than adequately discuss the sources of uncertainty. The choices, assumptions, and the impact of the various uncertainties have been clearly, objectively, and transparently described, and the consequences evaluated. The biggest uncertainty is the animal to human extrapolation, whether the key events in the MOA proposed for rodents: forestomach accumulation of acidic HAA, leading to irritation, leading to cytotoxicity, leading to compensatory cell proliferation, leading to forestomach tumors (in female mice), **and** RBC hemolysis leading to hemosiderin accumulation in the Kupffer cells of the liver (in male rats and mice), leading to oxidative stress, leading to cell apoptosis and compensatory cell proliferation, leading to tumorigenesis (only in male mice), are relevant to humans in both a qualitative and quantitative sense, and whether these adverse effects are likely or not likely (rationales provided support that it is not likely) to occur in humans at the RfD or RfC. The impact of the uncertainty on the assessment has been clearly, transparently, and objectively described, and the values for the RfD and RfC under different aspects of uncertainty have been calculated and discussed. There was consensus at the meeting that the hemosiderin deposition, per se, is not toxic (in fact, it is likely protective), but it may be a useful biomarker. However, it is not really ever quantified. RBC count and/or hemolysis is continuous, quantifiable, directly related to MOA, and may be a better biomarker and/or POD.

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

The selection of the two-year inhalation bioassay in rats and mice by the National Toxicology Program (NTP, 2000) was appropriate and fully justified. It was clearly, fully, transparently, and objectively described in this document. I am unaware of any other study which should even be considered, let alone selected, as the principal study. The 91-day drinking water studies in rats and mice (with adjustments from subchronic to chronic) should at least be considered.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

   The EPA selected hemosiderin staining (intracellular accumulation of iron from hemolysis) in the liver of male rats (which interestingly do not develop liver tumors from EGBE exposure) as the critical effect, because it is considered by EPA as a precursor to an adverse effect (hepatocellular tumors in male mice). The EPA has made a strong case for use of this "critical effect" since it is necessary (but not sufficient) for the subsequent tumor formation, and for the use of the rat (rather than the mouse) because the hemosiderin staining occurs at a lower EGBE dose in male rats than in male mice. The use of RBC count or hemolysis might be a better approach, and the females appeared more sensitive to hemosiderin deposition than did the males. The criteria, justification, and rationale for this effect in the more sensitive species and sex have been thoroughly, clearly, transparently, and objectively presented in the document. It is this reviewer's considered opinion that it is a defensible best choice for the critical effect and resulted in the lowest, most protective calculated RfD and RfC.

3. 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.

Benchmark Dose (BMD) modeling was applied to the incidence data for hemosiderin staining in the rat liver in the NTP study, to derive the Point of Departure (POD) for the calculation of the RfC. It is this reviewer's considered opinion that BMD modeling is the best approach for determining the POD and was appropriately conducted, and clearly, transparently and objectively described in terms of its use, its results, and the consequences of the outcome. The benchmark response (BMR) selected for use in the POD, 10% excess risk of hemosiderin deposition (staining) in the liver (necessary but not sufficient), was justified (as necessary, but not sufficient for the downstream liver tumor outcome in male mice, but present at lower EGBE doses in the male rat). It was also clearly, transparently, and objectively described. I am not aware of any better alternative approaches for the determination of the POD, and I concur with EPA's choice of the BMD modeling.

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

   PBPK modeling, to extrapolate the POD from rats to humans, is the model of choice to perform species-to-species extrapolation when the appropriate data are available in both species and the assumptions are explicitly presented and discussed. The model was scientifically justified and fully, transparently, and objectively described. We have toxicokinetic data from the rodent and human (a rarity!), and they were appropriately represented and applied. The model assumptions, parameter values, and selection of dose metrics were also clearly presented and scientifically supported. It would have been useful to compare and contrast the discussion in this document with that in the 1998 document.

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

- A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).

- Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

Comments on the selection of all of the uncertainty factors (Ufs) applied to the POD for the derivation of the chronic RfC (predominantly from Chapter 5.1.3, pages 86 ff).

- An UF(H) value of 10 was applied to account for the variability in sensitivity within the human population. This factor would cover potentially susceptible subpopulations (individuals with enhanced metabolism, decreased excretion of BAA, and/or individuals whose RBC membranes are more susceptible to lysis). However, it should be noted that in vitro assessments of RBC lysis from newborn, or elderly patients and patients with genetic blood dyscrasias, are <u>not</u> more sensitive to the hemolytic effects of EGBE. Perhaps a UF of 10 is too high (3?). In animal studies, older animals are more sensitive (perhaps due to older animals having older and therefore more vulnerable RBCs) than neonates, and females are more sensitive than males. Developmental toxicity studies in rodents do not indicate increased susceptibility in fetuses and/or neonates. However, there are no long-term human exposure studies in normal or predisposed individuals which drove the decision to use

an UF(H) of 10. These arguments were clearly, transparently explained and scientifically supported.

- A PK-UF of 1 was selected to cover potential differences in pharmacokinetic parameters between the animal models and humans. Since there are good PK data in both animals and humans, an HEC (Human Exposure Concentration) was calculated using known animal blood levels and PBPK modeling data. It appears that the human is much less sensitive to the hemolytic effects of EGBE; therefore, a PK-UF of 1 was considered sufficient. However, humans are sensitive to respiratory irritation. These arguments were clear and transparent and scientifically supported.

- A PD-UF of 1 was selected to cover potential differences in pharmacodynamic parameters between the animal models and humans. The PD-UF of 1 was selected based on clear evidence that the human is much less sensitive to EGBE concentrations that cause hemolytic effects in human red blood cells than are the animals. The chosen PD-UF is sufficiently conservative; a value less than 1 is not as protective, since we have not evaluated all sensitive subpopulations of humans, long-term human exposures, humans of different ages, or humans with all blood dyscrasias; at the meeting, we were told that as a matter of policy, UF of less than 1 are not used. There is one concern by this reviewer: The parameters assessed in the in vitro human blood tests with EGBE, etc., included MCV (mean corpuscular volume), i.e., the size of the red blood cell (RBC) (page 74, lines 7-8 described the in vivo animal studies; page 71, lines 14-17 described the human in vitro blood studies). MCV is a sensitive parameter in vivo, since animals with reduced RBC counts exhibit larger MCVs, from release into the circulatory system of younger, larger, more immature RBCs from the bone marrow (and rarely from extramedullary hematopoiesis in the spleen or liver). This release cannot occur in vitro using just blood samples. It is likely that the authors meant that there was RBC membrane deformation and/or RBC swelling (and bursting) due to induced membrane fragility. This distinction must be explicitly made. The UF is appropriate and protective, and was presented clearly and transparently, with strong scientific justification.

- A UF to account for extrapolation from subchronic to chronic exposure was not needed since the RfC was derived from a chronic study (page 87, lines 19-20). The suggestion to use the 91-day rat drinking water study would necessitate a UF.

