NTP Response to Expert Panels' Peer-Review Comments on Background Documents for Candidate Substances for the 12th Report on Carcinogens

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Commonly Used Abbreviations

CUNY	City University of New York
EPA	Environmental Protection Agency
ILSI	International Life Sciences Institute
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NTP	National Toxicology Program
NYU	New York University
RoC	Report on Carcinogens

Introduction

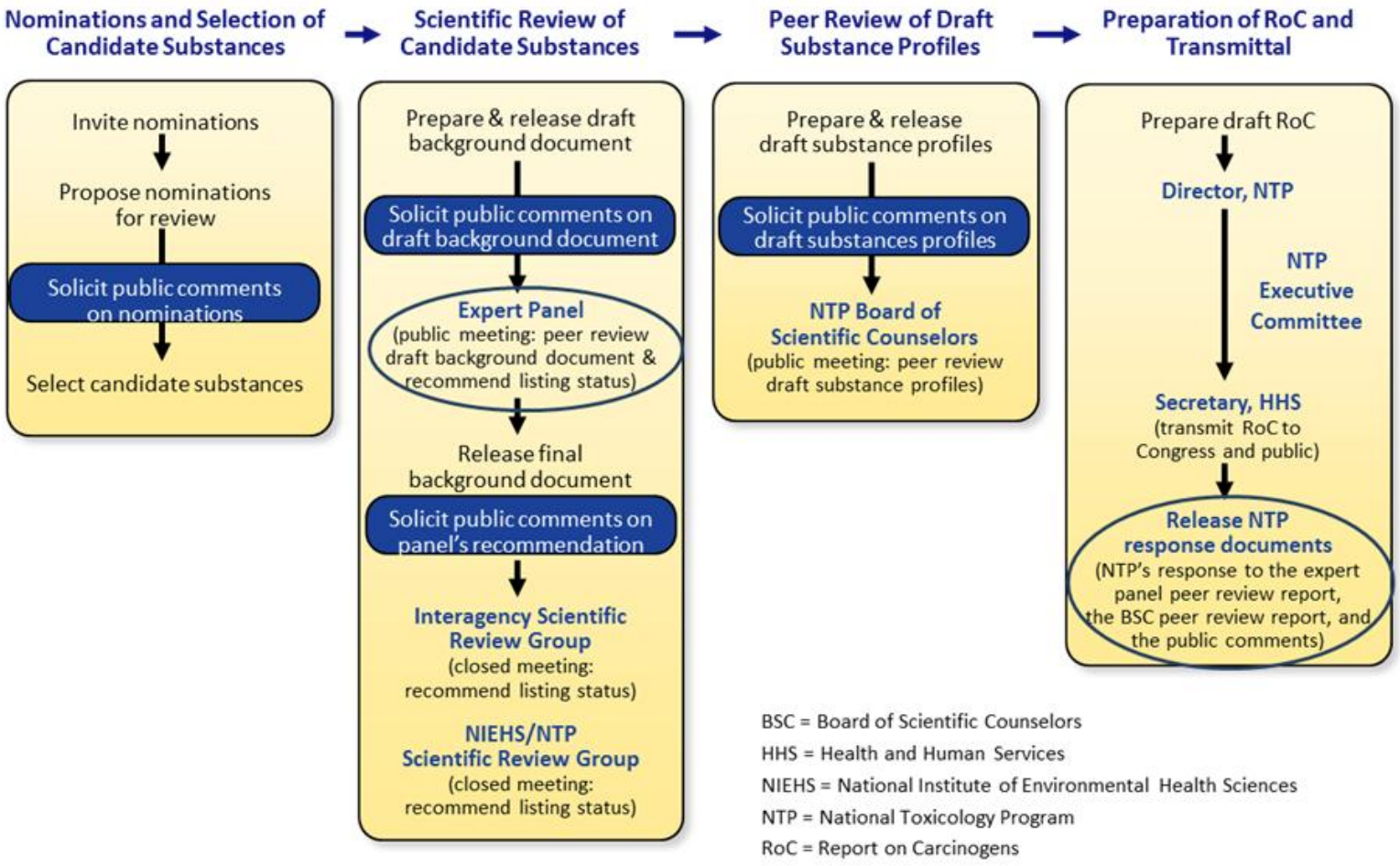
The National Toxicology Program (NTP) followed a formal process for the review of candidate substances for the *Report on Carcinogens, Twelfth Edition* (12th RoC) (see page 3 for a schematic of the review process[1]) that included peer review of the draft background document for each candidate substance by an expert panel in a public forum. A background document presents data for each substance on (1) use and production, (2) exposure including from environmental sources, to the general population and in the workplace, and (3) human epidemiology, animal, genotoxicity and mechanistic studies from the publicly available peer-reviewed literature.

The RoC Center convened six expert panels for eight candidate substances during the time period from October 2007 to November 2009. The charge to each expert panel was to "determine whether the information in the background document is presented in a clear and objective manner, identify any missing information from the body of knowledge presented in the document, and determine the utility of the body of knowledge in the background document for drawing conclusions about the carcinogenicity of a candidate substance and for applying the RoC criteria for listing."[2] The expert panel's peer-review comments for the draft background document on a specific candidate substance are captured in the "Expert Panel Report Part A: Peer-Review Comments on Draft Background Document."[3]

The NTP carefully reviewed the expert panel comments, and incorporated most of their suggested revisions in the final background documents.[4] As noted in the RoC review process (see part 4 of the review process in the schematic below), the NTP releases a report responding to each expert panel's peer-review report at the time the *12th RoC* is published. This report, organized by candidate substance, provides the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits. For each substance, the text includes (1) background information on the expert panel meeting including the members of the expert panel, and (2) responses to the relevant peer-review comments.

