

**National Toxicology Program**

**Peer Review of Draft Report on Carcinogens (RoC)**

**Monographs for Cumene and 1-Bromopropane**

**March 21–22, 2013**

**National Institute of Environmental Health Sciences**

**Peer Review Report**

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## **I. Attendees**

### **Peer Review Panel**

Lucy Anderson (Chair), Private Consultant  
Leo Thomas Burka, Private Consultant  
Michael Elwell, Covance Laboratories, Inc.  
Terry Gordon, New York University  
Lawrence Lash, Wayne State University  
Stephen Nesnow, Private Consultant  
Wayne Sanderson, University of Kentucky  
MaryJane Selgrade, Private Consultant  
Paul White, Health Canada

### **National Toxicology Program Board of Scientific Counselors Liaison**

Dale Hattis, Clark University

### **Other Federal Agency Staff**

Kevin Hanley, National Institute for Occupational Safety and Health (NIOSH), Technical advisor

### **National Institute of Environmental Health Sciences Staff**

Danica Andrews	Robin Mackar
Rebecca Boyles	David Malarkey
John Bucher	Diane Spencer
Gloria Jahnke	Lori White
Ruth Lunn	Mary Wolfe

### **Report on Carcinogens ContractSupport Staff**

Stanley Atwood, Integrated Laboratory Systems (ILS)  
Susan Dakin, Independent Consultant  
Ella Darden, ILS  
Andrew Ewens, ILS  
Sanford Garner, ILS  
Alton Peters, ILS  
Jennifer Ratcliffe, ILS

### **Public Attendees**

Karie Riley, Versar, Inc.  
Carr Smith, Albemarle Corporation

## II. Welcome and Introductions – Day 1

The National Toxicology Program (NTP) Peer Review Panel for the Draft Report on Carcinogens (RoC) Monographs for 1-Bromopropane and Cumene convened on March 21, 2013, in the Keystone Building, National Institute for Environmental Health Sciences (NIEHS), 530 Davis Drive, Morrisville, North Carolina. Dr. Lucy Anderson served as chair. Dr. Dale Hattis attended as the NTP Board of Scientific Counselors (BSC) liaison. Cdr. Kevin Hanley attended representing the National Institute for Occupational Safety and Health (NIOSH) and as a technical advisor to the Office of the RoC (ORoC). Representing the NTP were NTP Associate Director Dr. John Bucher; Dr. Mary Wolfe, Deputy Division Director for Policy; Dr. Ruth Lunn, Director, ORoC; Dr. Gloria Jahnke, Health Scientist, ORoC, and Ms. Diane Spencer, Health Scientist, ORoC. Dr. Lori White, Health Scientist Administrator, Office of Liaison, Policy and Review, served as the Designated Federal Official for the meeting.

Dr. Anderson called the meeting to order at 1:00 p.m., welcomed everyone to the meeting, and asked all attendees to introduce themselves. Dr. John Bucher also welcomed and thanked the attendees. Dr. Lori White read the conflict of interest policy statement and briefed the attendees on meeting logistics. Dr. Anderson briefed the panel and the audience on the format for the review.

## III. Process for Preparing the Draft RoC Monographs for 1-Bromopropane and Cumene

### III.A. Presentation

Dr. Lunn presented background information on the RoC and the process and methods used to prepare the draft RoC monographs for 1-bromopropane (1-BP) and cumene. She emphasized that the evaluation of substances for listing in the RoC as *known* or *reasonably anticipated to be human carcinogens* is a hazard identification activity and does not involve any other steps in the risk assessment process, such as formal dose response assessment or quantitative exposure assessment. She noted that the draft RoC monographs consist of two parts: (1) a literature-based cancer evaluation component and (2) the draft substance profile, which contains the preliminary listing recommendation and a summary of the scientific evidence considered to be key for reaching the recommendation.

The process for preparing the RoC was revised in January 2012, and 1-BP and cumene are the first substances to be evaluated under the new process, which consists of the following steps: (1) nomination and selection of the candidate substances, (2) scientific evaluation in draft monographs, (3) peer review of the draft monographs, and (4) submission of the substance profiles to the Secretary of Health and Human Services (HHS). Dr. Lunn noted that the process provides opportunities for public comment, scientific input, and peer review of the scientific information. She outlined the structure of the draft monographs, noting that the section on human exposure is not intended to be a quantitative exposure assessment, and that no cancer studies in humans were found for these two substances. She summarized the process for preparation of the cancer evaluation component of the monographs and reviewed the literature search strategy.

Dr. Lunn reviewed the criteria used to assess the literature in each discipline. With respect to exposure, she noted that the Public Health Service Act requires that the RoC list substances “to which a significant number of persons residing in the United States are exposed.” Because this information rarely is available, it typically has been inferred from data on uses, production volume, occupational monitoring, environmental occurrence, estimated daily intake, biomonitoring of the general public (e.g., National Health and Nutrition Examination Survey [NHANES] data), and past exposure. The peer reviewers were asked to use their judgment in deciding whether the exposure data in the draft monographs supported the conclusion that a significant number of U.S. residents are or have been exposed to these substances.

The preliminary listing recommendation is based on applying the RoC criteria to the data on cancer in humans, cancer in experimental animals, and mechanisms of carcinogenicity. These criteria are used to make decisions about the level of evidence for cancer in humans and in animals and to reach conclusions about potential mechanisms of action. This body of knowledge is integrated to form the basis for the listing recommendation. Because no human cancer studies were found that were specific to 1-BP or cumene, the listing recommendations were based on the evidence for carcinogenicity in animals and an evaluation of the mechanistic data. She noted that in most cases there is limited mechanistic data; however, conclusions regarding carcinogenicity of the substance include consideration of convincing or compelling data on whether the potential modes of actions could cause cancer in humans.

Dr. Lunn reviewed the RoC criteria regarding mechanistic data and noted that the criteria allow mechanistic data to be used for determining the listing category when there is (1) compelling evidence that the substance causes cancer in animals by a mechanism that would not operate in humans or (2) convincing evidence that the substance causes cancer by a mechanism that would operate in humans. She then reviewed the RoC criteria for listing of a substance as *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen*. She emphasized that conclusions regarding carcinogenicity in humans or experimental animals are based on consideration of all relevant scientific information, as outlined in the RoC listing criteria.

The charge to the Peer Review Panel was as follows:

- (1) To comment on the draft cancer evaluation components for 1-BP and cumene, specifically, whether they are technically correct and clearly stated, whether the NTP has objectively presented and assessed the scientific evidence, and whether the scientific evidence is adequate for applying the listing criteria.
- (2) To comment on the draft substance profiles, specifically, whether the scientific justification presented in the substance profile supports the NTP’s preliminary policy decision on the RoC listing status of 1-BP and cumene.

The panel would be asked to vote on the following questions:

- (1) Whether the scientific evidence supports the NTP’s conclusion on the level of evidence for carcinogenicity from experimental animal studies on 1-BP and cumene.
- (2) Whether the scientific evidence supports the NTP’s preliminary listing decision for 1-BP and cumene in the RoC.

Following the peer review, the draft monographs will be revised based on the peer review and public comments. This information will be provided to the BSC, and the monographs will be finalized. Once all reviews have been completed for the next edition of the RoC, the substance profiles will be submitted to the Secretary, HHS, for approval or disapproval.

#### **IV. Draft RoC Monograph for Cumene**

##### **IV.A. Peer Review Comments on the Process for Preparation of the Draft RoC Monograph for Cumene**

Dr. MaryJane Selgrade, first reviewer, had no major comments and felt the process was covered well. She noted that the diagram of the literature review process did not match the description in the text, and suggested the diagram be revised. Dr. Lawrence Lash, second reviewer, said the process was clearly described and he had no major comments. He questioned why the substance profile was called Part II of the monograph, because it is so small in relation to Part I. He suggested that the profile be included as an appendix. He also found it confusing that Appendix C discussed summarizing the quality of individual studies but in fact summarized only the NTP study. Dr. Selgrade asked whether the mechanistic studies also were evaluated for quality. Dr. Lunn said the mechanistic studies were evaluated for adequacy based on the reviewers' scientific judgment; the studies' strengths and limitations are discussed in the monographs, but formal evaluation questions for mechanistic studies have not been developed.

##### **IV.B. Oral public comments on the Draft RoC Monograph for Cumene**

No oral public comments were provided for the draft monograph on cumene.

##### **IV.C. Scientific Issues in Written Public Comments**

Dr. Mary Wolfe presented a summary of the two written public comment submissions, one from the American Chemistry Council by Dr. Richard Becker and Jonathan Busch, and one from Dow Chemical Company by Dr. James Bus. The written comments were provided to the Panel prior to the meeting and posted on the meeting page. The major scientific issues raised in the public comments were:

- The draft monograph does not document that a significant number of persons in the United States are exposed to cumene.
- The tumor findings in animals may be mediated through modes of action of questionable quantitative and/or qualitative relevance to human cancer outcomes.
  - Male rat specific kidney tumors are mediated through  $\alpha_{2u}$ -globulin.
  - Mouse lung tumors are mediated through mouse lung-specific metabolism by CYP2F2.
  - Mouse liver tumors are plausibly mediated through a phenobarbital-like liver enzyme induction.
- Cumene and its structural analogues are not genotoxic.

- Studies of structural analogues of cumene are important for understanding cumene's mode of action.
- The postulated genotoxic mode of action for cumene mediated through formation of a genotoxic epoxide metabolite of  $\alpha$ -methylstyrene is not highly plausible.

Dr. Wolfe encouraged the Panel to address these issues in their discussion.

#### **IV.D. Draft Cancer Evaluation Component**

##### **IV.D.1 Properties and Human Exposure**

###### **IV.D.1.1 Presentation**

Dr. Jahnke presented an overview of key information in the draft monograph. Cumene is a component of fossil fuels and is found in cigarette smoke. It is a high-production-volume chemical, used primarily in synthesis of acetone and phenol. It was selected as a candidate substance for the RoC based on widespread current and past U.S. exposure and the existence of an adequate database of studies in animals for evaluation of its potential carcinogenicity. The primary routes of occupational exposure are inhalation and dermal contact during production processes and during painting and car repair work. Environmental exposure results from combustion and evaporation of fossil fuels; emissions from production, use, and transport; and accidental spills. The primary route of environmental exposure is inhalation of ambient air. In 2010, over 1 million pounds of cumene were released into the environment from 300 facilities. In humans, cumene has been detected in expired air and in the blood of individuals (including nonsmokers) exposed environmentally and not occupationally. Based on the occupational and environmental exposure data, it was concluded that a significant number of U.S. residents are exposed to cumene.

