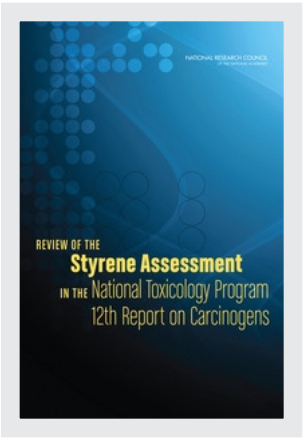

Review of the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens

DETAILS

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Committee to Review the Styrene Assessment in the
National Toxicology Program 12th Report on Carcinogens

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

National Research Council

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Preface

In 2011, the National Toxicology Program (NTP) listed styrene as "reasonably anticipated to be a human carcinogen" in its 12th Report on Carcinogens (RoC), marking the first time that the substance was listed in the RoC. Congress directed the Department of Health and Human Services to arrange for the National Academy of Sciences (NAS) to independently review the substance profile of styrene and its listing in the 12th RoC (112th Congress, 1st Session; Public Law 112-74). This report presents the findings and conclusions of the committee formed in response to the congressional request.

To address its statement of task, the committee first conducted a peer review of the styrene substance profile and listing in the NTP 12th RoC. It considered literature available to NTP up to the publication of the 12th RoC (that is, literature published by June 10, 2011). The committee then conducted an independent assessment of styrene and made a listing recommendation using the RoC listing criteria. In its independent assessment, the committee examined evidence published both before and after the publication of the 12th RoC. It considered presentations heard during its open-session meeting, comments submitted from the general public, and abstracts presented during conferences. It reviewed reports published by other authoritative bodies, and it examined primary literature, reviews, and meta-analyses that were publicly available in the peer-reviewed literature.

This report has been reviewed in draft form by persons chosen for their diverse disciplinary backgrounds and expertise in accordance with procedures approved by the National Research Council Report Review Committee. The purpose of the independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following for their review of the report: James S. Bus, Exponent; Samuel M. Cohen, University of Nebraska Medicine Center; Claude Emond, University of Montreal; William R. Fairweather, Flower Valley Consulting, Inc.; Mary Beth Genter, University of Cincinnati; Mark S. Goldberg, McGill University; Rogene F. Henderson, Lovelace Respiratory Research Insti-

tute; Richard D. Irons, Cinpathogen; Lawrence Loeb, University of Washington; Thomas M. Mack, University of Southern California; Roger O. McClellan, Toxicology and Human Health Risk Analysis; Steven R. Tannenbaum, Massachusetts Institute of Technology; and Martie Van Tongeren, Institute of Occupational Medicine.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the report's conclusions or recommendations. They did not see the final draft of the report before its release. The review of the report was overseen by the review coordinator, David L. Eaton, University of Washington, and the review monitor, Mark Cullen, Stanford University. Appointed by the National Research Council, they were responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the report rests entirely with the committee and the institution.

The committee gratefully acknowledges Wanda Jones, of the US Department of Health and Human Services, and John Bucher, of the National Toxicology Program, for making presentations to the committee. The committee appreciates all who supplied written documents or views during its open public session and throughout the study process. The committee thanks Ileana D'Andrea, Marcello Noli, Giannina Satta, and Michela Ursi, of the University of Cagliari, Italy, for providing the translation of a study. The committee also thanks Keith Soper of Merck Research Laboratories for acting as a consultant to provide the committee with input on targeted statistical questions.

On behalf of the committee, I want to acknowledge the diligence of each National Research Council staff member. Staff members who contributed to the effort are Heidi Murray-Smith, project director; Ellen Mantus, senior program officer; Keri Stoever, research associate; James Reisa, director of the Board on Environmental Studies and Toxicology; Norman Grossblatt, senior editor; Mirsada Karalic-Loncarevic, manager of the Technical Information Center; Radiah Rose, manager of editorial projects; and Ricardo Payne, program coordinator.

I thank members of the committee for their willingness to give their time, expertise, and energy to the task at hand. The members contributed greatly in their individual fields of scientific expertise. Moreover, all participated fully in the group's rigorous review of the evidence and deliberations that led to the committee's recommendations.

Jane E. Henney, *Chair*
Committee to Review the Styrene
Assessment in the National Toxicology
Program 12th Report on Carcinogens

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Summary

As part of the 2012 Consolidated Appropriations Act (112th Congress, 1st Session; Public Law 112-74), Congress directed the Department of Health and Human Services to arrange for the National Academy of Sciences to carry out an independent review of the styrene assessment in the National Toxicology Program's (NTP) 12th Report on Carcinogens (RoC).[1] In response, the Academy's National Research Council convened an expert committee that has prepared this report.

The committee approached its statement of task by first conducting a review of the substance profile for styrene as presented in the NTP 12th RoC. It considered literature published by June 10, 2011 (the date of publication of the 12th RoC), and it organized its review on the basis of the headings and subheadings of the substance profile. The committee then conducted its own independent assessment of the styrene literature, extending its review to include literature through November 13, 2013, and concluding with its own listing recommendation for styrene.

THE NATIONAL TOXICOLOGY PROGRAM AND STYRENE

NTP is an interagency program supported and managed by the National Institutes of Health's National Institute of Environmental Health Sciences (the administrative lead), the Centers for Disease Control and Prevention's National Institute for Occupational Safety and Health, and the Food and Drug Administration's National Center for Toxicological Research. Since 1980, NTP has published the RoC, which is a cumulative summary of substances that have been nominated for review and judged to meet two conditions. The first condition is that a significant number of people living in the United States are exposed to the substance of interest. The second condition is that there is evidence that the sub-

[1]NTP (National Toxicology Program). 2011a. Styrene. Pp. 383-392 in Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Styrene.pdf.

stance of interest is either known to be a human carcinogen or reasonably anticipated to be a human carcinogen on the basis of NTP's established listing criteria. The committee noted that the assessment of chemicals for the purpose of listing in the RoC constitutes a hazard assessment, not a risk assessment.

Styrene was first listed in the 12th Report on Carcinogens (RoC) as *reasonably anticipated to be a human carcinogen*, although a major metabolite of styrene, styrene-7,8-oxide, was first listed in the NTP 10th RoC as *reasonably anticipated to be a human carcinogen* in 2002. Styrene is a substance of interest because many people in the United States are exposed. Sources of environmental exposures include food (from migration of styrene from polymer packaging materials), cigarette smoke, vehicle exhaust, and other forms of combustion and incineration of styrene polymers. Occupational exposure to humans can occur during the industrial processing of styrene. It is used to create a broad spectrum of products, including latex paints and coatings; synthetic rubbers; construction materials, such as pipes, fittings, and lighting fixtures; packaging; household goods, such as synthetic marble, flooring, and molded furnishings; and automotive parts.

REVIEW OF THE STYRENE PROFILE IN THE NATIONAL TOXICOLOGY PROGRAM 12TH REPORT ON CARCINOGENS

To address the first part of its statement of task, this committee reviewed the styrene substance profile in the NTP's 12th RoC. The committee examined the primary literature cited in the background document for styrene[2] and other literature published by June 10, 2011 (the date when the 12th RoC was released). The headings and structure of the committee's review parallel the major headings that NTP used in the substance profile for styrene. As part of its review, the committee determined whether NTP had described and conducted its literature search appropriately, whether the relevant literature identified during the literature search was cited and sufficiently described in the background document, whether NTP had selected the most informative studies in making its listing determination, and whether NTP's arguments supported its conclusion that styrene is reasonably anticipated to be a human carcinogen.

Cancer Studies in Humans

The "Cancer Studies in Humans" section of the NTP substance profile for styrene considers whether the epidemiologic literature published by June 10,

[2]NTP (National Toxicology Program). 2008. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf.

2011, provides evidence of human carcinogenicity or whether that evidence reaches the level of being limited or sufficient for such a listing. Overall, NTP's background document and substance profile for styrene include appropriate literature reviews and identify the most informative studies. The text and tables in the background document clearly describe and critique the major strengths and limitations of the key epidemiologic studies, and the background document itself presents accurate data summaries.

On the basis of the studies available to NTP by June 10, 2011, the committee agrees with NTP's conclusion that there is limited but credible evidence that exposure to styrene in some occupational settings is associated with an increase in the frequency of lymphohematopoietic cancers. The evidence comes primarily from two occupational-cohort studies of reinforced-plastics workers in Europe.[3] The committee also agreed with NTP's conclusion that there is limited but credible evidence for esophageal and pancreatic cancers. The most informative studies were four cohort studies of the reinforced-plastics industry that covered subjects and controls in Washington state, the United States, Denmark, and combined European nations.[4] The strengths of those studies and the associations observed are credible because the studies were of high quality, of varied design (mortality and incidence), and consistent in their findings of associations of styrene with these cancers, especially when internal comparisons—many with an apparent exposure–response relationship—were presented. Examples of internal comparisons are incidence rate ratios and mortality rate ratios that compare workers with different levels of exposure in the same plant or industry. Kidney cancer should also have been mentioned in the styrene substance profile as having some evidence of styrene carcinogenicity on the basis of data published between 2008 and June 10, 2011. A case–control study of renal-cell cancer and

[3]Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261; Kolstad, H.A., E. Lynge, J. Olsen, and N. Breum. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand. J. Work Environ. Health 20(4):272-278.

[4]Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261; Kolstad, H.A., E. Lynge, J. Olsen, and N. Breum. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand. J. Work Environ. Health 20(4):272-278;Wong, O., L.S. Trent, and M.D. Whorton. 1994. An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup. Environ. Med. 51(6):386-396; Ruder, A.M., E.M. Ward, M. Dong, A.H. Okun, K. Davis-King. 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. Am. J. Ind. Med. 45(2):165-176.

occupational exposures, including exposure to styrene, was published in February 2011[5] after the background document was issued but before the publication of the substance profile. That study should have been included in the NTP evaluation for styrene.

The committee concludes that the description and analysis of literature presented in the background document and the substance profile support NTP's classification of styrene in the 12th RoC as "reasonably anticipated to be a human carcinogen". The committee's assessment is based on the following listing criterion: "there is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded".[6] Neither the background document nor the substance profile was explicit about how NTP defined the term *limited* in the context of the epidemiology evidence. So, the committee used its professional judgment (described in its independent assessment in Chapter 3) to develop and apply a set of factors that it used to evaluate the credibility of evidence on human carcinogenicity of styrene.

Cancer Studies in Experimental Animals

The section "Cancer Studies in Experimental Animals" in the NTP substance profile and supporting information in the background document summarized findings from several studies in which a carcinogenic response was evaluated in mice or rats after administration of styrene by various routes (inhalation, ingestion via gavage or drinking water, and injection). The committee is not aware of any informative studies of styrene carcinogenicity in animals that were available before June 10, 2011, and were not included in the NTP evaluation.

In the substance profile, NTP correctly focused on the key animal studies that provide evidence for and against styrene carcinogenicity. The substance profile states that lung tumors were not observed in styrene-treated rats and briefly summarized equivocal findings regarding mammary gland tumors. Findings of lung tumors in CD-1 mice after inhalation exposure[7] and supporting data

[5]Karami, S., P. Boffetta, P. Brennan, P.A. Stewart, D. Zaridze, V. Matveev, V. Janout, H. Kollarova, V. Bencko, M. Navratilova, N. Szeszenia-Dabrowska, D. Mates, J.P. Gromiec, R. Sobotka, W.H. Chow, N. Rothman, and L.E. Moore. 2011. Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. J. Occup. Environ. Med. 53(2):218-223.

[6]NTP (National Toxicology Program). 2011b. Listing Criteria [online]. Available: http://ntp.niehs.nih.gov/?objectid=47B37760-F1F6-975E-7C15022B9C93B5A6.

[7]Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C. Bevan, C.J. Hardy, D.W. Coombs, P.A. Mullins, and W.R. Brown. 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J. Appl. Toxicol. 21(3):185-198.

from a study of perinatal styrene exposure in mice[8] are appropriately described, as are the negative findings.

The background document and substance profile also described a National Cancer Institute oral (gavage) study,[9] which found that alveolar and bronchiolar adenomas and carcinomas combined were increased significantly in male B6C3F1 mice compared with concurrent study controls. The issue of controls is not discussed in the substance profile and appears only in the background document. It is easy for studies conducted in different laboratories, even under the same experimental protocol, to vary in subtle but important respects and consequently to yield different tumor incidences. Therefore, drawing historical controls from other laboratories is seldom justified. The committee considers the comparison of *concurrent* controls in the NCI styrene oral bioassay with *historical vehicle* control data from other laboratories to be of little value. The same concern applies to comparison with *historical untreated* controls in the NCI bioassay. Therefore, in the case of the NCI styrene bioassay, the interpretive value of comparison with *historical untreated* controls is also of limited value. Although limited in number, the *historical vehicle* controls from the same laboratory at about the same time are most relevant and are consistent with the *concurrent* controls. The committee finds that the use of *concurrent controls* reported by the National Cancer Institute is appropriate.

Studies on Mechanisms of Carcinogenesis

The section "Studies on Mechanisms of Carcinogenesis" in the NTP substance profile for styrene and supporting information in the background document summarize the mechanistic events that might link styrene exposure to cancer in experimental animals and humans. The mechanistic evidence on styrene and its major metabolites that was available to NTP is extensive and comes from a variety of studies in diverse model systems and from exposed humans. Although neither the substance profile nor the background document provides the exact search strategy that was used in collecting the evidence on the mechanisms of carcinogenesis, these documents present a balanced, comprehensive, and thorough review of the literature on the subject. Evidence tables and narrative descriptions of each study were used in the background document to present mechanistic evidence from primary studies and meta-analyses, and the committee finds the presentation of information to be inclusive and balanced.

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[8]Ponomarkov, V., and L. Tomatis. 1978. Effects of long-term oral administration of styrene to mice and rats. Scand. J. Work Environ. Health 4(suppl. 2):127-135.

[9]NCI (National Cancer Institute). 1979a. Bioassay of Styrene for Possible Carcinogenicity. Technical Report No. 185. NIH 79-1741. National Cancer Institute, National Institute of Health, Bethesda, MD [online]. Available: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr185.pdf.

The committee agrees with the conclusions presented in the background document and the substance profile that the mechanisms of styrene carcinogenicity are not fully understood. Overall, the background document and substance profile clearly stated that the carcinogenicity of styrene depends on its metabolism to styrene-7,8-oxide and other reactive intermediates and that such metabolism occurs in both rodents and humans. Even though species, tissue, and individual differences in metabolic capacity or in enzymes involved in styrene metabolism have been reported, strong evidence presented in the substance profile and the background document for styrene suggests that mechanistic events that may lead to carcinogenesis (such as genotoxicity) occur in both exposed rodents and humans. Furthermore, the listing correctly states that multiple mechanistic events may occur and that they are not necessarily mutually exclusive.

Summary and Conclusions for the Committee's Review of the Styrene Profile in the National Toxicology Program 12th Report on Carcinogens

The committee concludes that NTP correctly determined that styrene should be considered for listing in the RoC. There is sufficient evidence of exposure to a significant number of persons residing in the United States to warrant such consideration. NTP adequately documented that exposure to styrene occurs in occupational settings and in the general public regardless of smoking status.

After conducting a scientific review of the styrene assessment presented in the NTP 12th RoC, the committee finds that the overall conclusion reached by NTP in 2011, that styrene is "reasonably anticipated to be a human carcinogen", was appropriate. The following points of the listing criteria[10] support NTP's conclusion:

- "There is limited evidence of carcinogenicity from studies in humans". Publications available to NTP as of June 10, 2011, provided limited but credible evidence that exposure to styrene is associated with lymphohematopoietic, pancreatic, and esophageal cancers. The most informative human epidemiologic studies that support that conclusion are those by Ruder et al. (2004), Wong et al. (1994), Kolstad et al. (1994), and Kogevinas et al. (1994). The evidence is limited in that chance, bias, or confounding factors could not be adequately excluded.

[10]NTP (National Toxicology Program). 2008a. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf.

- "There is sufficient evidence of carcinogenicity from studies in experimental animals". Literature published by June 10, 2011, provided sufficient evidence that "there is an increased incidence of . . . a combination of malignant and benign tumors" in experimental animals induced by styrene administered by multiple routes of exposure (inhalation and oral gavage). The most informative experimental animal studies that support that conclusion are studies in mice (NCI 1979; Cruzan et al. 2001).
- "There is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans". Literature published by June 10, 2011, provided convincing evidence that genotoxicity is observed in cells from humans who were exposed to styrene. That evidence is derived from a large body of publications. In addition, styrene-7,8-oxide "was listed in a previous Report on Carcinogens as . . . reasonably anticipated as a human carcinogen". Styrene-7,8-oxide, a compound that is structurally related to styrene, is a major metabolite of styrene in both experimental animals and humans; it was first listed in the 10th RoC as reasonably anticipated to be a human carcinogen.

INDEPENDENT ASSESSMENT OF STYRENE

The second part of the committee's task was to conduct an independent assessment of styrene carcinogenicity. The committee started with the review it undertook in the first part of its task and the background document that supports the styrene profile in the 12th RoC. It searched for additional peer-reviewed literature published by November 13, 2013. Relevant human, experimental animal, and mechanistic studies were incorporated into this independent assessment. The cut-off date for the literature search was chosen to allow the committee time to review the literature within the time constraints of the project schedule. Details of the search strategy, exclusion criteria, and corresponding literature trees are provided in Appendix D of this report. Although the committee focused its attention on literature that contained primary data, it did examine review articles published in peer-review journals and reviews by other authoritative bodies to ensure that relevant literature was not missed and to ensure that all plausible interpretations of primary data were considered.

In accordance with the listing criteria, expert judgment was used to interpret and apply the RoC listing criteria to evidence in human and animal studies and to make an independent listing recommendation for styrene. A substance can be classified in the RoC as "reasonably anticipated to be a human carcinogen" if at least one of the following three criteria are fulfilled:

- "There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded."

- "There is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset."
- "There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans."

In the committee's peer review of the substance profile for styrene (Chapter 2), it discussed the information that was needed to meet the criterion for sufficient evidence in experimental animals. The type of information needed to meet the criterion for limited or sufficient evidence in humans required more interpretation and expert judgment on behalf of the committee. In its evaluation of the epidemiology literature, the committee described the information it used to identify informative studies and to evaluate those studies.

Metabolism and Toxicokinetics

The metabolism of styrene is key to its toxic and carcinogenic effects. Organ-specific tumorigenic responses to styrene will depend, in large part, on the balance between the rate of activation and the rate of detoxification in each organ. Thorough information on styrene activation and detoxification rates specific to target sites, particularly in the human, is not available. Given the wide array of CYP450 isozymes that can oxidize styrene, including forms that are known to be expressed in extrahepatic tissues (for example, CYP2E1 and CYP2A13), it is not possible to exclude the possibility that styrene bioactivation can occur in multiple target tissues. The presence of styrene-7,8-oxide in blood indicates that there is widespread tissue exposure to this genotoxic metabolite even in tissues that have low capacity for styrene activation. That highlights the importance of cellular detoxification capacities relative to organ-specific effects of styrene. In tissues that have low activity of epoxide hydrolase or glutathione-*S*-transferase, it might take only low levels of oxidation of styrene to produce cellular effects. The absence of marked toxicity in organs other than the liver or lung of mice suggests detoxification capacities in that species are sufficient to prevent overt toxicity, except in the liver and lung. However, specific information on capacities for detoxification of styrene metabolites (such as epoxide hydrolase and glutathione-*S*-transferase) in critical target tissues in humans is not available.

Therefore, the available information on styrene metabolism is insufficient to exclude any tissue from being a plausible target for styrene-induced cytotoxicity, which could contribute to carcinogenesis.

Epidemiologic Studies

As was mentioned in the committee's peer review of the substance profile, it used its professional judgment to develop and apply a set of factors to evaluate the credibility of evidence on the human carcinogenicity of styrene. Those factors were high estimates of relative risks or its surrogates; exposure–response relationships for any reliably established exposure metric; consistency of observations among independent cohort studies of the reinforced-plastics industry or between cohort and case–control studies; and at least two informative studies in independent populations or with varied study designs. The committee judged the evidence to be *limited* if the epidemiology evidence was credible but chance, bias, and confounding could not be adequately excluded. The evidence was judged to be *sufficient* if the epidemiology evidence was credible and chance, bias, and confounding could be excluded as an alternative explanation for the observed association.

The committee identified what it judged to be the eleven most informative epidemiologic publications: six studies that used four cohorts in the reinforced-plastics industry that were conducted in Europe[11] and the United States[12] and five case–control studies conducted in Europe[13] and Canada.[14] The results of

[11]Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261; Kolstad, H.A., E. Lynge, J. Olsen, and N. Breum. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand. J. Work Environ. Health. 20(4):272-278; Kolstad, H.A., K. Juel, J. Olsen, and E. Lynge. 1995. Exposure to styrene and chronic health effects: Mortality and incidence of solid cancers in the Danish reinforced plastics industry. Occup Environ Med. 52(5):320-327.

[12]Wong, O., L.S. Trent, and M.D. Whorton. 1994.An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup. Environ. Med. 51(6):386-396; Ruder, A.M., E.M. Ward, M. Dong, A.H. Okun, and K. Davis-King. 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. Am. J. Ind. Med. 45(2):165-176; Collins, J.J., K.M. Bodner, and J.S. Bus. 2013. Cancer mortality of workers exposed to styrene in the U.S. Reinforced plastics and composite industry. Epidemiology 24(2):195-203.

[13]Scélo, G., V. Constantinescu, I. Csiki, D. Zaridze, N. Szeszenia-Dabrowska, P. Rudnai, J. Lissowska, E. Fabiánová, A. Cassidy, A. Slamova, L. Foretova, V. Janout, J. Fevotte, T. Fletcher, A. Mannetje, P. Brennan, and P. Boffetta. 2004. Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe). Cancer Causes Control 15(5):445-452; Seidler, A., M. Mohner, J. Berger, B. Mester, E. Deeg, G.

those studies were evaluated to inform a judgment of whether the evidence of carcinogenesis in humans after exposure to styrene is sufficient, limited, or inconclusive. The following end points were evaluated:

- **Lymphohematopoietic Cancers.** The epidemiologic data provide credible but limited evidence that styrene is a risk factor for lymphohematopoietic cancers on the basis of two European cohort studies.[15] Studies of specific types of lymphohematopoietic cancer generate standardized mortality ratios, standardized incidence ratios, and relative risks with wider confidence intervals because of the smaller number of observed events (cancer incidence or deaths). Specifically, the epidemiologic data provide credible but limited evidence that styrene exposure is a risk factor for leukemia on the basis of the same two European cohort studies. The epidemiologic data provide credible but limited evidence that styrene exposure is a risk factor for non-Hodgkin lymphoma on the basis of a cohort study[16] and two case–control studies.[17] Be-

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Elsner, A. Nieters, and N. Becker. 2007. Solvent exposure and malignant lymphoma: A population-based case-control study in Germany. J. Occup. Med. Toxicol. 2:2; Cocco, P., A. t'Mannetje, D. Fadda, M. Melis, N. Becker, N., S. de Sanjose, L. Foretova, J. Mareckova, A. Staines, S. Kleefeld, M. Maynadie, A. Nieters, P. Brennan, and P. Boffetta. 2010. Occupational exposure to solvents and risk of lymphoma subtypes: Results from the Epilymph case-control study. Occup. Environ. Med. 67(5):341-347; Karami, S., P. Boffetta, P. Brennan, P.A. Stewart, D. Zaridze, V. Matveev, V. Janout, H. Kollarova, V. Bencko, M. Navratilova, N. Szeszenia-Dabrowska, D. Mates, J.P. Gromiec, R. Sobotka, W.H. Chow, N. Rothman, and L.E. Moore. 2011. Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. J. Occup. Environ. Med. 53(2):218-223.

[14]Gerin, M., J. Siemiatychi, M. Desy, and D. Krewski. 1998. Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: Results of a case-control study in Montreal. Am. J. Ind. Med. 34:144-156.

[15]Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261; Kolstad, H.A., E. Lynge, J. Olsen, and N. Breum. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand. J. Work Environ. Health 20(4):272-278.

[16]Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261.

[17]Gerin, M., J. Siemiatychi, M. Desy, and D. Krewski. 1998. Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: Results of a case-control study in Montreal. Am. J. Ind. Med. 34:144-156; Cocco, P., A. t'Mannetje, D. Fadda, M. Melis, N. Becker, N., S. de Sanjose, L. Foretova, J. Mareckova, A. Staines, S. Kleefeld, M. Maynadie, A. Nieters, P. Brennan, and P. Boffet-

cause Hodgkin's lymphoma and multiple myeloma are rarer and there is a paucity of data from existing studies, the committee concludes that there are insufficient data to assess whether exposure to styrene is associated with an increase in the frequency of these two malignancies.

- **Kidney Cancer:** The epidemiologic data provide credible but limited evidence that styrene is a carcinogen for the kidney on the basis of the US cohort studies[18] and a European case–control study.[19]
- **Pancreatic Cancer:** The epidemiologic data on pancreatic cancer constitute credible but limited evidence that styrene exposure is associated with pancreatic cancer on the basis of four cohort studies.[20]
- **Esophageal Cancer:** The epidemiologic data on esophageal cancer constitute credible but limited evidence that styrene exposure is associated with esophageal cancer on the basis of observations from three cohort studies.[21]

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ta. 2010. Occupational exposure to solvents and risk of lymphoma subtypes: Results from the Epilymph case-control study. Occup. Environ. Med. 67(5):341-347.

[18]Wong O., Trent L.S., Whorton MD. 1994. An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup Environ Med. 51(6):386-396; Ruder AM, Ward EM, Dong M, Okun AH, Davis-King K. 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: an update. Am J Ind Med. 45(2):165-176; Collins, J.J., K.M. Bodner, and J.S. Bus. 2013. Cancer mortality of workers exposed to styrene in the U.S. Reinforced plastics and composite industry. Epidemiology 24(2):195-203.

[19]Karami, S., P. Boffetta, P. Brennan, P.A. Stewart, D. Zaridze, V. Matveev, V. Janout, H. Kollarova, V. Bencko, M. Navratilova, N. Szeszenia-Dabrowska, D. Mates, J.P. Gromiec, R. Sobotka, W.H. Chow, N. Rothman, and L.E. Moore. 2011. Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. J. Occup. Environ. Med. 53(2):218-223.