[1] See http://ntp.niehs.nih.gov/go/15208 for a description of the NTP–RoC Review Process.
[2] Available at http://ntp.niehs.nih.gov/go/29711.
[3] Reports are available at http://ntp.niehs.nih.gov/go/29682 ; meetings are organized by candidate substance, see Expert Panel Report Part A.
[4] See http://ntp.niehs.nih.gov/go/roc12candidates; substances are listed in alphabetical order; see final background document for the substance of interest.

NTP Report on Carcinogens Review Process



Aristolochic Acids

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on January 24–25, 2008, at the Sheraton Chapel Hill Hotel, One Europa Drive, Chapel Hill, North Carolina.

Members of the expert panel included:

Arthur P. Grollman, M.D. (Chair)
State University of New York at Stony Brook

A. Morrie Craig, Ph.D.
Oregon State University

Patricia E. Ganey, Ph.D.
Michigan State University

Yanze Liu, Ph.D.
McLean Hospital
(Harvard Medical School Affiliate)

Albert B. Lowenfels, M.D.
New York Medical College

Joëlle L. Nortier, M.D.
Université Libre de Bruxelles

Brian T. Schaneberg, Ph.D.*
ChromaDex, Inc.

Bryan L. Stegelmeier, D.V.M., Ph.D.
U.S. Department of Agriculture

*non-member, technical expert

The expert panel voted (6 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of aristolochic acids and for applying the RoC listing criteria.[5] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Introduction; Comment 2 – Section 1. Table 1-1

For the entry for *A. contorta in Table 1-1*…check the accuracy for 6-MeO-AA methyl ester.

NTP Response: 6-MeO-AA methyl ester could not be confirmed in the Natural Products Alert (NAPRALERT)[6] database, so it was deleted from the table.

[5] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

[6] NAPRALERT (http://www.napralert.org) is a relational database of all natural products, including ethnomedical information, pharmacological/biochemical information of extracts of organisms *in vitro*, *in situ*, *in vivo*, in humans (case reports, non-clinical trials) and clinical studies.

Comment: Human Cancer Studies; Comment 1–Introduction

Delete the word "possible" (p. 36, line 10 of the draft background document) in statement "…possible relationship with aristolochic acid."

NTP Response: No change was made because it is not within the scope of the background document to state conclusions on whether Balkan endemic nephropathy is associated with exposure to aristolochic acid.

Comment: Human Cancer Studies; Comment 3 – Section 3.1.2, Table 3.1

Add information to table: …the large clinical study of AAN [aristolochic acid nephropathy] by Dr. Xiaomei Li (if published before the background document is finalized, manuscript in preparation).

NTP Response: The clinical study paper of AAN by Dr. Xiaomei Li was not available in the published literature and was not added to the table.

Comment: Human Cancer Studies; Comment 4-Section 3.2.2 Prevalence studies in the Belgian cases with herbal medicine nephropathy or AAN

Add the expert panel's calculated estimated risk ratio [RR = 22] for the study reported by Nortier *et al*. (2000), which used SEER data as the reference population. The assumptions for calculating the risk ratio are as follows: (1) approximately 25 cases of urothelial cancer reported in the study, (2) estimated group exposed to aristolochic acids is 1500, (3) estimated duration of exposure = 3 years, (4) cancer rate in exposed group = 550/100,000 per year, (5) estimated background cancer incidence rate (SEER) = 25 and (6) the estimated risk ratio is 550/25 = 22.

NTP Response: This analysis was not included in the background document because it was subject to several limitations including: (1) the exposed population in the study by Nortier *et al*. is from Belgian and the SEER data (reference population) is for the United States (urinary bladder cancer incidence appears to be higher, at least in males, in Belgium) (2) most of the cancer cases in the exposed population are ureter and renal pelvis tumors (with some urinary bladder tumors), whereas the calculation for the risk estimate uses cancer incidence rates for urinary bladder cancer (upper urothelial tumors are rarer than bladder tumors), and (3) the exposed cases (Belgium) are mainly women, whereas the background cancer rate is for both sexes (urinary bladder cancer incidence is higher in men than women).

Comment: Human Cancer Studies; Comment 6 – Section 3.4 Balkan endemic nephropathy and associated urothelial cancer

At the end of this section (p. 59, line 23 of the draft background document), add the information from The Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA 2006) review of the ochratoxin A (OTA) related adducts and genotoxicity in conjunction with the discussion of the controversial data published by Pfohl-Leszkowicz and her collaborators [Arlt *et al.* 2002a, Pfohl-Leszkowicz and Manderville 2007].

NTP Response: This information was in the draft background (p.135) in Section 5.3, "Genetic Damage and Related Effects." The text in the "Human Cancer" section of the draft background document already mentioned that this study is controversial and

refers the reader to "Other Relevant Data," which is the appropriate section of the background document to discuss these types of data (genotoxicity and mechanistic data).