###### **IV.D.1.2 Peer Review Comments and Panel Discussion**

Dr. Wayne Sanderson, first reviewer, commented that this section of the monograph was very clear and accurate and that he was impressed by the literature cited. He said he could not find any relevant publications that had been missed, and that many references had been included that are not easily accessible. He suggested adding information on sampling and analytical techniques, which is available from the Occupational Safety and Health Administration (OSHA) and NIOSH. He noted that the American Conference of Governmental Industrial Hygienists (ACGIH) has documented the basis for its threshold limit value (TLV); although no information pertinent to cancer is included, other health effects are documented and exposure is discussed. Also, OSHA has set a permissible exposure limit (PEL) for cumene and may have exposure measurements in its national database.

Dr. Sanderson said it is clear that cumene is ubiquitous in the environment and that virtually everyone in the United States is exposed at some time in his or her life. He asked for clarification on the use of the word "significant" in the RoC criteria for exposure. Based on numbers of people exposed to detectable levels of cumene, exposure is highly significant. However, no one is exposed to cumene at the current OSHA PEL, NIOSH recommended exposure level (REL), and ACGIH TLV of 50 ppm. Although the range of some samples

approached 30 ppm, virtually all measurements were below 10 ppm, and most were below 1 ppm. Dr. Jahnke noted that the RoC monograph is a hazard identification document, not a risk assessment document. She also commented that because of cancer's long latency, exposure at low levels over a lifetime could be an issue. Dr. Sanderson noted that risk assessment to date has largely looked at irritation effects; risk assessment for cancer would require different metrics.

Dr. Sanderson found the table of atmospheric concentration data in Appendix B to be valuable, especially because it showed the numbers of samples, and he suggested adding (where available) the standard deviation, the geometric mean and its standard deviation, and exceedance fractions (the fraction of measurements above some certain level), which allow estimation of the probability of exposure.

Dr. Anderson asked how exposure levels from car repair and painting, which would affect large numbers of people and not just in occupational settings, would compare with environmental exposure levels. Dr. Lunn said occupational exposure levels are generally orders of magnitude higher than environmental exposure levels. Dr. Leo Thomas Burka suggested looking for more information about exposure via aviation fuel. Dr. Terry Gordon noted that although the amount of cumene in gasoline products is proprietary information, it should be possible to figure out, and that the monograph should address exposure of gas station employees and self-service customers, who could be the largest population exposed. He suggested adding assessment of the quality of the data on environmental releases (i.e., from the Toxics Release Inventory database), to aid in interpretation of data. Dr. Sanderson noted that although no information is presented on exposure of gas station attendants or people pumping their own gas, the monograph does report on exposure of gasoline delivery truck drivers and on the cumene concentrations in various fuels (though it does not break out jet fuel).

Dr. Lunn noted that the NHANES database is always consulted during monograph preparation, and that if data were not cited, that means no data on the substance were available.

Dr. Lash addressed the public comment from Dow Chemical Co. that the draft monograph does not adequately address exposure and that exposure is insufficient to justify the listing of cumene. He felt that the draft monograph did a thorough job of presenting the exposure data in the appendix, and that the comment from Dow Chemical Co. was not an accurate portrayal. Dr. Sanderson echoed Dr. Lash's comment, noting that the discussion in the monograph is limited by what sampling data have been released to the public.

The Panel supported the statement that a significant number of persons in the United States are exposed to cumene.

## **IV.D.2 Disposition (ADME) and Toxicokinetics**

### **IV.D.2.1 Presentation**

Dr. Jahnke presented an overview of the key information in the Disposition and Toxicokinetics section of the draft monograph. Following either inhalation or oral exposure, cumene is readily absorbed, rapidly metabolized, and excreted primarily in the urine as 2-phenyl-2-propanol glucuronide in rats, mice, and humans.

The most informative study was by Chen *et al.* (2011), who studied metabolism of <sup>14</sup>C-labeled cumene in mice and rats exposed orally and by intravenous injection and in mouse and rat microsomes *in vitro*. Cumene was metabolized by cytochrome P450 oxidation primarily of the alkyl side chain, but also of the benzene ring. Metabolism using mouse or rat lung or liver microsomes produced  $\alpha$ -methylstyrene and 2-phenyl-2-propanol in all cases. In both mice and rats *in vivo*, small amounts of  $\alpha$ -methylstyrene were found in expired air. Based on detection of an *N*-acetylcysteine derivative of 2-phenyl-2-propanol,  $\alpha$ -methylstyrene oxide was proposed as an intermediate metabolite in side-chain oxidation. Both  $\alpha$ -methylstyrene and  $\alpha$ -methylstyrene oxide have genotoxic properties. Based on detection of phenyl sulfate derivatives, arene oxides and the quinone methide were proposed as intermediate metabolites of ring oxidation; both of these can form reactive oxygen species.

In the one study in humans (Seńczuk and Litewka, 1976), cumene absorption by inhalation was directly proportional to the concentration of 2-phenyl-2-propanol in the blood and urine. In the one *in vitro* study of cytochrome P450 metabolism of cumene (Henne *et al.*, 2001), rat CYP2B1 formed 2-phenyl-2-propanol, and rabbit CYP4B1 formed 2-phenyl-1-propanol.

Dr. Nesnow noted that in the draft monograph, the urinary metabolites of cumene in humans should be identified as conjugates.

#### **IV.D.2.2 Peer Review Comments and Panel Discussion**

Dr. Burka, first reviewer, noted that, especially in earlier studies, it is important to consider the methods used to identify metabolites, and that care should be taken in interpreting the results of studies involving treatment with acid, derivatization, and analysis by gas chromatography-mass spectrometry (which can identify only sufficiently volatile compounds). He noted that in the study by Chen *et al.*, no structures were given for two of the metabolites, three metabolites were characterized by mass spectrometry only, and only retention times were given for three more; only seven compounds actually were identified.

Dr. Burka suggested adding one pertinent reference to the monograph (Thompson *et al.*, 1996), which demonstrated that if ring hydroxylation occurs, quinone methides (reactive intermediates) are readily formed, which cause oxidative damage (prevented by glutathione).

Dr. Burka asked what would happen if species-specific P450 metabolism of cumene in mouse lung were eventually demonstrated. Dr. Jahnke said any substance listed in the RoC could be nominated for review if new data become available. She noted that while speculation is valuable for generating hypotheses, the RoC evaluation process is restricted to the available data. Drs. Nesnow and Burka discussed whether the one paper on human metabolism of cumene indicated convincing differences in cumene metabolism between rodents and humans; Dr. Nesnow noted that this study found 2-phenyl-2-propanol to be the major metabolite in both humans and rodents.

Dr. Nesnow suggested some corrections and clarifications to the monograph regarding the metabolites of cumene. He also stated that the text should make it clear that the hydroxylated metabolites shown in the figure as coming from  $\alpha$ -methylstyrene oxide can also result from hydroxylation of primary hydroxylated metabolites.

Summarizing the Panel's comments, Dr. Anderson stated that the metabolism of cumene is complex and not fully elucidated, but there are clear similarities across species and a variety of ways in which reactive intermediate metabolites can be generated.

### **IV.D.3 Studies in Experimental Animals**

#### **IV.D.3.1 Presentation**

Dr. Jahnke noted that no epidemiological studies were identified that examined the relationship between human cancer and exposure specifically to cumene. She then presented an overview of the key information in the draft monograph section on Studies in Experimental Animals. The only chronic cancer studies identified that evaluated preneoplastic or neoplastic lesions were the NTP two-year inhalation studies in rats and mice of both sexes (NTP, 2009).

Significant neoplastic lesions in rats were adenoma of the nasal respiratory epithelium in both sexes, renal tubule adenoma and carcinoma (combined) in males, and interstitial-cell adenoma of the testes in males. Because these benign tumors of the nasal respiratory epithelium and the testes typically do not progress to malignancy, these tumor types do not meet the RoC criteria. Significant neoplastic lesions in mice were alveolar/bronchiolar adenoma, carcinoma, or adenoma and carcinoma combined in both sexes; hemangiosarcoma in the spleen and adenoma of the thyroid gland in males; and hepatocellular adenoma or adenoma and carcinoma combined in females. The lung tumors in both sexes and the liver tumors in females showed significant dose-related trends. Because the incidence of hemangiosarcoma was within the historical control range and because thyroid adenoma typically does not progress to malignancy, these tumor types were not considered to meet RoC criteria.

Therefore, it was concluded that there is sufficient evidence of carcinogenicity in experimental animals based on increased incidences of (1) combined benign and malignant kidney tumors in male rats; (2) benign, malignant, and combined benign and malignant lung tumors in mice of both sexes; and (3) benign or combined benign and malignant liver tumors in female mice.

Dr. Elwell asked the basis for the statement that nasal epithelial adenoma typically does not progress to malignancy. Dr. Jahnke said she had consulted with the NTP pathologist, who concluded that these tumors typically do not progress based on studies with six different chemicals in which only one malignant nasal epithelial tumor was found.

#### **IV.D.3.2 Peer Review Comments and Panel Discussion**

Dr. Elwell, first reviewer, agreed with the overview of the studies in experimental animals, the conduct of which was adequate for evaluation of carcinogenicity. He stated that most of his comments were editorial or related to clarity of the text. He suggested that the rationale for dose setting in the rat study be clarified in this section, in particular, the relevance of the alpha 2-microglobulin ( $\alpha_{2\mu}$ -globulin) findings to dose setting.

Dr. Elwell raised the issue of the relationship of  $\alpha_{2\mu}$ -globulin nephropathy to tumor formation. The study findings were consistent with all but three of the International Agency for Research on Cancer (IARC) criteria for  $\alpha_{2\mu}$ -globulin nephropathy as the sole mechanism of carcinogenicity: lack of genotoxicity, male specificity, and lack of evidence of sustained increased cell proliferation. He requested further clarification as to why these three criteria for  $\alpha_{2\mu}$ -globulin

nephropathy as the only mechanisms for renal neoplasia were considered questionable. Questions on the relationship of  $\alpha_2\mu$ -globulin nephropathy to tumor formation in male rats were deferred until discussion of the section on mechanistic data. Dr. Elwell agreed with the findings of significant increases in tumors and hyperplasia in the kidneys of rats, the lungs of mice, and the livers of female mice.