[20]Ruder, A.M., E.M. Ward, M. Dong, A.H. Okun, K. Davis-King. 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. Am. J. Ind. Med. 45(2):165-176; Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261; Kolstad, H.A., K. Juel, J. Olsen, and E. Lynge. 1995. Exposure to styrene and chronic health effects: Mortality and incidence of solid cancers in the Danish reinforced plastics industry. Occup. Environ. Med. 52(5):320-327; Collins, J.J., K.M. Bodner, and J.S. Bus. 2013. Cancer mortality of workers exposed to styrene in the U.S. Reinforced plastics and composite industry. Epidemiology 24(2):195-203.

[21]Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261; Wong, O., L.S. Trent, and M.D. Whorton. 1994. An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup. Environ. Med. 51(6):386-396; Ruder, A.M., E.M. Ward, M. Dong, A.H. Okun, and K. Davis-King. 2004. Mortality patterns among

After identifying the most informative epidemiologic studies and evaluating their results, the committee found that there is limited evidence on the carcinogenicity of styrene on the basis of epidemiologic studies. A causal interpretation is credible, but alternative explanations—such as chance, bias, and confounding factors—cannot adequately be excluded.

Cancer in Experimental Animals

The committee did not identify any experimental animal studies using styrene or styrene-7,8-oxide that were not already cited in the background document. In general, the committee considered studies to be more informative when they included more than one dose, well-matched controls, chronic exposure, treatment groups of adequate size, the use of well-characterized test material of high purity, thorough necropsy and pathologic evaluation of tissues according to established criteria, and statistical evaluation of tumor data with accepted methods. The quality of the studies varied considerably; the value of some of them is limited by the numbers of animals treated, exposure duration, observation period, dose selection, or incomplete reporting of methods or results. Studies were considered less informative if any of those attributes were missing or could not be verified from the study description.

Positive findings of lung tumors in mice have been observed after both inhalation and oral administration of styrene in well-conducted chronic bioassays.[22] Results of another study that is more limited in value for assessing carcinogenicity[23] are also reasonably consistent with the production of lung tumors in mice after styrene exposure. Studies of rats exposed to styrene by both oral and inhalation routes have been consistently negative;[24] the tumorigenic response appears to be species-specific.

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workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. Am. J. Ind. Med. 45(2):165-176.

[22]NCI (National Cancer Institute). 1979. Bioassay of Styrene for Possible Carcinogenicity. Technical Report No. 185. NIH 79-1741. National Cancer Institute, National Institute of Health, Bethesda, MD [online]. Available: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr185.pdf.; Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C. Bevan, C.J. hardy, D.W. Coombs, P.A. Mullins, and W.R. Brown. 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J. Appl. Toxicol. 21(3):185-198.

[23]Ponomarkov, V., and L. Tomatis. 1978. Effects of long-term oral administration of styrene to mice and rats. Scand. J. Work Environ. Health 4(suppl. 2):127-135.

[24]Jersey, G.C., M.F. Balmer, J.F. Quast, C.N. Park, D.J. Schuetz, J.E. Beyer, K.J. Olson, S.B. McCollister, and L.W. Rampy. 1978. Two-Year Chronic Inhalation Toxicity and Carcinogenicity Study on Monomeric Styrene in Rats. Final Report. The Dow Chemical Company, Midland, MI.; NCI (National Cancer Institute). 1979. Bioassay of Styrene for Possible Carcinogenicity. Technical Report No. 185. NIH 79-1741. National Cancer Institute, National Institute of Health, Bethesda, MD [online]. Available: http://ntp.niehs.

A carcinogenic effect of exposure by more than one route in animals, even if in only one species, satisfies the criteria for "reasonably anticipated to be a human carcinogen". Thus, styrene-increased incidences of lung tumors in male mice after both inhalation and oral exposure constitute adequate evidence for listing under this classification.

Mechanistic and Other Relevant Data

The committee reviewed the evidence for styrene carcinogenicity via three modes of action: genotoxicity, immunosuppression, and cytotoxicity. The genotoxicity of styrene has been thoroughly and comprehensively investigated. Observations in various studies performed over the last 3 decades have been consistent. Temporal and exposure–response relationships have been established. Not only is the experimental evidence extensive, it is likely to be relevant to all target tissues that have been associated with cancer after exposure to styrene. A causal interpretation is strengthened by the large amount of evidence obtained from studies of exposed humans. Furthermore, the evidence reviewed by the committee indicates that styrene-7,8-oxide, a major reactive metabolite of styrene that is produced in exposed humans, reacts with DNA to form covalent adducts and other premutagenic forms of DNA damage, which result in genotoxic effects. The committee recognizes that styrene-7,8-oxide may not be the only genotoxic metabolite of styrene. For example, styrene-3,4-oxide may also be mutagenic.[25] However, to the committee's knowledge, the potential contribution of styrene-3,4-oxide to the carcinogenic response to styrene and the potential contribution of other aromatic-ring metabolites of styrene in addition to styrene-7,8-oxide have not been investigated.

Information pertaining to styrene exposure and immunosuppression is variable and inconsistent. In animals, the committee observed both inhibitory effects (such as decreases in lymphocyte counts, suppressed monocyte and macrophage activity, and suppressed natural killer cell activity) and stimulatory effects

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nih.gov/ntp/htdocs/LT_rpts/tr185.pdf; Beliles, R.P., J.H. Butala, C.R. Stack, and S. Makris. 1985. Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fundam. Appl. Toxicol. 5(5):855-868; Conti, B., P. Maltoni, G. Perino, and A. Ciliberti. 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. Ann. NY Acad. Sci. 534:203-234; Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C.J. Hardy, D.W. Coombs, P.A. Mullins, and W.R. Brown. 1998. Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol. Sci. 46(2):266-281.

[25]Watabe, T., N. Ozawa, and K.Yoshikawa. 1982. Studies on metabolism and toxicity of styrene. V. The metabolism of styrene, racemic, (*R*)-(+)-, and (*S*)-(–)-phenyloxiranes in the rat. J. Pharmacobiodyn. 5(2):129–133.

(such as increases in type IV hypersensitivity and increased cytokine production). In humans exposed to styrene, effects were more varied and both suppressive and stimulatory effects were observed. Additional research is needed to understand the effects of styrene on the immune system and to understand whether immunosuppression is a possible mechanism for styrene-induced carcinogenesis.

The evidence pertaining to cytotoxicity indicates that this mode of action and later proliferation at injured sites depends on the cellular, metabolic, and chemical processes involved in different organs and how their interactions modulate the toxic response. When the response in the liver was compared with that in the lung, it became clear that at least two organs are targets for styrene and its circulating metabolites. Studies of workers in the styrene industry found styrene or its metabolites in both blood and urine and identified additional target organs in at least three other systems—the lymphohematopoietic system (bone marrow, lymph nodes, and spleen), gastrointestinal system (esophagus and pancreas), and urinary system (kidney and bladder). The toxic responses of multiple organs may play a role in modulating the circulating concentrations of styrene, its metabolites, and other key compounds, such as glutathione, and in affecting the toxic response of other organs in the same individual.

Summary of Evidence and Conclusions

The statement of task (Appendix B) directed the committee to "integrate the level-of-evidence conclusions, and considering all relevant information in accordance with the RoC listing criteria, make an independent listing recommendation for styrene and provide scientific justification for its recommendation." As discussed throughout this report, a substance can be categorized as reasonably anticipated to be a human carcinogen on the basis of sufficient evidence in animals or limited evidence in humans and a substance can be categorized as known to be a human carcinogen on the basis of sufficient evidence in humans. Guided by the RoC listing criteria, the committee integrated data from individual studies to determine whether the evidence in experimental animals reached the level of limited or sufficient and to determine whether the evidence in humans reached the level of limited or sufficient. Supporting information was provided from mechanistic studies. The RoC listing criteria do not provide guidance on the integration of information across data streams (that is, across human, experimental animal, and mechanistic information) or the reconciliation of cross-data inconsistencies, so the committee only integrated information within data streams to derive a listing recommendation.

The committee identified evidence that styrene exposure could potentially lead to carcinogenicity through genotoxic and mutagenic mechanisms, and that evidence is considered strong, inasmuch as it has been found in vivo and in vitro in both humans and rodents. The genotoxic mechanism is probably relevant for all target tissues associated with cancer after exposure to styrene. Identification

of styrene metabolites, such as styrene-7,8-oxide, strongly supports the production of reactive intermediates in a variety of tissues in both humans and animals. The reactive metabolites, which may be produced in one organ and transported to produce toxicity in other sites, have been identified in the blood of humans exposed to styrene. Animal toxicology and carcinogenesis studies clearly support the possibility that multiple organs can be affected regardless of their capacity for metabolic activation. In humans, evidence of carcinogenicity in multiple organs is credible but limited. Those findings were based on large occupational cohort studies in the reinforced-plastics industry and on case–control studies.

In sum, the committee finds that compelling evidence exists to support a listing of styrene as, at a minimum, *reasonably anticipated to be a human carcinogen*. That conclusion is based on credible but limited evidence of carcinogenicity in traditional epidemiologic studies, on sufficient evidence of carcinogenicity in animals, and on convincing evidence that styrene is genotoxic in exposed humans.

The listing criteria state that a substance should be classified as known to be a human carcinogen if "there is sufficient evidence of carcinogenicity from studies in humans". The footnote associated with that sentence states that "this evidence can include data derived from the study of tissues or cells from humans exposed to [styrene] that can be useful for evaluating whether a relevant cancer mechanism is operating in people". The evidence of styrene genotoxicity in exposed humans is convincing, so a strong argument could be made to support the listing of styrene as a *known human carcinogen* if data derived from the study of tissues or cells from humans in and of themselves are considered sufficient for making such a determination**.** The committee notes that there is ambiguity with respect to weighing the mechanistic evidence in applying the listing criteria.

The types of evidence that are available to determine the listing and classification of substances in the RoC continue to evolve. In the future, there will probably be more powerful mechanistic evidence in exposed humans to use for cancer hazard evaluation. Similarly, improvements in exposure-assessment methods may be developed to improve the identification and characterization of exposed persons. This is true not only for styrene and styrene-7,8-oxide, but for all substances in the RoC. Thus, the committee finds that further clarification and expanded guidance by NTP regarding the types and strength of mechanistic evidence and the use of that evidence in the context of the RoC listing criteria are warranted.

1

Introduction

People in the United States are exposed to styrene from environmental sources and in occupational settings. Sources of environmental exposures include food (from migration of styrene from polymer packaging materials), cigarette smoke, vehicle exhaust, and "other forms of combustion and incineration of styrene polymers" (IARC 2002, p. 456). Occupational exposure to humans can occur during industrial processes that use styrene (ACGIH 2001; IARC 2002). To improve understanding of those exposures and potential adverse health outcomes, including cancer, scientists have studied styrene in vitro and in humans and animals for many years (IARC 2002; NTP 2011a).

The National Toxicology Program (NTP) assessed the potential carcinogenicity of styrene over the past decade and styrene was first listed in the 12th Report on Carcinogens (RoC) as *reasonably anticipated to be a human carcinogen* (NTP 2011a). Given some of the scientific controversies surrounding the assessment of styrene (Hegstad 2011, 2012) , Congress directed the Department of Health and Human Services (DHHS) to arrange for the National Academy of Sciences to carry out an independent review of the styrene substance profile in the 12th RoC (112th Congress, 1st Session; Public Law 112-74). This report presents findings and conclusions in response to the congressional request.

THE REPORT ON CARCINOGENS

NTP is an interagency program of the National Institute of Environmental Health Sciences (NIEHS), the administrative lead and a part of the National Institutes of Health; the National Institute for Occupational Safety and Health, a part of the Centers for Disease Control and Prevention; and the National Center for Toxicological Research, a part of the Food and Drug Administration. NTP publishes the RoC, which was congressionally mandated in 1978 as part of the Public Health Service Act (Section 262, Public Law 95-622, Part E). The act directed DHHS to publish an annual report that includes a list of all substances that meet two conditions: a significant number of people living in the United States are exposed and the substance is either known to be a human carcinogen or reasonably anticipated to be a human carcinogen. The RoC was required to include supporting information, such as the nature of exposure and an estimated number of persons exposed. The full congressional mandate is in Box 1-1. A

1993 amendment changed the RoC from an annual to a biennial report (42 US Code 241).

Nominations for substances to be added to, reclassified in, or removed from the RoC can come from anyone, but the submitter must include a rationale and, if possible, include background information to support the proposed change (NTP 2011a). Staff of the Office of the Report on Carcinogens review each submission and decide whether there is enough supporting evidence to move a substance forward for further evaluation. If so, the staff invite partnering agencies to review the substance, solicit public comments through the *Federal Register*, and develop a brief draft concept document with information on the substance, including exposure, major relevant issues, and approach to the cancer-evaluation component of the draft RoC. After consideration of comments from NTP's Board of Scientific Counselors and the public, the NTP director makes the final decision of whether the substance will be evaluated in a later RoC volume.

BOX 1-1 Congressional Language Mandating the Report on Carcinogens

A. a list of all substances
   i. which either are known to be carcinogens or may reasonably be anticipated to be carcinogens and
   ii. to which a significant number of persons residing in the United States are exposed;
B. information concerning the nature of such exposure and the estimated number of persons exposed to such substances;
C. a statement identifying
   i. each substance contained in the list under subparagraph (A) for which no effluent, ambient, or exposure standard has been established by a Federal agency, and
   ii. for each effluent, ambient, or exposure standard established by a Federal agency with respect to a substance contained in the list under subparagraph (A), the extent to which, on the basis of available medical, scientific, or other data, such standard, and the implementation of such standard by the agency, decreases the risk to public health from exposure to the substance; and
D. a description of
   i. each request received during the year involved
      I. from a Federal agency outside the Department of Health, Education, and Welfare for the Secretary, or
      II. from an entity within the Department of Health, Education, and Welfare to any other entity within the Department, to conduct research into, or testing for, the carcinogenicity of substances or to provide information described in clause (ii) of subparagraph (C), and
   ii. how the Secretary and each such other entity, respectively, have responded to each such request.

Source: Section 262, Public Law 95-622, Part E (pp. 3435-3436).

The RoC is cumulative and includes all substances known or reasonably anticipated to be human carcinogens that have been listed since the first RoC in 1980. The 12th RoC has 240 listings: 54 substances known to be human carcinogens and 186 substances reasonably anticipated to be human carcinogens. The criteria that are currently used to establish a listing as either known or reasonably anticipated to be a human carcinogen have been in use since the eighth RoC, which was published in 1998. Box 1-2 provides the specific listing criteria.

In preparation for a new RoC volume, the Office of the Report on Carcinogens creates a background document for each substance that is being considered for inclusion in the next ROC. Each background document includes an evaluation of the substance's properties, production, and use; human exposure to the substance; the substance's toxicokinetics; cancer studies of the substance in humans and animals; and possible mechanisms of cancer induction by the substance. The goal of the evaluation is to describe the strengths, limitations, and overall quality of the evidence. For the most recent RoC, the 12th, each background document that was created underwent review by an expert panel, and that panel was asked to recommend a listing status for the substance under review (see Figure 1-1). NTP also asked an Interagency Scientific Review Group and an NIEHS/NTP Scientific Review Group to recommend a listing status for the substances under review. The draft substance profile was then prepared and peer-reviewed by the NTP Board of Scientific Counselors. Public comments were solicited at multiple stages in the process. The draft was submitted to the NTP director for review and to the NTP Executive Committee[1] for consultation, review, and comment. It then went to the NTP director for final approval and finally to the secretary of health and human services for review, approval, and transmittal to Congress. The 12th RoC was released in 2011.

STYRENE

One substance profile in the 12th RoC that has drawn science, policy, and mass-media attention is that of styrene. Styrene is an oily or viscous, colorless to yellowish liquid (IARC 2002; O'Neil et al. 2006). It has a pungent odor that has been described as sweet or floral (IARC 2002; NTP 2008a). In 2000, 10.79 billion pounds of styrene were produced in the United States (HSDB 2005) and by

[1]The NTP Executive Committee is made up of the heads of the Consumer Product Safety Commission, the Department of Defense, the Environmental Protection Agency, the Food and Drug Administration, the National Cancer Institute, the National Center for Environmental Health, the Agency for Toxic Substances and Disease Registry, the National Institute of Environmental Health Sciences, the National Institute for Occupational Safety and Health, and the Occupational Safety and Health Administration. The committee gives programmatic and policy advice to the NTP director.

BOX 1-2 Listing Criteria for the Report on Carcinogens

Known To Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans,* which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated To Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans,* which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded,

or

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset,

or

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive subpopulations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question, which can be useful for evaluating whether a relevant cancer mechanism is operating in humans.

Source: NTP 2008a, p.v.

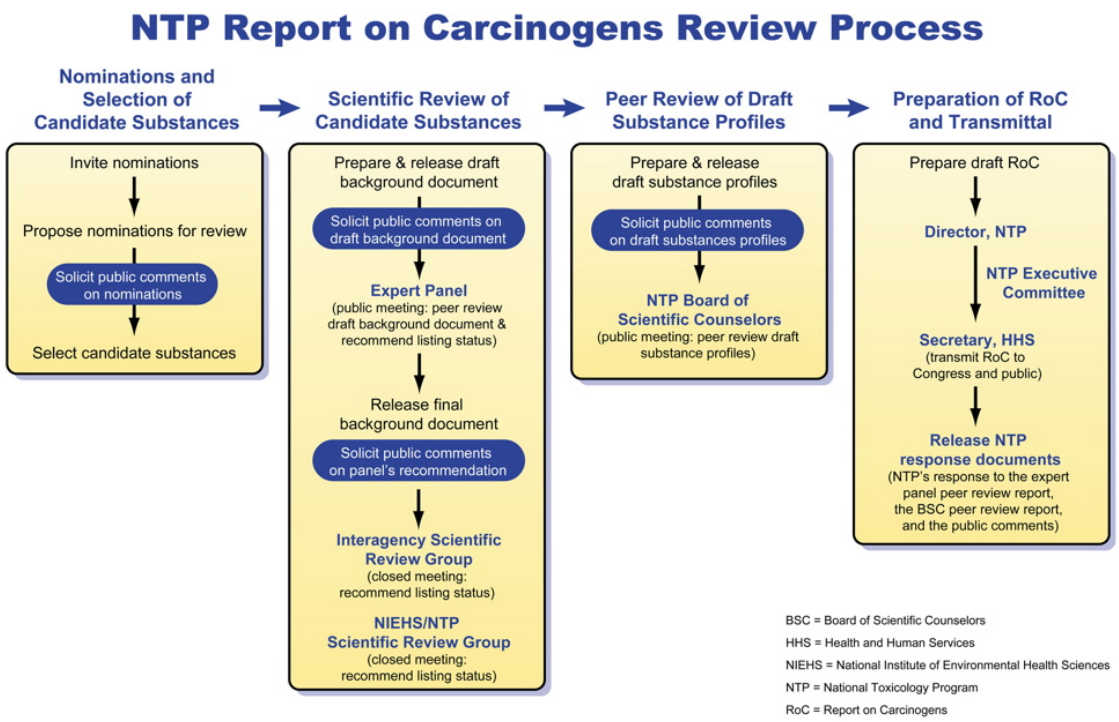

FIGURE 1-1 Schematic of the review process for the 12th Report on Carcinogens. Source: NTP 2011b.

2006, the production capacity was more than 13 billion pounds (ATSDR 2010). Over half the styrene that is produced in the United States is used for the manufacture of polystyrene, which is a component of such products as plastic packaging, building and refrigeration-equipment insulation, and disposable plates and cups (NTP 2008a). A smaller quantity is used as an intermediate in synthesizing styrene polymers and copolymers, such as styrene–acrylonitrile, acrylonitrile–butadiene–styrene, and styrene–butadiene rubber (ATSDR 2010). Styrene and styrene polymers and copolymers are used in the manufacture of a wide array of products, including food containers, toys, electric devices, automobile parts, construction items, waxes and polishes, adhesives, and personal-care products (WHO 2000; NTP 2008a). It is also used in polyester resins for fiber-reinforced plastics, such as boats, bathtubs, and tanks (NTP 2008a). In 2006, the US consumption of styrene was 9.6 billion pounds (Berthiaume and Ring 2006).

The general public is mainly exposed to styrene in indoor environments. Sampling indicates the median concentration of styrene in indoor air ranges from 0.07 to 11.5 parts per billion (ppb) (ATSDR 2010). Sources of emissions include off-gassing of building materials, skin contact with consumer products that contain styrene, and tobacco smoke (NTP 2008a; ATSDR 2010). People can also be exposed outdoors from combustion sources, such as automobiles, waste incinerators, wood stoves, and industrial facilities. Median styrene concentrations in urban air samples range from 0.07 to 4.6 ppb and rural and suburban samples range from 0.06 to 0.1 ppb (ATSDR 2010). In the general population, the average daily exposure to styrene in air is estimated to be 18 to 54 µg/person/day and the average daily exposure to styrene in food is estimated to be 0.2 to 1.2 µg/person/day (ATSDR 2010).

The largest occupational exposures are in the reinforced-plastics, styrene–butadiene, and styrene monomer and polymer industries (IARC 2002; NTP 2008a). Surveys conducted in 1962 and 1976 of US plants that manufactured styrene-based products indicated that the average exposure of employees to styrene was below 10 ppm (43 mg/m^3) (Ott et al. 1980). Another study indicated average occupational exposures to styrene rarely exceed 20 ppm (85 mg/m^3), but instances where such exposures do occur are usually the result of occasional bursts and leakages of reactors, tubing, and other equipment (Tossavainen 1978; IARC 2002).

A major metabolite of styrene is styrene-7,8-oxide, and there is evidence that this metabolite has genotoxic and mutagenic properties. Styrene-7,8-oxide was listed by the International Agency for Research on Cancer (IARC) as probably carcinogenic in humans (IARC 1994) and first listed in 2002 in the NTP RoC as reasonably anticipated to be a human carcinogen (NTP 2002). Because of the genotoxic properties of a major metabolite of styrene (styrene-7,8-oxide) and because the general population is exposed to styrene through several different routes, styrene is of concern to many scientific bodies, including NTP. NTP's Web site states that the chemical was nominated for inclusion by a private individual on the basis of the IARC finding that there is some evidence in humans and experimental animals of the carcinogenicity of styrene (NTP 2007).

After the multistep review process described above, styrene was listed as "reasonably anticipated to be a human carcinogen" (NTP 2011a, p. 383) in the 12th RoC.

Several IARC working groups have reviewed the potential carcinogenicity of styrene and the most recent review was published in 2002 (IARC 1979, 1987, 1994, 2002). In 1979, IARC determined that styrene was mutagenic, and it supported the need for additional epidemiologic research. In an update of IARC monograph volumes 1–42 in 1987, IARC listed styrene in group 2B (possibly carcinogenic in humans) on the basis of inadequate human data, limited evidence in animals, and supporting genotoxic data that showed its mutagenic potential. Although new information was incorporated into an IARC monograph in 1994, the listing of styrene continued to cite human data as inadequate and animal data as limited, and the listing of the substance remained categorized in group 2B. In the most recent IARC review of styrene (IARC 2002), the human, animal, and mechanistic evidence was updated, and the working group again came to the conclusion that styrene was possibly carcinogenic in humans (group 2B); however, IARC's categorization of styrene in this category is now based on limited evidence in humans and limited evidence in animals.

Other agencies and organizations have also reviewed the potential carcinogenicity of styrene. The Agency for Toxic Substances and Disease Registry undertook a review of styrene and determined that styrene may be a weak human carcinogen on the basis of studies in humans and animals (ATSDR 2010). In contrast, the American Conference of Governmental Industrial Hygienists (ACGIH) reviewed the epidemiologic evidence and animal bioassay data on styrene and determined that styrene exposure does not result in an excess risk of cancer. On the basis of the available data, ACGIH has listed styrene in category A4 (not classifiable as a human carcinogen). The European Union undertook a risk assessment of styrene and concluded that, "based on human studies, there is no clear and consistent evidence for a causal link between specific cancer mortality and exposure to styrene" (EU 2008, p. 271). The reason for different classifications from different organizations is partly a reflection of the criteria used and the process by which each organization integrates information to reach a conclusion on carcinogenicity, but it is also a reflection of the amount of evidence on styrene carcinogenicity in the scientific literature and some of the uncertainties surrounding that literature at the time of the assessment.

THE COMMITTEE'S TASK

In 2012, as part of the Consolidated Appropriations Act (112th Congress, 1st Session; Public Law 112-74), Congress directed the assistant secretary for health in the Department of Health and Human Services to contract with the National Research Council "to conduct a scientific peer review of the 12th Report on Carcinogens determinations related to formaldehyde and styrene. Included in the review should be all relevant, peer-reviewed research related to

formaldehyde and styrene." In response, the National Research Council convened the Committee to Review the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens, which wrote the present report. The committee included experts in epidemiology, exposure assessment, toxicology, toxicokinetic modeling, and mechanisms of carcinogenesis (see Appendix A for biographic information on the committee).

The committee's Statement of Task is presented in Appendix B. The committee was asked to conduct a peer review of the styrene assessment in the 12th RoC. As part of that review, it was asked to identify and evaluate relevant peer-reviewed literature, with particular emphasis on literature that had been published as of June 10, 2011, the release date of the 12th RoC. The committee was also asked to undertake an independent assessment of styrene, which was to include documentation of its decisions for inclusion or exclusion of literature, identification of the most critical studies and information, application of the RoC listing criteria to the scientific evidence, and independent level-of-evidence determinations with respect to the human and animal studies. Considering all relevant information in accordance with the RoC listing criteria, the committee was asked to make an independent listing recommendation for styrene and provided scientific justification of its recommendation.

THE COMMITTEE'S APPROACH

In writing its report, the committee reviewed documents pertaining to styrene that were written for or by NTP in preparation for the 12th RoC (see Table 1-1). It considered presentations heard during its open-session meeting, comments submitted by the general public,[2] and abstracts presented during recent conferences. It reviewed reports published by other authoritative bodies, and it examined primary literature, reviews, and meta-analyses publicly available in the peer-reviewed literature. The committee relied on its own expert judgment to assess the body of evidence related to the potential carcinogenicity of styrene. It was guided by the language and terminology of the RoC listing criteria (see Box 1-2), although some of the language in the criteria, such as *sufficient* and *limited*, required informed interpretation. The committee worked toward the goal of clearly describing its methods in writing this report, how it used the language of the listing criteria, and its analysis of the body of evidence related to styrene.

[2]A list and copies of presentations heard during the open-session meeting and comments submitted by the general public can be obtained by contacting the National Academies Public Access Records Office.