Comment: Other Relevant Data; Comment 4 – Section 5.2.2 Toxicity in experimental animals

Add Dong *et al*. (2006) and Shibutani *et al*. (2007) to the list of references cited for aristolochic acid toxicity in mice (p. 91, line 17 of the draft background document).

NTP Response: The Dong *et al*. (2006) reference was not added, as it reports only data on aristolochic acid adducts in rats and not in mice.

References

Arlt VM, Ferluga D, Stiborova M, Pfohl-Leszkowicz A, Vukelic M, Ceovic S, Schmeiser HH, Cosyns JP. 2002a. Is aristolochic acid a risk factor for Balkan endemic nephropathy-associated urothelial cancer? *Int J Cancer* 101(5): 500-502.

Dong H, Suzuki N, Torres MC, Bonala RR, Johnson F, Grollman AP, Shibutani S. 2006. Quantitative determination of aristolochic acid-derived DNA adducts in rats using ^{32}P-postlabeling/polyacrylamide gel electrophoresis analysis. *Drug Metab Dispos* 34(7): 1122-1127.

EFSA. 2006. *Opinion of the Scientific Panel on contaminants in the Food Chain on a Request from the Commission Related to Ochratoxin A in Food.* Question No. EFSA-Q-2005-154. 56 pp.

Nortier JL, Martinez MC, Schmeiser HH, Arlt VM, Bieler CA, Petein M, Depierreux MF, De Pauw L, Abramowicz D, Vereerstraeten P, Vanherweghem JL. 2000. Urothelial carcinoma associated with the use of a Chinese herb (Aristolochia fangchi). *N Engl J Med* 342(23): 1686-1692.

Pfohl-Leszkowicz A, Manderville RA. 2007. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. *Mol Nutr Food Res* 51(1): 61-99.

Shibutani S, Dong H, Suzuki N, Ueda S, Miller F, Grollman AP. 2007. Selective toxicity of aristolochic acids I and II. *Drug Metab Dispos* 35(7): 1217-1222.

Captafol

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on October 15–16, 2007, at the Sheraton Chapel Hill Hotel, One Europa Drive, Chapel Hill, North Carolina.

Members of the expert panel included:

Lauren Zeise, Ph.D. (Chair)
California EPA

Michael Elwell, D.V.M., Ph.D.
Covance Laboratories, Inc.

Penelope A. Fenner-Crisp, Ph.D., D.A.B.T.
Independent Consultant (Retired from ILSI and U.S. EPA)

Gregory L. Kedderis, Ph.D.
Independent Consultant

Steven Markowitz, M.D.
Queens College, CUNY

Robert C. Millikan, D.V.M., Ph.D.
The University of North Carolina at Chapel Hill

Shane S. Que Hee, Ph.D.
University of California, Los Angeles School of Public Health

Thomas J. Slaga, Ph.D.
University of Texas Health Science Center

Alexander W. Teass, Ph.D.
(Retired from NIOSH)

The expert panel voted (8 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of captafol and for applying the RoC listing criteria.[7] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Studies of Cancer in Experimental Animals; Comment 13 – Section 4.1 Mice, and Table 4-2.

> Resolve the discrepancy for male B6C3F_1 mouse liver tumors between the results reported by Ito *et al.* (1984) and the Carcinogenic Potency Database (CPDB)[8].

[7] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).
[8] See http://potency.berkeley.edu.

NTP Response: The values reported in the table for male $B6C3F_1$ mouse liver tumors and reported in Ito *et al.* are correct. The CPDB reported the data for $B6C3F_1$ mouse liver tumors inconsistently. For example, for female mice the combined incidence of liver adenoma and carcinoma was reported, but for male mice the incidence of hemangiosarcomas and hemangiomas in the liver was incorrectly included in the total liver tumor incidence. The NTP contacted Dr. Lois Gold of the CPDB and the error was identified and corrected in the CPDB.

Comment: Other Relevant Data; Comment 1 – Section 5.2 Metabolism

Insert (p. 41, line 10 of the draft background document) "Evidence also exists for conjugates for all the Phase I intermediates."

NTP Response: A reference in the scientific literature was not located to justify this statement; therefore, the suggested sentence was not added to the final background document.

References

Ito N, Ogiso T, Fukushima S, Shibata M, Hagiwara A. 1984. Carcinogenicity of captafol in $B6C3F_1$ mice. *Gann* 75(10): 853-865.

Cobalt–Tungsten Carbide: Powders and Hard Metals

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on December 9–10, 2008 at the Sheraton Chapel Hill Hotel, One Europa Drive, Chapel Hill, North Carolina.

Members of the expert panel included:

Max Costa, Ph.D. (Chair)
NYU Langone Medical Center

Marlies De Boeck, Ph.D.
Johnson & Johnson Pharmaceutical
Belgium

Kazimierz S. Kasprzak, Ph.D., D.Sc.
National Cancer Institute - Frederick

Dana Loomis, M.S.P.H., Ph.D.
University of Nevada

Steven Markowitz, M.D.
Queens College, CUNY

J. Michael Rigsbee, Ph.D.*
North Carolina State University

Wayne T. Sanderson, Ph.D., CIH
University of Iowa

Nancy Simcox, M.S.
University of Connecticut

*non-member, technical expert

The expert panel voted (6 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of cobalt–tungsten carbide: powders and hard metals and for applying the RoC listing criteria.[9] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Other Relevant Data; Comment 3 – Section 5.2.1 Humans – Respiratory effects

Add (p. 70, lines 10-16 of the draft background document) the reference Day *et al.* (2008).