- A UF to account for extrapolation from LOAEL to NOAEL was also not needed since this factor was one of the considerations in selecting the BMR of 10% increase in hemosiderin staining for BMD modeling. This 10% increase was considered "a biologically significant change" (page 87, lines 21-24).

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

   Specific comments on the UF for the database. A UF(D) of 1 was selected for the database, since there were adequate animal studies of appropriate sizes, doses, routes, durations, ages from prenatal to older than 2-years of age, and with sufficient range of endpoints to provide a robust animal database. There are limited human studies under short-term exposure conditions (with additional studies unlikely) and no human studies of long-term exposures(again, not likely to occur) (page 87, lines 25-28). Perhaps a UF(D) of 3 is more defensible. This UF(D) is clearly, cogently, and transparently explained and well supported scientifically.

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. The rationale provided (Chapter 5, Section 5.2, page 89 ff) for not deriving a chronic oral RfD, i.e., no chronic (or subchronic) oral human studies, and no chronic oral animal studies, is presented clearly and transparently and is obvious. Hemolysis is not viewed as progressive (it does not get worse), but it is accumulative (as the animals and their RBCs age). The two 91-day drinking water EGBE studies in rats and mice (NTP, 1993) are available. Use of the PBPK model for EGBE could allow their use. This would require a UF from subchronic to chronic if the effect is genuinely accumulative.

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

   A route-to-route extrapolation was done to derive the chronic RfD, using the chronic inhalation study in rats and PBPK modeling. The Human Equivalency Concentration (HEC) was based on "continuous" oral exposure to EGBE in the drinking water, that would yield the same AUC (area under the curve) for the metabolite BAA in arterial blood over 3 months as that estimated for the rat following external inhalation exposure to EGBE at the proposed POD (i.e., BMCL10). In this reviewer's opinion, using PBPK modeling for the route-to-route extrapolation to obtain an RfD was a very smart idea in the absence of adequate oral animal data or adequate human dose-response data. The rationale was well described. The extrapolation was correctly performed (to the best of my knowledge) and objectively and transparently documented and interpreted.

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether

the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

A UF for the database in the RfD derivation was selected as 1. The use of measured internal dose in rats (more sensitive than mice to the deposition of hemosiderin in the liver) and a human PBPK model were appropriate, given the data available, to derive the RfD. The selection of the UF (RfD) of 1 was clearly, transparently, and objectively described and scientifically justified.

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

   Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment, the Agency concluded that EGBE is not likely to be carcinogenic in humans at expected exposure concentrations. The effects of EGBE on hemosiderin deposition in the liver and forestomach irritation may both have qualitative relevance to humans. However, the exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC/RfD and well above the concentrations necessary to cause these effects in mice (the more sensitive species) (page 110, lines 25-29). Chapter 4.5 (Synthesis and Evaluation of Major Noncancer Effects and Mode of Action: Oral and Inhalation) and Chapter 4.6 (Evaluation of Carcinogenicity) provide detailed rationales for the weight of evidence for cancer (and noncancer) endpoints and excellent scientific justification for the evaluations. The weight of evidence "descriptor" has been sufficiently, transparently, and objectively described and scientifically justified.

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological

Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The EPA has proposed a MOA (mode of action) for male mouse liver cancer (the more sensitive species and sex). The sequence of events:

- EGBE metabolism to BAA
- Hemolysis of RBCs from BAA in the blood
- Hemosiderin deposition in the liver from bioaccumulation of iron from lysed RBCs
- Oxidative damage to hepatic cells
- Compensatory proliferation, leading to
- Tumor induction Chapter 4.6.3.1 (derived from Step Event, page 54)

The hypothesized MOA for the liver tumors following EGBE treatment involves exposure to high doses for prolonged periods of time. Each step in the proposed process has been confirmed in humans (first steps) and in animal models (last steps)(Chapter 4.6 ff). EGBE is not a genotoxic carcinogen, again supported by animal evidence. This analysis and proposed MOA are both scientifically sound, transparently and objectively described and fully supported by the data available. An analysis of the NTP database on chemicals which produce hemosiderin deposition in the liver in subchronic or chronic exposures (and which were carcinogens), indicated a highly statistically significant association, p less than 0.001, between the studies exhibiting deposition of hemosiderin (6) and those studies with liver carcinogenesis (hemangiosarcoma and hepatocarcinoma), Table 4-8 (page 57). Why is there no hemosiderin deposition in the spleen? In addition, tumor induction from initiated cells is speculation. No other viable MOAs have been identified that explain the existing laboratory animal and human observations (Chapter 4.6.3.3, page 67, lines 29-30). However, the hemosiderin deposition dose response is steeper in females (in all females, 10/10, in the top 3 doses) versus male mice (only 7/10 in the top dose). Again, hemosiderin deposition is a measure of exposure, <u>not</u> effect.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best

supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

EPA has proposed a MOA for female mouse forestomach tumors (the most sensitive species and sex). The sequence of events is:

- Deposition of EGBE/metabolite BAA in the stomach and forestomach (humans do not have a forestomach) via consumption or reingestion of EGBE-laden mucus, salivary excretions and fur material
- Retention of EGBE/BAA in food particles of the forestomach long after being cleared from other organs
- Metabolism of EGBE to BAL, which is rapidly metabolized to BAA systemically and in the forestomach
- Irritation of target cells by BAA leading to hyperplasia and ulceration
- Continued injury by BAA and degeneration leading to high cell proliferation and turnover, leading to
- Clonal growth of spontaneously initiated forestomach cells (estrogen-dependent event, speculation…?) (Chapter 4.6.3.2, Step Event, p. 62).

This analysis and MOA are both scientifically sound and transparently and objectively described. The first two steps have been demonstrated in animal studies. Step 3 requires ALD and ADH, which have been evaluated in the stomachs and forestomachs of mice. These enzymes have been shown to be heavily localized in the stratified squamous epithelim of the mouse and rat forestomach (while their distribution in the rodent and human stomach is more diffuse) (page 63, lines 16-22). Human stomach tissues, with less amounts and diffuse distribution of these deyhdrogenase enzymes, would be less capable of accumulating and localizing BAA than rat/mouse tissues, and would less likely be exposed to the irritating effects of BAA (Chapter 4.6.3.2, line 37, p. 63; and lines 1-5, p. 64). The process in rodents is well described and confirmed. The MOA also explains why humans

would not be expected to exhibit the full MOA and therefore the tumors in the (human) stomach. Interestingly, no hyperplasia or tumors were observed in the inhalation studies of EGBE in rats (NTP, 2000) or in the drinking water studies of mice (NTP, 1993), supporting the requirement for all of the steps above to occur prior to tumor formation (Chapter 4.6.3.2.1, page 64, lines 25-27). Again, the use of the 91-day drinking water studies in rats and mice is strongly suggested. One panelist suggested that it is "unwise to dismiss forestomach tumors out of hand." The discussion in the document was considered by many to be too speculative. The lack of use of historical control data on incidence and severity of the tumors by the initial authors and by the reviewers is regrettable. We don't know enough biologically; what is critical: dose, dose rate, accumulative dose (i.e., exposure duration, timing (specific vulnerable life stage(s)?