Dr. Anderson asked what weight should be given to the high incidence of neoplasms that typically do not progress. Dr. Elwell noted that the RoC criteria for sufficient evidence of carcinogenicity require a significant increase in malignant tumors or benign and malignant tumors combined. Nonetheless, the benign neoplasm response in the rat nasal cavity was remarkable. Dr. Gordon felt that the nasal cavity adenoma data lent credence to the other observations. Dr. Jahnke clarified that the adenoma data were not discounted, but were considered to be supportive of the call.

Dr. Gordon, second reviewer, agreed that strong evidence was presented for carcinogenicity in two species. He felt that the oral presentation was clearer than the presentation in the monograph, and mentioned issues affecting the clarity of the text, such as mixing discussion of rats and mice and of nonneoplastic and neoplastic effects. He felt that the monograph showed good objectivity. However, he suggested that the statistical analyses of trends should be discussed in the text, rather than just included in the tables. He also suggested that discussion of negative findings take into account high control values and raised the issue of how much weight should be given to historical controls, noting that the discussion in the monograph appeared to give more weight to historical controls than to current controls. Dr. Bucher clarified that comparison with the current control is most important, followed by comparison with historical controls. Dr. Selgrade noted that because the inhalation exposures were whole-body exposures, some oral and dermal exposure could also have occurred.

In summary, Dr. Anderson stated that the Panel largely accepted the results of the studies in experimental animals, with some issues about presentation and clarification of the statistics, and with some interpretation issues to be addressed in discussion of the mechanistic data.

#### **IV.D.3.3 Action**

Dr. Lunn noted that the vote on whether to accept the evidence of carcinogenicity from the experimental animal studies was separate from the vote on whether to accept the preliminary listing recommendation, and that the latter vote would take into account the mechanistic data as well as the evidence of carcinogenicity in animals. She reviewed the criteria for sufficient evidence of carcinogenicity in experimental animals.

The Panel agreed unanimously (8 yes, 0 no, 0 abstentions) that the scientific information presented from studies in experimental animals supports the NTP's level of evidence conclusion of *sufficient evidence of carcinogenicity*.

#### **IV.D.4 Mechanistic Data and Other Relevant Effects**

##### **IV.D.4.1 Presentation**

Dr. Jahnke presented an overview of the key information in the draft monograph section on Mechanistic Data and Other Relevant Effects. *In vitro* mutagenicity studies gave negative

results in bacteria, yeast, and mammalian cells. Limitations of studies in bacteria included the volatility of cumene and the use of solvents, such as alcohol and dimethylsulfoxide, that can act as competitive inhibitors of CYP2E1. Studies in yeast and mammalian cells were limited by incomplete reporting of methods and results. *In vitro* assays for chromosomal aberrations, cell transformation, and unscheduled DNA synthesis gave conflicting results. Limitations of these studies included nonreproducibility, incomplete reporting of methods, and high background cytotoxicity. *In vivo* studies of micronucleus formation gave negative results in mice and conflicting results in rats. Comet assays for DNA damage gave positive dose-related results in the livers of male rats and the lungs of female mice. There is some evidence that the cumene metabolite  $\alpha$ -methylstyrene is genotoxic, and  $\alpha$ -methylstyrene oxide has been shown to be mutagenic.

Mouse lung tumors induced by cumene exposure exhibit mutations in *K-ras* and *p53*, altered gene expression, loss of heterozygosity, and histone modifications. These changes differ from what is found in spontaneous tumors, and the mutation spectra and expression profiles are similar to those of human cancers. Because lung tumors have been observed in mice, but not in rats, it has been suggested that mouse lung tumors occur via a species-specific mechanism involving formation of cytotoxic metabolites via CYP2F2 metabolism in Clara cells. The relevance of this mechanism to human cancer has been questioned because human lungs have lower levels of the human ortholog CYP2F1 and fewer Clara cells than do mouse lungs. However, lung cytotoxicity was not observed in the NTP carcinogenicity studies. In the absence of cancer studies of this mode of action with mouse lung tumors as an endpoint and the absence of data on the metabolism of cumene by CYP2F2, no data are available that would discount the relevance of mouse lung tumors to humans. With respect to mouse liver tumors, no data are available that would discount their relevance to humans.

To answer the question of whether kidney tumors in male rats are caused exclusively by  $\alpha_{2\mu}$ -globulin nephropathy (a species- and sex-specific mode of action), a group of NTP scientists with specific expertise in nephropathy independently evaluated data from the NTP 90-day and two-year studies according to IARC and U.S. Environmental Protection Agency (EPA) criteria for  $\alpha_{2\mu}$ -globulin nephropathy as the sole mechanism of carcinogenicity. It was concluded that  $\alpha_{2\mu}$ -globulin nephropathy was present, but the possibility of an additional mechanism of carcinogenicity could not be excluded, because (1) there was weak evidence for the genotoxicity of cumene and its metabolite  $\alpha$ -methylstyrene, (2) nephropathy was observed in female rats, and (3) evidence for sustained cell proliferation was weak, with no significant increase in the labeling index. Dr. Jahnke showed slides of the tables from the NTP Technical Report summarizing the data for renal toxicity and nonneoplastic effects in male and female rats.

Dr. Selgrade asked whether the study data met the EPA criteria for  $\alpha_{2\mu}$ -globulin nephropathy. Dr. Jahnke said EPA's criteria were more of a description of the pathogenesis over time, and that the NTP data did not provide a detailed enough timeline for application of the criteria. Dr. Elwell asked if proliferating cell nuclear antigen (PCNA) staining was performed on 24-hour-fixed kidneys or retrospectively on formalin-fixed kidneys, and whether that could affect the sensitivity of the assay. He said the strength of the observed regenerative response in the kidney suggested some evidence for sustained cell proliferation. Dr. Anderson noted that the

public comments on lung tumors emphasized cytotoxicity in Clara cells caused by styrene. Dr. Jahnke confirmed that the NTP study of cumene showed no evidence of cytotoxicity in the lungs.

#### IV.D.4.2 Peer Review Comments and Panel Discussion

Dr. Nesnow, first reviewer, commented that the *in vitro* studies on genetic effects were clearly described and the strengths and weaknesses of each study clearly documented, particularly in Appendix D. As a minor correction, he suggested that the statement on page 39 concerning a limitation of the BALB/3T3 cell transformation study be changed to specify that an “exogenous” source of metabolic activation was not used, because these cells do contain limited cytochrome P450 activity and can be transformed by polycyclic aromatic hydrocarbons. Dr. Nesnow stressed the importance of the use of Aroclor-induced rat liver S9 for metabolic activation as a potential reason for negative results in the *in vitro* assays; rat liver S9 contains very low amounts of CYP2E1, which is known to be important in the metabolism of other small alkyl aromatics.

Dr. Nesnow emphasized the greater importance of the *in vivo* studies, in which cumene induced DNA damage in male rat liver and female mouse lung. He considered the critical study to be that of Hong *et al.* (2008), that sequenced the mutations in lung tumors from cumene-exposed and control mice. Tumors from cumene-exposed mice showed a clear increase in the numbers of tumors carrying mutations in the *ras* oncogene and the *p53* tumor-suppressor gene and a shift in the mutation spectra, which indicated that the mutations were truly induced. Furthermore, many of the specific transitions and transversions are also seen in human tumors. Thus, genetic alterations that could be related to human tumors have been observed *in vivo* both in normal tissues and in tumors.

Dr. Nesnow suggested a number of improvements that could be made to this section of the monograph. In the discussion of the study by Wakamatsu *et al.* (2008), he suggested adding information on the sequences of the mutations; one of the major sequence changes was a G to A change that is seen in both types of human lung cancer. Some of the *ras*-positive and *ras*-negative tumors also had *p53* mutations, but there were only two *ras*-negative tumor samples, which is an insufficient number for microarray studies. This limitation of the Wakamatsu study should be mentioned in the monograph and taken into account in the description of the study. In Figure 5-1, the mutations should be clearly identified by codon. He also suggested emphasizing throughout the monograph (and in the substance profile) the dose-related increases in the frequencies of mouse lung *K-ras* and *p53* mutations (in addition to the changes in the mutation profiles); these findings strengthen the argument for a genotoxic mode of action.

Dr. Nesnow noted that characterization of the evidence for genotoxicity (currently as “suggestive,” “equivocal,” or “some”) should be consistent throughout the monograph. The monograph should clearly state whether the *in vivo* data indicate a “real” effect. He expressed the opinion that the genotoxic effect was real and noted that the monograph came across as unsure of how to deal with the issue, given that cumene is not a “classic” genotoxic carcinogen. At the same time, it acknowledged that there could be multiple mechanisms of carcinogenicity, and that genotoxicity could be part of a larger mode of action. He suggested stating that cumene was genotoxic in some, but not all, tissues.

Other specific suggestions included changing the title of Section 5.2.3 to “Disposition and species-specific metabolism leading to tumors” (not “cytotoxic metabolites”), because no lung cytotoxicity was observed. Other references to “cytotoxic metabolites” also should be changed. The increase in S-phase renal proximal tubule cells in male rats occurred not at the two highest doses, but at the lowest and highest doses. Dr. Nesnow suggested the statement on page 45 concerning the potential role of methylation should be stricken, because it was based on a statement by Wakamatsu *et al.* for which no data were shown and the method was not identified.

Dr. Gordon asked Dr. Nesnow how he would address the public comment by Dow Chemical Co. that the increased incidences of K-ras and p53 mutations in mouse lung tumors sampled at terminal sacrifice were not necessarily due to a tumor process occurring earlier in the exposure period. Dr. Nesnow said the idea that these mutations were late forming was not supported by data from studies on tumor formation in mice following a single intraperitoneal injection; however, those data were based on studies with known genotoxic chemicals.

Dr. Nesnow noted that in calling the overall evidence for genotoxicity of cumene “equivocal,” the comment from Dow Chemical Co. was quoting the monograph. He argued that the monograph should not use the term “equivocal” to describe the overall evidence for genotoxicity. The *in vitro* test results were equivocal, and possibly the rat micronucleus results, but the other results were straightforward and not equivocal at all. Although numerous potential reactive metabolites of cumene were identified, and cumene hydroperoxide is used as a standard radical-initiating chemical, there were no hard data on the role of reactive metabolites. However, there were hard data on tumor mutations and from the comet assay.