NTP Response: The Day *et al.* paper does not contain any data on respiratory effects so was not added here. However, the paper does contain good information on dermal exposure, and this information has been added to the Human Exposure section (p. 19,

[9] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

after line 21 of the draft background document). [Note: the web publication is dated 2008, but the printed publication is dated 2009.]

References

Day GA, Virji MA, Stefaniak AB. 2009. Characterization of exposures among cemented tungsten carbide workers. Part II: Assessment of surface contamination and skin exposures to cobalt, chromium and nickel. *J Expo Sci Environ Epidemiol* 19(4): 423-434.

Formaldehyde

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on November 2–4, 2009, at the Hilton Raleigh-Durham Airport Hotel at Research Triangle Park, 4810 Page Creek Lane, Durham, North Carolina.

Members of the expert panel included:

Kenneth E. McMartin, Ph.D. (Chair)
Louisiana State University

Farhang Akbar-Khanzadeh, M.S.P.H., Ph.D., CIH
University of Toledo

Gary A. Boorman, D.V.M., Ph.D.
Covance, Inc.

Anneclaire DeRoos, M.P.H., Ph.D.
University of Washington

Paul Demers, Ph.D.
The University of British Columbia

Lisa Peterson, Ph.D.
University of Minnesota

Stephen M. Rappaport, Ph.D.
University of California, Berkeley
School of Public Health

David Barrie Richardson, M.S.P.H., Ph.D.
The University of North Carolina at Chapel Hill

Wayne T. Sanderson, Ph.D., CIH
University of Iowa

Martha S. Sandy, Ph.D.
California EPA

Technical experts to the panel: (non-voting)

Laura Beane Freeman, Ph.D.
National Cancer Institute

Michael DeVito, Ph.D.
NTP, NIEHS

Susan A. Elmore, M.S., D.V.M., DACVP
NTP, NIEHS

Luoping Zhang, Ph.D.
University of California, Berkeley
School of Public Health

The expert panel voted (9 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of formaldehyde and for applying the RoC listing criteria.[10] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

[10] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Human Cancer Studies; General Comment 1 – Section 3.3.1.7 Germany

Delete the study of Pesch *et al.* (2008) (p. 156 of the draft background document) because the study design is inappropriate for making inferences for the effects of formaldehyde.

NTP Response: The limitations in the study design were noted as bracketed comments rather than deleting the study.

Comment: Human Cancer Studies; Comment 5 – Section 3.2.1 National Cancer Institute (NCI) Cohort: mixed industries

Delete summary of earlier results from Hauptmann *et al.* (2004) on the following pages of the draft background document (p. 107, lines 19-30, p. 110, lines 23-30, p. 111, lines 1-5, Table 3.2, columns 5-7). Note that the analyses of lymphohematopoietic cancers are derived from follow-up through 2004 (reported by Beane Freeman *et al*. 2009).

NTP Response: The description of the earlier follow-up of the NCI study was shortened; however, the major findings are briefly included in the background document because the data may be useful for evaluating the later follow-up study published by Beane Freeman *et al*. 2009.

Comment: Human Cancer Studies. Comment 5 – Section 3.2.1 National Cancer Institute (NCI) Cohort: mixed industries

Delete (p. 112, lines 29-30 and p. 113, lines 1-5 of the draft background document) summary of findings on lung cancer.

NTP Response: Changes were not made; the findings on lung cancer were retained in the text for completeness.

Comment: Human Cancer Studies. Comment 5 – Section 3.2.1 National Cancer Institute (NCI) Cohort: mixed industries

Delete summary of reanalysis (p. 113, lines 10-25 of the draft background document) by Marsh and Youk (2004) since this discussion is about data that were subsequently updated by Beane Freeman *et al.* (2009).

NTP Response: The text describing the reanalysis by Marsh and Youk of Hauptmann *et al.* (2003) was retained because the background document includes a description of the original data (Hauptmann *et al.* [2003], see comment above).

Comment: Human Cancer Studies. Comment 11 – Section 3.2.8 Studies of health professionals, embalmers and funeral directors

Walrath and Fraumeni (1983, 1984) and Hayes *et al.* (1990) could be shortened (p. 138 of the draft background document), since parts of these studies have now been superseded by Hauptmann *et al.* (2009).

NTP Response: The description of the nested-case control study by Hauptmann *et al.* (2009) (both the text and the table) notes that the study includes the embalmers and funeral directors from the previous mortality studies (Walrath and Fraumeni 1983, 1984, Hayes *et al.* 1990), and thus clarifies that they are not independent populations. The background document includes a description of the original studies because of the differences in study methodologies (for example, case-control study versus proportional mortality study).

References

Beane Freeman LE, Blair A, Lubin JH, Stewart PA, Hayes RB, Hoover RN, Hauptmann M. 2009. Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute Cohort. *J Natl Cancer Inst* 101(10): 751-761.