4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

EPA has not proposed an MOA for female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as indicating "equivocal" evidence of carcinogenic activity. The pathology report for that study expressed concern whether the observed tumors met the established criteria used for the diagnosis of these tumors. Therefore, this tumor was not given "significant weight" in the qualitative or quantitative assessment of the carcinogenic potential of EGBE. This reviewer concurs with the EPA's (and NTP) concerns and conclusions. Examination of the NTP final report on 2-butoxyethanol (Appendix B, Table B1, Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Inhalation Study of 2-butoxyethanol) indicates that in the adrenal medulla, the incidence of malignant pheochromocytoma was 0 (0%) at control, 0 at 31.2 ppm (low), 0 at 62.5 ppm (mid), and 1 (2%) at 125 ppm (high). The incidence of benign

pheochromocytoma was 3 (6%) in controls, 4 (8%) at 31.2 ppm (low), 1 (2%) at 62.5 ppm (mid), and 6 (12%) at 125 ppm (high). A third entry was for benign pheochromocytoma, bilateral, reported only at 125 ppm (high concentration) in 1 (2%).

The report states (p. 58, left column) that: "The incidences of benign or malignant pheochromocytoma (combined) occurred with a positive trend in females; however, the incidence in females exposed to 125 ppm was not significantly increased relative to the chamber controls (Tables 10 and B3), but exceeded the range for historical controls from 2-year inhalation studies (Tables 10 and B4). One pheochromocytoma in the 125 ppm female group was malignant and another, while benign, was bilateral (Tables 10 and B1). The incidence of medullary hyperplasia was slightly, although not significantly, greater in females in the 125 ppm group than in the chamber controls (Tables 10 and B5). The primary criterion used to distinguish pheochromocytoma from medullary hyperplasia was the presence of mild to moderate compression of the adjacent tissue. Most of the pheochromocytomas were small and not substantially larger than the more severe grade of adrenal medullary hyperplasia." At best this is an uncertain, equivocal finding.

The analysis regarding the female rat pheochromocytomas is scientifically sound (and concurs with the concerns of the NTP Pathology Working Group for the 2-year inhalation bioassay of EGBE). I could not find a detailed discussion in the Toxicological Review of this tumor type. This tumor type (and incidence and severity) in the female rat is not considered to be adequate to support alternative analyses of qualitative and quantitative cancer risks to humans. Approaches to take to see if such analyses are warranted would include (but are <u>not</u> recommended) are:

- Search to see if these tumor types have occurred in other NTP bioassays and, if so, in what chemicals, by what routes, at what dose/concentration levels
- Search to see what the background incidence of this tumor type is in female rats
- Search to see what the background incidence of this tumor is in people (males and females)

- Determine whether there is a MOA for this tumor type in animal studies (I don't know of any), and see if the human is at risk based on MOA

My guess is that at the exposure levels to which humans are exposed, even if there were a MOA, human exposures would be below the effects level(s).

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations.

The choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE is the correct one. A number of the initiating and subsequent steps (each necessary but not sufficient in itself) are nonlinear with thresholds below which the adverse effect does not occur. This is true for the hepatocarcinomas in male mice and for the forestomach tumors in female mice. In fact, the strongest evidence for little or no risk to humans for these tumors (not counting that we do not have a forestomach) is that our exposures are below the determined RfC and RfD, and at these lower doses/concentration, the sequential progression of necessary adverse events does not happen. I concur completely that the EPA has provided clear, transparent objectives and cogent arguments, with strong scientific justification that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations.

D. Alan Warren

Charge Questions:

1. Is the Toxicological Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Yes, the Toxicological Review is a well-written, logically-organized and objective interpretation of the state-of-the-science regarding EGBE. It accurately reflects the increased understanding of EGBE gained from targeted research studies conducted since posting of the 1999 IRIS assessment. It also effectively incorporates many of the suggestions made in external peer reviews of past position papers and technical reports. Particularly impressive is the length to which the Toxicological Review went to allow comparison of RfCs and RfDs derived using alternative "choices" to those ultimately selected (e.g., Figures 5-3 and 5-4). Doing so increases confidence in the newly-derived toxicity constants, particularly in their health conservatism.

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of EGBE.

While recognizing the Toxicological Review is not intended to be a complete treatise on EGBE's toxicity, the issue of thrombosis and infarction might warrant more than the single paragraph afforded it (p. 35). Granted, it is clearly not the most sensitive endpoint for dose-response assessment, but it is believed to occur secondary to intravascular hemolysis, has been examined at inhalation concentrations used in the subchronic and chronic NTP studies, and exhibits age-, gender- and species specificity. In addition, it informs the issue of intrahuman variability as a source of uncertainty, as patients with some hemolytic conditions are prone to thrombosis and infarction. Several published studies on the subject exist that are not referenced in the Toxicological Review (see a few listed below), among them that of Yoshizawa et al. (2005) that evaluated atrial thrombosis in NTP studies and identified EGBE as one of 13 compounds with increased incidences (20-100%) among high-dose groups.

Yoshizawa et al. (2005). Chemical-induced atrial thrombosis in NTP rodent studies. *Toxicol. Pathol.* 33(5):517-32.

Ramot et al. (April 2007). Age and dose sensitivities in the 2-butoxyethanol F344 rat model of hemolytic anemia and disseminated thrombosis. *Exp. Toxicol. Pathol.* 58(5):311-22.

Nyska et al., (1999): Disseminated thrombosis and bone infarction in female rats following inhalation exposure to 2-butoxyethanol. *Toxicol. Pathol.* 27(3):287-94.

On a related note, Udden and Patton (2005) is cited as the lone study in a very brief discussion of the mechanism of BAA-induced RBC hemolysis (see p. 45). These authors indicate that preliminary studies in their laboratory have shown the movement of phosphatidylserine from the inner to the outer leaflet of the lipid bilayer of rat RBCs incubated with BAA. This "externalization" of phosphatidylserine is associated with adhesion of RBCs to endothelial cells and the generation of thrombin, which is relevant given reports of disseminated thrombosis and infarction in EGBE-treated rats. Perhaps this information would be a reasonable addition to the existing mechanistic discussion, despite what appears to be only an abstract detailing the findings [Tamirisa et al., 2002. Annexin V binding and hemolysis of rat RBCs exposed to butoxyacetic acid, *Blood* 100:7b].