Clarifying at Dr. Anderson’s request, Dr. Nesnow emphasized that the mutation profiles in tumors induced by cumene were similar not only to those observed following single exposures to known genotoxic chemicals, but also to those observed in human tumors. Therefore, a genotoxic mode of action for cumene could not be ruled out. Dr. Anderson wondered whether cumene might act both as an initiator and a promoter.

Dr. Paul White questioned the idea that the *in vitro* *Salmonella* mutation assays were limited by failure to use the chamber vaporization technique; based on its vapor pressure and boiling point, he did not consider cumene to be volatile. He suggested that the negative results were more likely due to the use of Aroclor-induced rat liver metabolic activation. Dr. Nesnow noted that the assays in sealed tubes also gave negative results.

Dr. Lash, second reviewer, noted that in hazard identification (as opposed to risk assessment), the issue is to demonstrate whether a mechanism is feasible or potentially occurs, not its extent. Given the good evidence for genotoxicity of some metabolites of cumene, cumene must be considered to be genotoxic. With respect to the issue of  $\alpha_{2\mu}$ -globulin nephropathy, Dr. Lash said that the discussion reminded him of the risk assessment for perchloroethylene (PERC), where  $\alpha_{2\mu}$ -globulin nephropathy clearly occurred, but there was evidence for other mechanisms as well. Based on the evidence of nephropathy in female rats and evidence of potential mechanisms for renal effects other than  $\alpha_{2\mu}$ -globulin nephropathy, he agreed with the conclusion that other mechanisms relevant to humans could not be excluded. He considered the mutation spectra discussions compelling and also noted that the dose response data were reminiscent of

what was seen with PERC, where tumor incidences were higher at medium doses than at high doses because of cell death at high doses. Dr. Lash thought the arguments presented in the monograph for not excluding the applicability of the animal findings to humans were reasonable (although some points could have been made more clearly), and that the criticisms made in the public comments, therefore, were not fair.

Dr. Anderson summarized the discussion as concluding that cumene could be carcinogenic by several different mechanisms; that there was fairly good evidence for genotoxicity, despite the negative results of *in vitro* assays, where metabolic activation probably was not adequate; and that there may or may not be several mechanisms for effects in the kidney.

Dr. Elwell asked whether the increased nephropathy in female rats at two years was looked at in relation to survival. He noted that survival was about 20 - 30% higher in the high dose group than in the controls, and wondered whether the increased nephropathy observed in the high dose females was due to their longer survival. Dr. Jahnke said she would look into the question.

#### **IV.D.5 Overall Cancer Evaluation**

##### **IV.D.5.1 Presentation**

Dr. Jahnke presented an overview of the Overall Cancer Evaluation. The evaluation concluded that there was sufficient evidence of cancer in experimental animals, based on observation of benign and malignant tumors in two species of rodent at multiple tissue sites:

- Lung tumors in male and female mice.
- Liver tumors in female mice.
- Kidney tumors in male rats.

No compelling evidence was identified to rule out the relevance of these tumors to humans. There was some evidence that cumene may cause DNA damage, and lung tumors from mice exposed to cumene showed molecular alterations similar to those found in human lung and other cancers. Therefore, the following preliminary listing recommendation was made:

Cumene is *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals.

##### **IV.D.5.2 Peer Review Comments and Panel Discussion**

Dr. Elwell, first reviewer, commented that the discussion of the genotoxicity evidence had changed his opinion on those results, and that he considered the evidence for genotoxicity to be positive, rather than equivocal, based on the relevant tests. He indicated that genotoxicity maybe the one exception to the listed criteria for  $\alpha_2\mu$ -globulin nephropathy required for this to be the sole mechanism for the rat kidney tumors. He agreed in principle with the preliminary listing recommendation, but noted that a question remained around the  $\alpha_2\mu$ -globulin nephropathy effect.

Dr. Nesnow, second reviewer, agreed with Dr. Elwell's remarks and made several specific suggestions. On page 55, the reference to "cytotoxic metabolites" should be changed to "reactive metabolites." He felt that the section contained nice summaries of the mechanistic

considerations for kidney and lung tumors, but should also include a summary for the liver tumors. He suggested that the following specific points be discussed: (1)  $\alpha$ -methylstyrene (4%) was detected in the expired air of female mice; (2) incubation of cumene with female mouse liver microsomes yields a small amount of  $\alpha$ -methylstyrene; (3)  $\alpha$ -methylstyrene has been shown to be converted to dihydrodiol, presumably through the oxide; (4) counter to that, the urinary levels of the  $\alpha$ -methylstyrene oxide mercapturic acid conjugate were undetectable, consistent with low levels of  $\alpha$ -methylstyrene oxide; (5)  $\alpha$ -methylstyrene induces liver tumors in female mice in inhalation studies; and (6) in conclusion, these data suggest a possible role for  $\alpha$ -methylstyrene in the induction of liver cancer in female mice by cumene, but the extent to which  $\alpha$ -methylstyrene oxide contributes is unknown.

Other suggested changes were to add the point about increased mutation frequencies in lung tumors, as well as the spectral changes and, in the preliminary listing recommendation, to state that “there is evidence that cumene causes DNA damage in some tissues.”

Dr. Selgrade suggested that the statement that no convincing evidence was identified to rule out the relevance of tumors to humans should be modified to concede the possibility that the kidney tumors were due to  $\alpha_{2\mu}$ -globulin nephropathy.

#### **IV.D.5.3 Action**

Dr. Anderson asked the Panel to vote on the preliminary policy decision that cumene should be listed in the RoC as a *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals, which includes the basic tumor endpoint results and consideration of the mechanisms and how they might translate from the animal to the human in terms of their fundamental biology. Dr. Gordon moved to approve the decision and Dr. Nesnow seconded the motion. The Panel agreed unanimously (8 yes, 0 no, 0 abstentions) with the NTP’s preliminary policy decision to list cumene in the RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals.

Dr. Lunn asked the panel for clarification as to which tumor sites met the criteria for the finding of sufficient evidence and which tumor sites, though not meeting the criteria, constituted supporting evidence.

With respect to the rat kidney tumors, Dr. Anderson noted that the panel was in agreement that cumene was genotoxic, but it was unknown whether it was genotoxic in the kidney. The Panel was also in agreement that cumene caused kidney tumors in male rats; the question was whether the mechanism was relevant to humans. She proposed that the panel vote on the latter question. Dr. Lunn reminded the panel that the RoC criterion was not whether the kidney tumors were relevant to humans, but whether there was compelling evidence that they were *not* relevant. She noted also that the criterion for potential relevance to humans was not whether the IARC criteria were met, but rather the panelists’ scientific judgment based on all of the evidence.

Dr. Nesnow noted that the EPA criteria for  $\alpha_{2\mu}$ -globulin nephropathy as the sole mechanism of renal carcinogenicity include negative results for genotoxicity in “a battery of tests,” not necessarily in the kidney; a substance that is highly active in some tissues is likely to be active

in other tissues. Because cumene is genotoxic in liver and in lung, this criterion is not met. The EPA criteria also include increased and sustained cell proliferation in the P2 segment of the proximal renal tubules. This criterion also was not met in the NTP study. Dr. Elwell stated that mild to moderate regeneration in the kidney was suggestive of sustained cell proliferation at this site, and that a more sensitive method might have detected cell proliferation. Dr. Bucher commented that if the evidence for sustained cell proliferation in males and against an effect in females is accepted, then the issue remains as to whether cumene is genotoxic. Dr. Sanderson questioned the significance of the kidney tumor incidence data. Dr. Elwell stated that regardless of mechanism ( $\alpha_{2\mu}$ -globulin nephropathy as potentially the sole mechanism of carcinogenicity) the results were consistent with clear evidence of a tumor effect in rats.

Dr. Lunn asked that the panel vote on whether the sufficient evidence supporting the listing as *reasonably anticipated to be a human carcinogen* was based on two or three tumor sites (i.e., lung and liver vs. lung, liver, and kidney), and noted again that the criterion for not including a tumor site was whether there was compelling evidence that the mechanism would not occur in humans. Dr. Gordon moved that the listing be based on sufficient evidence of carcinogenicity from studies in experimental animals based on lung tumors in male and female mice, liver tumors in female mice, and renal tumors in male rats. Dr. Lash seconded the motion. The Panel disagreed (4 yes, 5 no, 0 abstentions; chair broke the tie) that *sufficient evidence of carcinogenicity* from studies in experimental animals is based on lung tumors in male and female mice, liver tumors in female mice, and renal tumors in male rats.

The panel members who voted against the motion stated their reasons. Dr. Anderson said there were too many unsettled issues concerning the renal tumors, including the question of genotoxicity in the kidney and whether activation by a particular cytochrome P450 is required. Dr. Elwell cited uncertainty about the role of  $\alpha_{2\mu}$ -globulin in producing all of the kidney tumor effect. Drs. Selgrade, Gordon, and Sanderson agreed with Dr. Elwell. Dr. Sanderson stated that the data on renal tumors were not compelling enough and not statistically strong.

Dr. Anderson asked for a new motion on which tumor sites should be included as the basis for sufficient evidence from animal studies. Dr. Selgrade moved that the listing be based on sufficient evidence of carcinogenicity from studies in experimental animals based on lung tumors in male and female mice and liver tumors in female mice. Dr. Gordon seconded the motion. The Panel recommended (7 yes, 1 no, 0 abstentions) that there is *sufficient evidence of carcinogenicity* from studies in experimental animals based on lung tumors in male and female mice and liver tumors in female mice. Dr. Lash voted no, stating that the renal tumors in male rats could not be entirely explained by  $\alpha_{2\mu}$ -globulin nephropathy, and that there was therefore no compelling evidence that cumene could not cause kidney cancer in humans.

Dr. Gordon moved that renal tumors in rats and benign neoplastic lesions, particularly those of the nasal epithelium, be cited as additional evidence supporting the listing. Dr. Sanderson seconded the motion. The Panel recommended unanimously (8 yes, 0 no, 0 abstentions) that renal tumors in male rats and benign nasal tumors in male and female rats provide supporting evidence.