TABLE 1-1 Documents Pertaining to Styrene That Were Available to or Written by NTP

Document	Brief Description	Reference
Substance profile for styrene	The substance profile as presented in the 12th RoC.	NTP 2011a
Background document for styrene	Background information that was prepared by staff in the Office of the Report on Carcinogens to assist in the review of styrene for the 12th RoC.	NTP 2008a
Primary literature	Primary literature cited in the background document or obtained from other sources.	—
Expert panel reports	An expert panel was charged with doing a peer review of the draft background document on styrene and making a recommendation for the listing of styrene in the 12th RoC.	Phillips et al. 2008a,b
NTP Executive Committee Interagency Scientific Review Group (ISRG) report	The interagency scientific review group reviewed the body of literature on styrene and made a recommendation for the listing of styrene in the 12th RoC.	NTP 2008b
National Institute of Environmental Health Sciences (NIEHS)/NTP Scientific Review Group report	The NIEHS NTP scientific review group reviewed the body of literature on styrene and made a recommendation for the listing of styrene in the 12th RoC.	NIEHS/NTP 2008
Minutes from a Board of Scientific Counselors (BSC) meeting	The BSC assessed whether the scientific information in the draft substance profile was technically correct, was clearly stated, and supported NTP's preliminary listing of styrene in the 12th RoC.	NTP 2009
NTP's response to the expert panel reports and to the BSC	NTP's review and response to expert panel reports.	NTP 2011b, 2011c
Public comments	Comments from the public in response to *Federal Register* notices on May 19, 2004 (Vol. 69, No. 97), May 20, 2008 (Vol. 73, No. 98), September 8, 2008 (Vol. 73, No. 174), and December 22, 2008 (Vol. 73, No. 246), and additional public comments that were not associated with any *Federal Register* notices.	see http://ntp.niehs.nih.gov/go/9920

NTP's response to public comments	NTP's responses to public comments related to specific issues from the expert panel report that were applicable to the substance profile; comments on the final background document, the review process, or nontechnical or nonscientific issues were excluded by NTP.	NTP 2011d

The assessment of chemicals for the purposes of listing in the RoC constitutes a hazard assessment, not a risk assessment. A hazard assessment focuses on the identification of substances that may pose a hazard to human health and "makes a classification regarding toxicity, for example, whether a chemical is 'carcinogenic to humans' or 'likely to be'" (NRC 2009). A risk assessment[3] focuses on the likely degree of damage and requires much more information, including completion of a hazard identification, dose–response analysis, exposure quantification, and quantitative characterization of risk (NRC 1983). The committee approached its statement of task as an evaluation of hazard, not of risk. It evaluated measures of an association in a population (such as risk ratios, odds ratios, and incidence ratios) from epidemiology studies to inform its assessment of styrene, but it did not identify exposure scenarios that could pose cancer risk as part of a full risk assessment.

The committee worked in parallel with the Committee to Review the Formaldehyde Assessment in the NTP 12th RoC, which was also convened in response to the 2012 Consolidated Appropriations Act. Three persons served on both committees. The committees had identical statements of task except for the specific substance profile being reviewed, and the two committees met jointly for their first meeting. During the open session of that joint meeting, the committees heard presentations from and had an open discussion with representatives of DHHS and NTP. The committees also heard from several stakeholders who participated in the public session. During the meeting's closed session, members discussed the open-session presentations by the sponsor and the public and the committees' approach to the statements of task. The committees did not have another joint meeting. To accomplish its task, the present committee held four additional meetings, during which it discussed literature and other materials relevant to understanding styrene carcinogenicity and developed this report.

ORGANIZATION OF THE REPORT

The committee approached its statement of task by first doing a review, reported in Chapter 2, of the substance profile for styrene as presented in the

[3]"Risk assessment is the use of the factual base to define the health effects of exposure of individuals or populations to hazardous materials and situations…. Risk assessments contain some or all of the following four steps: Hazard identification: the determination of whether a particular chemical is or is not causally linked to particular health effects. Dose–response assessment: the determination of the relation between the magnitude of exposure and the probability of occurrence of the health effects in question. Exposure assessment: the determination of the extent of human exposure before or after application of regulatory controls. Risk characterization: the description of the nature and often the magnitude of human risk, including attendant uncertainty" (NRC 1983, p. 3).

12th RoC. It considered only literature that had been published as of June 10, 2011 (the release date of the 12th RoC). The background document did not include a description of NTP's literature search, so that information was obtained directly from NTP and presented in Appendix C. Chapter 2 is organized according to the headings and subheadings of the substance profile and concludes with findings on the appropriateness of NTP's listing for styrene on the basis of the RoC listing criteria.

Chapter 3 is the committee's independent assessment of the styrene literature, including literature pertaining to styrene carcinogenicity up to November 13, 2013. The assessment was accomplished by using the 12th RoC and the background document for styrene as supplemented by a search of the literature from 2008 on. The goal of the literature search was to capture literature published since the release of the background document for styrene (NTP 2008a). Details of the search are described in Appendix D. The committee did not do a systematic review of all literature published on styrene, because it determined in Chapter 2 that NTP cited and thoroughly described in the background document the relevant styrene literature that was published before 2008.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001. Styrene. Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Ed. American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2010. Toxicological Profile for Styrene. U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substance and Disease Registry [online]. Available: http://www.atsdr.cdc.gov/toxprofiles/tp53.pdf [accessed Aug. 15, 2013].

Berthiaume, S., and K.L. Ring. 2006. Styrene. P. 694.3000A in Chemical Economics Handbook Menlo Park, CA: SRI Consulting.

EU (European Union). 2008. European Union Risk Assessment Report: Styrene. Draft for publication RO34-0806hh-publication. June 2008 [online]. Available: http://echa.europa.eu/documents/10162/13630/trd_rar_uk_styrene_en.pdf [accessed June 2, 2014].

Hegstad, M. 2011. Manufacturing caucus seeks NAS study of styrene following cancer listing. Risk Policy Report 18(46):4. November 15, 2011.

Hegstad, M. 2012. Activists seek to defend administration's cancer listing for styrene. Risk Policy Report. 19(21). May 22, 2012.

HSDB (Hazardous Substances Data Bank). 2005. Styrene: Manufacturing/Use Information, U.S. Production [online]. Available: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~UoufIx:1 [accessed April 18, 2014].

IARC (International Agency for Research on Cancer). 1979. Styrene, polystyrene and styrene-butadiene copolymers. Pp. 231-274 in Some Monomers, Plastics and Synthetic Elastomers, and Acrolein. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans Vol. 19. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono19.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 1987. Styrene (Group 2B). Pp. 345-347.Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans Supplement 7. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/suppl7/Suppl7.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 1994. Styrene. Pp. 233-320 in Some Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 60. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol60/mono60.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 2002. Styrene. Pp. 437-550 in Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans Vol. 82. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf [accessed Feb. 12, 2014].

NIEHS/NTP (National Institute of Environmental Health Science/National Toxicology Program). 2008. Recommendation for Listing Status for Styrene in the Report on Carcinogens. Report on Carcinogens (RoC). NIEHS/NTP Scientific Review Group (NSRG), November 5, 2008.

NRC (National Research Council). 1983. Risk Assessment in the Federal Government: Managing the Process. Washington, DC: National Academy Press.

NRC (National Research Council). 2009. Science and Decisions: Advancing Risk Assessment. Washington, DC: National Academies Press.

NTP (National Toxicology Program). 2002. Report on Carcinogens, 10th Ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.

NTP (National Toxicology Program). 2007. Styrene: Nomination Information [online]. Available: http://ntp.niehs.nih.gov/index.cfm?objectid=03CA669F-E646-EDBC-287EFAC0676E8859 [accessed Aug. 8, 2013].

NTP (National Toxicology Program). 2008a. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2008b. Recommendation for Listing Status for Styrene. NTP Executive Committee Interagency Scientific Review Group, October 29, 2008.

NTP (National Toxicology Program). 2009. Summary Minutes February 24, 2009, Board of Scientific Counselors, National Institute of Environmental Health Sciences, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/About_NTP/BSC/2009/February/minutes022409.pdf [accessed Feb. 12, 2014].

NTP (National Toxicology Program). 2011a. Styrene. Pp. 383-392 in Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Styrene.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2011b. NTP Response to Expert Panels' Peer-Review Comments on Background Documents for Candidate Substances for the

12th Report on Carcinogens [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/2011/ResponseExpertPanelReport2011.pdf [accessed Oct. 28, 2013].

NTP (National Toxicology Program). 2011c. NTP Response to the NTP Board of Scientific Counselors (BSC) Peer Review Comments on the Draft Substances Profiles for the 12th Report on Carcinogens, February 24, 2009, BSC Meeting [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/2011/Response022409BSC2011.pdf [accessed Oct. 28, 2013].

NTP (National Toxicology Program). 2011d. NTP Response to Issues Raised in the Public Comments for Candidate Substances for the 12th Report on Carcinogens [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/2011/ResponsePublicComments2011.pdf#page=36 [accessed Oct. 28, 2013].

O'Neil, M.J., P.E. Heckelman, C.B. Koch, and R.J. Roman, eds. 1996. Styrene. Pp. 1519-1520 in The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 14th Ed. Whitehouse Station, NJ: Merck.

Ott, M.G., R.C. Kolesar, H.C. Scharnweber, E.J. Schneider, and J.R. Venable. 1980. A mortality survey of employees engaged in the development or manufacture of styrene-based products. J. Occup. Med. 22(7): 445-460.

Phillips, D., S. Snedeker, S. Eustis, M. Stone, P. Infante, E.M. Ward, G. Matanoski, G.S. Yost, S.S. Que Hee, L. Zeise, and T.J. Smith. 2008. Part A – Peer Review of the Draft Background Document on Styrene. Styrene Expert Panel Report [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2008/ExpertPanelMtgs/Styrene_PanelReportPartA_508.pdf [accessed July 17, 2013].

Phillips, D., S. Snedeker, S. Eustis, M. Stone, P. Infante, E.M. Ward, G. Matanoski, G.S. Yost, S.S. Que Hee, L. Zeise, and T.J. Smith. 2008. Part B – Recommendation for Listing Status for Styrene and Scientific Justification for the Recommendation. Styrene Expert Panel Report [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2008/ExpertPanelMtgs/Styrene_PanelReportPartB_508.pdf [accessed July 17, 2013].

Tossavainen, A. 1978. Styrene use and occupational exposure in the plastics industry. Scand. J. Work Environ. Health 4(suppl.2):7-13.

WHO (World Health Organization). 2000. Styrene. Chapter 5.12 in Air Quality Guidelines, Second Edition [online]. Available: http://www.euro.who.int/__data/assets/pdf_file/0018/123066/AQG2ndEd_5_12Styrene.pdf [accessed April 18, 2014].

2

Review of the Styrene Profile in the National Toxicology Program 12th Report on Carcinogens

To address part of its statement of task, the committee reviewed the substance profile for styrene in the National Toxicology Program (NTP) 12th Report on Carcinogens (RoC) (NTP 2011a). The committee's review was informed by many documents, including those in Table 1-1, and by comments submitted by stakeholder organizations and members of the general public. The committee also looked in detail at the primary literature cited in the background document for styrene and other literature identified by June 10, 2011 (the date on which the 12th RoC was released). To guide its review, the committee considered whether NTP described and conducted the literature search appropriately, whether all the relevant literature identified during the literature search was cited and sufficiently described in the background document, whether NTP had selected the most informative studies to support the listing determination, and whether NTP's arguments supported its conclusions.

This chapter is organized to follow the headings and organizational structure of the substance profile as presented in the 12th RoC (NTP 2011a). Those headings are "Cancer Studies in Humans", "Cancer Studies in Experimental Animals", "Metabolism of Styrene", and "Studies on Mechanisms of Carcinogenesis". The committee also reviewed the section in the substance profile that presents properties, use, and production of and exposure to styrene. On the basis of its review and analysis of the 12th RoC substance profile for styrene, the committee ends the chapter with a discussion about whether the evidence presented by NTP in the background document and the substance profile support the listing of styrene as "reasonably anticipated to be a human carcinogen".

CARCINOGENICITY

NTP began the substance profile for styrene with a clear statement of its conclusions—that styrene is reasonably anticipated to be a human carcinogen. That conclusion was based on limited evidence of carcinogenicity from studies

in humans, sufficient evidence from studies in experimental animals, and supporting mechanistic data. The committee finds this paragraph to be necessary, informative, and succinct.

Cancer Studies in Humans

The "Cancer Studies in Humans" section of the substance profile for styrene considers whether the epidemiologic literature published by June 10, 2011, provides limited evidence of human carcinogenicity or whether that evidence reaches the level of being sufficient for such a listing. Overall, the background document and the substance profile include appropriate literature reviews and identify the most informative studies (NTP 2008, 2011a). The text and tables in the background document clearly describe and critique the major strengths and limitations of the key epidemiologic studies, and the background document itself presents accurate data summaries with a few minor exceptions, which are mentioned below.

The committee concludes that the description and analysis of literature presented in the background document and the substance profile support NTP's classification of styrene in the 12th RoC as "reasonably anticipated to be a human carcinogen", as will be discussed in this section. The committee's assessment is based on the following RoC listing criterion: "there is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded" (NTP 2011b). Neither the background document nor the substance profile was explicit about how NTP defined the terms *limited* and *sufficient* in the context of the epidemiology evidence. So, the committee used its professional judgment to develop and apply a set of factors that it used to evaluate the credibility of evidence on the human carcinogenicity of styrene. As described in Chapter 3, those factors were high estimates of relative risks or its surrogates; exposure–response relationships for any reliably established exposure metric; consistency of observations among independent cohort studies of the reinforced-plastics industry or between cohort and case–control studies; and at least two informative studies in independent populations or with varied study designs. The committee judged the evidence to be *limited* if the epidemiology evidence was credible but chance, bias, and confounding could not be excluded. The evidence was judged to be *sufficient* if the epidemiology evidence was credible and chance, bias, and confounding could be excluded as an alternative explanation for the observed association.

Lymphohematopoietic Cancers, Including Leukemia, Non-Hodgkin Lymphoma, Hodgkin Lymphoma, and Multiple Myeloma

The classification of lymphohematopoietic cancers has evolved in recent decades, so there are some inconsistencies over time in the same cohort analyses

and inconsistencies among studies. For example, there have been changes in the diagnosis and classification of non-Hodgkin lymphoma—some cases of non-Hodgkin lymphoma would have been considered Hodgkin lymphoma before the 1980s (Banks 1992). According to a 1989 revision of the French–American–British classification criteria for leukemia, the M6 subtype of acute myeloid leukemia could be diagnosed if less than 30% of all nucleated cells in the bone marrow are blast cells (Bennett et al. 1985). Some of those cases would previously have been classified as refractory anemia with excess blast cells in transformation with a major erythroid component (Bennett et al. 1982), two conditions that were not considered malignancies.

The grouping of "all lymphohematopoietic cancers" includes many biologically distinct diagnoses in humans (NRC 2011), so it is not ideal to consider lymphohematopoietic cancers as constituting a homogeneous entity for etiologic assessment. However, the committee is limited by the methods used in the existing studies and is unable to separate lymphohematopoietic cancers into finer categories if the investigators of the studies did not separate them. That has been a challenge for many types of exposures in the epidemiologic literature and is not specific to studies of styrene exposure. In addition, lymphohematopoietic cancers are infrequent, so individual studies may have low statistical power, as reflected by low numbers of observed and expected cancer cases or deaths. Given those considerations, the committee thinks that to move forward with the review it is acceptable to consider lymphohematopoietic cancers as a group. The committee briefly summarizes below the evidence of carcinogenicity from traditional epidemiologic studies.

On the basis of the studies available to NTP by June 10, 2011, the committee judges there to be limited but credible evidence that exposure to styrene in occupational setting is associated with an increased frequency of lymphohematopoietic cancers. The evidence comes primarily from two occupational-cohort studies of workers in the reinforced-plastics industry in Europe (Kogevinas et al. 1994; Kolstad et al. 1994).

Kogevinas et al. (1994) compared workers in the reinforced-plastics industry with different levels of styrene exposure and showed that longer time since first exposure (at least 10 years vs less than 10 years) was associated with a significantly higher mortality due to lymphohematopoietic cancers combined. Compared with workers who had an average exposure of less than 60 ppm (seven deaths), the mortality rate ratios (MRRs) in those who had an average exposure of 110–119 ppm, 120–199 ppm, and at least 200 ppm were 3.11, 3.08, and 3.59, respectively, with a p value of 0.019 in a test of linear trend.

Kolstad et al. (1994) found that the standardized incidence ratio (SIR) for lymphohematopoietic cancers combined in workers at companies producing reinforced plastics was 1.20 (95% confidence interval [CI] 0.98–1.44). When the analysis was stratified by year of first employment, those who were first employed during 1964–1970 had a significantly higher incidence of lymphohematopoietic cancers (SIR = 1.32, 95% CI 1.02–1.67), whereas the SIRs for those first employed during 1971–1975 and during 1976–1988 were lower. That is

consistent with a possible exposure–response relationship, inasmuch as historical personal air samples from this cohort showed that average styrene concentrations decreased from 180 ppm during 1964–1970 to 43 ppm during 1976–1988 (Jensen et al. 1990). It should be noted that the Kogevinas et al. (1994) study included approximately one-third of the subjects in the Kolstad et al. (1994) study who worked at plants where the main product during the study period was reinforced plastics, although the analyses of the former were of mortality and included both male and female workers while the latter analyzed cancer incidence and excluded female workers.

Delzell et al. (2006) reported that exposure to styrene was associated with leukemia in a cohort of North American synthetic-rubber industry workers. Although the finding lends some credence to a possible leukemogenic role of occupational exposure to styrene, the committee does not give the study as much weight as NTP did, because of the workers' concomitant exposure to 1,3-butadiene, a known carcinogen, and the difficulty of teasing out the influence of 1,3-butadiene in assessing the independent effect of styrene (NTP 2011a).

Solid Tumors

On the basis of the studies published by June 10, 2011, the committee judged that there was limited but credible evidence that exposure to styrene in the occupational setting is associated with an increase in the frequency of some cancers in addition to those of the lymphohematopoietic system. The substance profile for styrene reported credible evidence of a cause–effect relationship between styrene exposure and cancers of the esophagus and pancreas (NTP 2011a). The committee thinks that the data regarding an association of styrene with kidney cancer should have been discussed in the substance profile.

Of the studies reviewed by NTP, four cohort studies of the reinforced-plastics industry that covered subjects and controls in Washington state (Ruder et al. 2004), the United States (Wong et al. 1994), Denmark (Kolstad et al. 1994), and combined European nations (Kogevinas et al. 1994) were the most informative. The strengths of those studies and the associations observed are credible because the studies were of high quality, of varied design (mortality and incidence), and consistent in their findings of associations of styrene with these cancers, especially when internal comparisons—many with an apparent exposure–response relationship—were presented.

Esophagus

The primary evidence of esophageal tumors is from cohort mortality studies in the reinforced-plastics industry (Kogevinas et al. 1994; Wong et al. 1994; Ruder et al. 2004), in which high exposures to styrene (especially from the 1940s through the 1970s) have been documented. The full study cohort of Ruder et al. (2004) in Washington state had an elevated rate of esophageal cancers

(standardized mortality ratio [SMR] = 2.30, 95% CI 1.19–4.02) on the basis of 12 deaths. The US cohort of Wong et al. (1994) had a similarly elevated SMR of 1.92 (95% CI 1.05–3.22) on the basis of 14 deaths. Those working in the high-exposure activity of open-mold processing had an SMR of 3.57 (two cases with exposure of at least 100 ppm-years,[1] 95% CI not reported). In an internal analysis in the European study by Kogevinas et al. (1994), exposure–response associations were observed in the most highly exposed subcohort (workers engaged in lamination; SMR = 1.81, 95% CI 0.87–3.34, 10 deaths). In the group with high cumulative exposure, the SMR rose to 5.82 (95% CI 1.0–33.9) after at least 20 years since first exposure.

Pancreas

The substance profile for styrene appropriately noted observations of high SMRs for pancreatic tumors, especially in high-exposure subcohorts of three of the reinforced-plastics cohorts, although the 95% CIs around some of the SMRs included 1.0. In Ruder et al. (2004), the SMR was 1.43 (95% CI 0.78–2.41) for the Washington state comparison group that was based on 14 deaths, and the SMR in high-exposure workers was 1.88 (95% CI 0.51–4.81). Kolstad et al. (1995) reported the incidence rate ratio for high-exposure workers to be 2.20 (95% CI 1.1–4.5) on the basis of 17 incident cases. Kogevinas et al. (1995) reported the SMR in laminators to be 1.48 (95% CI 0.76–2.58) on the basis of 12 deaths. In the latter study, after at least 20 years since first exposure, the SMR was 2.05 (95% CI 0.58–7.29), and the cumulative positive exposure trend had a p value of 0.068.

Kidney

There were multiple reports of associations of kidney cancers with styrene exposure in the reinforced-plastics industry, and some exhibit exposure–response relationships. Specifically, the relatively small study of Ruder et al. (2004) found elevated SMRs for kidney cancer (SMR = 1.43, 95% CI 0.57–2.95) on the basis of seven deaths, but in the high-exposure group the SMR was 3.60 (95% CI 0.98–9.20) on the basis of four deaths. The US study by Wong et al. (1994) found an SMR of 1.75 (95% CI 0.98–2.89) on the basis of 15 deaths; the association was higher in workers exposed for more than 2 years to open-mold processing: an SMR of 4.57 (95% CI not given). In the European cohort of Kogevinas et al. (1994), an exposure–response relationship of MRRs with increasing cumulative exposure to styrene was observed (p for trend = 0.12). Those studies did not attain the traditional level of statistical significance for

[1]"ppm–years" is the cumulative exposure calculated by multiplying the number of years by the (average) concentration in parts per million.

kidney cancer. However, kidney cancer should also have been mentioned in the styrene substance profile as having some evidence of styrene carcinogenicity. A case–control study of renal-cell cancer and occupational exposures, including exposure to styrene, was published in February 2011 (Karami et al. 2011), after the background document was issued but before the publication of the substance profile. That study should have been included in the NTP evaluation for styrene. It is discussed in more detail in Chapter 3.

Genetic Damage

The background document and the substance profile for styrene (NTP 2008, 2011a) cite informative studies that assessed the genetic damage caused by styrene. The brief "Genetic Damage" section under "Cancer Studies in Humans" summarized pertinent findings regarding adducts, single-strand DNA breaks, and an elevated frequency of chromosomal aberrations in workers exposed to styrene. Genetic damage may not result in clinical disease, but such information may inform underlying mechanisms of carcinogenicity. For this reason, NTP could consider describing the evidence of genetic damage in the section "Studies of Mechanisms of Carcinogenesis" rather than the section "Cancer Studies in Humans" if the background document and the substance profile for styrene are updated for a future edition of the RoC.

Cancer Studies in Experimental Animals

In the background document for styrene, NTP summarized findings from several studies in which a carcinogenic response was evaluated in mice or rats after administration of styrene by various routes (inhalation, ingestion via gavage or drinking water, and injection) (NTP 2008). The committee is not aware of any important studies of styrene carcinogenicity in animals that were available before June 10, 2011, that were not included in the background document. The characterization of styrene carcinogenicity findings in mice and rats as presented in the substance profile reflects the state of knowledge as of June 10, 2011.

In the substance profile, NTP correctly focused on the key animal studies that provide evidence for and against styrene carcinogenicity (NTP 2011a). The substance profile states that lung tumors were not observed in styrene-treated rats and briefly summarizes equivocal findings regarding mammary gland tumors. Findings of lung tumors in CD-1 mice after inhalation exposure (Cruzan et al. 2001) and supporting data from a study of perinatal styrene exposure in mice (Ponomarkov and Tomatis 1978) are appropriately described, as are the negative findings.

In the National Cancer Institute oral (gavage) study (NCI 1979), alveolar and bronchiolar adenomas and carcinomas combined were increased significantly in male B6C3F1 mice compared with *concurrent* study controls: *concurrent*

controls, 0/20 (0%); low dose, 6/44 (14%); high dose, 9/43 (21%). The authors noted the absence of lung tumors in the controls. *Historical vehicle* controls from the same laboratory (control male mice from similar studies in the same laboratory that also received only the vehicle, corn oil) were available for comparison and also showed no lung tumors (0/40). However, the authors considered the *historical vehicle* control population too small to provide useful perspective. A much larger number of *historical untreated* controls (controls from similar studies in the same laboratory that received no treatment) had an average lung tumor incidence of 12% (32/271) with incidences as high as 20% in two studies. If the *concurrent* control male mice in the NCI styrene bioassay had a lung tumor incidence similar to the average in the *historical untreated* controls, the incidences in the styrene-exposed animals would probably not have been significantly increased. Because the lung tumor incidence was increased only when compared with *concurrent* (and not *historical untreated*) controls, the NCI study authors concluded that the male mouse lung tumor findings were suggestive but that no convincing evidence of carcinogenicity was found.

In an effort to provide further insight into whether the incidence of lung tumors in the *concurrent* controls in the NCI oral bioassay was unusually low, NTP obtained *historical vehicle* control mouse lung tumor incidences from other laboratories used for NCI bioassays. The tumor incidence data came from studies that were carried out at about the same time as NCI (1979), under the same protocol, and with animals from the same source. NTP reported in the background document that the incidence of combined lung tumors in those *historical vehicle* controls was 4% (11/273) and concluded that the incidence in the *concurrent* controls in NCI's styrene gavage study was not unusually low. Because the increase in lung tumors in male mice was significant when compared with *concurrent* controls, NTP interpreted the study as positive, and it is so described in the substance profile. The issue of controls is not discussed in the substance profile and appears only in the background document.

The appropriate controls for the lung tumor incidences observed in male mice in the NCI oral bioassay constitute a pivotal issue in interpretation of the study as positive on one hand or negative, equivocal, or inconclusive on the other hand. It is a basic facet of the design of any scientific experiment that control animals need to be selected in such a way as to minimize, to the extent possible, variables that might influence the results other than the variables being specifically studied. With regard to cancer bioassays, it is well established that many characteristics of the conduct of a study unrelated to the treatment being evaluated can affect tumor rates (Haseman et al. 1984; Festing and Altman 2002; Keenan et al. 2009). Some characteristics related to the genetic makeup of the experimental animals used in the bioassay are the strain and substrain of the experimental animals, the supplier of the experimental animals, the breeding colony and the subpopulation of the colony from which the experimental animals were derived, procedures used for monitoring and controlling genetic drift in breeding populations, and the genetic homogeneity of animals used in the experiment. Husbandry practices are also important, both at the suppliers' sites

and at the experimental sites. Characteristics that can influence bioassay results related to husbandry practices include ventilation air (changes, filtration, monitoring, or exhaust), caging (structure or cleaning), bedding (frequency of replacement, source, chemical composition, or outgas monitoring), diet (availability to animals, vendor, nutrient composition, chemical composition, or quality-monitoring procedures), drinking water (availability to animals, sources, purity, or quality-monitoring procedures), and treatment vehicle (volume or dose, vendor, chemical composition, or quality-monitoring procedures). Controls and treated animals must be matched with respect to each of those characteristic to the greatest extent possible for the study to yield valid comparisons. That is best accomplished by using *concurrent* controls. If *historical vehicle* controls or *historical untreated* controls are used for comparison, the extent to which they might differ from treated animals with respect to the characteristics listed above must be considered.