Lastly, for those readers not well informed on the role of Kupffer cells in hepatotoxicity and carcinogenicity, the following reference provides an outstanding overview: Roberts et al. (2007). Role of the Kuppfer cell in mediating hepatic toxicity and carcinogenesis. *Toxicol. Sci.* 96(1):2-15. The publication is an outgrowth of a symposium held at the Annual Meeting of the Society of Toxicology in 2006. The article suggests that Kupffer cell activation may initially be protective and become injurious only with continued and higher dose exposure. This obviously has potential dose-response implications for EGBE-induced liver cancer that should be taken into consideration.

3. Please discuss research that you think would be likely to increase confidence in the database for future assessments of EGBE.

Given the paucity of data on the mechanism(s) by which BAA induces RBC hemolysis, this would be an informative line of research, particularly if it addressed whether the mechanism(s) was conserved across species. Such research would also inform the issue of sensitive subpopulations, potentially impacting the uncertainty factor for intrahuman variability in RfC/RfD derivation. One outstanding question is whether Udden and Patton's laboratory pursued the mechanistic issue of phospholipid externalization after preliminarily reporting the externalization of phosphatidylserine by BAA in rat RBCs *in vitro.*

The experimental evidence supporting the hypothetical mechanism by which EGBE produces hemangiosarcomas and hepatocellular carcinomas is discussed on p. 55. Based on this discussion, there are gains to be made by investigating the role of reactive oxygen species (ROS) in modulating gene expression specifically within endothelial cells and hepatocytes of male mice (and perhaps humans), the cell types that undergo neoplastic transformation. This line of research, coupled with *in vivo* studies designed to quantify the internal threshold doses that must be met to progress from one key precursor "event" in the mechanistic sequence to another (e.g., RBC hemolysis → hemosiderin deposition → ROS production or cytokine/growth factor release) would significantly enhance the EGBE database. In addition, determining the relative susceptibility of endothelial cells and hepatocytes to oxidative damage would be informative.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

The discussion of uncertainty surrounding RfC/RfD derivation is a strong suit of the Toxicological Review. The discussion is comprehensive and "choices and assumptions" are transparently and objectively described and supported by an exemplary section 4. As mentioned in my response to general charge question no. 1, the Toxicological Review is particularly impressive in its extensive effort to qualitatively and quantitatively present the impact of alternative "choices" on the derivation of toxicity constants. To some extent, the presentation of uncertainty can be seen as evidence that the precautionary principle can remain intact while deriving a mechanistically driven set of toxicity constants.

As for section 6 (Major Conclusions in the Characterization of Hazard and Dose Response), it would be reasonable to conduct a margin of exposure-type analysis for EGBE in which maximum inhalation concentrations and oral daily doses encountered by humans are compared to the newly-derived RfC/RfD values. Such an analysis, in which Hazard Quotients and Hazard Indices are computed, was previously published in USEPA's proposed rule removing EGBE from the Hazardous Air Pollutants list (Federal Register, Vol.68, No. 225, November 21, 2003).

Chemical-Specific Charge Questions:

(A) Inhalation reference concentration (RfC) for EGBE

1. The 2-year inhalation study by the National Toxicology Program (NTP, 2000) was selected as the basis for the chronic inhalation RfC. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

The selection of NTP (2000) as the principal study has been scientifically justified, and transparently and objectively described in the Toxicological Review. No other studies, to my knowledge, are better suited as the basis for RfC derivation.

2. The incidence of hemosiderin staining in the liver of male rats was selected as the critical effect because it is considered by EPA to be a precursor to an adverse effect. Please comment on whether the selection of this critical effect has been scientifically justified. Are the criteria and rationale for this selection transparently and objectively described in the document? Please provide a detailed discussion. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

The selection of hemosiderin staining in the liver of male rats as the critical effect, as opposed to one of several hematological parameters, has been scientifically justified. The basis for its selection is transparently and objectively described and includes the following: 1) its sensitivity to EGBE (a LOAEL of 31 ppm was identified in rats of both sexes, although Table 5-2 does not indicate a statistically significant difference from the control incidence at this concentration; indeed, the NTP Technical report indicates that hemosiderin deposition was increased relative to chamber controls at 62.5 and 125 ppm in the 2-year study); 2) its clear progression, as indicated by dramatic changes in incidence with continuing exposure, coupled with the lack of progression of hematological endpoints; 3) uncertainties surrounding what changes in hematological endpoints actually represent (toxicity vs. compensation), the mechanisms behind the changes, and their frequent lack of adherence to the fundamental tenet of dose-response (all of which preclude an informed decision as to which hematological endpoint is most appropriate); and 4) the recognition that all of the hematological endpoints discussed are considered precursors to hemosiderin deposition, a clear pathological finding with experimental evidence linking it to neoplastic transformation. The selection of hemosiderin deposition as the

critical endpoint is further justified, in a regulatory sense, by the fact that it represents the lower end of the RfC range when compared to other potential endpoints (see Figure 5-3). It also reflects an acknowledgement of hemosiderin deposition as a key mechanistic step or unifying mechanism behind liver tumor formation independent of exposure route.

While I can support hemosiderin deposition as the critical effect, it is not without significant reservation. While the incidence of this effect is increased in male and female rats at 2 years and 31 ppm, the increase is not statistically significant (Table 5-2). By contrast, RBC count is significantly decreased at 31 ppm in male and female rats at 14 weeks (prior to any increase in hemosiderin deposition; NTP Technical Report, Table 3) and in female rats at 3 and 6 months (NTP Technical Report, Table 9). Thus, an argument might be made for RBC count as the critical effect on the basis of sensitivity alone. However, as shown in Table 5-3 of the Review, its use as such does not result in a more health conservative RfC. In addition, hemosiderin deposition is treated as a quantal response for risk assessment purposes, when in reality it is not an all or none proposition. This is particularly noteworthy given that severity scores for hemosiderin deposition were reported in the subchronic drinking water study, but not in the 2-year inhalation study. Furthermore, the severity of hemosiderin deposition was "minimal" regardless of oral dose.

3. 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.

The data set to which BMD modeling was applied for determination of the POD is suitable for such an analysis. In this particular case, BMD modeling does represent the best approach, as the BMCL is informed by response information from all dose groups compared to the LOAEL/NOAEL that is constrained to one of the experimental doses. The BMD modeling is objectively described and its conduct is transparent. Based on the Toxicological Review's narrative, Tables 5-6 and 5-7, and review of model parameters and output (Appendix B-8 to B-

10), the modeling appears to have been correctly conducted, with any one of three potential model choices sufficing. In addition, the dose metric selected (i.e., AUC BAA) is appropriate, as the critical effect is more than likely a function of cumulative exposure rather than peak concentration. Comparison of the LOAEL/NOAEL and BMD/PBPK approaches was informative, with the comparable results increasing confidence in use of the latter. In addition, BMD modeling using an alternative endpoint (changes in RBC counts among rats of both sexes) and dose metric (Cmax BAA at 3 months) served to inform the debate over the most appropriate critical effect.

