# National Toxicology Program
# Response to the Report
# on the Peer Review of the Draft Report on Carcinogens Monographs for Cumene and 1-Bromopropane

Public Meeting
March 21-22, 2013

National Institute of Environmental Health Sciences

May 13, 2013

## Table of Contents

## Introduction

The NTP convened an *ad hoc* scientific panel ("Panel") to peer review the draft Report on Carcinogens (RoC) monographs for cumene and 1-bromopropane at a public meeting held March 21-22, 2013, at the National Institute of Environmental Health Sciences, Keystone Building, Research Triangle Park, NC (information on the meeting is available at http://ntp.niehs.nih.gov/go/38854). A draft RoC monograph consists of a cancer evaluation component and a substance profile. For each draft RoC monograph, the peer-review panel had a two-fold charge:

1. To comment on the draft cancer evaluation component, specifically, whether it was 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 RoC listing criteria,

2. To comment on the draft substance profile, specifically, whether the scientific justification presented in the substance profile supports the NTP's preliminary policy decision on the RoC listing status of the substance.

The Panel was asked to vote on each of the following for cumene and 1-bromopropane:

1. Whether the scientific evidence supports the NTP's conclusion on the level of evidence for carcinogenicity from experimental animal studies of the substance.

2. Whether the scientific evidence supports the NTP's preliminary listing decision for the substance in the RoC.

Per the process for preparation of the RoC, the NTP prepares a response to the peer review and posts it on the RoC website (see URLs as provided for monographs). The *NTP Response to the Report on the Peer Review of the Draft RoC Monographs for Cumene and 1-Bromopropane* ("Peer-Review Report") includes NTP's response to the Panel's recommendations and scientific and technical peer-review comments.

The NTP carefully reviewed and considered the Peer-review Report in revising the draft monographs. The revised draft RoC monographs[1] will be shared with the public and the NTP Board of Scientific Counselors (BSC) at their public meeting on June 25, 2013, and finalized following the meeting.

[1] Available at http://ntp.niehs.nih.gov/go/37895 [cumene] and http://ntp.niehs.nih.gov/go/37896 [1-bromopropane]

## Cumene and 1-Bromopropane Peer-Review Panel[2]

| | |
|---|---|
| Lucy Anderson, PhD, DABT (Chair) | Consultant for Biomedical Sciences and Toxicology<br>Catonsville, Maryland |
| Leo Thomas Burka, PhD | Consultant<br>Cary, North Carolina |
| Michael Elwell, DVM, PhD | Senior Pathologist<br>Department of Pathology<br>Nonclinical Safety Assessment<br>Covance Laboratories Inc.<br>Chantilly, Virginia |
| Terry Gordon, PhD | Professor<br>Department of Environmental Medicine<br>New York University School of Medicine<br>New York, New York |
| Lawrence H. Lash, PhD | Professor and Associate Chair<br>Department of Pharmacology<br>Wayne State University School of Medicine<br>Detroit, Michigan |
| Stephen Nesnow, PhD | Consultant<br>Chapel Hill, North Carolina |
| Wayne T. Sanderson PhD, CIH | Professor and Chair<br>Department of Epidemiology<br>College of Public Health<br>University of Kentucky<br>Lexington, Kentucky |
| MaryJane K. Selgrade, PhD | Senior Toxicologist<br>ICF International<br>Environmental Risk and Toxicology<br>Durham, North Carolina |
| Paul A. White, PhD | Leader, Genetic Toxicology Group<br>Environmental Health Sciences and Research Bureau<br>Health Canada<br>Ottawa, Ontario, Canada |

[2] The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and Federal policies and regulations. The panel members served as independent scientists, not as representatives of any institution, company, or governmental agency.

## Cumene

The Draft RoC Monograph for Cumene was peer reviewed at a public meeting held March 21-22, 2013, at the National Institute of Environmental Health Sciences, Keystone Building, Research Triangle Park, NC (for more information see the Introduction and Cumene and 1-Bromopropane Peer-Review Panel). The NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions and scientific issues supporting the recommendations, and (2) scientific and technical peer-review comments to improve the technical accuracy, clarity, and objectivity of the monograph. The Panel also provided several editorial comments, which are not included in their report or the NTP response to the Peer-review Report. These comments were also carefully considered in preparing the revised daft monograph for cumene.