NTP has been criticized for using *historical vehicle* control data from other laboratories to determine that the lung tumor incidences in the NCI styrene bioassay were not unusually low (Rhomberg et al. 2013). It is easy for studies conducted in different laboratories, even under the same experimental protocol, to vary in subtle but important respects (as outlined above) and consequently to yield different tumor incidences. Therefore, drawing historical controls from other laboratories is seldom justified (Haseman et al. 1984). The committee considers the comparison of *concurrent* controls in the NCI styrene oral bioassay (NCI 1979) with *historical vehicle* control data from other laboratories to be of little value. The same concern applies to comparison with *historical untreated* controls in the NCI bioassay (NCI 1979). The vehicle alone can influence tumor rates, and *historical vehicle* controls and *historical untreated* controls cannot be considered equivalent (Haseman et al. 1984). Therefore, in the case of the NCI styrene bioassay, the interpretive value of comparison with *historical untreated* controls is also of limited value. Although limited in number, the *historical vehicle* controls from the same laboratory at about the same time are most relevant and are consistent with the *concurrent* controls. The committee finds that the use of *concurrent* controls reported by NCI (1979) is appropriate.

Metabolism of Styrene

The section "Metabolism of Styrene" in the substance profile provides information on metabolites of styrene and on the specific CYP450 enzymes that are probably involved (NTP 2011a). Styrene, through formation of active metabolites, is thought to be capable of inducing genotoxic and cytotoxic effects. The metabolism of styrene is complex, and multiple metabolites are formed. One, styrene-7,8-oxide, is known to be genotoxic (see below), but the genotoxicity of others has not been thoroughly investigated. The contribution of specific metabolites to the genotoxic and cytotoxic effects of styrene may be organ-specific and the metabolites primarily responsible for cytotoxicity may not be

the same as those responsible for genotoxicity. An explicit statement of that circumstance would have enhanced the clarity of the metabolism section of the substance profile. As indicated in the substance profile, the strongest evidence of carcinogenicity in laboratory animals comes from studies in mice. In the mouse, lung tumors have been observed after either inhalation or oral exposure to styrene; this target-organ specificity is thought to be due to pulmonary activation of styrene in the mouse (see the discussion below and also the sections on Cancer Studies in Experimental Animals and Lung Cytotoxicity in Mice). Although the relevance of the mouse lung tumor response to humans has been questioned, the committee concludes that there was insufficient information when the substance profile was completed, so the mouse lung tumor response should not be excluded as irrelevant.

Thus, information on styrene metabolism in both the human and mouse is important for establishing a robust understanding of the carcinogenicity of this compound. The metabolism section of the substance profile provides a succinct overview of some aspects of styrene metabolism and, in general, is written clearly. The references provided are appropriately interpreted and described in the background document for styrene. However, the substance profile does not provide complete citations for the information that is presented; for example, the substance profile states without citation that over 90% of styrene is metabolized to styrene-7,8-oxide. Information is also provided on the state of knowledge regarding the specific CYP450s associated with styrene metabolism to styrene-7,8-oxide and their distribution in mice and humans, and some information is provided on metabolites other than styrene-7,8-oxide or downstream metabolites of styrene that have been detected in mice and humans.

A clear focus of the metabolism section of the substance profile is on the formation of styrene-7,8-oxide. Although not explicitly stated in the substance profile, that focus is presumably due to the facts that styrene-7,8-oxide is genotoxic and that styrene-7,8-oxide–protein and styrene-7,8-oxide–DNA adducts have been detected after styrene exposure. However, it is not known whether styrene-7,8-oxide is key to the cytotoxic response or whether it is the sole genotoxic metabolite of styrene. Minimal information is provided on the formation of 4-vinylphenol (presumably through the intermediate styrene-3,4-oxide), which is identified merely as a minor pathway.

Evidence available at the time of the release of the substance profile indicates that the role of metabolism may be more complex than portrayed in the substance profile for styrene. Specifically, although 4-vinylphenol is a minor metabolite of styrene, it is considerably more potent than styrene or styrene-7,8-oxide in inducing lung injury (Carlson et al. 2002; Carlson 2004; Cruzan et al. 2005), and it has been suggested that the aromatic ring–derived metabolites are important in the pulmonary toxicity of styrene in mice (Cruzan et al. 2005; Cruzan et al. 2009). To the extent that cytotoxicity and reparative cell proliferation play a critical role in pulmonary carcinogenesis in the mouse, a comprehensive description of styrene metabolism should include information on all metabolites that are thought to be important for cytotoxic responses.

The cellular balance of activation and detoxification pathways is an important consideration relative to target-organ sensitivity to any metabolically activated toxicant, including styrene. The substance profile includes some information on glutathione *S*-transferase in this regard. Information is also available from mouse studies on the potentially important role of epoxide hydrolase (Carlson 2010b), but that information is not included in the substance profile. The cellular response to a metabolically activated compound depends critically on both the activation rate and the detoxification rate. It is possible that the balance between activation and detoxification may differ among species and among target organs within a species. Thus, in accordance with fundamental toxicology principles, a comprehensive toxicologic evaluation requires detailed understanding of the full metabolic spectrum. The incompleteness of the information on metabolic detoxification pathways in the substance profile detracts from the quality of this section.

In summary, the substance profile for styrene provides much information on its metabolism. The information that is provided is correct, but the profile is not complete in its presentation of published information on styrene metabolism. There is a lack of clarity in the potential complexity of metabolism relative to styrene's cytotoxic and genotoxic effects. The information on styrene-metabolite phase II detoxification pathways is incomplete, and this weakens the scientific balance of this section of the substance profile. That information was included in the background document for styrene but was not provided clearly in the substance profile itself.

Studies on Mechanisms of Carcinogenesis

The section "Studies on Mechanisms of Carcinogenesis" in the substance profile for styrene (NTP 2011a) and supporting information in the background document (NTP 2008) summarize the mechanistic events that might link styrene exposure to cancer in experimental animals and humans. The mechanistic evidence on styrene and its major metabolites that was available to NTP is extensive and comes from a variety of studies in diverse model systems and from exposed humans. Although neither the substance profile nor the background document provides the exact search strategy that was used in collecting the evidence on the mechanisms of carcinogenesis, these documents present a balanced, comprehensive, and thorough review of the literature on the subject. Evidence tables and narrative descriptions of each study were used in the background document to present mechanistic evidence from primary studies and meta-analyses, and the committee finds the presentation of information to be inclusive and balanced.

The background document and the substance profile are in agreement with Part B of the styrene expert review panel (Phillips et al. 2008) that "the mechanisms of styrene carcinogenicity are not fully understood" (NTP 2011a, p. 385). Several potential mechanisms have been studied and are identified as separate

subsections below. Overall, it is clearly stated that the carcinogenicity of styrene depends on its metabolism to styrene-7,8-oxide and other reactive intermediates and that such metabolism occurs in both rodents and humans. Styrene-7,8-oxide has been listed as reasonably anticipated to be a human carcinogen since the 10th RoC (NTP 2002). Even though species, tissue, and individual differences in metabolic capacity or in enzymes involved in styrene metabolism have been reported, strong evidence presented in the substance profile and the background document for styrene suggests that mechanistic events that may lead to carcinogenesis (such as genotoxicity) occur in both exposed rodents and humans. Furthermore, the listing correctly states that multiple mechanistic events may occur and that they are "not necessarily mutually exclusive" (NTP 2011a, p. 385).

The substance profile for styrene identifies three modes of action: genotoxicity, cytotoxic effects, and immunosuppression. Genotoxicity is identified as relevant to all cancer sites, and cytotoxicity and immunosuppression are identified as site-specific. Those are reasonable categories, but they are somewhat inconsistent with the presentation and categorization of the mechanistic evidence in the background document and in Part B of the styrene expert panel report (Phillips et al. 2008). The executive summary of the background document, the body of the background document, and the substance profile present three styles of categorization of the same evidence; this may create some confusion with regard to the relative weight assigned by NTP to the mechanistic evidence in the overall cancer hazard classification of styrene. First, the executive summary of the background document includes separate sections on "genetic damage" and "mechanistic data", even though the latter section mentions "genotoxic pathway" as an important mechanistic event. Second, the body of the background document creates a poorly rationalized separation between sections "5.4 Genetic and related effects" and "5.5 Mechanistic studies and considerations", and the latter also contains subsection "5.5.1 Genotoxicity". Third, the substance profile for styrene, while maintaining the separate subsection "Genotoxicity" in accord with the executive summary and the main background document, emphasizes lung cytotoxicity in mice (information consistent with the background document subsection "5.5.4 Cytotoxic effects of styrene on mouse lung") and immunosuppression. Those mechanisms have not been clearly separated from among the likely mechanisms of styrene carcinogenesis in the background document. In contrast, "5.5.2 Gene expression and apoptosis" and "5.5.3 Oxidative stress" mechanisms are not mentioned in the substance profile. Although such inconsistencies are immaterial for the purpose of the cancer hazard classification of styrene in the 12th RoC, the committee suggests that NTP provide greater concordance among various documents to avoid confusion.

Genotoxicity

Both the background document and the substance profile identify genotoxicity of styrene as an important carcinogenesis mechanistic event that applies to

multiple potential target tissues and that operates in both experimental animals and humans exposed to styrene. The evidence that metabolism of styrene to styrene-7,8-oxide and other electrophiles that are capable of covalently binding to DNA, of forming adducts, and of causing other types of DNA damage that result in mutations and higher order cytogenetic damage is extensive and has been thoroughly reviewed and clearly presented. In the background document, over 20% of the entire document is dedicated to the presentation and critical evaluation of the data pertinent to this mechanism. The substance profile concludes correctly that styrene-associated "genotoxicity [is] relevant to all types of cancer" and that "a causal relationship between styrene exposure and cancer in humans is . . . supported by the finding of DNA adducts and chromosomal aberrations in lymphocytes from exposed workers" (NTP 2011a, p. 383). Similar conclusions regarding the role of metabolism and evidence of genotoxicity were drawn in Part B of the styrene expert panel report (Phillips et al. 2008). Hence, the overall role of genotoxicity of styrene as a key mechanistic event is well documented, supported by experimental evidence, and consistent with the recommendation of the expert panel.

Although the genotoxicity information in the substance profile for styrene is correct and comprehensive, the appropriateness of specific references selected to substantiate most of the arguments is somewhat difficult to ascertain because the literature base that is used to support these mechanisms and each specific event is large. The committee recognizes that no single study or even a collection of publications can represent the extent and diversity of mechanistic evidence; it therefore suggests that references included in this section be labeled as representative of similar studies. Perhaps the best way that the evidence can be summarized and presented is that depicted in "Table 5-18. Genetic and related effects of styrene" in the background document (NTP 2008). Such a systematic presentation of evidence may be amenable to being used as a guide for the brief summary included in the substance profile. The document could be further improved by pointing out, for each effect or model system, which evidence comes from studies of styrene and which from studies of styrene-7,8-oxide. As noted above, the role of styrene metabolism is necessary for its genotoxicity; however, because human evidence of genotoxic effects comes from subjects exposed to styrene, not to its metabolites, it is clear that styrene is metabolized in humans to genotoxic intermediates. Questions still remain as to which enzymatic systems may be responsible and as to the quantitative differences in styrene metabolism among species or individuals in the same species. Thus, the detailed attention devoted to human evidence in this and other sections concerned with genotoxicity end points is warranted and appropriate.

Lung Cytotoxicity in Mice

The objective of the section "Lung Cytotoxicity in Mice" of the substance profile for styrene is to address the mechanistic events by which exposure to

styrene leads to the formation of tumors, and this objective was clearly addressed by NTP. Establishing the mechanistic events for compounds—such as styrene, whose toxic and carcinogenic potential appears to require metabolic transformation by biologic tissues—depends on several issues. Some of those issues are identification of target tissues and cell populations; definition of the cellular response to exposure; establishment of the pattern of toxicity on the basis of exposure characteristics, including route, duration, and concentration; definition of variations in cytotoxic response between species and between strains of species; comparison of changes in cytotoxic response produced by altering the function of relevant enzyme systems through either chemical inhibitors or molecular alteration; and comparison of changes in cytotoxic response produced by the parent compound and its potentially toxic metabolites.

NTP identified 26 references that were published before the release date of the substance profile for styrene, June 10, 2011, and were relevant to lung cytotoxicity in mice and the role of specific activating enzyme systems and styrene metabolites. All except one (Cruzan et al. 2009) were cited and discussed in the background document for styrene. The substance profile for styrene addresses the most relevant of the studies, including that by Cruzan et al. (2009). For issues on which studies disagreed, such as the cytotoxic response in Clara and alveolar type II cells in the lungs of rats, all the relevant studies identified in the background document are cited and discussed in the substance profile for styrene. With respect to the cytotoxicity of different styrene metabolites, the most detailed, rigorous, and informative studies cited in the background document are discussed in the substance profile. However, in an independent literature search the committee identified six additional studies that could have added strength to the discussion of the mechanisms by which styrene produces cytotoxicity in the lungs (Harvilchuck and Carlson 2009; Harvilchuck et al. 2008, 2009; Carlson 2010a,b; Meszka-Jordan et al. 2009). Thus, the background document could be strengthened by providing a full evaluation of the strengths and limitations of the literature relevant to lung cytotoxicity and the potential roles of specific metabolites and activating enzymes. The studies are discussed in more detail in Chapter 3.

Of the material that was included in the background document, NTP selected the most appropriate for inclusion in the substance profile for styrene. As emphasized in the metabolism section, cytotoxicity produced by a bioactivated compound is the result of the balance between enzymatic capability for activating the compound and cellular capacity for detoxifying the reactive metabolites. When the metabolites are transported by the circulation, organs that have little activation capability may also become targets if their detoxification systems are overwhelmed. The substance profile for styrene could have been strengthened by broadening the discussion to include studies that assess oxidative stress produced by styrene and its metabolites, modulation of styrene toxicity by detoxification pathways, and toxicity produced in other organ systems, such as the gastrointestinal, urinary, and lymphohematopoietic systems. Broadening that discussion may provide insights into species differences (for example mouse vs

rat vs human) related to styrene sensitivity and mechanisms of target organ sensitivity to styrene or styrene-7,8-oxide.

Immunosuppression

An intact immune system is critical if an organism is to recognize or counteract the damaging effects of chemical substances, infectious agents, or neoplastic cells. Epidemiologic studies have shown that several chemical substances, particularly vapor chemicals, may alter immune function in humans and animals (Weill et al. 1975; Aranyi et al. 1986; Boverhof et al. 2013). Immunodeficiency or immunosuppression is theorized to be a possible mechanism by which exposure to a chemical substance might lead to carcinogenesis.

In the substance profile, NTP included a brief section on immunosuppression. The substance profile indicates that CYP2E1, the enzyme involved in converting styrene to styrene-7,8-oxide, is expressed in lymphocytes and hematopoietic stem cells (Kousalova et al. 2004; Siest et al. 2008); this suggests that cytotoxicity might occur in these cells and might damage the immune system after exposure to styrene. Genotoxicity has been detected in peripheral lymphocytes of styrene-exposed workers (Biro et al. 2002), and NTP discusses this point in several places: the section "Genotoxicity" in the substance profile and the corresponding sections "Genetic and Related Effects" and "Mechanistic Studies and Considerations" in the background document. The committee found that NTP provided inadequate evidence and few citations to support the argument that exposure to styrene may cause immunosuppression. Basic but critical data pertaining to immunity—such as the number of lymphocytes, the weight of lymphoid organs, the function of systemic and localized lymphoid organs, and effects on innate vs specific immunity after styrene exposure in experimental animals or humans—were not fully reviewed in the background document or the substance profile for styrene. In addition, the committee identified some typographic errors in the substance profile for styrene that should be corrected. In the sentence in the substance profile that states, "Veraldi et al. (2006) concluded that there was immediate evidence for the immunotoxicity of styrene oxide", "immediate" should be changed to "intermediate", and the sentence should refer to styrene, not styrene-7,8-oxide.

PROPERTIES

The section "Properties" of the substance profile for styrene details major physicochemical characteristics of the compound, including chemical stability, reactivity, and flammability (NTP 2011a). Overall, this brief section serves its purpose well and provides necessary information on the chemical itself. The substance profile also includes information on styrene-7,8-oxide, so a reference should be made to the substance profile for styrene-7,8-oxide (NTP 2011c).

USE

The substance profile and background document provide a comprehensive review of industrial uses of styrene (NTP 2008, 2011a). This section makes it clear that styrene is used primarily in the production of polymer products and resins and that humans may therefore come into contact with styrene through a variety of consumer products and diverse manufacturing processes. Overall, the section demonstrates that "a significant number of persons residing in the United States are exposed," which is one of the requirements for a substance to be listed in the RoC.

PRODUCTION

The section "Production" in the substance profile covers the chemical processes that are used to manufacture styrene and provides estimates of domestic production and import and export volumes (NTP 2011a). Styrene is a high-volume production chemical, and its manufacture is increasing. This section further supports the notion of potentially wide exposure to styrene in the United States inasmuch as nearly 40 lb of styrene were produced per person in the United States in 2006.

EXPOSURE

The section "Exposure" of the background document is critical for the interpretation of much of the epidemiologic data (NTP 2008). The substance profile makes it clear that exposure to styrene has been documented in both occupational settings and the general population (NTP 2011a). In addition, smoking is identified as an important source of exposure to styrene, and it is correctly noted that workers who are exposed to styrene may also suffer effects of nonoccupational sources of styrene, such as cigarette smoke, outdoor and indoor air, food, and water.

Exposure sources, duration, frequency, and concentrations; measures of exposure (for example, average, duration, or cumulative exposure); and biomarkers of exposure assist in determining the linkages between exposure and adverse health effects. Exposures may occur in the workplace and in community settings, although sources are not the same and occupational exposures may differ from environmental exposures in intensity, duration, frequency, and route of exposure. Because of these differences, occupational epidemiology studies sometimes inform environmental epidemiology studies. Such information provides critical context for the hazard classification of any chemical, including styrene. The background document for styrene contains relevant information and is comprehensive and well organized. The committee concludes that ample evidence of widespread exposure to styrene justifies its consideration for listing in the RoC.

The committee identified several revisions that would improve the clarity and conciseness of the presentation of the information. First, different types of industries may be compared in a quantitative manner, and the most logical way of presenting the information is perhaps by magnitude of exposure. Where information on smoking status is available, special attention should be paid to differences between smokers and nonsmokers in the risks posed by workplace exposure. Time trends, if any, need to be identified. A clear description of methods of exposure assessment is especially important for the interpretation and clarification of the exposure component in the epidemiologic studies. Second, the subsection "General population" should be divided into exposures from smoking, including second-hand exposure (that is, environmental tobacco smoke), and exposures from other sources. NTP could also consider including information on whether there is a relationship between pack-years of smoking and styrene exposure and on the magnitudes of styrene exposure from tobacco-smoking vs exposure in various occupations. Third, NTP could consider including a discussion of the effects of various other sources of potential exposure in the nonsmoking general population. This section devotes considerable space to the discussion of food as a source of styrene, but it is listed last among all possible sources. All possible units of exposure (μg/kg of body weight, $\mu g/m^3$, μg/L, and ppm) are mentioned. To improve clarity, the units of exposure could be standardized. Fourth, the subsection "General population" could begin by pointing out that nearly all members of the general population, not only those exposed in a workplace or through smoking, have detectable styrene in their biologic fluids (for example, blood and breast milk). That information makes it clear not only that there is a high potential that "a significant number of persons residing in the United State are exposed" to styrene but that there are detectable concentrations of styrene in most people tested.

REGULATIONS AND GUIDELINES

This section in the substance profile provides a comprehensive list of rules, regulations, and advisory notices that pertain to styrene (NTP 2011a). Many government agencies in the United States have set quantitative limits of styrene exposure in various scenarios (such as the Food and Drug Administration's maximum permissible level of styrene in bottled water or the Occupational Safety and Health Administration's ceiling concentration and permissible exposure limit for styrene) and many agencies regulate the production, use, distribution, and disposal of styrene. The level of detail provided on these varied greatly, and it is not clear whether the appropriate source of the information can be easily identified. For example, the Department of Homeland Security regulation is identified by a clear reference to the *Code of Federal Regulations*, whereas other regulations are noted without proper links to the appropriate documents. If this section is to provide not only information but proper references to other sources, this part of the discussion could be improved in future editions of the RoC.

SUGGESTED REVISIONS FOR THE LISTING OF STYRENE IN THE REPORT ON CARCINOGENS

Through its review of the background document and substance profile for styrene, the committee identified several revisions that could be made to improve future iterations of the listing of styrene in the RoC (see Table 2-1). The committee recognizes that the Office of the Report on Carcinogens is managing an open and public process and that it must produce RoC editions that are scientifically sound, consistent, and timely. Its comments and suggestions for future revisions are not intended to lead to additional layers of complexity or to delay future editions of the RoC. Addressing the suggestions in Table 2-1 would add clarity and improve the presentation of information in NTP's assessment of styrene, but making the revisions would not likely change the overall conclusion of carcinogenicity presented in the substance profile.

The committee identified two overarching improvements that could be made to the presentation of information in the background document and substance profile. The first observation pertains to NTP's identification of relevant literature in support of the assessment of styrene. Although the committee did not identify many relevant studies that NTP missed in its assessment of styrene, there was no discussion of the literature-search strategy in the background document. The committee was able to obtain information about the literature searches directly from NTP (Bucher 2013; see Appendix C). There are several organizations who have developed or have commented on best practices for evidence identification (Higgins and Green 2008; CRD 2009; AHRQ 2011; IOM 2011; NRC 2014). Describing NTP's literature identification process in greater detail in the background document for styrene, including inclusion and exclusion criteria, the date of the search, the publication dates searched, and the roles of experts involved in reviewing the literature, would have provided additional transparency to the development of the background document. In spite of that deficiency, the essential literature appears to be cited and discussed appropriately in the substance profile and the background document.

The second area of improvement identified by the committee pertains to the way in which NTP used the listing criteria to reach the determination that styrene is "reasonable anticipated to be a human carcinogen". The substance profile states that styrene is "reasonably anticipated to be a human carcinogen based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity from studies in experimental animals, and supporting data on mechanisms of carcinogenesis" (NTP 2011b). The committee is clear about NTP's determination of "sufficient evidence of carcinogenicity from studies in experimental animals" on the basis of the listing criteria instruction that experimental animal evidence is considered sufficient if there is an increase in "(1) multiple species or to multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of

tumor, or age at onset" (NTP 2008, p. v). However, the background document and substance profile are less clear how NTP came to its conclusion that the listing criteria for "limited evidence of carcinogenicity from studies in humans" (NTP 2008, p. v) had been fulfilled. The introductory section of the 12th RoC describes the multiple layers of expert judgment involved in the RoC process, but neither the background document nor the substance profile for styrene transparently describe the way in which the different studies and the attributes of those studies were integrated to support a conclusion of limited evidence. The background document would be more transparent if it stated the considerations that were used to evaluate evidence from studies in humans (such as study population characteristics, approach used for exposure assessment, potential for bias and confounding, and precision of estimate of effect); if it described the structured approach that experts used in their application of the listing criteria to reach a conclusion; and if it explicitly defined *limited* evidence and *sufficient* evidence in terms of the number of studies and attributes of those studies that would lead to such conclusions. NRC (2014) discusses organizing principles and qualitative and quantitative approaches for integrating evidence that NTP might find helpful in its application of the RoC listing criteria to the scientific literature for styrene.

CONCLUSIONS

The committee concludes that NTP correctly determined that styrene should be considered for listing in the RoC. There is sufficient evidence of exposure to a significant number of persons residing in the United States to warrant such consideration. NTP adequately documented that exposure to styrene occurs in occupational settings and in the general public regardless of smoking status.

After conducting a scientific review of the styrene assessment presented in the NTP 12th RoC, the committee finds that the overall conclusion reached by NTP in 2011, that styrene is "reasonably anticipated to be a human carcinogen", was appropriate. The following points of the listing criteria support NTP's conclusion:

- "There is limited evidence of carcinogenicity from studies in humans" (NTP 2008). Publications available to NTP as of June 10, 2011, provided limited but credible evidence that exposure to styrene is associated with lymphohematopoietic, pancreatic, and esophageal cancers. The most informative human epidemiologic studies that support that conclusion are those by Ruder et al. (2004), Wong et al. (1994), Kolstad et al. (1994), and Kogevinas et al. (1994). The evidence is limited in that "chance, bias, or confounding factors could not be adequately excluded" (NTP 2008).

TABLE 2-1 Suggested Clarifications and Updates for the Styrene Substance Profile and Background Document in Future Editions of the Report on Carcinogens

Sections in the Substance Profile for Styrene	Suggested Revisions
Carcinogenicity	• Add a discussion of the literature-search strategy in the background document for styrene.
Cancer Studies in Humans	• Include a review of Karami et al. (2011) and add a discussion of the evidence of an association of styrene with kidney cancer on the basis of that study and findings in the reinforced-plastics industry cohort studies. • Include and discuss a case–control study of non-Hodgkin lymphoma by Cocco et al. (2010). • Describe the evidence of genetic damage in the section "Studies of Mechanisms of Carcinogenesis" rather than the section "Cancer Studies in Humans".
Metabolism	• Include a more complete presentation of information on styrene metabolism in the substance profile, particularly with respect to detoxification pathways.

Studies on Mechanisms of Carcinogenesis	• Provide greater concordance among the headings of the executive summary of the background document, the body of the background document, and the substance profile.
	• Include information in the substance profile on the potential role of oxidative damage to DNA and the important role that DNA repair, or individual variability in DNA-repair response, may play in the genotoxicity of styrene to augment and strengthen the overall genotoxicity evidence.
	• Consider including studies that assess
	  o toxicity produced in organ systems other than the respiratory system, such as the gastrointestinal, urinary, and lymphohematopoietic systems.
	  o toxic responses in animals whose bioactivation potential has been modified by gene manipulation or administration of specific inhibitors.
	  o cellular oxidative stress responses to styrene or its metabolites.