#### **IV.E. Draft RoC Substance Profile**

Dr. Jahnke summarized the contents of the draft substance profile as containing NTP's preliminary listing status recommendation, summarizing the scientific information key to reaching a recommendation, and providing information on properties, use, production, exposure, and existing federal regulations and guidelines.

Dr. Sanderson, first reviewer, stated that the draft profile supported a strong argument that a large proportion of the U.S. population is exposed to cumene, and that some exposure levels are well above normal environmental levels; exposure therefore qualifies as "significant."

Dr. Elwell, second reviewer, stated that with the revisions suggested by the Panel, the profile's discussion of carcinogenicity studies in experimental animals was clear, technically correct, and objectively stated. He noted that the kidney tumors would be moved to the paragraph on supporting evidence, and that he considered the nasal tumors to provide stronger supportive evidence than the kidney tumors.

Dr. Nesnow, third reviewer, stated that his earlier comments on the draft monograph should carry over to the draft profile, including (1) identifying the human urinary metabolites as conjugates, (2) adding a paragraph summarizing the mechanistic data for liver cancer, (3) adding the information that lung cancer could not have been caused by  $\alpha$ -methylstyrene, (4) correcting "cytotoxic" intermediates to "reactive" intermediates, and (5) adding the references for the data.

In summary, Dr. Anderson stated that the Panel was happy with the draft substance profile if modified as discussed.

#### **V. Closing Comments and Adjournment**

Dr. Jahnke acknowledged and thanked those who contributed to putting together the draft monograph and organizing the meeting. The meeting was adjourned at 5:00 p.m.

#### **VI. Call to Order and Introductions – Day 2**

The meeting was reconvened on March 22, 2013. Dr. Anderson called the meeting to order at 8:32 a.m. and asked the attendees to introduce themselves. Dr. Lori White stated the conflict of interest guidelines.

#### **VII. Draft RoC Monograph for 1-Bromopropane (1-BP)**

##### **VII.A. Peer Review Comments on the Process for Preparation of the Draft RoC Monograph for 1-BP**

Dr. Selgrade, first reviewer, had mainly editorial comments, which she provided in written form. Again, she noted that the diagram of the literature review process in Appendix A could be improved. She also wondered why the key scientific questions did not include a question about the role of inflammation, given that inflammation is discussed in the mechanisms section of the monograph and can play an important role in the development of cancer. Dr. Lunn noted that

the key questions came from the concept document and did not reflect how the questions had expanded during development of the monograph; this approach can be reevaluated. Dr. Lash, second reviewer, had comments similar to those on the process for preparation of the draft RoC monograph for cumene, in particular, the inclusion of the draft substance profile as Part II of the monograph and the need for clarification of Appendix C.

## VII.B. Oral Public Comments

Dr. Adam Finkel, of the University of Pennsylvania Law School, made a public comment by telephone on his own behalf, and not on behalf of his employer. Dr. Finkel nominated 1-BP for testing nearly 14 years ago when he was Director of Health Standards Programs for OSHA. OSHA had just spent 10 years regulating methylene chloride and had dealt with many purported tradeoffs between health risks and the consequences of not using this substance. He became concerned when, shortly thereafter, it came to OSHA's notice that 1-BP was being imported into the United States as a substitute for methylene chloride. The NTP completed testing of 1-BP four or five years ago, and he encouraged the NTP to complete the evaluation process.

Dr. Finkel noted that this review was a hazard identification proceeding, and that questions of risk, potency, and exposure were secondary and should not divert the Panel from the task at hand. He said that he would nonetheless address some issues of risk, potency, and exposure, because other commenters were invoking them incorrectly. Dr. Finkel commented that the NTP had conducted a "low-dose" bioassay, in that the lowest carcinogenic dose in the study was in fact comparable to or lower than the airborne levels at which many workers currently are exposed. Therefore, questions of extrapolation, of thresholds, and of mechanisms that operate only at very high doses do not apply.

Dr. Finkel said 1-BP has been clearly shown to be an animal carcinogen and should at least be classified as *reasonably anticipated to be a human carcinogen*. The NTP listing criteria are clear and require this outcome; they require only the finding of cancer at multiple sites or in multiple species, and this case there were both. The occupational exposures make it urgent that, after 14 years, a determination be made expeditiously.

Dr. Finkel stated that the RoC review panels would have to come to grips with the upgrading of animal carcinogens to human carcinogens, just as they have considered the downgrading of animal carcinogens if there is compelling evidence to the contrary. The NTP bioassay showed 1-BP to be a powerful immunosuppressant in rodents. Immunosuppression is a mechanism that can be very relevant to animal and human cancer, both by reducing immune surveillance and by causing proliferation of B cells. Dr. Finkel cited a new paper (Strauss and Heiger-Bernays, 2012) that suggested that the traditional bioassay might be inefficient at detecting some immune system and hematopoietic tumors.

Dr. Finkel stated that the only written public comments submitted on 1-BP were diversionary and should be ignored. He was confident that the Panel understood the role of the ancillary data on mechanisms; the listing criteria that allow positive results for carcinogenicity in animals to be ignored are clear that such data must be "compelling" and sweeping, in the sense that they must be qualitative. Therefore, arguments that the results were weaker quantitatively in humans than in animals, or that the substance may be safe at low doses but not at high doses,

or that mechanisms may not operate in some human subpopulations, should not be entertained, even if they had merit.

He said, in this case, it is clear that nothing even vaguely resembling a compelling counterargument has been offered. The written comment from Albemarle Corp. discusses nonmutagenicity; this argument is incorrect for at least five reasons: (1) 1-BP is mutagenic in assays in closed systems, (2) there are reasonably anticipated and known human carcinogens that have not been demonstrated as mutagenic in standard assays, (3) there is no logical connection between nonmutagenicity and the existence of a threshold, (4) even if there were compelling information that there was a threshold, it would have to be at the population level, not the individual level, and (5) the listing criteria are oblivious to the possibility of a threshold, because this is a hazard identification determination.

Dr. Finkel found it troubling that anyone would posit the existence of a threshold in light of the actual bioassay exposure levels and the actual worker exposure levels. Many workplace exposures have been and continue to be well above 62.5 ppm and 100 ppm. Efforts are being made to reduce exposure levels, and some manufacturers have recommended fairly low limits. The TLV is now 10 ppm, and it has been proposed that it be reduced to 0.1 ppm, but a major manufacturer, Enviro Tech International, is still recommending 25 ppm. Dr. Finkel found it hard to believe that anyone would be talking about a threshold when an 8-fold excess of lung tumors was seen at 62.5 ppm, and workers clearly are exposed to that level and higher levels today.

Dr. Finkel quoted from Rozman and Doull (2002) regarding structure-activity relationships of the brominated low-molecular-weight alkanes, “it can be expected with confidence that if a carcinogenicity bioassay were to be conducted with *n*-propyl bromide [1-BP] at levels used in the ethyl bromide bioassay, the outcome would be negative.” When the NTP tested ethyl bromide in 1989, the lowest exposure level at which tumors were significantly increased was 400 ppm; at roughly one-tenth that level, 1-BP produced an 8-fold excess of tumors.

### **VII.C. Scientific Issues in Written Public Comments**

Dr. Wolfe said the NTP had received two written public comments on behalf of Albemarle Corporation, from Dr. Carr Smith and Tina Craft. These were provided to the Panel to be carefully considered in their review and were posted to the meeting page. The following major scientific issues raised in the public comments were identified:

- 1-BP is not a direct-acting mutagen.
- The tumor response observed in the NTP mouse and rat inhalation study can be assumed to possess a threshold under which 1-BP would not be expected to be carcinogenic.

Dr. Wolfe encouraged the Panel to address these issues in their discussion.

## **VII.D. Draft Cancer Evaluation Component**

### **VII.D.1 Properties and Human Exposure**

#### **VII.D.1.1 Presentation**

Ms. Diane Spencer presented an overview of the key information in the Properties and Human Exposure section of the draft monograph. 1-BP is used as a solvent in several industrial processes, including as a degreaser for electronics and metal, as a vehicle for aerosolized adhesives (e.g., in foam cushion manufacturing), in dry cleaning, as a spot remover in the textile industry, and as an intermediate. It was selected as a candidate substance for the RoC based on the potential for substantial human exposure and the existence of an adequate database for evaluation of its potential carcinogenicity. It is a high-production-volume chemical. Occupational exposure has increased in recent years because of its increased use in open processes and its use as an alternative to likely carcinogens and ozone-depleting chemicals. The primary route of human exposure is inhalation, though dermal exposure also is possible. Metabolites of 1-BP have been detected in urine of exposed workers at levels proportionate to the levels of 1-BP in ambient air. The highest occupational exposures occur among factory workers who use spray adhesives, but many exposures in other industries exceed the ACGIH TLV of 10 ppm. Little information is available on environmental exposure. Based on the occupational exposure data and production data, it was concluded that a significant number of U.S. residents are exposed to 1-BP.

Dr. Anderson asked what the rationale was for the ACGIH TLV of 10 ppm. Cdr. Kevin Hanley said it was based on neurological and reproductive developmental concerns from animal studies and some case studies of neuropathy in exposed workers. Dr. Sanderson said some data on neurological effects in humans were used.

#### **VII.D.1.2 Peer Review Comments and Panel Discussion**

Dr. Sanderson, first reviewer, stated that the information provided in this section of the monograph was quite clear and accurate, well written, and consistent with information that has been provided in other sources. He suggested adding a citation for the ACGIH TLV of 10 ppm; however, the recommendation to change the TLV to 0.1 ppm cannot yet be cited. He commented that the information on production and human exposure was quite clear and accurate. He noted that while 1-BP is not ubiquitous in the environment, it is a high-impact chemical used in a variety of industries, sometimes in closed systems, where exposures are fairly low, but sometimes in open systems, where exposures are quite high. Even where exposures are controlled, they can be above the 10-ppm ACGIH TLV. Currently, there is no OSHA PEL or NIOSH REL, but both agencies have published sampling and analytical techniques for 1-BP, which should be cited. OSHA also has in its Integrated Management Information System database about 90 samples for 1-BP documented across various industries, which could be added to the monograph.

Dr. Sanderson noted that the monograph contains no mention of the numbers of workers exposed, and that he could not find any estimates himself. He thought it was reasonable to

believe that a sizeable number of workers are exposed, probably in the few thousands range, and that because the use of 1-BP is increasing, the number could be expected to increase.