Hauptmann M, Lubin JH, Stewart PA, Hayes RB, Blair A. 2004. Mortality from solid cancers among workers in formaldehyde industries. *Am J Epidemiol* 159(12): 1117-1130.

Hauptmann M, Stewart PA, Lubin JH, Beane Freeman L E, Hornung RW, Herrick RF, Hoover RN, Fraumeni JF, Blair A, Hayes RB. 2009. Mortality from lymphohematopoietic malignancies and brain cancer among embalmers exposed to formaldehyde, *J Natl Cancer Inst* 101(24): 1696-1708.

Hayes RB, Blair A, Stewart PA, Herrick RF, Mahar H. 1990. Mortality of U.S. embalmers and funeral directors. *Am J Ind Med* 18(6): 641-652.

Marsh GM, Youk AO. 2004. Reevaluation of mortality risks from leukemia in the formaldehyde cohort study of the National Cancer Institute. *Regul Toxicol Pharmacol* 40(2): 113-124.

Pesch B, Pierl CB, Gebel M, Gross I, Becker D, Johnen G, Rihs HP, Donhuijsen K, Lepentsiotis V, Meier M, Schulze J, Bruning T. 2008. Occupational risks for industry. *Occup Environ Med* 65(3): 191-196.

Walrath J, Fraumeni JF, Jr. 1983. Mortality patterns among embalmers. *Int J Cancer* 31(4): 407-411.

Walrath J, Fraumeni JF, Jr. 1984. Cancer and other causes of death among embalmers. *Cancer Res* 44(10): 4638-4641.

Glass Wool Fibers

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on June 9–10, 2009 at the Sheraton Chapel Hill Hotel, One Europa Drive, Chapel Hill, North Carolina.

Members of the expert panel included:

Karl Kelsey, M.D., M.O.H. (Chair)
Brown University

Aaron Blair, Ph.D., M.P.H.
National Cancer Institute

Michael Elwell, Ph.D., D.V.M.
Covance Laboratories

Andrij Holian, Ph.D.
University of Montana

Marie-Claude Jaurand, Ph.D.
INSERM U67 Paris

Peter Lees, Ph.D., CIH
The Johns Hopkins University

Morton Lippmann, Ph.D.
NYU School of Medicine

J. Michael Rigsbee, Ph.D.*
North Carolina State University

Allan Smith, M.D., Ph.D.
University of California, Berkeley

Kyle Steenland, Ph.D.
Emory University

*non-member, technical expert

The expert panel voted (8 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of glass wool fibers and for applying the RoC listing criteria.[11] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Other Relevant Data; Comment 6 – Section 5.3.1 Studies of fiber characteristics and tumorigenicity

Insert (p. 179, line 19 of the draft background document) "testing fibers by implantation" and change "tested fibers by" to "relied on."

NTP Response: Sentence revised to read: "After the studies by Stanton and co-workers, most investigators have tested fibers by intraperitoneal injection."

[11] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

Comment: Other Relevant Data; Comment 10 – Section 5.7.1 Summary – Deposition, clearance, and retention

Revise the second sentence (p. 250, first paragraph of this section in the draft background document) to "fibers that are inhalable but non-respirable…can cause adverse effects, but the effect of these fibers are beyond the scope of this review."

NTP Response: Sentence revised, but without the clause, "but the effect of these fibers are beyond the scope of this review," because health effects discussed in the toxicology section of the background document are not limited to those potentially caused by respirable fibers.

Comment: Additional references

The expert panel provided twenty-three additional references.

NTP Response: Two of the references (Coussens and Werb 2001, 2002) provided by the expert panel were not included in the background document. The first was not included because it is a commentary and the second because it is a general review article that is not specific for fiber-induced carcinogenicity.

References

Coussens LM, Werb Z. 2001. Inflammatory cells and cancer; think different! *J Exp Med* 193(6): F23-26.

Coussens LM, Werb Z. 2002. Inflammation and cancer. *Nature* 420(6917): 860-867.

ortho-Nitrotoluene

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on October 16, 2007, at the Sheraton Chapel Hill Hotel, One Europa Drive, Chapel Hill, North Carolina.

Members of the expert panel included:

Lauren Zeise, Ph.D. (Chair)
California EPA

Michael Elwell, D.V.M., Ph.D.
Covance Laboratories, Inc.

Penelope A. Fenner-Crisp, Ph.D., DABT
Independent Consultant (Retired from ILSI and U.S. EPA)

Gregory L. Kedderis, Ph.D.
Independent Consultant

Steven Markowitz, M.D.
Queens College, CUNY

Robert C. Millikan, D.V.M., Ph.D.
The University of North Carolina at Chapel Hill

Shane S. Que Hee, Ph.D.
University of California, Los Angeles

Thomas J. Slaga, Ph.D.
University of Texas Health Science Center

Alexander W. Teass, Ph.D.
(Retired from NIOSH)

The expert panel voted (7 yes/0 no/1 absent) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of *ortho*-nitrotoluene and for applying the RoC listing criteria.[12] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Human Exposure; Comment 3 – Section 2.3.1 Air

> Insert (p. 9, line 23 of the draft background document) the following statements from additional studies identified by the expert panel: "Smog in Japan and China has been shown to contain *ortho*-nitrotoluene (Takahara and Hayakawa, 1984; Li *et al.* 2005; Wu *et al.* 2006)."