	○ the role of cellular antioxidant pools in modulating the toxic response. ○ the role of enzymatic detoxification pathways in modulating the toxic response. ○ the effect of modulation of bioactivation and detoxification pathways and antioxidant pools on the cytotoxic response in whole animals or specific organs. Provide a more complete review of data pertaining to immunity, such as the number of lymphocytes, the weight of lymphoid organs, the function of systemic and localized lymphoid organs, and effects on innate vs specific immunity after styrene exposure in experimental animals or humans.
Properties	• Add a reference in the substance profile for styrene for the substance profile for styrene-7,8-oxide.
Exposure	• Consider comparing different types of industries in a quantitative manner, perhaps by magnitude of exposure. • Where information on smoking status is available, special attention should be paid to differences between smokers and nonsmokers in the risks posed by workplace exposure. • Identify time trends, if any. • Add a clear description of methods of exposure assessment to support the interpretation and clarification of the exposure component in the epidemiologic studies. • Divide the subsection "General population" into exposures from smoking, including second-hand exposure (that is, environmental tobacco smoke), and exposures from other sources. • Include information on whether there is a relationship between pack-years of smoking and styrene exposure and on the magnitudes of styrene exposure from tobacco-smoking vs exposure in various occupations. • Include a discussion of the effects of various other sources of potential exposure in the nonsmoking general population. • All possible units of exposure (μg/kg of body weight, $\mu g/m^3$, μg/L, and ppm) are mentioned and clarity could be improved by standardizing the units of exposure.

(Continued)

TABLE 2-1 Continued

Sections in the Substance Profile for Styrene	Suggested Revisions
	• Make a point at the beginning of the subsection "General population" that nearly all members of the general population, not only those exposed in a workplace or through smoking, have detectable styrene in their biologic fluids (for example, blood and breast milk).
Regulations and Guidelines	• Provide more information on regulations and include references to the sources.

- "There is sufficient evidence of carcinogenicity from studies in experimental animals" (NTP 2008). Literature published by June 10, 2011, provided sufficient evidence that "there is an increased incidence of . . . a combination of malignant and benign tumors" (NTP 2008) in experimental animals induced by styrene administered by multiple routes of exposure (inhalation and oral gavage). The most informative experimental animal studies that support that conclusion are studies in mice (NCI 1979; Cruzan et al. 2001).
- "There is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans" (NTP 2008). Literature published by June 10, 2011, provided convincing evidence that genotoxicity is observed in cells from humans who were exposed to styrene. That evidence is derived from a large body of publications. In addition, styrene-7,8-oxide "was listed in a previous Report on Carcinogens as . . . reasonably anticipated as a human carcinogen" (NTP 2008). Styrene-7,8-oxide, a compound that is structurally related to styrene, is a major metabolite of styrene in both experimental animals and humans; it was first listed in the 10th RoC (NTP 2002) as reasonably anticipated to be a human carcinogen.

REFERENCES

AHRQ (Agency for Healthcare Research and Quality). 2011. The Effective Health Care Program Stakeholder Guide. Publication No. 11-EHC069-EF. Rockville, MD: Agency for Healthcare Research and Quality.

Aranyi, C., W.J. O'Shea, J.A. Graham, and F.J. Miller. 1986. The effects of inhalation of organic chemical air contaminats on murine lung host defenses. Fundam. Appl. Toxicol. 6(4):713-720.

Banks, P.M. 1992. Changes in diagnosis of non-Hodgkin's lymphomas over time. Cancer Res. 52(19 suppl.):5453s-5455s.

Bennett, J.M., D. Catovsky, M.T. Daniel, G. Flandrin, D.A. Galton, H.R. Gralnick, and C. Sultan. 1982. Proposals for the classification of the myelodysplastic syndromes. Br. J. Haematol. 51(2):189-199.

Bennett, J.M., D. Catovsky, M.T. Daniel, G. Flandrin, D.A. Galton, H.R. Gralnick, and C. Sultan. 1985. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. Ann. Intern. Med. 103(4):620-625.

Berthiaume, S., and K.L. Ring. 2006. Styrene. P. 694.3000A in Chemical Economics Handbook Menlo Park, CA: SRI Consulting.

Biró, A., E. Pállinger, J. Major, M.G. Jakab, T. Klupp, A. Falus, and A. Tompa. 2002. Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed solvents. Immunol. Lett. 81(2):133-140.

Boverhof, D.R., S.M. Krieger, J.A. Hotchkiss, K.E. Stebbins, J. Thomas, and M.R. Woolhiser. 2013. Assessment of the immunotoxic potential of trichloroethylene and percholoroethylene in rats following inhalation exposure. J. Immunotoxicol. 10(3):311-320.

Bucher, J.R. 2013. Follow-up Questions Submitted by the NAS Committee on Review of the Formaldehyde Assessment in the NTP 12th RoC and the NAS Committee on Review of the Styrene Assessment in the NTP 12th RoC, April 2, 2013.

Carlson, G. 2004. Influence of selected inhibitors on the metabolism of the styrene metabolite 4-vinylphenol in wild-type and CYP2E1 knockout mice. J. Toxicol. Environ. Health A 67(12):905-909.

Carlson, G.P. 2010a. Depletion by styrene of glutathione in plasma and bronchioalveolar lavage fluid of non-Swiss albino (NSA) mice. J. Toxicol. Environ. Health A 73(11):766-772.

Carlson, G.P. 2010b. Metabolism and toxicity of styrene in microsomal epoxide hydrolase-deficient mice. J. Toxicol. Environ. Health A 73(24):1689-1699.

Carlson, G.P., M. Ullman, N.A. Mantick, and P.W. Snyder. 2002. 4-Vinylphenol-induced pneumotoxicity and hepatotoxicity in mice. Toxicol. Pathol. 30(5):565-569.

Cocco, P., A. t'Mannetje, D. Fadda, M. Melis, N. Becker, N., S. de Sanjose, L. Foretova, J. Mareckova, A. Staines, S. Kleefeld, M. Maynadie, A. Nieters, P. Brennan, and P. Boffetta. 2010. Occupational exposure to solvents and risk of lymphoma subtypes: Results from the Epilymph case-control study. Occup. Environ. Med. 67(5):341-347.

CRD (Centre for Reviews and Dissemination). 2009. Systematic Reviews: CRD's Guidance for Undertaking Reviews in Health Care. York, UK: York Publishing Services, Ltd [online]. Available: http://www.york.ac.uk/inst/crd/pdf/Systematic_Reviews.pdf [accessed Feb. 24, 2013]

Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C. Bevan, C.J. Hardy, D.W. Coombs, P.A. Mullins, and W.R. Brown. 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J. Appl. Toxicol. 21(3):185-198.

Cruzan, G., G.P. Carlson, M. Turner, and W. Mellert. 2005. Ring-oxidized metabolites of styrene contribute to styrene-induced Clara-cell toxicity in mice. J. Toxicol. Environ. Health A 68(3):229-237.

Cruzan, G., J. Bus, M. Banton, R. Gingell, and G. Carlson. 2009. Mouse specific lung tumors from CYP2F2-mediated cytotoxic metabolism: An endpoint/toxic response where data from multiple chemicals converge to support a mode of action. Regul. Toxicol. Pharmacol. 55(2):205-218.

Delzell, E., N. Sathiakumar, J. Graff, M. Macaluso, G. Maldonado, and R. Matthew. 2006. An Updated Study of Mortality Among North American Synthetic Rubber Industry Workers. HEI Research Report No. 132. Boston, MA: Health Effects Institute [online]. Available: http://pubs.healtheffects.org/getfile.php?u=366 [accessed Feb. 21, 2014]

Festing, M.F., and D.G. Altman. 2002. Guidelines for design and statistical analysis of experiments using laboratory animals. ILAR J. 43(4):244-258.

Harvilchuck, J.A., and G.P. Carlson. 2009. Effect of multiple doses of styrene and R-styrene oxide on CC10, bax, and bcl-2 expression in isolated Clara cells of CD-1 mice. Toxicology 259(3):149-152.

Harvilchuck, J.A., R.J. Zurbrugg, and G.P. Carlson. 2008. CC10 mRNA and protein expression in Clara cells of CD-1 mice following exposure to styrene or its metabolites styrene oxide or 4-vinylphenol. Toxicol. Lett. 183(1-3):28-35.

Harvilchuck, J.A., X. Pu, J.E. Klaunig, and G.P. Carlson. 2009. Indicators of oxidative stress and apoptosis in mouse whole lung and Clara cells following exposure to styrene and its metabolites. Toxicology 264(3):171-178.

Haseman, J.K., J. Huff, and G.A. Boorman. 1984. Use of historical control data in carcinogenicity students in rodents. Toxicol. Pathol. 12(2):126-135.

Higgins, J.P.T., and S. Green, eds. 2008. Chochrane Handbook for Systematic Reviews of Interventions. Chichester, UK: John Wiley & Sons.

IOM (Institute of Medicine). 2011. Finding What Works in Health Care: Standards for Systematic Reviews. Washington, DC: The National Academies Press.

Jensen, A.A., N.O. Breum, J. Bacher, and E. Lynge. 1990. Occupational exposures to styrene in Denmark 1955-88. Am. J. Ind. Med. 17(5):593-606.

Karami, S., P. Boffetta, P. Brennan, P.A. Stewart, D. Zaridze, V. Matveev, V. Janout, H. Kollarova, V. Bencko, M. Navratilova, N. Szeszenia-Dabrowska, D. Mates, J.P. Gromiec, R. Sobotka, W.H. Chow, N. Rothman, and L.E. Moore. 2011. Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. J. Occup. Environ. Med. 53(2):218-223.

Keenan, C., S. Elmore, S. Francke-Carroll, R. Kemp, R. Kerlin, S. Peddada, J. Pletcher, M. Rinke, S.P. Schmidt, I. Taylor, and D.C. Wolf. 2009. Best practices for use of historical control data of proliferative rodent lesions. Toxicol. Pathol. 37(5):679-693.

Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261.

Kolstad, H.A., E. Lynge, J. Olsen, and N. Breum. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand. J. Work Environ. Health 20(4):272-278.

Kolstad, H.A., K. Juel, J. Olsen, and E. Lynge. 1995. Exposure to styrene and chronic health effects: Mortality and incidence of solid cancers in the Danish reinforced plastics industry. Occup. Environ. Med. 52(5):320-327.

Kousalova, L., E. Anzenbacherova, J. Baranova, P. Anzenbacher, I. Skoumalova, and J. Vondrakova. 2004. Presence of cytochrome P450 enzymes in human CD34+ haematopoietic progenitor cells. Gen. Physiol. Biophys. 23(2):251-257.

Meszka-Jordan, A., R. Mahlapuu, U. Soomets, and G.P. Carlson. 2009. Oxidative stress due to (R)-styrene oxide exposure and the role of antioxidants in non-Swiss albino (NSA) mice. J. Toxicol. Environ. Health A 72(10):642-650.

NCI (National Cancer Institute). 1979. Bioassay of Styrene for Possible Carcinogenicity. Technical Report No. 185. NIH 79-1741. National Cancer Institute, National Institute of Health, Bethesda, MD [online]. Available: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr185.pdf [accessed Feb. 21, 2014].

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington, DC: The National Academies Press.

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) Process. Washington, DC: The National Academies Press.

NTP (National Toxicology Program). 2002. Report on Carcinogens, 10th Ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.

NTP (National Toxicology Program). 2008. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2011a. Styrene. Pp. 383-392 in Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Styrene.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2011b. Listing Criteria [online]. Available: http://ntp.niehs.nih.gov/?objectid=47B37760-F1F6-975E-7C15022B9C93B5A6 [accessed Feb. 24, 2014].

NTP (National Toxicology Program). 2011c. Styrene-7, 8-oxide. Pp. 383-392 in Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/StyreneOxide.pdf [accessed Aug. 14, 2013].

Phillips, D., S. Snedeker, S. Eustis, M. Stone, P. Infante, E.M. Ward, G. Matanoski, G.S. Yost, S.S. Que Hee, L. Zeise, and T.J. Smith. 2008. Part B – Recommendation for Listing Status for Styrene and Scientific Justification for the Recommendation. Styrene Expert Panel Report [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2008/ExpertPanelMtgs/Styrene_PanelReportPartB_508.pdf [accessed July 17, 2013].

Ponomarkov, V., and L. Tomatis. 1978. Effects of long-term oral administration of styrene to mice and rats. Scand. J. Work Environ. Health 4(suppl. 2):127-135.

Rhomberg, L., J.E. Goodman, and R.L. Prueitt. 2013. The weight of evidence does not support the listing of styrene as "reasonably anticipated to be a human carcinogen" in NTP's Twelfth Report on Carcinogens. Hum. Ecol. Risk Assess. 19(1):4-27.

Ruder, A.M., E.M. Ward, M. Dong, A.H. Okun, K. Davis-King. 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. Am. J. Ind. Med. 45(2):165-176.

Siest, G., E. Jeannesson, J.B. Marteau, A. Samara, B. Marie, M. Pfister, and S. Visvikis-Siest. 2008. Transcription factor and drug-metabolizing enzyme gene expression in lymphocytes from healthy human subjects. Drug Metab. Dispos. 36(1):182-189.

Veraldi, A. A.S. Costantini, V. Bolejack, K. Miligi, P. Vinesis, and H. van Loveren. 2006. Immunotoxic effects of chemicals: A matrix for occupational and environmental epidemiological studies. Am. J. Ind. Med. 49(12):1046-1055.

Wong, O., L.S. Trent, and M.D. Whorton. 1994. An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup. Environ. Med. 51(6):386-396.

Weill, H., J. Salvaggio, A. Neilson, B. Butcher, and M. Ziskind. 1975. Respiratory effects in toluene diisocyanate manufactore: a multidisciplinary approach. Environ. Health Perspect. 11:101-108 .

3

Independent Assessment of Styrene

The committee's task had two parts. The first was to review the styrene substance profile as it was presented in the National Toxicology Program (NTP) 12th Report on Carcinogens (RoC) (NTP 2011a). In Chapter 2 of this report, the committee considered only literature that was available to NTP (literature published by June 10, 2011). It reviewed the primary literature, assessed NTP's description and analysis of that literature, and determined whether NTP's arguments support the conclusion that styrene is "reasonably anticipated to be a human carcinogen".

To address the second part of its task, the committee carried out an independent assessment of styrene carcinogenicity, which is the focus of the present chapter. The committee used its peer review in Chapter 2 and the background document that supports the styrene profile in the 12th RoC as a starting point. The present chapter provides a brief summary of informative studies and highlights key data that informed its independent assessment of styrene. The reader is also referred to the background document (NTP 2008) for a more detailed discussion of study methodologies, strengths, and weaknesses for literature published prior to 2011 and to the primary literature.

The committee's independent assessment of styrene carcinogenicity was based on literature that included primary data; however, the committee used published peer-reviewed review articles and reviews by other authoritative bodies to ensure that relevant literature was not missed and that all plausible interpretations of primary data were considered. The committee also considered comments and arguments that were presented during its first meeting, comments received from outside stakeholders during the study process, and independent literature searches carried out by National Research Council staff. The goal of the literature searches was to identify relevant literature that may have missed inclusion in the 12th RoC and relevant literature that was published after the release of the 12th RoC. Each search began on January 1, 2008, the year in which the background document for styrene was published (Bucher 2013). The search was first run on May 28, 2013, and it was updated on November 13,

2013.[1] Databases searched were PubMed, Medline (Ovid), Embase (Ovid), Scopus, and Web of Science. The search strategy for each database and the exclusion criteria are described in greater detail in Appendix D. After identifying the relevant body of literature up to November 13, 2013, the committee reviewed the primary data and applied the RoC listing criteria to human, experimental animal, and mechanistic studies. It then integrated the evidence to develop its own independent listing recommendation for styrene. Consideration was given to all relevant information, including "dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance" (NTP 2011b).

In accordance with the listing criteria, expert judgment was used to interpret and apply the RoC listing criteria to evidence in human and animal studies. A substance can be classified in the RoC as "known to be a human carcinogen" if "there is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer" (NTP 2008, p. v). A substance can be classified in the RoC as "reasonably anticipated to be a human carcinogen" if at least one of the following three criteria are fulfilled (NTP 2008, p. v):

- "There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded."
- "There is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset."
- "There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans."

[1]The cut-off date for the literature search was chosen to allow the committee time to review the literature within the time constraints of the project schedule.

As discussed in Chapter 2, the type of information needed to meet the criterion for sufficient evidence in experimental animals is clear and transparent. The type of information needed to meet the criterion for limited or sufficient evidence in humans required more interpretation and expert judgment on behalf of the committee. In its evaluation of the epidemiology literature, the committee described the information it used to identify informative studies and to evaluate those studies.

This chapter begins with a section on metabolism and toxicokinetics. It then reviews cancer studies in humans, cancer studies in experimental animals, and mechanistic data. The chapter ends with a section that summarizes the evidence and provides a final conclusion and listing recommendation for styrene that is based on the listing criteria published in the 12th RoC.

METABOLISM AND TOXICOKINETICS

The absorption, distribution, metabolism, and excretion of styrene have been reviewed by several organizations (Sumner and Fennell 1994; IARC 2002; Vodicka et al. 2006; NTP 2008). In brief, as expected for a lipid-soluble hydrocarbon, styrene is absorbed after inhalation, ingestion, or dermal exposure. Increased blood concentrations of styrene or styrene metabolites have been observed in experimental subjects and workers exposed to styrene. Concentrations of styrene in the blood increase rapidly after the onset of exposure and decay over the course of several hours after termination of the exposure (see the background document for styrene [NTP 2008] for more information). Styrene is extensively metabolized, and metabolites are excreted in urine. Humans and rodents differ quantitatively in whole-body metabolism and excretion, but styrene metabolic pathways are qualitatively similar in rodents and humans (IARC 2002; Vodicka et al. 2006). Several pharmacokinetic and physiologically based pharmacokinetic models of inhaled styrene absorption and metabolism have been developed (Filser et al. 2002; Sarangapani et al. 2002; Chen et al. 2008; NTP 2008; Verner et al. 2012).

Metabolic activation is thought to be essential for styrene toxicity and carcinogenicity (IARC 2002; Vodicka et al. 2006; NTP 2008, 2011a). The balance between the metabolic activation rate and the detoxification rate in a specific target tissue is critical in determining the ultimate response. This section provides information on styrene phase I metabolism (metabolic activation), information on phase II (detoxification) pathways, and then a summary.

Multiple target sites are relevant to the carcinogenic hazard posed by styrene. In humans, styrene exposure is associated with cancer of the lymphohematopoietic system, esophagus, pancreas, and kidney (see the section "Epidemiologic Studies" below). In mice, styrene causes lung tumors, but statistically significant increases in tumor burden were not observed for other sites. The metabolic pathways that are likely to be important in the carcinogenic response are fairly well defined (and described below), but there is no comprehensive information on activation and detoxification rates in potential target tissues in the human.

Phase I: Metabolism

Styrene is metabolically activated by cytochrome P450 monooxygenase (CYP450)-dependent oxidation on the side chain to form styrene-7,8-oxide (see Figure 3-1). Because this molecule contains an asymmetric carbon, there are two enantiomers, R- and S-styrene-7,8-oxide. Styrene aromatic ring metabolites are also formed, including 4-vinylphenol (presumably through styrene-1,2-oxide) and 2-vinylphenol (presumably through styrene-2,3-oxide). Of those pathways, styrene-7,8-oxide and 4-vinylphenol have been the most studied. It is possible that styrene-7,8-oxide and its initial detoxification products are further metabolized, perhaps through ring oxidation, and that 4-vinylphenol is also metabolized (Carlson et al. 2001; Carlson 2004, 2012; Cruzan et al. 2012, 2013). In the rodent, ^{14}C-labeled CO_2 is exhaled after ^{14}C-styrene administration; this suggests that aromatic ring–opened metabolites are formed (Boogaard et al. 2000a). The precise structures and toxicologic roles of those downstream metabolites are not fully characterized.

In humans, styrene-7,8-oxide-based metabolic products form over 90% of the excreted metabolites of styrene (see below). That is clear evidence that styrene-7,8-oxide is the primary phase I metabolite in humans. Ring oxidation to 4-vinylphenol also occurs but to a much smaller extent. Sulfate and glucuronide conjugates of 4-vinylphenol have been identified in urine of humans but at low concentrations (less than 1% of the excreted metabolites) (IARC 2002; Vodicka et al. 2002a; NTP 2008). Aromatic ring metabolites may be critical with respect to cytotoxic or genotoxic effects in specific target organs even though they constitute only a minor pathway with respect to whole-body metabolism of styrene.

Multiple forms of human CYP450 are reported to catalyze styrene oxidation, albeit with different activities, including CYP1A2, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2E1, CYP2F1, CYP2S1, CYP3A3, CYP3A4, CYP3A5, and CYP4B1 (Nakajima et al. 1994; Vodicka et al. 2006; Carlson 2008; Fukami et al. 2008; NRC USCG 2008; Bui and Hankinson 2009). Multiple recent studies have shown associations of polymorphisms in selected CYPs with the pattern of urinary styrene metabolite excretion; although the studies suggest a role of CYPs in styrene metabolism, they did not examine associations with disease outcome. Two CYP2E1 binding sites with allosteric interactions result in a shift to more efficient metabolism as styrene concentration increases. Many of the aforementioned CYPs are expressed in nonhepatic tissues, and this indicates that styrene may be metabolically activated in multiple organs in humans. Because styrene produces lung tumors in mice, a focus of investigation has been on pulmonary metabolism in mice. Styrene is extensively metabolized in the mouse liver and lung (primarily in Clara cells) by multiple forms of CYP, including CYP2E1 and CYP2F2 (Carlson 2004, 2008, 2012; Shen et al. 2010; Cruzan et al. 2012, 2013). Both side chain and aromatic ring metabolites are probably formed in the lung. In the mouse, CYP2F2 may be particularly important in activation of styrene (Cruzan et al. 2012, 2013). Investigations of the role of CYP2F2 in the pulmonary response to styrene have focused solely on

cytotoxicity and short-term cell proliferation, and its role is critical in both these processes in the mouse lung. It is important to consider, however, that cytotoxicity and short-term cell proliferation responses may not be the sole determinants of the ultimate tumorigenic response and, in this context, the role of this CYP in lung tumorigenicity in mice remains uncertain.

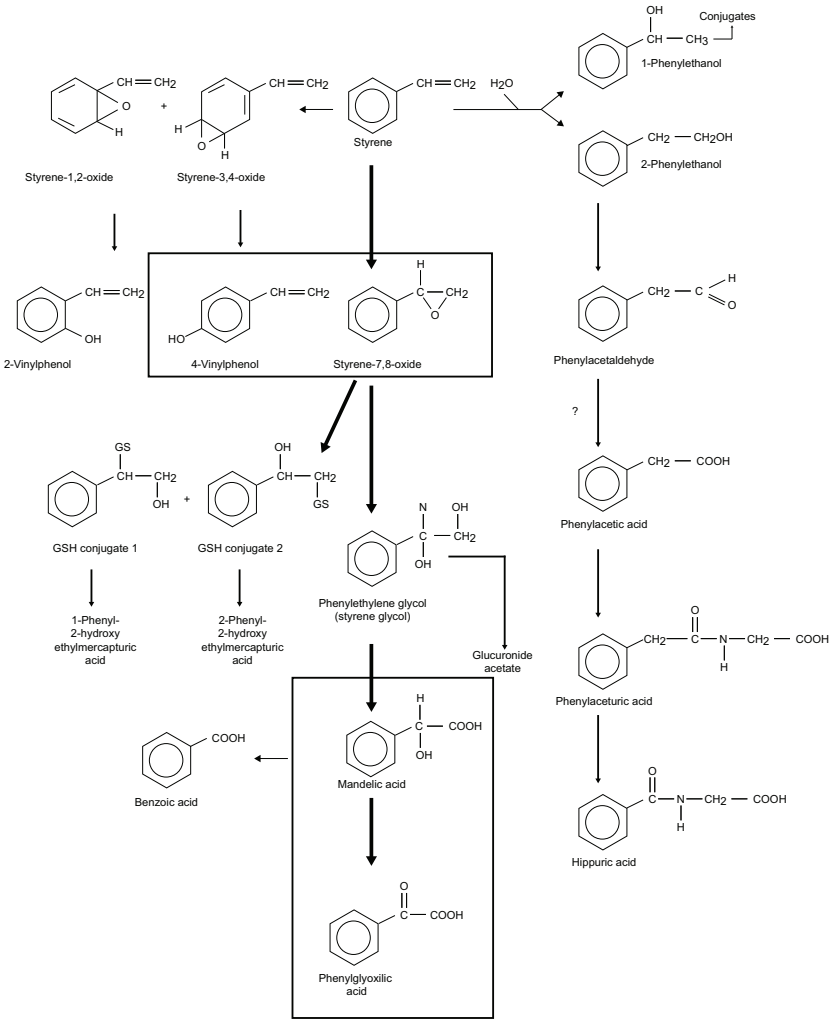

FIGURE 3-1 Primary metabolic pathways of styrene. Main pathways are indicated by thick arrows. Metabolites that have been extensively studied are highlighted in the boxes. This figure is not a complete depiction of all known metabolites. A similar figure can be found in the background document for styrene (NTP 2008). GSH, glutathione. Source: Adapted from IARC (2002).

Styrene-7,8-oxide is genotoxic (see below). Metabolites derived from styrene-7,8-oxide are excreted in urine after styrene exposure; this is a clear indication that it is formed in humans. Moreover, styrene-7,8-oxide is detected in venous blood in humans and rodents after styrene exposure (NTP 2008), so it can probably migrate from the organ in which it was formed. The presence of styrene-7,8-oxide in the blood indicates widespread exposure to this genotoxic metabolite throughout the body. In humans, styrene-7,8-oxide–hemoglobin adducts and styrene-7,8-oxide–DNA adducts in lymphocytes have been observed, possibly because of circulating styrene-7,8-oxide or the generation of styrene-7,8-oxide in circulating blood cells (NTP 2008, 2011a). Nonenzymatic epoxidation of styrene, perhaps via oxyhemoglobin, may occur in erythrocytes (Tursi et al. 1983).