### *Panel Recommendations and NTP Response*

Panel Recommendations

*The NTP's conclusion regarding U.S. exposure*

The Panel agreed that a significant number of people in the United States are exposed to cumene.

*The NTP's preliminary listing decision for cumene in the RoC*

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.

*The NTP's conclusion regarding the level of evidence for carcinogenicity from studies 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.*

*Scientific basis for sufficient evidence in experimental animals and mechanistic data*

The Panel disagreed (4 yes, 5 no, 0 abstentions; chair broke the tie) that the NTP's level of evidence conclusion of *sufficient evidence of carcinogenicity of cumene in experimental animals* was based on lung tumors in male and female mice, liver tumors in female mice, and renal tumors in male rats.

The Panel agreed with the finding of sufficient evidence for lung tumors in male and female mice and liver tumors in female mice but felt there was uncertainty about the role of $\alpha_{2u}$-globulin nephropathy in producing all of the renal tumor effects. $\alpha_{2u}$-Globulin nephropathy is a recognized mode of action associated with kidney tumors in male rats and its relevance to humans cancer hazard is questionable. 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 of carcinogenicity. Their recommendation about the nasal tumors was consistent with the NTP's proposed conclusions. Some panel members noted that although the nasal tumors are benign, the response was remarkable, adding credence to the observations of tumors at other sites.

The NTP concluded in the draft RoC monograph that although cumene exposure induces $\alpha_{2u}$-globulin-associated nephropathy in male rats, other mechanisms of carcinogenesis could not be unequivocally ruled out and human carcinogenicity could not be dismissed. The International Agency for Cancer Research (IARC)[3] has developed criteria for assessing whether $\alpha_{2u}$-globulin nephropathy is the sole mechanism for causing renal tumors in male rats. The NTP used these criteria in its assessment of the renal tumors and concluded that three of the seven criteria were supported by evidence for $\alpha_{2u}$-globulin-associated nephropathy concomitant with cumene exposure in male rats; however, the evidence was questionable as support for the other three criteria: (1) nongenotoxicity, (2) male-rat specificity for nephropathy, and (3) evidence of sustained cell proliferation in the renal cortex. (No data were available to evaluate one of the criteria.)

The Panel commented on NTP's conclusions for these three criteria. Several panel members stated that although cumene was not a classic genotoxic carcinogen there was evidence of genotoxicity, especially in some tissues. However, some members felt that the extent of genotoxicity in the kidney is not known. With respect to the other two criteria, several panel members noted the following: (1) the findings of nephropathy observed in the female rats at the high dose may be inflated by the low survival of the control group and (2) although there was no increase in the labeling index by proliferative cell nuclear antigen (PCNA) staining, there was histologic evidence of renal tubular regeneration.

NTP Response

The NTP concurs with the Panel that cumene should be listed in the RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals. The NTP also agrees with the Panel that the level of evidence for carcinogenicity from studies in experimental animals is based on lung tumors in male and female mice and liver tumors in female mice, with supporting evidence for benign nasal tumors in rats of both sexes and renal tumors in male rats.

The revised draft RoC monograph addresses the uncertainty of the relevance of the renal tumors to humans. The NTP concludes that the data provide evidence that cumene causes renal tumors largely via $\alpha_{2u}$-globulin nephropathy; however, is unclear whether other mechanisms, such as genotoxicity, also contribute to renal carcinogenicity. Although it is likely that genotoxicity plays a role in cumene-induced carcinogenicity at some tissues, the strongest evidence for genotoxicity was found for lung and liver tumors, and the extent to which genotoxicity contributes to the renal tumors is unknown. Thus, the relevance of the renal tumors in rats to human cancer is uncertain, and the renal tumor

[3] IARC. 1999. Species Differences in Thyroid, Kidney and Urinary Bladder. *Carcinogenesis*. IARC Scientific Publications no. 147. Lyon, France: International Agency for Research on Cancer. pp. 1-14.

findings are considered as supportive, rather than contributing directly to the sufficiency of evidence for the carcinogenicity of cumene from studies in experimental animals.