Dr. Sanderson found Figures 1-2 and 1-3 confusing and recommended that the figures be removed and the exposure data be presented in tabular format. He noted that the reports cited provide the raw data. Information could be reported by industry or workers within industries and include the number of samples, arithmetic mean and standard deviation, geometric mean and standard deviation, range, and exceedance fractions, which could be used to predict probabilities of exposures above certain levels.

His main concern was that both arithmetic and geometric means are presented and are hard to differentiate; tabular presentation would help in that regard. Dr. Sanderson noted that Figure 1-3 was intended to show changes within the same industry (in primary and follow-up NIOSH evaluations), but no statistical analysis was presented. If the data were in tabular format, reviewers could do their own analyses of whether exposure had changed over time, and could compare exposures across industries. Dr. Lunn noted that the data are presented in tabular format separately for each industry in Appendix B, and that the figure was intended to compare exposure across industries. Dr. Sanderson suggested that Appendix B could be improved by addition of the data suggested above.

Cdr. Hanley noted that in 2007, EPA, under the Significant New Alternatives Policy Program, published a final rule to accept the use of 1-BP in vapor degreasing and immersion cleaning operations, but proposed not to accept its use in more emissive applications, such as spray adhesives and aerosols. He believed that many of the adhesive manufacturers selling to the foam fabricating industries had recommended searching for replacements for 1-BP; therefore, exposure may have decreased for this sector of workers in the last several years. 1-BP solvents are largely used in vapor degreasing, particularly in aerospace industries. 1-BP has also been marketed to replace PERC in dry cleaning, where it can be used more or less as a drop-in replacement. Cdr. Hanley noted that if the use of 1-BP in dry cleaning increases, the possibility of commercial and residential exposures (beyond occupational exposures) will increase.

The Panel agreed that a significant number of persons in the U.S. are exposed to 1-BP.

## **VII.D.2 Disposition and Toxicokinetics**

### **VII.D.2.1 Presentation**

Ms. Spencer presented an overview of the key information in the Disposition and Toxicokinetics section of the draft monograph. 1-BP can be metabolized in two ways: (1) via CYP-catalyzed oxidation (primarily by CYP2E1), which leads to 1-bromo-2-propanol, which can be further metabolized to several reactive metabolites, and (2) via glutathione conjugation, which leads to *N*-acetyl-*S*-propylcysteine, which can also be further metabolized. The available data suggest that some of the metabolic pathways for 1-BP may be similar in humans and rodents. The 16 urinary metabolites identified in rodents include the four urinary metabolites that have been identified in studies of biomarkers in exposed workers. CYP2E1 is expressed in human lung and other tissues; however, no studies in humans have adequately tested for oxidative metabolites or likely intermediates. Dr. Nesnow asked why propylene, which was inferred as an intermediate by Jones and Walsh, was not shown in the figure presented. Ms. Spencer clarified

that the first figure showed only the metabolic pathways identified by Garner *et al.* following inhalation exposure of rats and mice.

### VII.D.2.2 Peer Review Comments and Panel Discussion

Dr. Burka, first reviewer, thought that this section of the monograph was well done. He cautioned that postulated metabolites that have not been rigorously characterized (such as propylene) should be shown in brackets. He also noted that the half-life for clearance of 1-BP from plasma is only about 8 to 10 minutes, and it is gone in about 45 minutes, leaving not much time for metabolism. However in response to a question he said the half-life of 1-BP would allow for several cycles of metabolism. With respect to the Jones and Walsh (1979) studies, he noted that Udenfriend's reagent (a mixture of iron, citric acid, EDTA, and oxygen) was used to oxidize 1-BP, which he characterized as a chemical reaction and not an *in vitro* metabolism study.

Dr. Lash noted that one of the metabolites is a sulfoxide; he thought it should be mentioned that sulfoxides could be quite reactive. Dr. Burka noted that the reactive sulfoxides are an example of what could be missed by methods such as those used by Jones and Walsh; he cautioned that the older metabolic studies might be showing less than is really there. Dr. Gordon wondered whether this section of the monograph should discuss the rapid metabolism of 1-BP with respect to mechanisms of carcinogenicity.

### VII.D.3 Studies in Experimental Animals

#### VII.D.3.1 Presentation

Ms. Spencer noted that no epidemiological studies were identified that examined the relationship between human cancer and exposure specifically to 1-BP. She presented an overview of the key information in the draft monograph section on Studies in Experimental Animals. The only chronic studies identified that evaluated preneoplastic or neoplastic endpoints were the NTP two-year inhalation studies in rats and mice of both sexes (NTP, 2011a).

Significant neoplastic lesions in rats included benign and malignant skin tumors in male rats; these included keratoacanthoma (which can progress to squamous-cell carcinoma), squamous-cell carcinoma, and these two tumor types combined with basal-cell adenoma and carcinoma. The incidence of adenoma of the large intestine (a rare tumor, which can progress to carcinoma) was significantly increased in female rats, but not in male rats, and the incidence exceeded the historical control range for both sexes. Significant neoplastic lesions in mice were alveolar/bronchiolar adenoma, carcinoma, and adenoma and carcinoma combined in females. The incidences of adenoma and of adenoma and carcinoma combined showed significant dose-related trends. Other neoplastic lesions that may have been exposure-related were (1) malignant mesothelioma of tunica vaginalis of epididymis in male rats, which was significantly increased at the high dose and showed a significant dose-related trend; (2) pancreatic islet-cell adenoma and adenoma and carcinoma combined in male rats, which were significantly increased but within the historical control range and not dose-related; and (3) skin tumors in

female rats, for which there were no significant pairwise differences between exposed and control groups, but which showed a significant dose-related trend for all tumor types combined.

Therefore, it was concluded that there is *sufficient evidence of carcinogenicity* in experimental animals based on increased incidences of benign and/or malignant skin tumors in male rats, benign and malignant lung tumors in female mice, and benign large-intestine tumors (a rare tumor) in rats of both sexes.

Dr. Anderson asked whether there were multiplicity data for these tumor types; it might be useful to add this information for the large-intestine tumors, as it would strengthen the case based on the occurrence of a rare tumor. After checking the NTP technical report, Dr. Lunn said no statistics on tumor multiplicity were reported. Dr. Elwell asked whether there was information on the locations of the skin tumors, and Dr. Gordon asked whether there was information on time to tumor; Dr. David Malarkey said that this information was available and could be added to the monograph.

Dr. Gordon asked why the “other” neoplastic lesions (mesothelioma of the tunica vaginalis, pancreatic islet-cell tumors, and skin tumors in female rats) were placed in a lower category of evidence for carcinogenicity. He wondered in particular about the pancreatic islet-cell tumors, as their incidence was significantly increased relative to current controls. Dr. Anderson asked whether the lower incidence of pancreatic tumors at the high dose could be related to an effect on body weight. Dr. Lunn said there were no body weight effects. Dr. Bucher noted that body weight has not been associated with pancreatic islet-cell tumor incidence, and that these tumors have only rarely been considered to be exposure-related; therefore, they have been considered to be evidence of carcinogenicity only if they are clearly dose-related.

#### **VII.D.3.2 Peer Review Comments and Panel Discussion**

Dr. Gordon, first reviewer, commented that this section of the monograph was very clear, technically correct, and presented objectively. He suggested adding a discussion of gender effects either to this section or to the section on mechanisms. He felt that the evidence for carcinogenicity in animals was clear-cut, and noted that Dr. Finkel had been prescient in nominating this substance.

Dr. Elwell, second reviewer, said his comments might be considered editorial, but he felt they were needed for clarity. In the second paragraph of page 29, “gastrointestinal” should be changed to “intestinal,” as the stomach was not involved, and the conclusion that colon tumors are “thus a concern for human cancer” should be omitted, as this section of the document concerns carcinogenicity in experimental animals, not human risk assessment.

Dr. Lash suggested that in the discussion of trends, the statistical analysis should be discussed in the text (not just shown in the tables), and that characterization of results as positive or negative should be consistent with the results of statistical analyses.

Dr. Anderson summarized the general sense of the Panel as being in agreement with the general conclusions from the studies in experimental animals.

### VII.D.3.3 Action

Dr. Anderson asked for a vote on the question of whether there was *sufficient evidence of carcinogenicity* of 1-BP from studies in experimental animals. Dr. Gordon so moved and Dr. Elwell seconded the motion. The Panel agreed unanimously (8 yes, 0 no, 0 abstentions) that the scientific information presented from studies in experimental animals supports the NTP's level of evidence conclusion of *sufficient evidence of carcinogenicity* based on skin tumors in male rats, large intestine tumors in female and male rats, and lung tumors in female mice. The Panel supported including malignant mesothelioma of the abdominal cavity and pancreatic islet tumors in male rats and skin tumors (squamous-cell papilloma, keratoacanthoma, and basal-cell adenoma or carcinoma) in female rats as supporting evidence.

### VII.D.4 Mechanistic Data and Other Relevant Effects

#### VII.D.4.1 Presentation

Ms. Spencer presented an overview of the key information on Mechanistic Data and Other Relevant Effects. No studies were identified that evaluated mechanisms of carcinogenicity for the tumor sites observed in experimental animals. However, mechanistic studies of toxicity in experimental animals indicate that 1-BP causes molecular alterations typically associated with carcinogenicity, including genotoxicity, oxidative stress due to glutathione depletion, and  $\gamma$ -amino butyric acid (GABA) dysfunction.

The only *in vitro* bacterial mutagenicity study that used a protocol adequate for testing volatile substances (Barber *et al.*, 1981) found positive results with or without mammalian metabolic activation. Using the comet assay, Toraason *et al.* (2006) found limited evidence for DNA damage in leukocytes from exposed workers. Strengths of this study were use of individual exposure measurements, consideration of confounding factors, and the wide range of exposures; limitations were the small number of exposed workers, the use of multiple comparisons, and the lack of unexposed controls. S-propylcysteine adducts were found in globin from 1-BP-exposed workers. Genotoxicity was also observed for known and proposed metabolites of 1-BP.