As in the human, styrene-7,8-oxide is present in blood of both rats and mice exposed to styrene. Styrene exposure does not induce tumors in the rat and a statistically significant increase in tumors was only observed in the lungs of mice. The reasons for this are unclear. Styrene-7,8-oxide-based DNA adducts are formed in mouse lung after styrene exposure (Boogaard et al. 2000b), but their role in lung tumorigenesis in mice is not known. The amount of DNA adducts found in the lung vs liver in the mouse or in the rat lung vs mouse lung does not correlate with the target organ or a specific-species tumor response (Cruzan et al. 2009). However, inasmuch as adduct formation is the first of multiple steps of tumor development, a direct relationship between adduct concentrations and tumor response among species or organs is not necessary. The lack of direct concordance between styrene-7,8-oxide-adduct concentrations and tumor formation does not exclude the potential role of these adducts in the pulmonary carcinogenic response in mice.

Styrene is also metabolized via oxidation of its aromatic ring. Although a minor component, 4-vinylphenol-derived metabolites are present in urine after styrene exposure (NTP 2008, 2011a; Linhart et al. 2010, 2012). The aromatic ring–derived metabolite 4-vinylphenol is more potent than styrene or styrene-7,8-oxide in inducing pulmonary toxicity (Carlson 2002, 2004; Cruzan et al. 2005). This metabolite is thought to be formed by CYP2E1 and CYP2F2 in rodents (Carlson et al. 2001). 4-Vinylphenol is further metabolized by epoxidation in the rodent liver and lung (Zhang et al. 2011). Although it is not known with absolute certainty, aromatic ring–derived metabolites are likely important in the pneumotoxicity of styrene in mice (Cruzan et al. 2009, 2012, 2013). Information is not available on target organ–specific formation of aromatic ring metabolites in humans. The genotoxicity of these metabolites has not been extensively investigated.

Phase II: Detoxification

The side-chain–based or aromatic ring–based phase I metabolic oxidative products of styrene can be detoxified by hydrolysis via epoxide hydrolase or glutathione conjugation. Styrene-7,8-oxide is metabolized by epoxide hydrolase to

form styrene glycol, which can be converted to mandelic acid and phenylglyoxylic acid (Figure 3-1). Those two products are excreted in urine and account for more than 90% of the excreted styrene metabolites in humans (Vodicka et al. 2006; NTP 2008, 2011a). Information is not available on the pharmacogenetics of epoxide hydrolase relative to styrene disposition in humans; however, microsomal epoxide hydrolase knockout mice are more sensitive to styrene-induced cytotoxicity, and this highlights its potentially important role (Carlson 2010b, 2011a). Glutathione-based conjugates of styrene metabolites are also formed; they account for less than 10% of the excreted metabolites in humans but may account for more than 30% of the excreted metabolites in rodents (NTP 2008; Vodicka et al. 2006). Studies in mice that are deficient in glutathione-*S*-transferase P1P2 (-/-) suggest that this form is not important in styrene detoxification, but other forms of glutathione-*S*-transferase may still have a role in detoxification (Carlson 2011b). Some evidence suggests that expression of glutathione-*S*-transferase M1 and T1 in humans may contribute to individual variability in the fraction of styrene-7,8-oxide that is conjugated to glutathione (Haufroid et al. 2002; Teixeira et al. 2004; Fustinoni et al. 2008; Vodicka et al. 2006). Further information on the precise phase II activities of styrene in critical target cells is not available.

Summary of Styrene Metabolism and Toxicokinetics

Metabolism of styrene is key to its toxic and carcinogenic responses. The organ-specific tumorigenic responses to styrene will depend, in large part, on the balance between the rate of activation and the rate of detoxification in each organ. Thorough information on styrene activation and detoxification rates specific to target sites, particularly in the human, is not available. Given the wide array of CYP450 isozymes that can oxidize styrene, including forms that are known to be expressed in extrahepatic tissues (for example, CYP2E1 and CYP2A13), it is not possible to exclude the possibility that styrene bioactivation can occur in multiple target tissues. The presence of styrene-7,8-oxide in blood indicates that there is widespread tissue exposure to this genotoxic metabolite even in tissues that have low capacity for styrene activation. That highlights the importance of cellular detoxification capacities relative to organ-specific effects of styrene. In tissues that have low activity of epoxide hydrolase or glutathione-*S*-transferase, it might take only low levels of oxidation of styrene to produce cellular effects. The absence of marked toxicity in organs other than the liver or lung of mice (see the "Cytotoxicity" section) suggests detoxification capacities in that species are sufficient to prevent overt toxicity, except in the liver and lung. However, specific information on capacities for detoxification of styrene metabolites (such as epoxide hydrolase and glutathione-*S*-transferase) in critical target tissues in humans is not available. Therefore, the available information on styrene metabolism is insufficient to exclude any tissue from being a plausible target for styrene-induced cytotoxicity, which could contribute to carcinogenesis.

EPIDEMIOLOGIC STUDIES

The literature was searched to identify relevant epidemiologic studies that had been published by November 13, 2013 (see Appendix D). The committee established exclusion criteria for the literature search and identified studies that were most informative for determining whether an association exists between styrene exposure and carcinogenesis. The committee then reviewed and evaluated the methods and results of those informative studies and applied the RoC listing criteria to the evidence.

Identification of Informative Studies

The committee established a set of attributes for identifying the most informative cohort and case–control studies. The most informative cohort studies were ones that had

- Large cohorts that were exposed to high and varied concentrations of styrene.
- Systematically assigned exposure estimates (such as the years in which exposure began and ended or job exposure matrices that coupled worker histories with exposure estimates according to occupation).
- Styrene exposures that could be assessed apart from exposures to other potentially carcinogenic chemicals.
- Systematically and reliably assigned cancer end points.
- Internal comparisons, for example incidence rate ratios (IRRs) and mortality rate ratios (MRRs) that compared workers with different levels of exposure in the same cohort.

The most informative case–control studies were ones that had

- Relatively large numbers of cases and controls.
- Reliable assessments of styrene exposure based on specific job histories or other sources of information.
- Interviews of cases and controls or their next of kin to collect occupational histories and data related to lifestyle (for example, smoking) and other important potential confounders.

On the basis of those attributes, the committee identified what it judged to be the 11 informative publications: six studies that used four cohorts in the reinforced-plastics industry that were conducted in Europe (Kogevinas et al. 1994; Kolstad et al. 1994, 1995) and the United States (Wong et al. 1994; Ruder et al. 2004; Collins et al. 2013) and five case–control studies conducted in Europe (Scélo et al. 2004; Seidler et al. 2007; Cocco et al. 2010; Karami et al. 2011) and

Canada (Gerin et al. 1998). See Table 3-1 for descriptions of the studies, including the salient strengths and weaknesses of each study. It should be noted that approximately one-third of the Kolstad et al. (1994) cohort in Denmark was included in the Kogevinas et al. (1994) European cohort. However, the two studies assessed different cancer outcomes (incidence and mortality, respectively), and the Kolstad et al. (1994) study only included male workers whereas the Kogevinas et al. (1994) study included male and female workers. It should also be noted that the Collins et al. (2013) study was an extension of the study by Wong et al. (1994). However, Wong et al. (1994) assessed somewhat different exposure metrics and necessarily focused on mortality that occurred closer to the time of high exposure.

The committee reviewed all epidemiologic studies that reported human exposure to styrene and an assessment of cancer end points and it identified several epidemiologic studies that it judged to be less informative compared to the 11 studies listed above. Reasons why the studies were judged as less informative studies and were excluded from Chapter 3 include small numbers of subjects, low concentrations of exposure to styrene, and simultaneous exposure of cohorts to other chemicals in addition to styrene (especially 1,2-butadiene), which prevented a clear characterization of styrene exposures. For an in-depth description of all epidemiology studies that were reviewed by NTP, see NTP (2008).

Evaluation of Informative Studies

After identifying studies that it deemed most informative, the committee evaluated the study data to inform its judgment of whether the evidence of carcinogenesis in humans after exposure to styrene is sufficient, limited, or inconclusive. The following factors were judged by the committee to increase the credibility of evidence on human carcinogenicity of styrene:

- High estimates of MRRs, IRRs, standardized mortality ratios (SMRs), standardized incidence ratios (SIRs), or odds ratios (ORs). The committee considered a study to be particularly credible if a relative risk or its surrogate was ≥2.0 in an entire study cohort or a subset with high exposure (that is, a doubling of cancer mortality or incidence compared to the less exposed or unexposed comparison group). However, a relative risk estimate ≥1.5 also added credibility to the overall body of evidence, particularly if the relative risk was unlikely to be due to chance on the basis of the confidence interval (CI) around the estimate.
- Exposure–response relationships for any reliably established exposure metric.
- Consistency of the above two types of observations among independent cohort studies of the reinforced-plastics industry or between cohort and case–control studies. Some inconsistencies among findings in different populations is typical in the epidemiologic literature and can be the

TABLE 3-1 Summary of Most Informative Epidemiologic Studies Related to Styrene Exposure and Cancer

Study Design, Population, Outcomes, and Analytic Strategy	Exposure Assessment and Exposure Metrics	Strengths and Limitations
Kogevinas et al. 1994 (Included approximately one-third of subjects from Kolstad et al. 1994). A retrospective cohort of 40,688 male and female workers ever employed in 660 reinforced-plastics plants in six countries (Denmark, Finland, Italy, Norway, Sweden, United Kingdom). Mortality from all causes and specific causes. SMRs and 95% CIs: External comparisons based on data from WHO, standardized by sex, age (5-year age groups), and calendar period (5-year periods). Rate ratios and 95% CIs from Poisson regression: Internal comparisons limited to exposed subjects.	Cumulative exposure and average exposure assessed for each worker on the basis of individual job histories and country-, period-, and job-specific exposure estimates from personal sampling measurements and urine measurements. • 16,500 personal sampling measurements from 1955 to 1990. • 18,500 measurements of styrene metabolites in urine conducted in the 1980s. *Cumulative exposure* (<75, 75–199, 200–499, ≥500 ppm–years). *Average exposure* (<60, 60–99; 100–119; 120–199; ≥200 ppm). *Longest-held job* collapsed into 5 job groups (laminators, n = 10,629; workers with unspecified tasks, n = 19,408; workers in other exposed jobs, n = 5,406; workers not exposed to styrene, n = 4,044; and workers with unknown job titles, n = 1,201). *Duration of exposure* (assessed by combining data from payroll records and plant records showing the dates of production of reinforced plastics [in Denmark, pension-fund records were used]). *Time since first exposure* (<10, 10–19, ≥20 years).	Strengths • Large cohort with many workers involved in lamination. • Relatively long duration of followup (period of followup and employment varied by country, average followup = 13 years, 539,479 person–years at risk), with little loss to followup (3.0% of the cohort). • Cumulative exposure computed with and without a 5-year lag period. • Internal comparisons made—*Poisson* regression models included cumulative exposure, age, sex, calendar period, and time since first exposure. Limitations • About 60% of the cohort was employed in the reinforced-plastics industry for <2 years. • No information on smoking, alcohol use, or other lifestyle factors.

Kolstad et al. 1994 A retrospective cohort study of 36,525 male workers in 386 reinforced-plastics plants ever employed during 1964–1988 in Denmark and 14,254 workers not exposed to styrene in 166 industries not producing reinforced plastics, or company unknown. Incidence of all cancers and specific lymphohematopoietic cancers. SIRs and 95% CIs: External comparisons based on national incidence rates standardized for sex, age, and year of diagnosis. SIRs and 95% CIs from Poisson regression (internal comparisons to unexposed workers; authors reported that results were similar to results based on external comparisons but data were not provided).	Pension-fund records were used to determine duration of employment (for exposed workers, only payments recorded during exposed employment were included). *Type of company* (ever producing reinforced plastics, never producing reinforced plastics, and unknown production) and *years since first employment* (<10 vs ≥10 years). For workers employed in plants producing reinforced plastics (n=36,525): • *First year of employment* (1964–1970, 1971–1975, 1976–1988) and *years since first employment* (<10 vs ≥10). • *Years since first employment* (<10 vs ≥10) and *years of employment* (<1 vs ≥1). Analyses of a subset of workers in plants with styrene measurements (9,335 workers employed during the years of sampling). 2,473 personal air samples (not linked to workers or job titles) collected during 1964–1988; 1,814 of which were sampled in the 128 companies included in the study. These were averaged by company and dichotomized as follows: <50 ppm vs ≥50 ppm.	Strengths • Large cohort with relatively long followup (followup during 1970–1989, range of followup 0 to 20 years, mean = 10.9 years, 584,556 person–years at risk) and little loss (<2%) to followup. • Outcome of interest was cancer incidence instead of mortality, which avoids the issues related to cause of death categorization and different lengths of survival after cancer diagnosis. • Internal comparisons made and yielded similar results. Limitations • Exposure assessment at plant level, with 12,837 workers from 287 companies in which it was estimated that ≥50% of workers were involved in reinforced-plastics production (included in Kogevinas et al. 1994) and 23,748 workers from 99 companies in which 1–49% of the workforce produced reinforced plastics); 60% of workers employed <1 year. • Personal sampling data available on a subset of the cohort, but not linked to workers or job titles. • No information on smoking, alcohol use, or other lifestyle factors.

(Continued)

TABLE 3-1 Continued

Study Design, Population, Outcomes, and Analytic Strategy	Exposure Assessment and Exposure Metrics	Strengths and Limitations
Wong et al. 1994 Update of Wong 1990. A retrospective cohort study of 15,826 male and female workers in 30 US reinforced-plastics facilities who were employed in areas exposed to styrene for ≥6 months during January 1, 1948–December 31, 1977. Mortality from all causes and specific causes. SMRs and 95% CIs: External comparisons based on US national age-, sex-, cause-, race-, and year-specific data (race missing from employment records, so the entire cohort was assumed to be white). Coefficients and standard deviations from Cox proportional hazards model; internal comparisons for selected causes of death.	Because of scant historical monitoring data (Wong 1990), contractors collected monitoring data around 1980. Coupling the monitoring data to information about process changes, engineering controls, and personal protective equipment and employment histories, a job-exposure matrix (JEM) was used to estimate exposure for each plant, accounting for calendar time with 6 process categories: 1. Open-mold processing. 2. Mixing- and closed-mold processing. 3. Finish and assembly. 4. Plant office and support. 5. Maintenance and preparation. 6. Supervisory and professional. *Time since first exposure to styrene* (<10, 10–19, ≥20 years). *Duration of employment* (<1, 1–1.9, 2–4.9, 5–9.9, ≥10 years). Sensitivity analyses were done for workers employed >2 years in the 6 process categories. *Duration of exposure (*<1, 1–1.9, 2–4.9, 5–9.9, ≥10 years). *Cumulative exposure (*<10, 10–29.9, 30–99.9, ≥100 ppm–years). *Cumulative exposure and time since first exposure.*	Strengths • 307,932 person–years at risk in the cohort during the followup period (through 1989), with little loss (3.5%) to followup (due to unknown vital status). • Internal comparisons made—*Cox* proportional hazards models with cumulative exposure, duration of exposure, sex, and age included as independent variables. Limitations • 24% of the cohort was employed for <1 year and 27% for >5 years. • "Conservative" historical estimates of styrene exposure were reported by AD Little, Inc. (Little 1981). Exposures not assessed after 1977 (affecting 27% of the cohort). No assessment of average exposure. No information on departments or jobs worked for 3% of the cohort who were assigned the lowest exposure levels. • No information on smoking, alcohol use, or other lifestyle factors.

Kolstad et al. 1995 A retrospective-cohort study of 36,610 male workers in 386 reinforced-plastics plants ever employed during 1964–1988 in Denmark and 14,293 workers not exposed to styrene in similar industries. Mortality from nonmalignant causes and incidence of total and specific solid cancers. SMRs, SIRs, and 95% CIs: External comparisons based on age and calendar-specific national rates. MRRs, IRRs, and 95% CIs from Poisson regression: Internal comparisons based on workers not exposed to styrene in similar industries.	Pension-fund records used to determine duration of employment. *Type of company*. Companies classified as (1) high-probable exposure to styrene (producing reinforced plastics with ≥50% of the workforce involved in production) or (2) low-probable exposure to styrene (<50% of the workforce involved in production). *Year of first employment* (≤1970 and >1970). *Duration of employment (year)* (<1 and ≥1). *Years since first employment* (<10 and ≥10).	Strengths • Large cohort with relatively long followup (followup during 1970–1990, 618,900 person–years at risk) and little loss to followup (<2.1%). • Internal comparisons made—*Poisson* regression models included exposure probability (unexposed, low, and high), age, year of first employment, duration of employment, and time since first employment, but results on exposure probability not reported. • Outcome of interest was cancer incidence instead of mortality, which avoids the issues related to cause of death categorization and different lengths of survival after cancer diagnosis. Limitations • Exposure assessment at plant level. • No information on smoking, alcohol use, or other lifestyle factors.
Gerin et al. 1998 A population-based case–control study of men ages 35–70 years living in the metropolitan area of Montreal, Canada (3,730 cancer cases diagnosed during 1979–1986 in 19 major hospitals; 533 population controls age-stratified to cases; 533 cancer controls and 1,066 pooled controls).	Detailed questionnaires on working histories, including each job held. For each job, information about the company's activities, the raw materials and final product, the machines used and responsibility for machine maintenance, the type of room or building in which the person worked, activities of surrounding workers, and presence of gases, fumes, or dusts. A team of chemists and industrial hygienists estimated exposure for each job:	Strengths • High participation rates of cases—82% of cases agreed to participate, 82% of responses were obtained from study subjects and the rest from next of kin. • Population controls—71% of controls who were selected to participate were interviewed.

(Continued)

TABLE 3-1 Continued

Study Design, Population, Outcomes, and Analytic Strategy	Exposure Assessment and Exposure Metrics	Strengths and Limitations
12 cancer sites. ORs and 95% CIs from unconditional logistic regression.	• Confidence that the exposure actually occurred (possible, probable, definite). • Frequency of exposure during a normal work week (<5%, 5–30%, >30% of the time). • Concentration in the environment (low, medium, high; relative to certain occupations that were used as reference points). • Frequency and concentration coded on an ordinal 1, 2, 3 scale and transformed to 1, 4, and 9 scores for estimating cumulative exposure. *Ever vs never exposed.* *Cumulative exposure index* estimated as the sum over all jobs of the product of duration, frequency, and concentration. Categorized as low, medium, or high (defined by cut-off points at the 70th and 90th percentiles of the distribution of all subjects; medium and high groups were collapsed when numbers were small).	• Detailed retrospective exposure assessment, chemists and industrial hygienists responsible for exposure assessments were blinded to case–control status. • Adjustment for age, family income, ethnic group, cigarette smoking, and respondent status (self or proxy). • Separate analyses using cancer controls, population controls, and pooled controls (generally, authors reported that results were similar). • Single and multiple (styrene, benzene, toluene, and xylene) exposure models. Limitations • Relatively low prevalence of exposure—2% of study population exposed to styrene. Era of first exposure: 0.6% before 1950; 0.8% during 1950–1960; 0.6% after 1960. • Sparse numbers of cases or controls by exposure status for some outcomes.
Ruder et al. 2004 Update of Okun et al. 1985. A retrospective-cohort study of 5,204 male and female workers in two US reinforced-plastics boatbuilding plants who worked ≥1 day during 1959–1978.	Personnel records used to determine departments in which workers were employed and for which periods (no information on job titles). Industrial hygiene surveys were conducted to classify jobs and departments within plants according to level of styrene exposure (Okun et al. 1985).	Strengths • 135,588 person-years at risk in the cohort during the followup period (through 1998) with little loss to followup (n = 72) and few participants excluded because of missing data (n = 3). • Latency analysis conducted.

Mortality from all causes and specific causes (including cancers). SMRs and 95% CI: External comparisons based on rates for Washington state and the United States standardized for sex, race, age, and calendar period. SMRs reported for the entire cohort, the high-exposure and low-exposure cohorts, and for workers who were employed for >1 year.	High-exposure subcohort (n = 2,063) included persons who ever worked in the fibrous-glass or lamination departments (TWA = 42.5 ppm/day in company A or TWA of 71.7ppm/day in company B). Low-exposure subcohort (n = 3,141) included workers who never worked in fibrous-glass or lamination departments (TWA = 5ppm/day). *Latency* (<15 years latency and ≥15 years latency defined as date of first exposure to date of death, date last known alive, or December 31,1998). *Years of employment* (for high-exposure group, <1year vs ≥1 year). *Cumulative exposure* (5 to <500, ≥500 to <5,000, ≥5,000 ppm*). *The publication reported incorrect units for cumulative exposure.	Limitations • Cumulative exposure assessed for two departments at each plant and up to 1978; no information on previous or subsequent employment. • Mean duration of employment for the entire cohort = 1.59 ± 3.0 years; mean duration of employment for the high-exposure subcohort = 1.10 ± 2.1 years and for the low-exposure subcohort = 1.80 ± 3.4 years. • No information on smoking, alcohol use, or other lifestyle factors.
Scélo et al. 2004 A multicenter case–control study in six central and eastern European countries and the United Kingdom (Liverpool). 2,861 newly diagnosed cases and 3,118 hospital-based controls that were frequency-matched to cases on age and sex. Two centers (Poland and Liverpool) recruited population-based controls. Lung cancer. ORs and 95% CIs from unconditional logistic regression.	In-person interviews on jobs held at ≥1 year using standardized questionnaires (specialized questionnaires were used for jobs and industries likely to entail exposures to known or suspected lung carcinogens). Industrial hygienists at each center evaluated the frequency and intensity of exposure to 70 agents and indicated the level of confidence in their assessment. *Duration of exposure* (not exposed and 1–6, 7–14, >14 years).	Strengths • Large case–control study with prospective ascertainment of cases during 1998–2002 from hospitals covering entire population except in Russia (exclusion of Russian cases did not alter results). • Hospital controls excluded cancer and tobacco-related diseases. • Detailed retrospective exposure assessment with standardized protocols across centers and industrial hygienists blinded to case–control status.

(Continued)

TABLE 3-1 Continued

Study Design, Population, Outcomes, and Analytic Strategy	Exposure Assessment and Exposure Metrics	Strengths and Limitations
	Weighted years of exposure (weighted by frequency of exposure in each job) (not exposed and 0.01–0.50, 0.51–3.00, >3.00). *Cumulative exposure* (ppm-years). Frequency and intensity of exposures (2.5, 26, 100 ppm) were based on assigned midinterval weightings (2.5%, 17.5%, 65.0%). Categorical analyses were based on tertiles of the distribution among exposed controls (subjects never exposed made up the referent category).	• Reliability study (of a small number of jobs) indicated comparability among expert teams, although different levels of misclassification by agent. • Adjustment for center, sex, age, tobacco consumption, vinyl chloride, acrylonitrile, formaldehyde, and inorganic pigment dust. • Analyses also conducted with a 20-year lag and for jobs with high-confidence assessments (data not shown in publication). Limitations • Use of hospital-based controls. • Low prevalence of styrene exposure (1.8% of cases and 1.5% of controls).
Seidler et al. 2007 A population-based case–control study of men and women ages 18–80 years living in 6 regions in Germany (710 lymphoma patients diagnosed during 1979–1986; 710 controls matched on sex, region, and age [±1 year of birth]). Lymphoma (and lymphoma subentities). ORs and 95% CIs from conditional logistics regression.	Interviewer-administered questionnaire to obtain information on jobs held for ≥ 1year; start and end dates of employment; and job title, industry, and specific job tasks. For specific occupations, job task-specific supplementary questions were administered. Industrial physician assessed the intensity and frequency of exposure for each job held. • Intensity of exposure assessed as low (0.5 to 5 ppm), medium (>5 to 50 ppm), and high (>50 ppm).	Strengths • Population-based case–control study with prospective ascertainment of cases and a case participation rate of 87.4%. • Detailed retrospective exposure assessment with industrial physician responsible for exposure assessment blinded to case–control status. • High prevalence of styrene exposure in controls (23.8%).

	• Frequency of exposure assessed as low (1 to 5%), medium (>5 to 30%), and high (>30%). • Confidence of exposure assessment (possible but not probable, probable, certain). *Cumulative exposure* (ppm–years). For every job held, the sum of the product of the intensity (2.5 ppm for "low" intensity; 25 ppm for "medium" intensity; 100 ppm for "high" intensity), frequency (3% of the time for "low" frequency; 17.5% of the time for "medium" frequency; 65% of the time for "high" frequency), and duration of employment in the job. Cumulative exposure was categorized as 0; >0 to ≤1.5; 1.5 to ≤67.1; >67.1 ppm-years.	• Adjustment for age, sex, region, smoking (pack years), and alcohol consumption (g/day) in unmatched analyses and adjustment for smoking and alcohol consumption in matched analyses. Limitations • Relatively low participation rate of controls (44.3%). • Sparse numbers of cases or controls by exposure status for some outcomes.
Cocco et al. 2010 Multicenter case–control study in Czech Republic, France, Germany, Ireland, Italy, Spain (2,348 incident cases of lymphoma diagnosed during 1998–2004). 2,462 controls were randomly selected from the general population (Germany and Italy) and matched to cases by sex, 5-year age intervals, and residence areas or selected from hospital controls who were limited to diagnoses other than cancer, infectious diseases, or immune-deficient disease. Lymphoma (by subtype). ORs and 95% CIs from unconditional logistic regression.	In-person interviews on full-time jobs held for ≥1 year; information on activity of the company, tasks performed, machines used, and potential exposures were ascertained. There were14 modules for specific occupations to gather additional details. Occupations were coded using the 1968 International Labour Organisation Standard Classification of Occupations and 4-digit codes of the 1996 European Economic Community Classification of Economic Activities, Revision 1. *Intensity of exposure* (0 = unexposed, 1 = low, 2 = medium, 3 = high). *Frequency of exposure* (proportion of work time involving contact with the agent; 0 = unexposed, 1 = 1–5% work time, 2 = 5–30% work time, 3 = >30% work time).	Strengths • Large case–control study with high participation rates of cases (88%) and hospital controls (81%), although lower participation rates of population controls (52%). • Specific histologic outcomes were studied. • Detailed retrospective exposure assessment. • Adjustment for age, sex, education, and center. • Correction for multiple comparisons. Limitations • A relatively low percentage of subjects had styrene exposure assessed with high confidence (27% of cases and 33% of controls).