### *Scientific and technical peer-review comments on the draft RoC monograph*

The Panel provided comments regarding the Draft RoC Monograph for Cumene that would add to its clarity and completeness. The specific comments and NTP response to comments are discussed below.

*Comments and NTP's response related to improving the clarity of the RoC monograph.*

*The following comments were related to improving the clarity and technical accuracy of information reported in the draft monograph.*

Panel Comments

- Clarify in the metabolism section of the monograph that hydroxylated metabolites of cumene can also result from hydroxylation of the primary hydroxylated metabolites as well as from α-methylstyrene oxide. Urinary metabolites of cumene should be identified as conjugates in the monograph.
- Clarify the rationale for dose setting in the NTP chronic bioassay of cumene in the rat, as reported in the NTP Technical Report no. 542[4].
- Clarify the description of the Wakamatsu *et al.* (2008)[5] study in the monograph. The monograph should discuss the study's limitation, specifically the small sample size for conducting microarray analyses and that some of the *ras* positive and negative mutations lung tumors also had *p53* mutations. It should also note that one of the major sequence changes is seen in both types of human lung cancers (small cell and non-small cell carcinoma). The text on the potential role of methylation should be removed because it is based on statements in the publication for which no details or data are provided.
- Emphasize, in both the cancer evaluation component and the substance profile, that the increases in the frequencies of mouse lung K-*ras* and *p53* mutations were dose-related in the study reported by Hong et al. (2008)[6]. These finding and the changes in the mutation profiles strengthen the argument for a genotoxic mode of action.
- Use consistent wording for the evidence of genotoxicity and clearly state when the data indicate a real effect.

---

[4] NTP. 2009. *Toxicology and Carcinogenesis Studies of Cumene (CAS No. 98-82-8) in F344/N Rats and B6C3F1 Mice (Inhalation Studies)*. NTP Technical Report Series no. 542, NIH Publication no. 09-5885. Research Triangle Park, NC: National Toxicology Program. 206 pp.

[5] 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.

[6] Hong HH, Ton TV, Kim Y, Wakamatsu N, Clayton NP, Chan PC, Sills RC, LahousseSA. 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.

- Change the title of the section "Disposition and species-specific metabolism leading to cytotoxic metabolites" so that it does not reference "cytotoxic metabolites" because lung cytotoxicity was not observed. Use the term reactive metabolites rather than cytotoxic metabolites throughout the document.

NTP Response: The draft RoC monograph was revised to clarify information regarding metabolism, toxicology, genotoxicity, and mechanisms. The NTP concurs that the genotoxic evidence is not equivocal for some endpoints and that findings for the K-*ras* and *p53* mutations strengthen the genotoxicity data. The NTP also agrees the term "reactive metabolites" is a more appropriate description than "cytotoxic metabolites."

*Comments and NTP response related to enhancing the completeness of the monograph*

- Add information on exposure sampling and methodology.

NTP Response: A description of analytical sampling methods is beyond the scope of the RoC monograph; however, available references or links are cited.

- Add information on exposure to cumene (1) from its presence in aviation fuel, and (2) among workers in the gasoline industry, specifically gas station attendants, and gasoline delivery personnel.

NTP Response: No exposure information was located on exposures to cumene and use or transport of aviation fuel or gasoline.

- Discuss the study by Thompson *et al.* (1995)[7] on the metabolism and formation of quinone methides, which are proposed reactive intermediates in cumene metabolism.

NTP Response: A discussion of this study was added to the metabolism section of the monograph.

- Add a discussion of the mechanistic data for liver cancer, including the evidence for a potential role of $\alpha$-methylstyrene, presumably through $\alpha$-methylstyrene oxide in the induction of liver cancer in female mice by cumene.

NTP Response: A summary of the available mechanistic data on liver cancer was added to both the cancer evaluation component and substance profile of the revised draft RoC monograph for cumene.

[7] Thompson DC, Perera K, London R. 1995. Quinone methide formation from para isomers of methylphenol (cresol), ethylphenol, and isopropylphenol: relationship to toxicity. *Chem Res Toxicol* 8(1): 55-60.