In rodents, 1-BP causes glutathione depletion and oxidative stress, and studies have shown links between the molecular alterations and toxic end points. Although no studies have evaluated the role of oxidative stress in 1-BP-induced carcinogenicity, oxidative stress is a relevant mechanism for human carcinogenicity. There is also evidence that 1-BP causes GABA dysfunction in rats, and that GABA has a role in carcinogenicity, through its involvement in cell proliferation, differentiation, and migration. It is unclear whether immunomodulation plays a role in 1-BP carcinogenicity. 1-BP has been shown to cause immunosuppression in rats and mice. The NTP two-year bioassay found respiratory tract inflammation (lesions with Splendore-Hoeppli bodies) in rats but not in mice, and lung tumors were observed in mice but not in rats.

Although studies of 1-BP metabolism in humans have been limited to identification of potential biomarkers in urine, there is some evidence that humans have similar metabolic pathways as animals. It was concluded that the available data support the relevance of the cancer studies in experimental animals to human carcinogenicity.

Dr. Anderson asked for more information on the Splendore-Hoeppli bodies observed in rats. Dr. Malarkey explained that in areas of chronic inflammation, there might be a buildup of proteinaceous material that may come from eosinophils or immunoglobulins; these visible lesions are an end stage of a certain type of chronic inflammation. In the NTP study, these were found in the nasal cavity and airway. Their occurrence may represent an immunocompromised state.

#### VII.D.4.2 Peer Review Comments and Panel Discussion

Dr. Paul White, first reviewer, found this section of the monograph to be complete, clear, technically correct, and objectively presented, but asked for improved clarity. He suggested highlighting the difficulties of testing volatile substances *in vitro*, citing papers by Claxton and Hughes (1985) that compared the standard assay techniques with the chamber vaporization technique and concluded that chamber vaporization is the only reliable way to test volatile compounds. He suggested that the monograph emphasize that the one study that was conducted properly (Barber *et al.*, 1981) gave positive results showing that 1-BP is a direct-acting mutagen. He also noted that the study by Barber *et al.* was extremely well conducted, going to great lengths to validate the chamber technique, using radiolabeled volatile substances and chemical analyses to look at the kinetics of vapor transfer from the chamber to the agar plate. This study found a strong positive response without the need for metabolic activation. He noted that a 2-fold increase was found even in *Salmonella* strain TA98 revertants, although the dose response was somewhat erratic. Barber *et al.* also compared several compounds and found 1-BP to be among the most active. He noted that the Elf Atochem study summarized in the NTP technical report supposedly used a chamber vaporization technique and did not find a positive response; however, the details of the chamber vaporization technique are not available. The Elf Atochem study did find positive results in the mouse lymphoma mutagenesis assay.

Addressing the written public comment from Albemarle Corp. stating that 1-BP did not induce DNA damage, Dr. White said it was not clear whether this comment was referring to the *Salmonella* or human studies. Dr. White considered the *Salmonella* and mouse lymphoma studies to very clearly indicate that 1-BP induced mutations in both mammalian and bacterial cells *in vitro*. He noted that the mouse lymphoma study is an older study; since new data acceptance and test evaluation criteria were adopted at a 2009 meeting of the International Working Group on Genetic Toxicity Testing Protocols (see Moore *et al.*, 2011), it might be desirable to evaluate these data with respect to the newer criteria.

Dr. White considered it accurate to characterize the Toraason *et al.* (2006) study as providing limited evidence of DNA damage in occupationally exposed humans. He considered this to be a fairly weak study. For example, the data showed a modest increase in DNA damage for sprayers vs. non-sprayers in one facility, but a non-significant decrease in the other facility. He did not consider the evidence to be extremely compelling, noting that the study authors had difficulty relating the DNA damage level to exposure level.

Dr. White commented that the summary of information related to inflammatory effects of 1-BP on page 42 did not effectively summarize all of the mechanistic phenomena that were discussed. He suggested including a complete list of inflammatory changes.

Dr. White noted that the Lee *et al.* (2007) study was really looking at glutathione conjugation in relation to immunotoxicity and hepatotoxicity, and mentioned the formation of alkyl-deoxyguanosine adducts only in one or two sentences at the end of the paper, without any details on methods. The study on adducts was published only as an abstract from a Society of Toxicology meeting (Lee *et al.*, 2003), but since no details on the method were provided, the evidence was weak.

Dr. White suggested caution in how metabolic activation is discussed in the monograph. The fact that *in vitro* tests do not require mammalian microsomes does not mean that there is no metabolic alteration or processing of the compound before adducts are formed.

Dr. Anderson asked whether information was available on the degree of the mutagenic effect in lymphoma cells. Dr. White checked the NTP technical report, which said that the criterion for a positive response was a reproducible 2-fold increase and that the study was conducted according to current guidelines and had no perceived weaknesses. A positive response was found in two separate studies in the absence of metabolic activation. However, the detailed data were not presented.

Dr. Selgrade, second reviewer, suggested adding a table summarizing the genotoxicity of the metabolites of 1-BP. She felt the data on immune suppression were good, demonstrating suppression of the T-cell-mediated antibody response, which is considered the most predictive assay for immune suppression and correlates well with effects in host resistance models that include tumor models. She would not relate this to the Splendore-Hoeppli lesions, which are probably just due to chronic inflammation in the nose. She also noted that although the whole-body exposures result in a fair amount of dermal exposure, which would probably affect the skin barrier; she would not suggest that the immune suppression was related to the skin tumors. She also wondered why no immune suppression data were included in the appendices to the 1-BP monograph. She suggested including the data on antibody forming cell suppression data in the appendix.

Dr. Gordon suggested that the first paragraph of Section 5.3, discussing the complexity of mechanistic considerations in carcinogenesis, should also be included in other monographs, including the cumene monograph. He suggested that type of interpretive text found in the section on GABA, describing its other roles, should also be provided in the sections on the other potential mechanisms.

Dr. Anderson asked for discussion about what was happening in systems where effects were seen without the addition of mammalian microsomes. Dr. Burka said the end products would be mercapturic acids derived from the glutathione conjugates. Dr. Anderson noted that this pathway would lead to oxidative stress. Dr. Burka said that would be the case especially if CYP2E1 is involved in the metabolism, as it is well known to generate reactive oxygen species. Dr. Anderson suggested that this would be consistent with the occurrence of unusual tumor types, because carcinogenesis would not be dependent on large amounts of a particular cytochrome P450. Dr. Burka agreed that would be the case as long as the compound itself was a potent alkylating agent. Dr. Nesnow thought that a combination of events was occurring, including formation of reactive metabolites, glutathione depletion to various degrees in various tissues, and formation of reactive oxygen species.

Summarizing the Panel's discussion, Dr. Anderson stated that there was one strong study on bacterial mutagenesis, adequate data for genotoxicity in mammalian cells, and adequate pathways to explain the observed effects.

## **VII.D.5 Overall Cancer Evaluation**

### **VII.D.5.1 Presentation**

Ms. Spencer presented an overview of the Overall Cancer Evaluation. The evaluation concluded that a significant number of persons residing in the United States are exposed to 1-BP, and that the level of evidence of carcinogenicity from studies in experimental animals is sufficient; tumors occurred in both rats and mice and at multiple tissue sites, including lung, skin, and large intestine. In addition, 1-BP caused molecular alterations in experimental systems that are relevant to possible mechanisms of human carcinogenicity and consistent with the available mechanistic data in humans. Therefore, the following preliminary listing recommendation was made:

1-BP is *reasonably anticipated to be a human carcinogen* based on sufficient evidence in experimental animals.

### **VII.D.5.2 Peer Review Comments and Panel Discussion**

Dr. Gordon, first reviewer, commented that the overall evaluation was clear and did a more than adequate job of integrating the metabolic, genotoxic, and mechanistic data with the carcinogenicity study results. The integration was objective and pointed out that there were no relevant mode-of-action studies for the tumor sites of interest. The data on similar metabolic pathways in humans and rodents are limited, but the adduct data and the importance of reactive metabolites support the carcinogenicity findings in rodents and their relevance to potential carcinogenicity in humans.

As noted earlier, Dr. Gordon said that it would be helpful if the definite gender differences in the tumor profiles were discussed mechanistically. Responding to a question from Dr. Anderson, Dr. Lunn said no data were found to address that issue. Dr. Gordon suggested that simply stating that fact would be sufficient. Dr. Malarkey mentioned that in a recent retrospective study of intestinal tumors, about 20 chemicals were identified that caused intestinal tumors in rats, in both the large and small intestine, but only 5 chemicals that caused intestinal tumors in mice, mainly in the small intestine, and none of the chemicals caused intestinal tumors in both species. This study also has information on gender differences, which could be incorporated into the monograph, as well as discussion of why the rat is more susceptible to cancer of the large intestine and the mouse to cancer of the small intestine. Dr. Anderson asked whether there was any information on gender differences in antioxidant capabilities of these target tissues. Dr. Burka said that CYP2E1 is considered to be female-dominant in rats (with higher levels in females than in males), but not in mice.

Dr. Paul White, second reviewer, thought the section provided an effective synthesis of the information presented in the monograph, but had a few specific comments. In the first paragraph, the list of molecular alterations typically associated with carcinogenesis should include immunosuppression (or evasion of immune surveillance) and inflammation. The next

paragraph mentions the *N*<sup>7</sup>-guanine adducts; as noted earlier, the cited paper does not actually include any data on adduct formation, which is found only in the Society of Toxicology meeting abstract from 2003. Also, a paper from the same group showed formation of *N*<sup>7</sup>-guanine adducts by 2-bromopropane.

Dr. White noted that while the paragraph at the top of page 46 notes evidence for the importance of metabolic activation in 1-BP-induced genotoxicity and toxicity, the next sentence highlights the *Salmonella* mutagenicity results, which did not require exogenous metabolic activation. This could be somewhat confusing for the reader. He also reiterated the importance of explicitly stating that the *Salmonella* results were positive in the absence of “exogenous post-mitochondrial mammalian metabolic activation.”

### **VII.D.5.3 Action**

Dr. Anderson asked the Panel to vote on the preliminary policy decision that 1-BP should be listed in the RoC as a *reasonably anticipated to be a human carcinogen* based on sufficient evidence from studies in experimental animals. Dr. Selgrade moved to accept the decision and Dr. Paul White seconded the motion. The Panel agreed unanimously (8 yes, 0 no, 0 abstentions) with the NTP’s preliminary policy decision to list 1-BP in the RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals.