4. PBPK modeling was used to extrapolate the POD from rats to humans. Please comment on whether the PBPK modeling for interspecies extrapolation is scientifically justified, and transparently and objectively described in the document. Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Are the model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

Based on Table 5-3, Summary of PBPK models, it appears the model of Corley et al. (1994, 1997) is the only one capable of extrapolating between rats and humans. Corley et al.'s model is essentially a coupling of two models, one describing the disposition of EGBE and the other BAA. The model includes the introduction of EGBE via IV infusion, inhalation, ingestion and dermal absorption; the distribution of EGBE to the liver; the metabolism of EGBE to BAA solely in the liver following Michaelis-Menten kinetics; protein binding of BAA in the blood; and distribution of BAA to tissues, including the kidney where it is excreted via an active transport process in the urine.

It is appropriate for the experimentally validated model of Corley et al. to be exercised to convert the BMCL for the RfC (based on AUC BAA in rats, for example) to a human equivalent concentration (HEC) or oral human equivalent dose (HED). Footnote 8 on p. 78 indicates that a review of PBPK models was conducted prior to their use in the 1999 EGBE Toxicological Review. Furthermore, the text on p. 78 notes that established EPA methods and procedures were used to review, select and apply the chosen PBPK models. It can therefore be assumed that any errors in model structure, parameterization or application would have been remedied prior to its

use in the current context. My review of the basic components of the Corley et al. model, including the parameters listed in Appendix A, Table A-1, found nothing to suggest otherwise.

5. Please comment on the selection of all of the uncertainty factors applied to the POD for the derivation of the chronic RfC. For instance, are they scientifically justified, and transparently and objectively described in the document? An UF of 10 for extrapolation from animals to humans (UF_A) is generally applied when data are not available to inform potential pharmacokinetic (PK-UF) and pharmacodynamic (PD-UF) differences. In this assessment, an UF_A of 1 was applied.

- A PBPK model was used to inform pharmacokinetic differences and a PK-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether there are sufficient scientific data and support for the use of this PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$).
- Evidence from human and animal in vitro and in vivo studies was used to inform pharmacodynamic differences and a PD-UF of 1 was selected. Please comment on whether this selection is scientifically justified. Is the rationale transparently and objectively described? Please comment on whether a higher value for the PD-UF should be used (e.g., to account for the limited information available on the potential for effects in human cell types other than red blood cells) or alternatively, should a lower (i.e., fractional) PD-UF be used (e.g., to account for the 40 - 150 fold difference in the concentrations that cause pre-hemolytic effects in human red blood cells (RBCs), including RBCs from potential susceptible populations such as the elderly, and patients suffering from anemia and RBC disorders that weaken the cellular membrane such as hereditary spherocytosis).

Please identify and provide the rationale for any alternative approaches for the selection of the uncertainty factors.

As mentioned in the response to general charge question no. 4, the discussion of uncertainty surrounding RfC derivation is a strong suit of the Toxicological Review. In my opinion, all of the uncertainty factors (UF) applied to the POD for RfC derivation are valued appropriately, scientifically justified, and transparently and objectively described. This includes the overall UF for interspecies extrapolation of 1 and both of its component parts (i.e., interspecies differences in toxicokinetics and toxicodynamics). The use of the experimentally validated PBPK model of Corley et al. for animal to human extrapolation obviates the need for any interspecies UF for toxicokinetic differences. As for an UF to account for toxicodynamic differences, any uncertainty stemming from the lack of chronic, human exposure data is offset by

the decreased susceptibility of human RBCs to hemolysis as demonstrated *in vitro* and in acute poisoning incidents.

Despite my comments above, I believe that some consideration should be given to reducing the intraspecies UF from 10 to 3 given the outcome of studies conducted with RBCs from what were suspected to be potentially sensitive human subpopulations. At the same time, however, I would consider an increase in the database UF from 1 to 3 given USEPA's medium-to-high confidence in the RfC (and RfD) assessment and in the database that supports it. Such a confidence level is seemingly inconsistent with a database UF of 1. If both changes mentioned above were made, the overall UF applied to RfC (and RfD) derivation would obviously remain unchanged from that currently proposed.

6. Please comment specifically on the database uncertainty factor of 1 applied in the RfC derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Please comment on the body of information regarding the hemato and hepatic toxicity of EGBE and the use of the toxicokinetic data in the determination of the database uncertainty factor. Please comment on whether the selection of the database uncertainty factor for the RfC has been scientifically justified. Has this selection been transparently and objectively described in the document?

A database UF of 3 or 10 is generally used when extrapolating from valid results in experimental animals when data are "incomplete." It is intended to account for what some believe is the inability of any single study to adequately address all possible adverse outcomes. As indicated in the response to charge question no. 5 above, I can support a database UF of 1, particularly as an UF for intrahuman variability of 10 exist to account for the outstanding questions surrounding potentially sensitive subpopulations. The data base UF of 1 is scientifically justified, and its basis transparently and objectively described in the Toxicological Review. The Toxicological Review expresses medium-to-high confidence in the RfC (and RfD) assessment and in the database that supports it (which is seemingly inconsistent with a database UF of 1). I am somewhat more optimistic and believe the bioassays detailed in NTP (2000), coupled with existing studies of hemato- and hepatotoxicity, are sufficient to identify the critical effect and develop a high confidence estimate of its sub-threshold concentration or dose. USEPA indicates that its confidence in the database in not high since the potential for effects in

humans from repeat, long-term exposures has not been investigated. USEPA does acknowledge, however, that the existing data suggest long-term exposures in humans would be no more adverse, and likely less so, than long-term rat exposures. As opportunities to examine humans after repeated, long-term exposures are rare, if they exist at all, USEPA's reservations over the completeness of the database are not likely to be resolved.

Regarding the body of literature specific to the hemato- and hepatotoxicity of EGBE, in my opinion it is sufficiently robust to formulate with a high degree of confidence a hypothetical, threshold-dependent mechanism beginning with EGBE's metabolic activation to a hemolytic metabolite and ending with neoplastic transformation of two liver cell types. This high degree of confidence should not be misconstrued as approaching absolute certainty. The toxicokinetics of EGBE have been well investigated in terms of their species-, dose-, age- and route-dependency, and have been used to develop and validate predictive PBPK models for rats, mice and humans. Internal doses of BAA at which hemolysis is seen in rodents have been measured and provide a basis for model predictions of the exposure circumstances, if any, under which such an effect might be seen in humans. As for the role of toxicokinetics in determining the database UF, I suppose the qualitative similarity in kinetics across species (and the ability for interspecies extrapolation via a PBPK model) makes the numerous rodent studies relative to the prediction of human risk, barring toxicodynamic differences that suggest otherwise. This increases the utility of the database for human health risk assessment and lessens the concern for the lack of chronic human data.