# 1-Bromopropane

The Draft RoC Monograph for 1-Bromopropane was peer reviewed at a public meeting held March 21-22, 2013, at the National Institute of Environmental Health Sciences, Keystone Building, Research Triangle Park, NC (for more information see the Introduction and Cumene and 1-Bromopropane Peer-Review Panel). The NTP's response addresses the Panel's (1) recommendations concerning NTP's draft conclusions and scientific issues supporting the recommendations, and (2) scientific and technical peer-review comments to improve the technical accuracy, clarity, and objectivity of the monograph. The Panel also provided several editorial comments, which are not included in their report or the NTP response to the Peer-review Report. These comments were also carefully considered in preparing the revised draft monograph for 1-bromopropane.

### *The Panel's recommendations and NTP Response*

Panel Recommendations

*The NTP's conclusion regarding U.S. exposure*

The Panel agreed that a significant number of people in the United States are exposed to 1-bromopropane.

*The NTP's preliminary listing decision for 1-bromopropane in the RoC*

The Panel agreed unanimously (8 yes, 0 no, 0 abstentions) with the NTP's preliminary policy decision to list 1-bromopropane in the RoC as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals.

*The NTP's conclusion regarding the level of evidence for carcinogenicity from studies 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* based on (1) skin tumors in male rats, (2) tumors of the large intestine in female and male rats, and (3) 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.

*Mechanism data*

In general, the Panel also agreed with (1) the assessment on genotoxicity including that there is evidence indicating that 1-bromopropane is mutagenic in bacteria and mammalian cells and (2) the overall synthesis of the animal and mechanistic data.

NTP Response

The NTP concurs that the scientific information supports the conclusion of sufficient evidence of carcinogenicity of 1-bromopropane from studies in experimental animals

based on skin tumors in male rats, large intestine tumors in female and male rats, and lung tumors in female mice, and with supporting evidence from 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.

### *Scientific and technical peer-review comments on the draft RoC monograph*

The Panel provided comments regarding the Draft RoC Monograph for 1-Bromopropane that would add to its clarity and completeness. The specific comments and NTP's response to the comments are discussed below.

*Comments and NTP's response related to improving the clarity of the RoC monograph.*

Panel Comments

- Clarify the key scientific question (in the introduction) related to immune effects so that it is consistent with the discussion of inflammation in the mechanistic section.
- Clarify that both the arithmetic and geometric means for occupational exposure levels are presented in Figure 1-2, TWA 1-bromopropane air concentrations across industry sectors.
- Clarify the statistical analyses of trends.
- Clarify that Jones and Walsh (1979)[8] study used a harsh reagent to oxidize 1-bromopropane; thus it reflects more of a chemical reaction than an *in vitro* metabolism.
- Clarify the role of metabolic activation in genotoxicity studies. The fact that *in vitro* tests do not require mammalian microsomes does not mean there is no metabolic alteration or processing of the compound before adducts are formed.
- Exclude information (also in substance profile) on the formation of 1-bromopropane DNA adducts, which was referenced from a secondary source (Lee *et al.* 2007)[9]; the primary source was a meeting abstract published by the same author (Lee *et al.* 2003)[10].
- Include additional details on the mouse lymphoma assay results, as reported in NTP (2003)[11], in the draft monograph.
- Provide a summary table of 1-bromopropane metabolite genotoxicity.

[8] Jones AR, Walsh DA. 1979. The oxidative metabolism of 1-bromopropane in the rat. *Xenobiotica* 9(12): 763-772.

[9] Lee SK, Jeon TW, Kim YB, Lee ES, Jeong HG, Jeong TC. 2007. Role of glutathione conjugation in the hepatotoxicity and immunotoxicity induced by 1-bromopropane in female BALB/c mice. *J Appl Toxicol* 27(4): 358-367.

[10] Lee ES, Moon YS, Zhao LX, Kim E, Lim HT, Basnet A, Jeong TC, Chae W. 2003. Synthesis, characterization, *in vitro* and calf thymus DNA identification of N7-guanine adducts of 1- and 2-bromopropane. *Toxicol Sci* 72(S–1): 996.