[12] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

NTP Response: All English abstracts for the above citations (non-English papers) do not support insertion of the suggested statement; these references are for technical papers on how to measure *ortho*-nitrotoluene in air.

Comment: Human Exposure; Comment 6 – Section 2.4 General population exposure

Insert after "…HSDB 2007": (p. 12, line 25 of the draft background document) "as well as via skin contact with contaminated surfaces and via oral ingestion of contaminated food water, or dust."

NTP Response: Human exposure to *ortho*-nitrotoluene via dust and food was not noted in available references. Therefore, the sentence was modified as follows: "The general population may be exposed to *ortho*-nitrotoluene via inhalation of ambient air in the vicinity of production sites, and by oral ingestion, particularly of contaminated water (HSDB 2008), as well as via skin contact with contaminated substances."

Comment: Human Exposure; Comment 9 – Introduction and Summary

Insert (p. 7, line 11 and p. 15, line 24 of the draft background document) "*ortho*-Nitrotoluene has been detected in workplace and ambient air, surface water, ground water, fish, and soils."

NTP Response: Inserted amended text but with "fish" removed from above sentence and from the summary, as references do not support the finding of *ortho*-nitrotoluene in fish in the environment.

Comment: Additional references

The expert panel provided thirty-five additional references.

NTP Response: Five of these references were not included in the document for the reasons stated below:

Takahara and Hayakawa (1984), Wu *et al.* (2006), and Li *et al.* (2005): As stated above, the abstracts for these three citations do not support the statement regarding *ortho*-nitrotoluene in smog in Japan and China; all are technical papers on how to measure *ortho*-nitrotoluene in air.

Best *et al.* (2000): The reference was unobtainable, but information given in the text is supported by Best *et al.* (2001), which is included in the background document.

Ewers *et al.* (2000): This reference is not specific for *ortho*-nitrotoluene.

References

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Riddelliine

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on January 24–25, 2008, at the Sheraton Chapel Hill Hotel, One Europa Drive, Chapel Hill, North Carolina.

Members of the expert panel included:

Arthur P. Grollman, M.D. (Chair)
State University of New York at Stony Brook

A. Morrie Craig, Ph.D.
Oregon State University

Patricia E. Ganey, Ph.D.
Michigan State University

Yanze Liu, Ph.D.
McLean Hospital
(Harvard Medical School Affiliate)

Albert B. Lowenfels, M.D.
New York Medical College

Joëlle L. Nortier, M.D.
Université Libre de Bruxelles

Brian T. Schaneberg, Ph.D.
ChromaDex, Inc.

Bryan L. Stegelmeier, D.V.M., Ph.D.
U.S. Department of Agriculture

The expert panel voted (7 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of riddelliine and for applying the RoC listing criteria.[13] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Human Exposure; Comment 3 – Section 2.3.3 Food

> Meat (p. 22, lines 1–15 of the draft background document): This method (GC-MS) has been problematic. It is inconsistent and no one has been able to make it truly quantitative. It is useful as a qualitative indicator of exposure.
>
> *NTP Response:* The following bracketed comment was added to Section 2.4.3 after discussion of GC-MS methodology: "[Although this method is useful as a qualitative indicator of exposure, quantitation of metabolites has been problematic.]".

[13] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

Comment: Other Relevant Data; Comment 5 – Section 5.3.1 DNA adducts and mutations

Add a new paragraph in Section 5.3.1 to discuss riddelliine cross-linking using the following references: Hoorn and Roth, 1992; Hoorn *et al.* 1993; Kim *et al.* 1999 and Wagner *et al*. 1993.

NTP Response: All new references suggested by the expert panel in their report were added to the background document except Hoorn and Roth (1992) because this paper did not report results for cross-linking. Two additional papers (Petry *et al.* 1984, 1986) cited by Hoorn and Roth (1992), which describe DNA-DNA and DNA-protein cross-linking, were also included in Section 5.3.1.

References

Hoorn CM, Roth RA. 1992. Monocrotaline pyrrole alters DNA, RNA and protein synthesis in pulmonary artery endothelial cells. *Am J Physiol* 262(6 Pt 1): L740-L747.

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Petry TW, Bowden GT, Huxtable RJ, Sipes IG. 1984. Characterization of hepatic DNA damage induced in rats by the pyrrolizidine alkaloid monocrotaline. *Cancer Res* 44(4): 1505-1509.

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Styrene

Expert Panel Meeting

The NTP Report on Carcinogens (RoC) Center convened an expert panel of scientists from public and private sectors on July 21–22, 2008, at the Radisson Hotel, 150 Park Drive, Research Triangle Park, North Carolina.

Members of the expert panel included:

David Phillips, Ph.D., DSc., FRCPath (Chair)
Institute of Cancer Research, U.K.

Scot Eustis, D.V.M., Ph.D., DACVP
(Retired from Pfizer)

Peter Infante, Dr.P.H., M.P.H., D.D.S.
Peter F. Infante Consulting, LLC.