Despite my comments above, I will reiterate a portion of my response to charge question no. 5, as it is applicable here as well. I believe that some consideration should be given to increasing the database UF from 1 to 3 given USEPA's medium-to-high confidence in the RfC (and RfD) assessment and in the database that supports it. Such a confidence level is seemingly inconsistent with a database UF of 1. However, should this be done, I would advocate for a reduction in the intraspecies UF from 10 to 3 given the outcome of studies conducted with RBCs from what were suspected to be potentially sensitive human subpopulations. If both changes mentioned above were made, the overall UF applied to RfC (and RfD) derivation would obviously remain unchanged from that currently proposed.

(B) Oral reference dose (RfD) for EGBE

1. A conclusion was reached that the available oral toxicity data are inadequate to support derivation of a chronic oral RfD value. Is the rationale for not developing an RfD from the available database of oral studies transparently and objectively described? If other oral studies are identified that would be suitable for the derivation of the RfD, please identify and provide the rationale for their use.

The rationale for performing a route-to-route extrapolation from inhalation data to derive an RfD is transparent and objectively described. The Toxicological Review notes that the oral database for EGBE is quite limited relative to that of inhalation, with no chronic oral studies in any species. It also accurately points out that the hematological effects considered precursors to hemosiderin deposition are consistent between the oral and inhalation exposure routes. Nonetheless, it is noteworthy that RBC count was significantly reduced and MCV significantly elevated in female rats at the lowest dose administered in the 13-week drinking water study of NTP (1993). Furthermore, 13 weeks was sufficient to generate a dose-response for minimal to mild Kuppfer cell pigmentation (hemosiderin deposition) among female rats of 0/10 (control), 0/10 (69 mg/kg-day), 2/10 (129 mg/kg-day), 10/10 (281 mg/kg-day), 10/10 (367 mg/kg-day) and 10/10 (452 mg/kg-day).

As the hematological effects of EGBE/BAA are not progressive, the subchronic nature of the NTP (1993) study should not be a major consideration when determining the value of these endpoints for RfD derivation. It was therefore appropriate that the Toxicological Review would apply the BMD/PBPK approach to hematological data for RfD derivation. Unfortunately, the Toxicological Review failed to do likewise for the hemosiderin deposition data in female rats from the same study. Granted, the Corley et al. PBPK model is based on male rat kinetic data and use of the NTP (1993) study may necessitate an UF for subchronic to chronic exposure duration, but these considerations did not preclude the application of BMD/PBPK methodology to RBC count data. It is therefore suggested that the same methodology be applied to the hemosiderin deposition data using AUC BAA as the dose metric. Doing so would further inform the decision of whether route-to-route extrapolation is preferred to the use of subchronic data, albeit data from the opposite sex to that which served as the basis for RfC derivation. At present, I am not opposed to the use of the route-to-route extrapolation for RfD derivation. Nor am I convinced, however, that the subchronic data have no utility in this regard. This is only

reinforced by the demonstration that BMDL(HEDs) based on subchronic RBC counts and route-to-route extrapolation are comparable (3 vs. 1.4 mg/kg-day).

2. A route-to-route extrapolation was performed to derive the chronic RfD, using the chronic inhalation study and PBPK modeling. The Human Equivalent Concentration (HEC) was based on a continuous oral exposure to EGBE in drinking water that would yield the same AUC for the metabolite BAA (in the arterial blood over three months) as that estimated for the rat following an external inhalation exposure to EGBE at the level of the proposed POD (i.e., the $BMCL_{10}$). Please comment on whether the PBPK model is adequate for use to conduct a route-to-route extrapolation for EGBE to derive an RfD in the absence of adequate oral animal or human dose-response data to derive the RfD directly. Was the extrapolation correctly performed and objectively and transparently documented?

The PBPK model is thought to be adequate for the conduct of route-to-route extrapolation in the derivation of the RfD, despite it being a potential source of uncertainty. The extrapolation appears to have been correctly performed and is transparently documented. Please refer to my response to the charge question immediately above for reservations about accepting the route-to-route extrapolation outright in lieu of using data from the subchronic study of NTP (1993).

3. Please comment specifically on the database uncertainty factor of 1 applied in the RfD derivation. Are the criteria and rationale for the selection of the database uncertainty factor transparently and objectively described in the document? Measured internal doses in rats and a human PBPK model were used to perform a route-to-route extrapolation to derive the RfD. Please comment on the use of the PBPK model and the inhalation database in the determination of the database uncertainty factor for the RfD. Please comment on whether the selection of the database uncertainty factor for the RfD has been scientifically justified. Has this selection been transparently and objectively described in the document?

In the Toxicological Review, the descriptions of UFs applied to RfC and RfD derivations are essentially the same. This might be expected, as the RfD reflects the inhalation database for EGBE more so than the oral database given the means by which it was derived (i.e., inhalation to oral extrapolation). Accordingly, most of my comments on the database UF applied in RfC derivation are applicable here. As for the uncertainty potentially introduced by route-to-route extrapolation, the similarity between HEDs derived using this method (1.4 mg/kg-day), the NOAEL/LOAEL method (7.6 mg/kg-day) and back-calculation from a rat BMDL (3 mg/kg-day), suggest that it is not of sufficient magnitude to disqualify the model's use in this regard.

(C) Carcinogenicity of EGBE

1. Under the EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/background.htm), the Agency concluded that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations. Please comment on the scientific justification for the cancer weight of evidence characterization and describe the basis for your view. Has the scientific justification for the weight of evidence descriptor been sufficiently, transparently and objectively described?

Yes, the scientific justification for describing EGBE as "not likely to be carcinogenic to humans" at expected exposure concentrations is sufficiently, transparently and objectively described in the Toxicological Review. In other words, the descriptor is appropriately presented in the context of a weight-of-evidence narrative. As pointed out in the *Guidelines for Carcinogen Risk Assessment*, the descriptor is appropriate when the available data, as in this case, are considered robust for deciding that there is not a basis for human hazard concern. Use of the descriptor obviously does not depend upon the absence of positive bioassay data or cancer mechanism(s) that are likely operable only in experimental animals. Rather, the descriptor can be applied to chemicals such as EGBE that are clearly animal carcinogens by at least one mechanism that, in theory, might be operable in humans at extreme doses rarely, if ever, encountered. Use of the descriptor under these circumstances, however, is contingent on the unlikelihood that human doses above the threshold for precursor effects essential to tumor formation would ever be realized. In other words, such a description as the one applied to EGBE and qualified by exposure concentration or dose is typically reserved for carcinogens for which sufficient evidence of a non-linear mechanism exist. Such is the case for EGBE. The descriptor applied to EGBE is further supported by 1) the chemical's general lack of mutagenicity and clastogenicity; 2) a PBPK exercise demonstrating that vapor pressure limitations preclude inhalation exposures sufficient to achieve hemolytic blood levels of BAA in humans; 3) the experimental demonstration of the relative insensitivity of humans to RBC hemolysis, an essential precursor to liver tumor formation; 4) a BMD/PBPK analysis confirming that the RfC and RfD derived on the basis of hemosiderin deposition were also protective against forestomach hyperplasia and tumors; and 5) the likelihood that high doses of EGBE in humans would result in metabolic acidosis before hemolysis, which would require treatment and likely result in discontinuation of the exposure scenario.