### **VII.E. Draft RoC Substance Profile**

Ms. Spencer summarized the contents of the draft substance profile as containing NTP’s preliminary listing status recommendation, summarizing the scientific information key to reaching a recommendation, and providing information on properties, use, production, exposure, and existing federal regulations and guidelines.

Dr. Sanderson, first reviewer, found the information on use, production, and human exposure to be clear and accurate.

Dr. Gordon, second reviewer, agreed with respect to the information on carcinogenicity and cancer studies in experimental animals, and noted that the profiles were much tighter, well-written, and clearer than the text of the monographs in general.

Dr. Paul White, third reviewer, found that the information on mechanisms of carcinogenicity and other relevant data was very clearly presented, but he had a few comments. The second sentence notes that unmetabolized 1-BP has been detected in human urine but not in rodent urine. Although this is important, it is also important to emphasize that occupational exposure studies have consistently reported a correlation between ambient air levels of 1-BP and levels of 1-BP or its metabolites in urine. The first sentence of the third paragraph uses the wording “directly and by its metabolism”; again, increased clarity is needed, so that readers are not confused about the lack of need for metabolism in the *in vitro* studies. Also, there is the question of whether evidence of adduct formation presented at a scientific meeting, published as an abstract, and mentioned in one line of a peer-reviewed paper, which was not about adduct formation, constitutes sufficient evidence.

Dr. Nesnow questioned why a substantial write-up on immunosuppression was included in the cancer evaluation but not in the profile. Dr. Bucher said the view on the potential role of immunosuppression in the carcinogenesis of 1-BP evolved during the writing of the monograph, and that he would like to hear the panel's opinion on how much emphasis should be placed on it. Dr. Selgrade noted that immune suppression in humans is associated with lymphomas, but it is not clear whether there is cause or effect. There is a good correlation with suppression of the antibody-forming cell response in some host-resistance models that involve tumor challenges, but it is hard to say whether this applies to these specific types of tumors. She felt immunosuppression was worth mentioning, as it probably does contribute in some way. Dr. Anderson asked whether skin tumors are particularly likely to be controlled by immune surveillance. Dr. Selgrade noted that ultraviolet light causes tumors through immune suppression, and that immune-suppressed people have an increased incidence of skin cancers, but also of other cancers. Dr. Nesnow noted that immunosuppression is part of the overall mechanism of induction of skin tumors by benzo[a]pyrene, in addition to genotoxicity and reactive oxygen species.

The Panel confirmed that mesothelioma of tunica vaginalis of the epididymis in male rats, pancreatic islet-cell tumors in male rats, and skin tumors in female rats should be retained in the profile as supporting evidence for carcinogenicity.

Ms. Spencer acknowledged and thanked those who contributed to putting together the draft monograph and organizing the Panel meeting.

### **VIII. Closing Remarks on Draft RoC Monographs**

Dr. Anderson asked for a general discussion by the Panel on the draft monographs and the process used for their development. Dr. Wolfe asked in particular for comments on the documents' organization and content, to strengthen the process.

Dr. Lunn said that the RoC team would welcome input on what to include in appendices vs. the main text. Dr. Bucher noted that for the 12<sup>th</sup> RoC, a background document was created for each substance that abstracted factual information but did not explain how conclusions were reached. The peer-reviewed monographs are meant to outline not only the data, but also the thinking that went into developing the conclusions. This means reaching a balance between providing the detail that would allow an adequate peer review and showing how the information fit together to produce a conclusion.

Dr. Gordon felt that the RoC team came close to hitting the target; he agreed that it was appropriate to put much of the toxicology data for 1-BP in the appendix, and he liked including the figures showing exposure levels in the text. However, he thought that statistical evaluations should have received more discussion in the text.

Dr. Sanderson said this was the best review panel he had been on, and that the RoC team had hit the target. He commented that the monographs were well written and incredibly thorough, that he had only minor editorial comments, and that he had already provided additional references and data sources that might be considered. He felt that the figures were unusable,

and that it would be more valuable to improve Appendix B, so that the data could be used for other purposes as well. He considered both monographs to be first-rate products.

Dr. Nesnow agreed that the monographs were well written. He asked whether it would be possible to provide hyperlinks to the references, to make it easier to look at the original data. Dr. Wolfe said that this would not be possible with the documents on the public meeting site, but that it might be possible to arrange it for future reviews for the panelists.

Dr. Anderson noted that this Panel had a great depth of expertise in all areas relevant to this process, so the fact that, by and large, the Panel was happy with the monographs could be taken as a positive sign.

Dr. Lunn thanked the panel for their valuable comments and their time. Dr. Lori White described the next steps in the review process and noted that meeting presentations (and other meeting documents) would be available at (<http://ntp.niehs.nih.gov/go/38854>). Dr. Bucher thanked the chair and the Panel for doing an outstanding job at reviewing the draft monographs. He stressed the high public profile of the RoC and the importance of having access to technical expertise to ensure that no errors are made and that the correct conclusions are reached. He also thanked Dr. Hattis, who would report on the proceedings at the next BSC meeting, and the RoC staff.

The meeting was adjourned at 11:15 a.m. on March 22, 2013.

## IX. References Cited

- Barber ED, Donish WH, Mueller KR. 1981. A procedure for the quantitative measurement of the mutagenicity of volatile liquids in the Ames *Salmonella*/microsome assay. *Mutat Res* 90(1): 31-48.
- Chen LJ, Wegerski CJ, Kramer DJ, Thomas LA, McDonald JD, Dix KJ, Sanders JM. 2011. Disposition and metabolism of cumene in F344 rats and B6C3F1 mice. *Drug Metab Dispos* 39(3): 498-509.
- Claxton, LD. 1985. Assessment of bacterial bioassay methods for volatile and semi-volatile compounds and mixtures. *Environ Int* 11: 375-382.
- Garner CE, Sumner SC, Davis JG, Burgess JP, Yueh Y, Demeter J, Zhan Q, Valentine J, Jeffcoat AR, Burka LT, Mathews JM. 2006. Metabolism and disposition of 1-BP in rats and mice following inhalation or intravenous administration. *Toxicol Appl Pharmacol* 215(1): 23-36.
- Henne KR, Fisher MB, Iyer KR, Lang DH, Trager WF, Rettie AE. 2001. Active site characteristics of CYP4B1 probed with aromatic ligands. *Biochemistry* 40(29): 8597-8605.
- Hughes TJ, Simmons DM, Monteith LG, Claxton LD. 1987. Vaporization technique to measure mutagenic activity of volatile organic chemicals in the Ames/Salmonella assay. *Environ Mutagen* 9: 421-441.
- Hong HH, Ton TV, Kim Y, Wakamatsu N, Clayton NP, Chan PC, Sills RC, Lahousse SA. 2008. Genetic alterations in K-ras and p53 cancer genes in lung neoplasms from B6C3F1 mice exposed to cumene. *Toxicol Pathol* 36(5): 720- 726. (Supported by the National Institutes of Health, National Institute of Environmental Health Sciences. Authors affiliated with National Institute of Environmental Health Sciences, NC; North Carolina State University, NC; Louisiana State University, LA.)
- Jones AR, Walsh DA. 1979. The oxidative metabolism of 1-BP in the rat. *Xenobiotica* 9(12): 763-772.
- Lee E, Moon Y, Zhao L, Kim E, Lim H, Basnet A, Jeong T, Chae W. 2003. Synthesis, characterization, *in vitro* and calf thymus DNA identification of N<sup>7</sup>-guanine adducts of 1- and 2-bromopropane. *Toxicologist* 77: 996.
- Lee SK, Jeon TW, Kim YB, Lee ES, Jeong HG, Jeong TC. 2007a. Role of glutathione conjugation in the hepatotoxicity and immunotoxicity induced by 1-BP in female BALB/c mice. *J Appl Toxicol* 27(4): 358-367.
- Moore MM, Honma M, Clements J, Awogi T, Douglas GR, van Goethem F, Gollapudi B, Kimura A, Muster W, O'Donovan M, Schoeny R, Wakuri, S. 2011. Suitable top concentration for tests with mammalian cells: Mouse lymphoma assay workgroup. *Mutat Res–Genet Toxicol Env Mutagen* 723(2): 84-86.
- NTP. 2009. *Toxicology and Carcinogenesis Studies of Cumene (CAS No. 98-82-8) in F344/N Rats and B6C3F<sub>1</sub> Mice (Inhalation Studies)*. NTP TR 542, NIH Publication No. 09-5885. Research Triangle Park, NC: National Toxicology Program. 206 pp.

- NTP. 2011a. NTP *Technical Report on the Toxicology and Carcinogenesis Studies of 1-BP (CAS No. 106-94-5) in F344/N Rats and B6C3F1 Mice (Inhalation Studies)*. NTP TR 564, NIH Publication No. 11-5906. Research Triangle Park, NC: National Toxicology Program. 195 pp.
- Rozman KK, Doull J. 2002. Derivation of an occupational exposure limit (OEL) for *n*-propyl bromide using an improved methodology. *Appl Occup Environ Hyg* 17(10): 711-716.
- Seńczuk W, Litewka B. 1976. Absorption of cumene through the respiratory tract and excretion of dimethylphenylcarbinol in urine. *Br J Ind Med* 33(2): 100-105.
- Strauss HS, Heiger-Bernays W. 2012. Methodological limitations may prevent the observation of non-Hodgkin's lymphoma in bioassays for polychlorinated biphenyls. *Toxicol Pathol* 40: 995-1003.
- Thompson DC, Perera K, London R. 1996. Quinone methide formation from para isomers of methylphenol (cresol) ethylphenol and isopropylphenol: Relationship to toxicity. *Chem Res Toxicol* 5: 55-60.
- Toraason M, Lynch DW, DeBord DG, Singh N, Krieg E, Butler MA, Toennis CA, Nemhauser JB. 2006. DNA damage in leukocytes of workers occupationally exposed to 1-BP. *Mutat Res* 603(1): 1-14.
- Wakamatsu N, Collins JB, Parker JS, Tessema M, Clayton NP, Ton TV, Hong HH, Belinsky S, Devereux TR, Sills RC, Lahousse SA. 2008. Gene expression studies demonstrate that the K-ras/Erk MAP kinase signal transduction pathway and other novel pathways contribute to the pathogenesis of cumene-induced lung tumors. *Toxicol Pathol* 36(5): 743-752.