Genevieve Matanoski, M.D., Dr.P.H.
Johns Hopkins Bloomberg School of Public Health

Shane S. Que Hee, Ph.D.
University of California, Los Angeles School of Public Health

Thomas J. Smith, Ph.D., CIH
Harvard School of Public Health

Suzanne Snedeker, Ph.D.
Cornell University College of Veterinary Medicine

Michael P. Stone, Ph.D.
Vanderbilt University School of Medicine

Elizabeth M. Ward, Ph.D.
American Cancer Society

Garold S. Yost, Ph.D.
University of Utah College of Pharmacy

Lauren Zeise, Ph.D.
California EPA

The expert panel voted (10 yes/0 no) to accept that the draft background document (with the proposed changes suggested by the expert panel) was adequate for drawing conclusions about the carcinogenicity of styrene and for applying the RoC listing criteria.[14] The NTP accepted most of the expert panel's suggestions for changes to the draft background document and they are not discussed here. Provided below is the NTP's response to relevant scientific and technical issues raised in the expert panel's peer-review comments for which the NTP did not accept the panel's suggested edits.

NTP Response to the Expert Panel's Peer-Review Comments

The comments are organized by the location in the peer-review report and draft background document; the comment number refers to the number (under a specific section) in the peer-review report.

Comment: Introduction; Comment 4 – Section 1.3 Metabolites

Add information on stereochemistry of styrene-3,4-oxide to background document.

[14] See http://ntp.niehs.nih.gov/go/29682 for the draft background document and the expert panel peer-review comments on it (Expert Panel Report Part A).

NTP Response: No information was found on the stereochemistry of styrene-3,4-oxide.

Comment: Introduction; Comment 4 – Section 1.3 Metabolites

Include IUPAC names for the *R*- and *S*-styrene-7,8-oxide isomers on p. 4 [of the draft background document].

NTP Response: No reference that specifically provided the IUPAC names for the *R*- and *S*-isomers was identified.

Comment: Human Cancer Studies; Comment Part C 2 – Section 3.1.4

The expert panel for Section 2 (Human Exposure) recommended that a discussion of the data in Kolstad *et al.* 2005 be added to this section.

NTP Response: Kolstad *et al*. 2005 describes a semi-quantitative exposure assessment that appears to have been developed for nested case-control analyses of several chronic diseases among the Danish cohort of reinforced plastic workers (Kolstad *et al.* 1994, 1995). No publications of these nested case-control studies on cancer were identified in the peer-reviewed literature. The exposure information in Kolstad *et al.* 2005 is discussed in the human exposure section but not in the human cancer section because it is not relevant for evaluating the cancer findings for studies described in the background document. No reanalysis of the cancer findings for workers in this cohort was reported using this exposure assessment.

Comment: Studies of Cancer in Experimental Animals; Comment 2 – Section 4. General

Statistical analyses: …For the NCI studies where mortality is significant and could substantially impact the outcome of a statistical test, NTP should perform and report the results using poly-3 pairwise comparisons and trend tests, if individual animal data can be obtained. This is the standard for statistical analyses of animal data in NTP's current reports. It was not applied 30 years ago when the NCI bioassays for the styrene mixture and styrene alone were published.

For cases where there are elevated findings of note that are not statistically significant but between the cut-off of $P = 0.05$ and $P = 0.1$, the exact *P*-value should be given.

NTP Response: Statistical analyses were performed by NTP for pairwise and trend tests. In some cases, e.g., mammary gland data, the exact *P* values were noted in the background document. However, poly-3 pairwise comparisons and trend tests were not done, as individual animal data were not available in the NTP testing database.

Comment: Studies of Cancer in Experimental Animals; Comment 4 – Section 4.1.1 Mice, Oral

In response to a request during the peer review of the background document, NTP provided, for the sites in Table 4-1, the exact Cochran-Armitage trend tests. The notable values should be given in Table 4-1, to one significant figure. Thus, (i) For the trend value for the male lung carcinoma, $P = 0.08$. This should be included in the row indicating trend values in Table 4-1. (ii) For the adenoma and carcinoma combined, the trend test value is $P = 0.02$. (iii) For the female hepatocellular adenoma, the *P*-value is 0.03. (iv) The *P*-value for the female combined adenoma and

carcinoma, the *P*-value (and incidence) is the same as for the female adenoma, $P = 0.03$ (NCI 1979a).

NTP Response: The NTP added the trend values for the lung tumors (i. and ii.), but not for the hepatocellular tumors in females because the *P*-value for the female combined hepatocellular adenoma and carcinoma is 0.13, not 0.03.

Comment: Studies of Cancer in Experimental Animals; Comment 7. Table 4-3.

The expert panel suggested replacement text for Table 4-3 (Lung tumors in CD-1 mice exposed to styrene by inhalation for 98 to 104 weeks; Sources: Cruzan *et al.* 1998 and Cohen *et al.* 2002) that included (1) more precise statistics than those reported by the study authors; for example, the study authors only reported values as $P < 0.05$, and the expert panel request that exact Fisher *P*-values be calculated and the values be reported as $P = 0.1$, $P \leq 0.01$, $P \leq 0.001$ and $P \leq 0.0001$, (2) the *P* value for trend be calculated using the Exact Cochran-Armitage test and provided in the table, and that (3) the tumor incidence be reported only for 98–104 weeks.