Based on the above discussion, it is not anticipated that the descriptor applied to EGBE will be the subject of debate. Nonetheless, as suggested in my response to general charge question no. 4, it would be reasonable to add a margin of exposure-type analysis for EGBE to the Toxicological Review in which maximum inhalation concentrations and oral daily doses encountered by humans were compared to the newly-derived RfC/RfD values.

2. EPA has proposed a mode of action (MOA) for male mouse liver cancer involving metabolism, hemolysis of RBCs, hemosiderin deposition in the liver, oxidative damage and proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for liver cancer is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The analysis regarding the MOA for liver hemangiosarcoma and hepatocellular carcinoma is scientifically sound, and transparently and objectively described in the Toxicological Review. In particular, the stepwise progression from metabolic activation of EGBE to neoplasm formation (pp. 54-55) is a nice way of bringing disparate data sources together in the form of a single MOA, that while hypothetical, nonetheless enjoys considerable experimental support. In addition, the specific experimental support for each of the nine "steps" in the hypothesized MOA is discussed in the text, independent of the section on biological plausibility. This impressive compilation of supportive studies alone is sufficient to increase confidence and decrease uncertainty in the MOA. Confidence in the MOA is further increased by a discussion of other chemicals that, like EGBE, increase the incidence of both liver tumors and hemosiderin deposition among male mice. Knowledge of the hypothesized MOA is more than sufficient to select an appropriate dose metric (AUC BAA), critical effect (hemosiderin deposition) and low-dose extrapolation method (BMD modeling with back-extrapolation via a PBPK model to a human equivalent concentration), with the latter being the source of uncertainty with the greatest potential impact on EGBE's RfC. Lastly, the Toxicological Review contains a statement to the effect that no other viable MOAs have been identified to explain the hemato- and hepatotoxicological observations among laboratory animals and humans following EGBE exposure.

Despite the above statement, I remain somewhat hesitant to fully embrace hemosiderin deposition as the critical effect given the hypothetical nature of the MOA. Might reactive oxygen species be generated without Kupffer cell involvement? If so, hemosiderin deposition within Kupffer cells might be more of a biomarker of exposure rather than effect, and given the "minimal" severity of the deposition regardless of dose, not a good one at that. Selection of RBC hemolysis as the critical effect, while not making the hypothesized MOA any less viable, would avoid having to place more confidence in the hypothesized MOA than might arguably be justified.

3. EPA has proposed a MOA for female mouse forestomach tumors involving metabolism, irritation and regenerative proliferation leading to tumor induction as key events best supported by the data. Please provide detailed comments on whether this analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Considerations include the scientific support regarding the plausibility for the hypothesized MOA and the characterization of uncertainty regarding this MOA.

The analysis regarding the MOA for forestomach tumors is scientifically sound, and transparently and objectively described in the Toxicological Review. Similar to that for the liver, the Toxicological Review presents the MOA for forestomach tumors as a stepwise progression from deposition and metabolic activation of EGBE in the forestomach to the promotion of initiated forestomach cells via a proliferative response to cell injury (p. 62). Again, this is a nice way of bringing disparate data sources together in the form of a single MOA, that while hypothetical, nonetheless enjoys considerable experimental support. The experimental support for each of the six "steps" in the hypothesized MOA is discussed in the text, independent of a section on biological plausibility. This impressive compilation of supportive studies alone is sufficient to increase confidence and decrease uncertainty in the MOA. Confidence in the MOA is further increased by acknowledgment that several other chemicals, like EGBE, are capable of inducing forestomach hyperplasia after inhalation exposure. Knowledge of the hypothesized MOA is more than sufficient to select an appropriate dose metric (Cmax of blood BAA), critical effect (epithelial hyperplasia of the forestomach) and low-dose extrapolation method (BMD modeling with back-extrapolation via a PBPK model to a human equivalent oral dose and air concentration). Extensive uncertainty surrounding the relevance of the MOA in mice for humans persists and is due to the absence of a forestomach in humans and differences in enzyme

distribution and kinetics between the glandular and forestomach tissues of the two species. The Toxicological Review has, however, effectively eliminated any concern for forestomach tumors created by opting for a critical effect related to the liver, as a BMD/PBPK analysis demonstrated RfC and RfD values for EGBE are protective against forestomach hyperplasia. Lastly, the Toxicological Review contains a statement to the effect that no other viable MOAs have been identified to explain the toxicity of EGBE to the forestomach.

4. EPA has not proposed a MOA for the female rat pheochromocytomas of the adrenal medulla. NTP rated the female rat pheochromocytomas as providing equivocal evidence of carcinogenic activity and the pathology report expressed concern as to whether the observed tumors met the criteria used to diagnose pheochromocytomas. For these reasons, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential. Please provide detailed comments on whether this analysis regarding the female rat pheochromocytomas is scientifically sound, and transparently and objectively described in the Toxicological Review. Please comment on whether and the extent to which the female rat pheochromocytomas are adequate to support alternative analyses of qualitative and quantitative cancer risks to humans and discuss approaches to consider if such analyses are warranted.

Page 53 of the Toxicological Review (which is part of the overall weight of evidence summary for EGBE's carcinogenicity) contains a one paragraph justification as to why pheochromocytomas in female rats were not significantly weighted in the assessment of EGBE's cancer potential. The paragraph is an accurate reflection of the concerns expressed in NTP's Technical Report. Though concise, it provides a scientifically sound, transparent and objective basis for USEPA's dismissal of the pheochromocytoma data. After all, there were no increased incidences among males; the incidence even at the highest exposure concentration (16%) barely exceeded the highest incidence observed in any one historical inhalation (13%) or non-inhalation control group (14%); while a trend for combined benign and malignant tumor incidence was seen among females, it was not strictly concentration dependent, nor were there any statistically significant pairwise comparisons; and, the time to first tumor incidence was not inversely related to concentration. Therefore, NTP's characterization of the incidences of pheochromocytoma as <u>equivocal</u> findings not clearly related to EGBE exposure is justified, as is USEPA's decision to exclude them from consideration in quantitatively assessing EGBE's hazard potential.