(Continued)

TABLE 3-1 Continued

Study Design, Population, Outcomes, and Analytic Strategy	Exposure Assessment and Exposure Metrics	Strengths and Limitations
	Confidence. Based on the probability of exposure (1 = possible but not probable, 2 = probable, 3 = certain) and proportion of workers exposed in a given job (1 = <40%; 2 = 40–90%; 3 = >90%) (low, medium, high). *Cumulative exposure score*, $C_i = \sum (y_i * f_i/3)^{x_i}$ where c_i = cumulative exposure score, i = study subject, y = duration of exposure, x = exposure intensity level, f = exposure frequency level; categorized into quartiles (unexposed, low, medium, and high).	• Possibility for subjects to be occupationally exposed to multiple chemicals. • No adjustment for smoking. • Sparse numbers of cases or controls by exposure status for some outcomes.
Karami et al. 2011 A hospital-based case–control study with controls frequency-matched to cases on age, sex, place of residence in seven centers in central and eastern Europe (1,097 renal-cancer cases and 1,476 controls). Renal cell cancer. ORs and 95% CIs from unconditional logistic regression.	*Jobs held ≥1 year* (questionnaires were used to ascertain lifetime occupational histories: job title, tasks, working environment, time spent on each task, type of employer, and starting and ending dates of employment). Specialized occupational questionnaires were administered for nine specific jobs and eight industries. Industrial hygienists evaluated the frequency and intensity of exposure to PAHs and "plastics" specific to the dates of employment. They assigned a confidence score to their exposure assessment (possible <40%, probable 40–90%, or definite >90% exposure). *Ever vs never exposed.* *Duration of exposure* (years). *Cumulative exposure* (ppm–years). Product of duration in each job, the midpoint of the frequency of exposure	Strengths • Large case–control study with high participation rates of cases (90–99%) and controls (90% –96%) across study centers, with additional controls from a study of head and neck cancer that likely increased power. • Specific histologic type of renal cancer was studied. • Detailed retrospective exposure assessment with industrial hygienists blinded to case–control status. • Adjustment for sex, age, center, smoking status, self-reported hypertension, body mass index, and family history of cancer. • Assessment of lag period and sensitivity analysis (restricted to exposures with a high level of confidence).

	(3%, 17.5%, 65%), and the intensity weight of the job (low, 2.5 ppm; medium, 25 ppm; high, 100 ppm)**, summed across all of the subjects' jobs. *Average exposure* (ppm). Computed by dividing cumulative exposure by the number of years exposed. ** Weights not specified in Karami et al. (2011); assumed to be the same as those reported in Scélo et al. (2004).	Limitations • Use of hospital-based controls. • Relatively low prevalence of styrene exposure in cases (2.1%) and controls (1.2%). • Additional controls from a study of head and neck cancer made it difficult to assess the representativeness of the study. • Sparse numbers of cases or controls by exposure status for some exposure metrics.
Collins et al. 2013 Update of Wong et al. 1994. A retrospective-cohort study of 15,826 male and female workers in 30 reinforced-plastics facilities in 16 US states who were employed in areas exposed to styrene for ≥6 months during 1948–1977. Mortality from all causes and specific causes. SMRs and 95% CIs: comparisons based on the US population standardized for sex, age, time interval. Hazard ratios and 95% CIs from proportional-hazards models (for internal comparisons; cumulative exposure only).	See Wong et al. (1994) for details about the exposure assessment. *Time since first exposure to styrene* (<15 vs ≥15 years). *Cumulative exposure* (0–149.9, 150–399.9, 400–1,199.9, ≥1,200 ppm–months). *Peak exposure* (0, 1–719***, 720–1,799, ≥1,800 days with 100 ppm or higher for 15 min). *Average exposure* (cumulative exposure divided by duration; results reported in the text for pancreatic cancer and diabetes only). *Duration of exposure* (years). Text indicates no increasing trends for any cause of death and duration of exposure. ***Corrected from publication: "1–179".	Strengths • Long duration of followup (1948–2008) with 561,530 person–years at risk and little loss to followup (<1%). • Relatively long duration of exposure (mean = 4.3 years). • Proportional hazards models included adjustment for sex, year of hire, and year of birth. Limitations • Exposure not assessed after 1977 (relevant for 27% of the cohort); average exposure in 1977 was 25 ppm vs 34 ppm a decade earlier. • Peak exposure defined as the average number of peaks over 100 ppm for 15 min of a working day with no details provided as to whether monitoring data or expert judgment was used to construct this exposure metric. • No information on smoking, alcohol use, or other lifestyle factors.

Abbreviations: CI, confidence interval; IRR, incidence rate ratio; MRR, mortality rate ratio; OR, odds ratio; SIR, standardized incidence ratio; SMR, standardized mortality ratio; TWA, time-weighted average; WHO, World Health Organization. Source: committee-generated.

result of the variability across populations, the difficulty in controlling for all potential variables that could influence an observed association or lack thereof, and methods for selecting or retaining individuals in a study and collecting information from them.

- At least two informative studies in independent populations or with varying study designs were needed for the committee to consider evidence for a particular cancer outcome to be credible. In the committee's view, the presence of negative findings in other studies did not negate positive findings. The existence of conflicting findings was one reason why the committee considered the evidence for an association between styrene exposure and carcinogenesis to be limited instead of sufficient.

The committee judged the evidence to be *limited* if the epidemiology evidence was credible but chance, bias, and confounding could not adequately be excluded. The evidence was judged to be *sufficient* if the epidemiology evidence was credible and chance, bias, and confounding could be excluded as an alternative explanation for the observed association.

In addition, the committee considered the implications of traditional statistical significance, which is usually thought of in terms of a p value less than 0.05 and the exclusion of 1.0 in the 95% CI around an effect estimate. The committee considered some observations of increased frequency of disease to be informative in smaller studies if they did not reach traditional statistical significance but were consistent with those of other studies. For example, the National Institute for Occupational Safety and Health cohort described by Ruder et al. (2004) involved fewer person–years than the Kogevinas et al. (1994), Kolstad et al. (1994), and Wong et al. (1994) cohorts and in some instances found similar SMRs, albeit with wider CIs because of the lower statistical power. Given the nature of the styrene exposure assessment in many of the cohort studies, such misclassifications are likely. For example, there were no individual-based exposure data in Kolstad et al. (1994); rather, assigned exposure status was based on the proportion of employees in specific plants who were working in the production of reinforced plastics. Furthermore, as in most occupational studies, it is possible that the "healthy-worker effect" influenced observations of the cohort studies in ways that cannot be determined.

The committee also looked at the presence or absence of isolated associations. Each of the six cohort studies that the committee determined to be most informative included a multitude of statistical analyses of exposures and cancer outcomes, as is typical in occupational cohort studies. Similarly, many different comparisons were conducted in the five case–control studies that were judged informative by the committee, often involving different exposure metrics and specific types of cancer. Because of issues potentially related to multiple comparisons, associations that appeared to be isolated among the multiple studies were not judged to constitute evidence of human carcinogenicity. However, the committee notes that nondifferential exposure misclassification and other data errors that are independent of exposure or disease status tend to result in an at-

tenuation of observed relative risks and its surrogates; therefore, any strong associations repeatedly found in informative studies need to be given more weight.

Findings on Different Types of Cancers

In this section, the committee describes specific findings on cancers of the lymphohematopoietic system, kidney, pancreas, and esophagus. Tables 3-2 through 3-8 and the pages that follow present the salient observations in those studies on specific types of cancer.

Lymphohematopoietic Cancers Combined

As discussed in Chapter 2, the definition of lymphohematopoietic neoplasm has advanced in recent decades. Some of the advance has come from recognition of subtypes that were previously lumped together, some from reclassification of subtypes, and some from revisions of knowledge and capabilities for classification of the broad array of lymphohematopoietic cancers. Recognizing that the grouping of "all lymphohematopoietic cancers" includes many biologically distinct diagnoses in humans (NRC 2011), the committee has adopted a general approach of focusing on more detailed classification when it is feasible but using broader categories when needed. When detailed classification is possible, looking at the finer categories may reveal specific associations if an effect is present in some subcategories but not others. Thus, the committee discussions below begin with the broadest classification of lymphohematopoietic cancers and follow with more detailed classifications. The epidemiologic data provide credible but limited evidence that styrene is a risk factor for lymphohematopoietic cancers on the basis of two European cohort studies (Kogevinas et al. 1994; Kolstad et al. 1994), as the role of chance, bias, or confounding cannot be adequately excluded.

Kogevinas et al. (1994) studied 40,688 workers in Denmark, Finland, Italy, Norway, Sweden, and the United Kingdom and followed up in various countries during 1945–1991 (539,479 person–years and an average duration of followup of 13 years). The SMR for lymphohematopoietic cancers combined was 0.93 (95% CI 0.71–1.20, 60 deaths) (see Table 3-2). Their internal analysis, which used data obtained from workers within the study (instead of an external standard population) and compared workers with different levels of styrene exposure to each other, showed that a longer time since first exposure (at least 10 years vs less than 10 years) was associated with a significantly higher mortality due to combined lymphohematopoietic cancers (10–19 years: MRR = 2.90, 95% CI 1.29–6.48, 25 deaths; at least 20 years: MRR = 3.97, 95% CI 1.30–12.13, nine deaths; p value for the test of linear trend = 0.012). Compared with workers who had an average exposure of less than 60 ppm (seven deaths), the MRRs for those who had an average exposure of 60–99 ppm, 100–119 ppm, 120–199 ppm, and at least 200 ppm were 1.68 (95% CI 0.59–4.79, nine deaths), 3.11 (95% CI 1.07–9.06, 10 deaths), 3.08 (95% CI 1.04–9.08, 13 deaths), and 3.59 (95% CI

TABLE 3-2 Summary of Observations for Lymphohematopoietic Cancers Combined

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 0.93 (0.71–1.20), n = 60 Subgroups by job category: Laminators: SMR = 0.81 (0.43–1.39), n = 13 Unspecified task: SMR = 1.19 (0.80–1.70), n = 30 Other exposed jobs: SMR = 0.65 (0.26–1.34), n = 7 Unexposed: SMR = 0.91 (0.41–1.72), n = 9 Cumulative exposure (ppm–years): <75 as reference: n = 20 75–199: MRR = 0.98 (0.43–2.26), n = 8 200–499: MRR = 1.24 (0.57–2.72), n = 10 ≥ 500: MRR = 0.84 (0.35–2.02), n = 9 p for trend = 0.65 Time since first exposure (years): <10 as reference: n = 13 10–19: MRR = 2.90 (1.29–6.48), n = 25 ≥20: MRR = 3.97 (1.30–12.13), n = 9 p for trend = 0.012 Average exposure (ppm): <60 as reference: n = 7 60–99: MRR = 1.68 (0.59–4.79), n = 9 100–119: MRR = 3.11 (1.07–9.06), n = 10 120–199: MRR = 3.08 (1.04–9.08), n = 13 ≥200: MRR = 3.59 (0.98–13.14), n = 8 p for trend = 0.019
Kolstad et al. 1994	Full study cohort: SIR = 1.20 (0.98–1.44), n = 112 Employees of companies with 1–49% reinforced-plastics workers: SIR = 1.24 (0.99–1.54), n = 81 Employees of companies with 50–100% reinforced plastics workers: SIR = 1.09 (0.74–1.55), n = 31

	Year of first employment: 1964–1970: SIR = 1.32 (1.02–1.67), n = 6 1971–1975: SIR = 1.12 (0.75–1.62), n = 28 1976–1988: SIR = 0.97 (0.57–1.53), n = 18 Time since first employment ≥10 years: Overall: SIR = 1.20 (0.92–1.53), n = 64 Duration of employment <1 year: SIR = 1.65 (1.18–2.26), n = 39 Duration of employment ≥1 year: SIR = 0.84 (0.54–1.24), n = 25
Wong et al. 1994	Full study cohort (CIs not reported): SMR = 0.82 (0.56–1.17), n = 31 Subgroups by latency (that is, time since first exposure in years) (CIs not reported): <10: SMR = 0.81, n = 9 10–19: SMR = 0.66, n = 10 ≥20: SMR = 1.04, n = 12 Subgroups by cumulative exposure (ppm–years) (CIs not reported): <10: SMR = 1.05, n = 9 10–29.9: SMR = 0.56, n = 5 30–99.9: SMR = 0.76, n = 8 ≥100: SMR = 0.94, n = 9 Employed for ≥2 years by processing category (CIs not reported): Open-mold processing: SMR = 1.41, n = 4 Mixing and closed-mold processing: SMR = 0.71, n = 2 Finish and assembly: SMR = 0.62, n = 4 Plant office and support: SMR = 0.65, n = 3 Maintenance and preparation: SMR = 0.93, n= 5 Supervisory and professional: SMR = 1.02, n = 2 In proportional-hazard models, cumulative exposure and duration of exposure to styrene were not significant (n = 31).

(Continued)

TABLE 3-2 Continued

Reference	Observations (95% CI)
Collins et al. 2013	Full study cohort: SMR = 0.84 (0.69–1.02), n = 106 Latency ≥15 years: SMR = 0.87 (0.70–1.07), n = 93 Subgroups by cumulative exposure (ppm–months): 0–149.9: SMR = 0.85 (0.56–1.25), n = 26 150–399.9: SMR = 0.80 (0.51–1.21), n = 23 400–1199.9: SMR = 0.90 (0.60–1.29), n = 29 ≥1,200: SMR = 0.80 (0.53–1.16), n = 28 Cumulative exposure (ppm–months) p for trend = 0.819, hazard ratio = 0.994 (0.983–1.006)
Ruder et al. 2004 Reference population = Washington state	Full study cohort: SMR = 0.74 (0.42–1.20), n = 16 High exposure: SMR = 0.72 (0.20–1.84), n = 4 Low exposure: SMR = 0.74 (0.38–1.30), n = 12 Workers who were employed >1 year: Overall: SMR = 0.54 (0.17–1.26), n = 5 High exposure: SMR = 0.56 (0.01–3.09), n = 1 Low exposure: SMR = 0.53 (0.15–1.37), n = 4 High exposure by duration of employment: <1 year: SMR = 0.76 (0.15–2.50), n = 3 ≥1 year: SMR = 0.58 (0.01–4.70), n = 1

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

0.98–13.14, eight deaths), respectively, with a p value of 0.019 for the test of linear trend. Cumulative exposure (ppm-years) did not appear to be associated with an increase in mortality due to combined lymphohematopoietic cancers in this cohort (Kogevinas et al. 1994).

The study by Kolstad et al. (1994) included 36,525 male workers who were employed in 386 reinforced-plastics plants in Denmark during 1964–1988 and 14,254 employees of similar industries who were not exposed to styrene, with followup from 1970 through 1989 (584,556 person–years and an average duration of followup of 10.9 years). Between this study and the one by Kogevinas et al. (1994), there was an overlap of 12,837 male workers who were employed in 287 Danish plants where more than 50% of the workforce manufactured reinforced plastics. Kolstad et al. (1994) found that the SIR for combined lymphohematopoietic cancers in workers in companies that produced reinforced plastics was 1.20 (95% CI 0.98–1.44, 112 observed cases) (see Table 3-2). When the analysis was stratified by year of first employment, those who were first employed during 1964–1970 had a significantly higher incidence of combined lymphohematopoietic cancers (SIR = 1.32, 95% CI 1.02–1.67, 6 cases), whereas the SIR was 1.12 (95% CI 0.75–1.62, 28 cases) for those first employed during 1971–1975 and 0.97 (95% CI 0.57–1.53, 18 cases) for those first employed during 1976–1988. That observation is consistent with a possible exposure–response relationship, inasmuch as 2,473 historical personal air samples from the cohort showed that average styrene concentrations decreased from 180 ppm in 1964–1970 to 43 ppm in 1976–1988 (Jensen et al. 1990). Workers employed for less than 1 year and with at least 10 years since first employment had a significantly higher incidence than the standard population (SIR = 1.65, 95% CI 1.18–2.26, 39 cases). The SIR in those who were employed for at least 1 year with at least 10 years since first employment was not increased. The phenomenon of high disease frequency in short-term workers is a frequent finding in occupational cohort studies of cancer outcomes. While Kolstad et al. (1994) acknowledged that it was possible for short-term workers to have carcinogenic exposures in other industries or less favorable lifestyle factors, they considered it "less likely since a comparison between the exposed and unexposed short-term employees does not yield lower ratios for leukemia" (p. 277). An internal analysis with Poisson regression was also conducted, but no details were provided except for a statement that the rate ratios were close to the results presented (Kolstad et al. 1994).

Wong et al. (1994) studied a cohort of 15,826 male and female employees who were exposed to styrene for at least 6 months during 1948–1977 in 30 participating reinforced-plastics manufacturing plants in the United States and included followup through 1989 (307,932 person–years). The study observed an SMR of 0.82 (95% CI 0.56–1.17, 31 deaths) for lymphohematopoietic cancers combined in the overall cohort (see Table 3-2). Additional subgroup analyses by latency, duration of employment, duration of exposure to styrene, cumulative styrene exposure (ppm–years), and latency and cumulative styrene exposure simultaneously did not suggest an association between styrene exposure and

mortality due to lymphohematopoietic cancers. An internal analysis with Cox proportional hazard regression—including age, sex, cumulative exposure, and duration of exposure to styrene as independent variables—did not support an association either (Wong et al. 1994).

The study by Collins et al. (2013) is an extension of the Wong et al. (1994) cohort with an additional 19 years of followup (through 2008) and a total number of 561,530 person–years. The SMR for lymphohematopoietic cancers combined was 0.84 (95% CI 0.69–1.02, 106 deaths). Additional analyses by latency, cumulative exposure (ppm–months), number of peak exposures, cumulative duration, and average exposure and an internal analysis did not indicate an association between occupational styrene exposure and mortality due to lymphohematopoietic cancers (Collins et al. 2013).

The study by Ruder et al. (2004) included 5,204 workers exposed to styrene during 1959–1978 in two reinforced-plastic boatbuilding plants in Washington state and followup through 1998 (135,707 person–years). Using the Washington state population as the standard, the investigators observed an SMR of 0.74 (95% CI 0.42–1.20, 16 deaths) for lymphohematopoietic cancers combined (see Table 3-2). The SMRs for people who had high and low exposures were 0.72 (95% CI 0.20–1.84, four deaths) and 0.74 (95% CI 0.38–1.30, 12 deaths), respectively. An additional analysis by duration of employment and another analysis focusing on workers who were employed for over 1 year did not support an association between styrene exposure and mortality due to lymphohematopoietic cancers combined. However, the cohort had a smaller sample and smaller number of person–years of followup than the other studies discussed here. The total number of deaths due to lymphohematopoietic cancers combined was only five in workers who were employed for more than 1 year (Ruder et al. 2004).

Studies of specific types of lymphohematopoietic cancer generate SMRs, SIRs, and MRRs with wider CIs because of the smaller number of observed events (cancer incidence or deaths). Findings on leukemia and non-Hodgkin lymphoma (NHL) are discussed below. Because Hodgkin lymphoma and multiple myeloma are rare and there is a paucity of data from existing studies, the committee concludes that there are insufficient data to assess whether exposure to styrene is associated with the frequency of these two malignancies.

Leukemia

The epidemiologic data provide credible but limited evidence that styrene exposure is associated with an increase in the frequency of leukemia on the basis of two European cohort studies (Kogevinas et al. 1994; Kolstad et al. 1994), as the role of chance, bias, or confounding cannot be adequately excluded. As reported by Kogevinas et al. (1994), workers in the reinforced-plastics industry who had a higher average exposure to styrene or a longer time since first exposure appeared to have a higher probability of dying from leukemia although none of the MRRs reached statistical significance (see Table 3-3). In the Danish cohort (Kolstad et al. 1994), workers who were first employed during 1964–

TABLE 3-3 Summary of Observations for Leukemia

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 1.04 (0.69–1.50), n = 28 Subgroups by job category: Laminators: SMR = 0.48 (0.10–1.39), n = 3 Unspecified task: SMR = 1.40 (0.79–2.28), n = 16 Other exposed jobs: SMR = 0.94 (0.26–2.40), n = 4 Unexposed: SMR = 0.99 (0.27–2.54), n = 4 Cumulative exposure (ppm–years): <75 as reference: n = 11 75–199: MRR = 0.46 (0.10–2.09), n = 2 200–499: MRR = 0.69 (0.19–2.53), n = 3 ≥500: MRR = 0.86 (0.26–2.83), n = 5 p for trend > 0.52 Time since first exposure (years): <10 as reference: n = 5 10–19: MRR = 3.01 (0.90–10.08), n = 12 ≥20: MRR = 3.79 (0.70–20.59), n = 4 p for trend = 0.094 Average exposure (ppm) <60 as reference: n = 3 60–99: MRR = 1.58 (0.32–7.79), n = 4 100–119: MRR = 4.43 (0.98–20.03), n = 8 120–199: MRR = 1.36 (0.22–8.48), n = 3 ≥200: MRR = 2.16 (0.29–16.24), n = 3 p for trend = 0.47

(Continued)

TABLE 3-3 Continued

Reference	Observations (95% CI)
Kolstad et al. 1994	Full study cohort : SIR = 1.22 (0.88–1.65), n = 42 Employees of companies with 1–49% reinforced plastic workers: SIR = 1.15 (0.77–1.67), n = 28 Employees of companies with 50–100% reinforced plastics workers: SIR = 1.38 (0.75–2.32), n = 14 Year of first employment: 1964–1970: SIR = 1.54 (1.04–2.19), n = 30 1971–1975: SIR = 1.00 (0.46–1.90), n = 9 1976–1988: SIR = 0.51 (0.11–1.50), n = 3 Time since first employment ≥10 years: Overall: SIR = 1.57 (1.07–2.22), n = 32 Duration of employment <1 year: SIR = 2.34 (1.43–3.61), n = 20 Duration of employment ≥1 year: SIR = 1.01 (0.52–1.77), n = 12
Wong et al. 1994	Full study cohort: SMR = 0.74 (0.37–1.33), n = 11 Subgroups by latency (time since first exposure in years) (CIs not reported): <10: SMR = 1.11, n = 5 10–19: SMR = 0.68, n = 4 ≥20: SMR = 0.46, n = 2 Subgroups by cumulative exposure (ppm–years) (CIs not reported): <10: SMR = 0.30, n = 1 10–29.9: SMR = 1.12, n = 4 30–99.9: SMR = 0.73, n = 3 ≥100: SMR = 0.80, n = 3 Employed for ≥2 years by processing category (CIs not reported): Open-mold processing: SMR = 0.90, n = 1 Mixing and closed-mold processing: n = 0 Finish and assembly: SMR = 0.80, n = 2

	Plant office and support: SMR = 0.56, n = 1 Maintenance and preparation: SMR = 0.48, n= 1 Supervisory and professional: SMR = 1.33, n = 1 In proportional-hazard models, cumulative exposure and duration of exposure to styrene were not significant (n = 11).
Collins et al. 2013	Full study cohort: SMR = 0.84 (0.60–1.14), n = 40 Latency ≥15 years: SMR = 0.88 (0.61–1.22), n = 35 Subgroups by cumulative exposure (ppm–months): 0–149.9: SMR = 0.61 (0.25–1.26), n = 7 150–399.9: SMR = 1.30 (0.71–2.18), n = 14 400–1,199.9: SMR = 0.66 (0.28–1.30), n = 8 ≥1,200: SMR = 0.83 (0.42–1.49), n = 11 Cumulative exposure (ppm–months) p for trend = 0.908, hazard ratio = 0.996 (0.979–1.014)
Ruder et al. 2004 reference population = Washington state	Full study cohort: SMR = 0.60 (0.19–1.40), n = 5 High exposure: SMR = 0.47 (0.01–2.63), n = 1 Low exposure: SMR = 0.64 (0.18–1.65), n = 4

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

1970 had a significantly higher incidence of leukemia (SIR = 1.54, 95% CI 1.04–2.19, 30 cases) whereas the SIRs for workers first employed after 1970 were not above 1 (see Table 3-3). Given the substantial change in the concentration of styrene exposure in this cohort over time (Jensen et al. 1990), such a finding is consistent with a possible exposure–response relationship. In addition, workers who had more than 10 years of followup since their first employment in a participating plant also had a significantly higher incidence of leukemia (SIR =1.57, 95% CI 1.07–2.22, 32 cases) although the observed higher leukemia incidence was limited to short-term workers whose duration of employment was less than 1 year (SIR = 2.34, 95% CI 1.43–3.61, 20 cases) (Kolstad et al. 1994).

The findings by Wong et al. (1994) and Collins et al. (2013) did not support a leukemogenic role of styrene. The SMR for leukemia was 0.74 (95% CI 0.37–1.33, 11 deaths) and 0.84 (95% CI 0.60–1.14, 40 deaths), respectively, in the studies by Wong et al. (1994) and Collins et al. (2013). Additional analyses by latency, duration of exposure, and cumulative exposure also did not suggest an association between styrene and leukemia in these two studies.

There were only five deaths due to leukemia in the study by Ruder et al. (2004). The SMR for leukemia was 0.60 (95% CI 0.19–1.40) when the Washington state population was used as the standard for the overall cohort and similar for people who had high or low exposures. No additional analysis was conducted, probably because of the small number of deaths.