NTP Response: The NTP verified the Fisher and Exact Cochran-Armitage trend tests as requested by the panel; the NTP used the convention (similar to its usual practice) of $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, and $P \leq 0.0001$ to report the statistical calculations. The NTP corrected the Fisher Exact Test results (as suggested in the expert panel report) for female styrene-exposed incidences as follows: for adenoma at 20 ppm and 40 ppm and for adenoma and carcinoma combined at 20 ppm, all are $P < 0.05$ not $P < 0.01$; for adenoma at 160 ppm, $P < 0.0001$ not $P < 0.001$; and for carcinoma at 160 ppm, $P < 0.01$ not $P < 0.001$.

Comment: Studies of Cancer in Experimental Animals; Comment 12 – Section 4.2.1 Rats, Oral

Effective numbers of treated animals are not given in the description of the Conti *et al.* (1988) study. This should be noted in the background document. (pp. 171-2 and Table 4-4 in the draft background document).

NTP Response: The effective numbers of treated animals, i.e., those animals surviving until the first target tumor is detected, were not reported in the Conti *et al.* (1988) study. However, the total number of animals in each group at study start is given, as well as the percentage of animals with tumors in each treatment group; these values are included in the background document.

Comment: Studies of Cancer in Experimental Animals; Comment 20 – Section 4.4 Mixtures containing styrene

If NTP can obtain the individual animal data, it should conduct and report the results for the poly-3 test for pairwise comparisons and trend for lung tumors in male mice.

NTP Response: NTP did not do the poly-3 test because individual animal data are not publically available.

Comment: Other Relevant Data; Comment 2 – Section 5.1 Absorption, distribution, metabolism and excretion

The NTP should review additional studies on dermal exposure supplied by the Section 2 subgroup and include information relevant to Section 5.1.1.1 (Sandell *et al.* 1978, Smith *et al.* 2006, Stewart *et al.* 1968).

NTP Response: Sandell *et al.* (1978) was the only one of these references with relevant information, and it was added to the document. Smith *et al.* (2006) examined the correlation between octanol/water partition coefficients of chemicals in tobacco smoke and tumorigenic potential; Stewart *et al.* (1968) did not address dermal exposure but exposed a human volunteer to styrene vapor by inhalation and measured styrene in exhaled breath and urinary hippuric acid.

Comment: Other Relevant Data; Comment 11 – Section 5.4.3.1 In vivo *studies in experimental animals, DNA adducts, Styrene*

In the description of the study of adducts in rats and mice by Boogaard *et al.* (2000) given on pp. 237-238 [of the draft background document], the use of metabolite standards, and the fact that some of these co-elute with radioactive peaks from the digests of rodent DNA, suggests that the peaks do not contain DNA adducts, so the material should not be described as an "adduct" on p. 238, lines 12 and 15. The reference should be checked and "adduct" should be changed to "unidentified compound" on lines 8, 12, and 15 as appropriate. Also revise Table 5-7 as needed.

NTP Response: The reference was checked, and the authors consistently referred to these peaks as *unidentified adducts*; therefore, the change was not made. The existing text does provide an explanation that these "adducts" may have been an artifact associated with benzoic acid.

Comment: Other Relevant Data; Comment 16 – Section 5.5 Mechanistic studies and considerations (General)

Add discussion of styrene metabolism to 4-vinylphenols that are further oxidized to quinone reactive intermediates. Quinones participate in reactive oxygen-mediated damage and cytotoxicity. Similar to naphthalene, this cytotoxicity could be a mechanism of clonal expansion of initiated cells.

NTP Response: No references were found to confirm that 4-vinylphenol is metabolized to quinone intermediates; however, additional information was added regarding cytotoxicity of 4-vinylphenols and their metabolites as a possible mechanism.

Comment: Other Relevant Data; Comment 17 – Section 5.5.3 Cytotoxic effects of styrene oxide on mouse lung

Add discussion of proposed mechanism (p. 331, line 17 of the draft background document) that discusses a possible role of styrene-induced elevation in prolactin levels as a potential mechanism for the induction of breast cancer.

NTP Response: This is discussed in Section 5.2.1 Toxicity, Humans and is not included in the mechanism section because no literature was identified that discussed it as a mechanism related to styrene.

References

Boogaard PJ, de Kloe KP, Wong BA, Sumner SC, Watson WP, van Sittert NJ. 2000. Quantification of DNA adducts formed in liver, lungs, and isolated lung cells of rats and mice exposed to ^{14}C-styrene by nose-only inhalation. *Toxicol Sci* 57(2): 203-216.

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Conti B, Maltoni C, Perino G, Ciliberti A. 1988. Long-Term Carcinogenicity Bioassays on Styrene Administered by Inhalation, Ingestion and Injection and Styrene Oxide Administered by Ingestion in Sprague-Dawley Rats, and *para*-Methylstyrene Administered by Ingestion in Sprague-Dawley Rats and Swiss Mice. In *Living in a Chemical World*, Annals of the New York Academy of Sciences, vol. 534. Maltoni C, Selikoff IJ, eds. New York, NY: New York Academy of Sciences. pp. 203-234.

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Stewart RD, Dodd HC, Baretta ED, Schaffer AW. 1968. Human exposure to styrene vapor. *Arch Environ Health* 16(5): 656-662.