5. Please comment on the choice of the nonlinear threshold approach for the quantitative assessment of the carcinogenic potential of EGBE. Please comment on whether this approach is scientifically sound, and transparently and objectively described. Please comment on whether the example calculations using linear low-dose extrapolation for cancer as discussed in section 5.4.1 represent useful characterizations of the potential quantitative uncertainty associated with exposure to EGBE. Please comment on whether the linear analysis should be presented as an alternative to the threshold approach considering the Agency conclusion that EGBE is not likely to be carcinogenic to humans at expected exposure concentrations.

Whether applying it to data on hematological factors such as RBC count, hemosiderin deposition or forestomach hyperplasia, I support the choice of the non-linear threshold approach employed in the Toxicological Review. The approach appears to be scientifically sound, and transparently and objectively described. It is clearly applicable based on the hypothesized MOAs of EGBE within the two target tissues in which tumors were clearly elevated. As a result, key "steps" in the MOA that include critical effects and all downstream events (including cancer) are unlikely to occur at or below the RfC or RfD. I favor retaining the analysis in section 5.4.1 as a means of reinforcing the importance of reducing uncertainties and strengthening the database to the point where a mechanistically-driven assessment of hazard potential is possible. Its retention could be further justified in those cases where a minority of genotoxicity data or structural analogy suggests the possibility of low-dose linearity and direct interaction with DNA. I do not, however, support its presentation as an alternative to the threshold approach clearly warranted in the case of EGBE.

Appendix A

List of Reviewers

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

US EPA Conference Facilities
Campus Building 3 (Room C113)
Research Triangle Park, NC
October 16, 2008

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Appendix B

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

US EPA Conference Facilities
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Research Triangle Park, NC
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Appendix C

Meeting Agenda

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

US EPA Conference Facilities
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Research Triangle Park, NC
October 16, 2008

Agenda

8:00 a.m. **Registration**

8:30 a.m. **Welcome, Introductions, Meeting Purpose & Agenda**..... *Jan Connery, ERG*

8:40 a.m. **EPA Welcome Remarks**..... *John Vandenberg, Director EPA, NCEA-RTP*

8:50 a.m. **Public Comment**..... *Jan Connery*

9:00 a.m. **Discussion Process and Overarching Comments**..... *David Jollow (Chair) & Panel*

9:15 a.m. **Inhalation RfC for EGBE** ..... *David Jollow & Panel*

A1) **Use of NTP (2000) as principal study for the chronic inhalation RfC**. Scientifically justified as principal study? Transparently and objectively described? Should any other studies be selected as the principal study?

A2) **Selection of incidence of hemosiderin staining in the liver of male rats as critical effect.** Selection scientifically justified? Criteria and rationale for selection transparently and objectively described? Should any other endpoints be considered for the critical effect?

A3) **Use of BMD modeling to derive POD for the RfC.** Is BMD modeling the best approach? Has it been appropriately conducted and objectively and transparently described? Was the BMR selection scientifically justified and transparently and objectively described? Should EPA consider any alternative approaches for deriving the POD? Are the alternatives preferred to EPA's approach?

A4) **Use of PBPK modeling to extrapolate the POD from rats to humans.** Use for interspecies extrapolation scientifically justified and transparently and objectively described? Does the model properly represent the toxicokinetics of the species under consideration? Was the model applied properly? Model assumptions, parameter values, and selection of dose metrics clearly presented and scientifically supported?

Agenda (cont.)

A5) **Uncertainty factors applied to the POD.** Selection and application scientifically justified and transparently and objectively described? Should EPA consider any alternative approaches for selection of UFs? **UF_A of 1 for animal-to-human extrapolation:** Data sufficient to support use of PBPK model to estimate interspecies toxicokinetic differences and to replace the default interspecies factor for toxicokinetic differences (i.e., $10^{1/2}$)? **PD-UF:** Should a higher or lower value than 1 be used?

A6) **Database UF of 1 applied in the RfC derivation.** Criteria and rationale for selection of UF scientifically justified and transparently and objectively described? Comment on the body of information re hemato and hepatic toxicity and use of toxicokinetic data in determining the database UF.

10:30 a.m. BREAK

10:45 a.m. **Inhalation RfC for EGBE** (cont.) ..... *David Jollow & Panel*

Noon LUNCH

1:00 p.m. **Oral RfD for EGBE** ..... *David Jollow & Panel*

B1) **Conclusion that available oral toxicity data are inadequate to support derivation of chronic oral RfD.** Rationale for not developing an RfD transparently and objectively described? Are other oral studies suitable for deriving the RfD? What is the rationale for their use?

B2) **Use of PBPK model to conduct route-to-route extrapolation to derive an RfD.** PBPK model adequate for route-to-route extrapolation in absence of adequate oral animal or human dose-response data? Extrapolation correctly performed and objectively and transparently documented?

B3) **Database UF of 1 applied in the RfD derivation.** Criteria and rationale for selection scientifically justified and transparently and objectively described? Comment on the use of the human PBPK model and the inhalation database in determining this UF.

2:00 p.m. **Carcinogenicity of EGBE** ..... *David Jollow & Panel*

C1) **Cancer weight-of-evidence characterization.** Scientific justification sufficiently, transparently and objectively described?

C2) **Mode of action analysis for male mouse liver cancer.** Scientifically sound and transparently and objectively described, considering scientific support for plausibility of and uncertainty characterization for the hypothesized MOA?

C3) **Mode of action analysis for female mouse forestomach tumors.** Scientifically sound and transparently and objectively described, considering scientific support for plausibility of and uncertainty characterization for this MOA?

C4) **Analysis of female rat pheochromocytomas of the adrenal medulla.** Scientifically sound and transparently and objectively described? To what extent are female rat pheochromocytomas adequate to support alternative analyses of qualitative and quantitative cancer risks to humans? What approaches should be considered if such analyses are warranted?

C5) **Nonlinear threshold approach for quantitative assessment of carcinogenic potential of EGBE.** Scientifically justified and transparently and objectively described? Do the example calculations using linear low-dose extrapolation for cancer (section 5.4.1) represent useful characterizations of the potential quantitative uncertainty associated with EGBE exposure? Should the linear analysis be presented as an alternative to the threshold approach considering EPA's conclusion that EGBE is *not likely to be carcinogenic to humans* at expected exposure concentrations?

2:30 p.m. BREAK

2:45 p.m. **Carcinogenicity of EGBE** (cont.)..... *David Jollow & Panel*

3:15 p.m. **General Questions** ..... *David Jollow & Panel*

1) Is the Review logical and clear? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

2) Should any additional studies be considered in the assessment of the noncancer and cancer health effects?

3) What research would likely increase confidence in the database for future assessments.

4) **Sources of uncertainty in sections 5 and 6:** Key uncertainty sources adequately discussed? Choices and assumptions transparently and objectively described? Impact of uncertainty on the assessment transparently and objectively described?

4:10 p.m. **Reviewer Final Comments** ..... *David Jollow & Panel*

4:25 p.m. **Closing Remarks** ..... *Jan Connery & EPA/NCEA*

4:30 p.m. ADJOURN