[11] NTP. 2003. *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of 1-Bromopropane*. Research Triangle Park, NC: National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction. 88 pp.

- Add data on the T cell mediated antibody response reported in the study by Anderson *et al.* (2010)[12], which studied immunosuppression in rodents exposed to 1-bromopropane. The conclusions of this study were summarized in the text; however, details on the findings are not reported. This assay is considered to be the most predictive assay for immune suppression.

NTP Response: The NTP concurs with the Panel's recommendations. The scientific question was changed to "Does immunomodulation play a role in 1-bromopropane carcinogenicity?" The discussion of information regarding exposure, genotoxicity, and potential mechanisms of carcinogenicity was revised to address the Panel's specific recommendations, and a summary table of the genotoxic findings for 1-bromopropane metabolites was added to the Other Relevant Data section of the revised monograph.

*Comments and NTP response related to enhancing the completeness of the monograph*

- Add additional occupational exposure information to the tables in Appendix B, including the number of samples, arithmetic mean and standard deviation, geometric mean and standard deviation, range, and exceedance fractions. This information would be useful to predict probabilities of exposures above certain levels.
- Add information about the methods and techniques utilized by NIOSH and OSHA for exposure assessments.

NTP Response: The purpose of the human exposure section in the monograph is to provide information needed for evaluating whether a significant number of people residing in the United States are exposed to 1-bromopropane and to provide information on how people are exposed. It is beyond the scope of the monograph to provide a detailed analysis of the occupational exposure data or describe analytical methods for measuring exposure.

- Add information from OSHA's Integrated Management Information System on occupational exposure to 1-bromopropane.

NTP Response: A summary of 1-bromopropane concentration industrial hygiene sampling data from OSHA compliance monitoring program from 1998 to 2011 was added to the exposure section of the RoC monograph for 1-bromopropane.

- If available, add more information on the tumors of the large intestine in male and female rats in the NTP study (as reported in the NTP Technical Report no. 564, 2010)[13], which would strengthen the discussion of these rare tumors. Specifically, add information on multiplicity and 'time to tumor' after treatment. Research why the intestinal adenomatous polyp (in the rectum of male rats exposed to 125 ppm

[12] Anderson SE, Munson AE, Butterworth LF, Germolec D, Morgan DL, Roycroft JA, Dill J, Meade BJ. 2010. Whole-body inhalation exposure to 1-bromopropane suppresses the IgM response to sheep red blood cells in female B6C3F1 mice and Fisher 344/N rats. *Inhal Toxicol* 22(2): 125-132.
[13] NTP. 2011. *NTP Technical Report on the Toxicology and Carcinogenesis Studies of 1-Bromopropane (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.

1-bromopropane), reported in the appendix of the NTP technical report, is not included in the statistical analysis of the intestinal tumors.

NTP Response: Some of the requested data, such as 'time to tumor,' from the NTP bioassay in rats was added to the monograph; however, multiplicity data was not reported in the NTP technical report, indicating that the affected animals did not have multiple tumors. In addition, the NTP investigated why the intestinal adenomatous polyp was not reported in the analysis of the technical report. A NTP pathologist reviewed the data and concluded that the adenomatous polyp should have been included in the analyses. The RoC monograph was revised to note the additional adenoma induced in male rats exposed to 125-ppm 1-bromopropane.

- Add information on the location of the skin tumors in rats from the NTP study.

NTP Response: No information on location of skin tumors was found in NTP Technical Report no.564 (referenced above) for 1-bromopropane.

- Provide a discussion on gender differences in the tumor profiles. Specifically, whether there was a stronger response of intestinal tumors in female rats compared with males and whether the difference could be due to metabolism.

NTP Response: The revised RoC monograph contains a short discussion on the sex differences in intestinal tumors in rats and lung tumors in mice from the data reported for NTP studies, and sex differences in CYP2E1 expression.

- Add a discussion of immunosuppression to the substance profile; it is included in the cancer evaluation component of the draft monograph.

NTP Response: A short discussion of immunomodulatory effects of 1-bromopropane was added to the substance profile. The profile discusses the immunosuppression finding and concludes that it is unclear whether induction of immunotoxicity by 1-bromopropane plays a role in tumor development.