Non-Hodgkin Lymphoma

The epidemiologic data provide credible but limited evidence that styrene exposure is a risk factor for NHL on the basis of a cohort study (Kogevinas et al. 1994) and two case–control studies (Gerin et al. 1998; Cocco et al. 2010), as the role of chance, bias, or confounding cannot be adequately excluded. In the study by Kogevinas et al. (1994), the SMR for NHL was 0.77 (95% CI 0.43–1.28, 15 deaths) for the general cohort and 1.40 (95% CI 0.56–2.88, seven deaths) for laminators, who were expected to have greater exposure to styrene. Workers who had a higher average exposure to styrene or a longer time since first exposure consistently had higher mortality due to malignant lymphomas (the term probably meant both Hodgkin lymphoma and NHL), as reflected by MRRs in the range of 1.65–7.15 although only one of the six MRRs reached statistical significance (see Table 3-4). The p values for the test of linear trend were 0.052 and 0.072 for average exposure and time since first exposure, respectively. In the Kolstad et al. (1994) study, the SIR for NHL was 1.33 (95% CI 0.96–1.80, 42 cases) (see Table 3-4). The SIRs for different periods of first employment were all above 1 but imprecise in that all 95% CIs included 1 (Kolstad et al. 1994). The study by Wong et al. (1994) listed a total of four deaths due to lymphosarcoma and reticulosarcoma in Table 2 of the publication but 10 deaths due to NHL in Table 9. It is unclear which subtypes of hematopoietic cancers were

TABLE 3-4 Summary of Observations for Non-Hodgkin Lymphoma

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 0.77 (0.43–1.28), n = 15 Subgroups by job category: Laminators: SMR = 1.40 (0.56–2.88), n = 7 Unspecified task: SMR = 0.55 (0.15–1.39), n = 4 Other exposed jobs: SMR = 0.30 (0.01–1.67), n = 1 Unexposed: SMR = 1.01 (0.21–2.94), n = 3 Time since first exposure (years): <10: SMR = 0.51 (0.11–1.49), n = 3 10–19: SMR = 0.76 (0.25–1.78), n = 5 ≥20: SMR = 1.55 (0.42–3.97), n = 4 Duration of exposure (years): <2: SMR = 0.60 (0.19–1.40), n = 5 ≥2: SMR = 1.05 (0.42–2.17), n = 7 Malignant lymphomas (probably include both NHL and Hodgkin lymphoma) Average exposure (ppm): <60 as reference: n = 3 60–99: MRR = 2.51 (0.49–12.87), n = 4 100–119: MRR = 1.65 (0.15–18.57), n = 1 120–199: MRR = 7.15 (1.21–42.11), n = 8 ≥200: MRR = 4.40 (0.42–45.99), n = 2 p for trend = 0.052
Kolstad et al. 1994	Full study cohort: SIR = 1.33 (0.96–1.80), n = 42 Employees of companies with 1–49% reinforced-plastic workers: SIR = 1.65 (1.15–2.28), n = 36 Employees of companies with 50–100% reinforced-plastics workers: SIR = 0.62 (0.23–1.35), n = 6

(Continued)

TABLE 3-4 Continued

Reference	Observations (95% CI)
	Year of first employment: 1964–1970: SIR = 1.28 (0.79–1.96), n = 21 1971–1975: SIR = 1.19 (0.57–2.18), n = 10 1976–1988: SIR = 1.64 (0.82–2.94), n = 11 Time since first employment ≥10 years: Overall: SIR = 1.12 (0.69–1.70), n = 21 Duration of employment <1 year: SIR = 1.27 (0.63–2.28), n = 11 Duration of employment ≥1 year: SIR = 0.98 (0.47–1.81), n = 10
Wong et al. 1994	It is unclear how many deaths due to NHL were included in this study. Table 9 of the publication indicated 10, but earlier tables did not show this. In proportional-hazard models, cumulative exposure and duration of exposure to styrene were not significant (n = 10).
Collins et al. 2013	Full study cohort: SMR = 0.72 (0.50–1.00), n = 36 Latency ≥15 years: SMR = 0.75 (0.52–1.06), n = 33 Subgroups by cumulative exposure (ppm–months): 0–149.9: SMR = 1.08 (0.58–1.85), n = 13 150–399.9: SMR = 0.17 (0.02–0.64), n = 2 400–1,199.9: SMR = 0.94 (0.49–1.64), n = 12 ≥1,200: SMR = 0.65 (0.30–1.23), n = 9 Cumulative exposure (ppm–months) p for trend = 0.766, hazard ratio = 0.994 (0.976–1.013)
Ruder et al. 2004 Reference population = Washington state	Referred to as lymphosarcoma and reticulosarcoma, not NHL Full study cohort: SMR = 0.39 (0.01–2.19), n = 1 High exposure: n = 0 Low exposure: SMR = 0.53 (0.01–2.93), n = 1

Case–Control Studies	
Gerin et al. 1998	NHL, not otherwise specified. Ever occupationally exposed to styrene: Adjusted OR = 2.0 (0.8–4.8), number of exposed cases = 8; unadjusted OR = 2.1 Adjusted for age, family income, ethnic group, cigarette smoking, and respondent status.
Seidler et al. 2007	B-cell NHL (ppm-years): >0 to ≤1.5: adjusted OR = 0.8 (0.6–1.2), number of exposed cases = 53; unadjusted OR = 0.8 >1.5 to ≤67.1: adjusted OR = 1.2 (0.8–1.7), number of exposed cases = 62; unadjusted OR = 1.2 >67.1: adjusted OR = 0.8 (0.4–1.8), number of exposed cases = 12; unadjusted OR = 0.9 Test for trend: p = 0.18 T-cell NHL (ppm-years): >0 to ≤1.5: adjusted OR = 1.3 (0.5–3.6), number of exposed cases = 6; unadjusted OR = 1.7 >1.5 to ≤67.1: adjusted OR = 1.6 (0.5–4.8), number of exposed cases = 4; unadjusted OR = 1.4 Test for trend: p = 0.41 Large diffuse B-cell lymphoma (ppm-years): >0 to ≤1.5: adjusted OR = 0.8 (0.4–1.5), number of exposed cases = 15; unadjusted OR = 0.8 >1.5 to ≤67.1: adjusted OR = 1.3 (0.7–2.3), number of exposed cases = 19; unadjusted OR = 1.3 >67.1: adjusted OR = 1.5 (0.5–4.4), number of exposed cases = 5; unadjusted OR = 1.4 Test for trend: p = 0.03 Follicular lymphoma (ppm-years): > 0 to ≤1.5: adjusted OR =1.1 (0.5–2.1), number of exposed cases = 12; unadjusted OR = 1.3 > 1.5 to ≤ 67.1: adjusted OR = 2.2 (1.2–4.0), number of exposed cases = 17; unadjusted OR = 2.3 >67.1: adjusted OR = 1.6 (0.5–6.0), number of exposed cases = 3; unadjusted OR = 1.6 Test for trend: p = 0.20 Chronic lymphocytic leukemia (ppm-years): > 0 to ≤1.5: adjusted OR = 1.0 (0.5–2.2), number of exposed cases = 10; unadjusted OR = 0.8 > 1.5 to ≤ 67.1: adjusted OR = 1.1 (0.5–2.2), number of exposed cases = 11; unadjusted OR = 1.1 >67.1: adjusted OR = 0.5 (0.2–2.3), number of exposed cases = 2; unadjusted OR = 0.8 Test for trend: p = 0.37

(Continued)

TABLE 3-4 Summary of Observations for Non-Hodgkin Lymphoma

Reference	Observations (95% CI)
	Marginal-zone lymphoma (ppm-years): > 0 to ≤1.5: adjusted OR = 1.0 (0.3–3.0), number of exposed cases = 4; unadjusted OR = 0.8 > 1.5 to ≤ 67.1: adjusted OR = 0.8 (0.2–2.6), number of exposed cases = 3; unadjusted OR = 0.8 Test for trend: p = 0.28 All analyses used "no exposure, i.e., cumulative exposure = 0" as the reference group. All ORs adjusted for age, sex, region, smoking, and alcohol consumption.
Cocco et al. 2010	B-cell NHL: Ever exposed to styrene occupationally: Adjusted OR = 1.6 (1.1–2.3), number of exposed cases = 66; unadjusted OR = 1.6 Cumulative exposure score based on confidence, intensity of exposure, frequency of exposure: p for trend = 0.000096 Adjusted for age, sex, education, and center

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; NHL, non-Hodgkin lymphoma; OR, odds ratio; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

considered NHL. In the proportional-hazard model that included 10 NHL deaths (Table 9 of the publication), duration of styrene exposure and cumulative exposure to styrene were not associated with mortality due to NHL (Wong et al. 1994). In the study by Collins et al. (2013), the SMR for NHL was 0.72 (95% CI 0.50–1.00, 36 deaths). Additional analyses by latency, cumulative exposure (ppm–months), number of peak exposures, cumulative duration, and average exposure and an internal analysis that used Cox proportional-hazard regression did not suggest an association between occupational styrene exposure and mortality due to NHL. The cohort study by Ruder et al. (2004) had only one observed death due to lymphosarcoma and reticulosarcoma and did not use the term *non-Hodgkin lymphoma*. The SMR for lymphosarcoma and reticulosarcoma was 0.39 (95% CI 0.01–2.19) when the Washington state population was used as the standard. No additional analysis was conducted.

Gerin et al. (1998) conducted a population-based case–control study in Montreal, Canada, that ascertained newly diagnosed incident cancer cases from 19 sites between 1979 and 1986 and population controls. For cases with a specific type of cancer (for example, 215 cases of NHL), three different control groups were used: cancer controls who had cancers other than NHL (n = 2,341), population controls (n = 533), and pooled controls (n = 1,066) that consisted of 533 cancer controls randomly selected from the total of 2,341 cancer controls and 533 population controls. All three control groups were used for comparisons with cases, but most of the findings presented by the authors were derived from the comparisons between cases and pooled controls. Based on a logistic regression model adjusted for age, family income, ethnic group, cigarette smoking, and respondent status, subjects who had occupational exposure to styrene appeared to have a higher odds of NHL than those who were not occupationally exposed to styrene (adjusted odds ratio [OR] = 2.0), although the association did not reach statistical significance (95% CI 0.8–4.8). It should be noted that the crude, unadjusted OR was 2.1, which was very close to the adjusted OR derived from the model that controlled for multiple covariates.

A population-based case–control study of lymphoma was conducted in six regions of Germany during 1998–2003 (Becker et al. 2004). Seidler et al. (2007) analyzed the relationship between solvent exposure and malignant lymphoma in the Becker et al. (2004) study, which included 554 incident cases of B-cell NHL and 35 incident cases of T-cell NHL who were diagnosed at the age of 18–80 years and an equal number of gender-, region-, and age-matched population controls (Seidler et al. 2007). Using job task-specific supplementary questionnaires, a trained industrial physician assessed the exposure to styrene and other solvents. The intensity and frequency of exposure to styrene were categorized semi-quantitatively as low, medium, and high, and a cumulative exposure was calculated by incorporating duration, intensity, and frequency of exposure. Compared with subjects without occupational styrene exposure, those who had varying levels of cumulative exposure to styrene had similar frequencies of B-cell NHL and T-cell NHL based on an unconditional logistic regression model that adjusted for age, sex, region, smoking, and alcohol consumption (Table 3-

4). Additional analyses by specific subtypes of B-cell NHL produced similar findings in general, although the trend test for diffuse large B-cell lymphoma (n = 158) was significant (p = 0.03), and elevated ORs for follicular lymphoma were observed for subjects with higher levels of cumulative exposure to styrene (Table 3-4). It should be noted that multiple myeloma was included in this study as a subtype of B-cell NHL, while it is usually considered a separate entity and a distinct type of cancer by itself.

Cocco et al. (2010) conducted a multicenter case–control study of lymphomas in the Czech Republic, France, Germany, Ireland, Italy, and Spain from 1998 to 2004. The study included 1,127 cases of B-cell NHL; 66 of the cases had occupational exposure to styrene. Three independent exposure metrics were used: intensity, frequency, and duration of exposure. Statistical analyses adjusted for age, sex, education, and center. Compared with people who had no exposure to styrene in an occupational setting, those who were exposed to styrene at work had a higher odds of B-cell NHL (adjusted OR = 1.6, 95% CI 1.1–2.3). The test for trend by increasing levels of the three independent exposure metrics yielded a p value of 0.000096, which was lower than a preset p value of 0.000125 chosen by the authors as the threshold for rejecting the null hypothesis. The authors chose a lower threshold than the usual 0.05 to account for multiple comparisons. Supplementary tables available online showed significant trends with frequency and duration of exposure to styrene (p = 0.04 and p = 0.03, respectively) (Cocco et al. 2010). It should be noted that subjects exposed to styrene could have been exposed to other solvents, but of the different subgroups of solvents evaluated in this study, exposure to styrene showed the highest OR of 1.6, with the ORs for exposure to other groups of solvents ranging from 1.0 to 1.2. In addition, the list of covariates adjusted for in Cocco et al. (2010) was not as extensive as in Gerin et al. (1998) and Seidler et al. (2007), but the unadjusted and adjusted ORs in the latter studies were very close for NHL (Gerin et al. 1998) or B-cell NHL (Seidler et al. 2007) (Table 3-4), which suggests that the covariates adjusted for did not have a strong confounding effect. Overall, the findings of Cocco et al. (2010) are consistent with an association between occupational exposure to styrene and B-cell NHL.

Kidney Cancer

The epidemiologic data provide credible but limited evidence that styrene is a carcinogen for the kidney (see Table 3-5) on the basis of the US cohort studies and a European case–control study. The US study of Wong et al. (1994) found a kidney-cancer SMR of 1.75 (95% CI 0.98–2.89); the strongest association was in workers who had been exposed for at least 2 years to open-mold processing (SMR = 4.57, CI not given, three cases). Collins et al. (2013) published an update of the Wong et al. (1994) study with about twice as many person–years of followup. The authors analyzed cumulative exposures (ppm–months), duration of exposure,

TABLE 3-5 Summary of Observations for Kidney Cancer

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 0.77 (0.44–1.25), n=16 Industrial process SMRs (95% CI) Laminators: 0.90 (0.25–2.32), n = 4 Unspecified task: 0.75 (0.30–1.54), n = 7 Other exposed jobs: 0.29 (0.01–1.61), n = 1 Unexposed: 0.69 (0.08–2.51), n = 2 Cumulative exposure (ppm–years): <75 reference: n=2 100–199: MRR = 4.40 (0.71–27.2), n = 3 200–499: MRR = 3.30 (0.42–25.6), n = 2 ≥500: MRR = 6.04 (0.74–49.5), n = 3 trend p = 0.12
Wong et al. 1994	Full study cohort: SMR= 1.75 (0.99–2.89), n = 15 SMRs by latency (years) (CIs not reported): <10: 1.67, n = 3 10–19: 1.41, n = 5 ≥20: 2.18, n = 7 SMRs by duration of exposure to styrene (years) (CIs not reported): <1: 1.89, n = 3 1–1.9: 1.96, n = 3 2–2.9: 1.51, n = 3 5–9.9: 1.26, n = 2 ≥10: 2.15, n = 4 SMRs by cumulative styrene exposure (ppm–years) (CIs not reported): <10: 0.54, n = 1 10–29.9: 2.06, n = 4 30–99.9: 1.67, n = 4 ≥100: 2.55, n = 6

(Continued)

TABLE 3-5 Continued

Reference	Observations (95% CI)
	SMRs employed ≥2 years in processing categories (CIs not reported): Open-mold processing: 4.57, n = 3 Mixing and closed-mold processing: n = 0 Finish and assembly: 1.94, n = 3 Plant office and support: 1.72, n = 2 Maintenance and preparation: 2.14, n = 3 Supervisory and professional: 1.85, n = 1 Proportional-hazard models, cumulative exposure, duration of exposure to styrene were not significant.
Kolstad et al. 1995	Full study cohort: SIR = 0.93 (0.65–1.28), n = 37
Ruder et al. 2004 reference population = Washington state	Full study cohort: SMR = 1.43 (0.57–2.95), n = 7 High exposure: SMR = 3.60 (0.98–9.20), n = 4 Low exposure: SMR = 0.80 (0.16–2.33), n = 3 SMRs for those employed >1 year: Total: 1.38 (0.28–4.04), n = 3 High exposure: 5.11 (0.62–18.4), n = 2 Low exposure: 0.56 (0.01–3.12), n = 1 SMRs for duration of exposure in high-exposure department (year) <1: 2.35 (0.26–10.2), n = 2 >1: 4.91 (0.55–21.3), n = 2
Collins et al. 2013	Total cohort: SMR = 1.18 (0.83–1.62), n = 38 ≥15 years latency: SMR= 1.18 (0.82–1.65), n = 34 Cumulative exposure SMRs (ppm–months): 0.0–149.9: 0.76 (0.28–1.66), n = 6 150–399.9: 1.09 (0.47–2.15), n = 8 400–1,199.9: 0.98 (0.42–1.94), n = 8 ≥1,200: 1.79 (1.02–2.91), n = 16 Test for trend: p = 0.045 Proportional-hazard model: hazard ratio for styrene exposure = 1.009 (1.000–1.017) SMR (days with ≥15 min of styrene at >100 ppm):

	0: 0.88 (0.50–1.42), n = 16 1–719*: 1.08 (0.49–2.04), n = 9 720–1,799: 2.73 (1.17–5.38), n = 8 ≥1,800: 1.82 (0.59–4.24), n = 5 Test for trend: p = 0.054 *Corrected from publication: "1–179".
Case–Control Studies	
Gerin et al. 1998	Kidney cancer: all histologies combined Ever exposed: Adjusted OR = 0.3 (0.0-2.0), n=1; unadjusted OR = 0.3 Adjusted for age, family, income, ethnic group, cigarette smoking, and respondent status.
Karami et al. 2011	Renal-cell carcinoma All exposed cases: Adjusted OR = 1.7 (0.8–3.6), n = 17; unadjusted OR = 1.76 Cumulative exposure** (years x frequency x ppm): ≤1.40: adjusted OR = 0.56 (0.19-1.67), n = 5; unadjusted OR = 0.66 >1.40: adjusted OR = 6.65 (1.82–24.27), n = 12; unadjusted OR = 5.79 Average exposure** (frequency x ppm): ≤0.175:adjusted OR = 1.09 (0.41–2.93), n = 8; unadjusted OR = 1.29 >0.175: adjusted OR = 3.05 (0.99–9.42), n = 9; unadjusted OR = 2.61 Duration of exposure** (years): ≤10:adjusted OR = 1.26 (1.47–3.39), n = 8; unadjusted OR = 1.29 >10: adjusted OR = 2.57 (0.83–7.95), n = 9; unadjusted OR = 2.61 Adjusted for center, sex, age, body-mass index, self-reported hypertension, smoking status (ever/never), and family history of cancer. **OR obtained from S. Karami on July 29, 2013, in response to a request from the Committee to Review the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens.

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; OR, odds ratio; CI, confidence interval; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

average exposure, and number of days with at least 15 min exceeding 100 ppm ("number of peak exposure days") and calculated proportional hazard ratios. Although the SMR in the combined cohort of Collins et al. (2013) was 1.18 (95% CI 0.83–1.62), the authors observed an increased and positive association between styrene exposure and kidney cancer (proportional hazards ratio = 1.009, 95% CI 1.000–1.017) and exposure–response trends for cumulative exposure (ppm–months) ($p = 0.045$) and for number of peak exposure days ($p = 0.054$). The relatively small study of Ruder et al. (2004) in Washington state found an SMR of 1.43 with a wide 95% CI (0.57–2.95) on the basis of seven cases. However, the SMRs for the high-exposure subsets were 3.60 (95% CI 0.98–9.20) overall and 5.11 (95% CI 0.62–18.4) for workers who had been employed for more than 1 year. The European cohort studies (Kogevinas et al. 1994; Kolstad et al. 1995) were either inconsistent with the above observations or had sparse but suggestive data that were based on cumulative exposure–response analysis, as noted in Table 3-5.

An interview-based case–control study focusing on occupational exposure to polycyclic aromatic hydrocarbons and plastics was published by Karami et al. (2011) as part of the Central and Eastern European Renal Cell Carcinoma study. It fell outside the time window for NTP's background document for styrene (NTP 2008) and was not reviewed in the substance profile for styrene (NTP 2011a). The interviews that were part of this study design enabled the authors to control for factors, such as smoking, which could not be controlled for in the cohort studies described above. The study also had the advantage that it included only renal-cell carcinomas whereas the cohort studies described previously in this section apparently also included other histologic types of kidney cancer, such as transitional-cell carcinomas. For study participants who were ever exposed vs never exposed to styrene, an increased OR was observed (OR = 1.7, 95% CI 0.8–3.6). Relative to the unexposed group and after adjustment for location, smoking, family history, hypertension, body-mass index, sex, and age, the ORs were 0.6 (95% CI 0.2–1.7) for exposed persons below the median value of cumulative exposure and 6.7 (95% CI 1.8–24.3) for exposed persons above the median value of cumulative exposure; the p for trend of ORs with cumulative exposure was 0.02. Analysis by average concentration and by duration also yielded higher associations with greater styrene exposure (Table 3-5), including some associations that approached or reached statistical significance and some associations with high ORs. The committee notes that the adjusted ORs, including the one with adjustment for smoking, were similar to or higher than the unadjusted ORs. Limitations of the study included the use of hospital-based controls, excluding patients who had other urologic conditions or diagnoses related to smoking; past cancer diagnosis of nonurologic cancer in the controls was possible. Kidney-cancer survival rates are relatively high and have been increasing over the last several decades (NCI 2014), so studies based on death certificates have the limitation that they do not capture some incident cases, and this can introduce a bias if case survival rates vary among groups with varied degrees of exposure. A smaller population-based case–control study in a Canadian popula-

tion included 177 cases in all of various histological types of kidney cancer and found no association with styrene exposure. The adjusted OR was the same as the unadjusted OR.

Overall, the observations for kidney cancer include repeated observations of associations with styrene exposure for various metrics, including independent populations and contrasting study designs, high estimates of risk, and exposure–response relationships. However, the role of chance, bias, or confounding cannot be adequately excluded. Therefore, the evidence fulfills NTP's listing criteria for limited evidence and not sufficient evidence for an association between exposure to styrene and kidney cancer.

Pancreatic Cancer

The epidemiologic data on pancreatic cancer constitute credible but limited evidence that styrene exposure is associated with pancreatic cancer on the basis of four cohort studies (see Table 3-6). High case-fatality rates in pancreatic cancer make mortality a reliable index of incidence. Kogevinas et al. (1994) found an SMR of 1.48 (95% CI 0.76–2.58) for the highest-exposure group (laminators). The cumulative ppm–years analysis showed an exposure–response trend of p = 0.068 (MRR = 2.56 for exposures greater than 500 ppm, 95% CI 0.90–7.31, 10 cases). Kolstad et al. (1995) found a statistically significant pancreatic-cancer excess incidence in the subgroup that had the highest probability of exposure (IRR = 2.2, 95% CI 1.1–4.5). Ruder et al. (2004) found an SMR of 1.43 (95% CI 0.78–2.41) in the overall cohort and 1.88 (95% CI 0.51–4.81) in the high-exposure subgroup. The Wong et al. (1994) study did not find an association of styrene with pancreatic cancer. The update by Collins et al. (2013) found an overall SMR close to expected (SMR = 0.96, 95% CI 0.73–1.22), but found a significantly increased proportional hazard ratio of 1.008 (95% CI 1.002–1.015) that was based on cumulative exposure and a monotonic "increasing risk with increasing average exposure…with SMRs of 0.75, 0.83, 1.46, and 1.52" (Collins et al. 2013, p. 201). No large case–control studies of pancreatic cancer that include an assessment of styrene have been reported, but the committee did review a small population-based case–control study in Canada that included 116 cases of pancreatic cancer (Gerin et al. 1998). The authors did not find an association of cancer with exposure to styrene.

Overall, the observations for pancreatic cancer demonstrated exposure–response relationships with styrene exposure estimates in cohort mortality studies conducted in both the United States and Europe and in the Danish incidence study. However, the role of chance, bias, or confounding cannot be adequately excluded. Therefore, the evidence fulfills NTP's listing criteria of limited evidence, not sufficient evidence, for an association between exposure to styrene and pancreatic cancer. The committee notes that the study by Collins et al. (2013) has been recently published and was not included in the background document or substance profile.

TABLE 3-6 Summary of Observations for Pancreatic Cancer

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 1.00 (0.71–1.38), n = 37 ≥20 years after first exposure: SMR = 2.05 (0.58-7.29), n = 9 Industrial process SMRs: Laminators: 1.48 (0.76–258), n = 12 Unspecified task: 1.17 (0.68–1.88), n = 17 Other exposed jobs: 0.30 (0.04–1.10), n = 2 Unexposed: 0.79 (0.26–1.86), n = 5 Cumulative exposure (ppm–years): <75 reference: n = 9 100–199: MRR = 1.44 (0.48–4.34) n = 5 200–499: MRR = 1.90 (0.65–5.53), n = 6 ≥500: MRR = 2.56 (0.90–7.31), n = 10 Trend p = 0.068
Wong et al. 1994	Full study cohort: SMR = 1.13 (0.68–1.77), n = 19 SMRs by latency (years) (CIs not reported): <10: 1.45, n = 5 10–19: 0.87, n = 6 ≥20: 1.25, n = 8 SMRs by duration of exposure to styrene (years) (CIs not reported): <1: 2.03, n = 6 1–1.9: 1.04, n = 3 2–4.9: 1.29, n = 5 5–9.9: n = 0 ≥10: 1.30, n = 5

	SMRs by cumulative styrene exposure (ppm–years) (CIs not reported): <10: 1.40, n = 5 10–29.9: 1.61, n = 6 30–99.9: 0.63, n = 3 ≥100: 1.06, n = 5 SMRs employed ≥2 years in processing categories (CIs not reported): Open-mold processing: 0.80 n = 1 Mixing and closed-mold processing: 1.57, n = 2 Finish and assembly: 0.93, n = 3 Plant office support: 0.44, n = 1 Maintenance and preparation: 0.34, n = 1 Supervisory and professional: n = 0
Kolstad et al. 1995	Full study cohort: SIR = 1.20 (0.86–1.63), n = 41 Incidence rate ratio based on exposure probability: Low: 1.1(0.6–2.2), n = 24 High: 2.2 (1.1–4.5), n = 17
Ruder et al. 2004	Full study cohort: SMR = 1.43 (0.78–2.41), n = 14 High exposure: SMR = 1.88 (0.51–4.81), n = 4 Low exposure: SMR = 1.31 (0.63–2.41), n = 10 SMRs for those employed >1 year: Total: 1.54 (0.62–3.17), n = 7 High exposure: 1.23 (0.03–6.85), n = 1 Low exposure: 1.60 (0.59–3.49), n = 6
Collins et al. 2013	Total cohort: SMR = 0.96 (0.73–1.22), n = 63 ≥15 years latency: SMR= 0.90 (0.67–1.17), n = 53 Cumulative exposure SMRs (ppm-months): 0.0–149.9: 0.90 (0.49–1.51), n = 14 150–399.9: 1.15 (0.67–1.84), n = 17

(Continued)

TABLE 3-6 Summary of Observations for Pancreatic Cancer

Reference	Observations (95% CI)
	400–1,199.9: 0.53 (0.24–1.01), n = 9 ≥1,200: 1.24 (0.78–1.86), n = 23 Test for trend: p = 0.274 Proportional-hazards model: hazard ratio for styrene exposure = 1.008 (1.002–1.015) SMR (number of days with ≥15 min of styrene at >100 ppm): None: 0.84 (0.58–1.19), n = 32 1–719*: 1.21 (0.74–1.87), n = 20 720–1,799: 0.52 (0.11–1.51), n = 3 ≥1,800: 1.45 (0.63–2.85), n = 8 Test for trend: p = 0.337 *Corrected from publication "1–179". SMRs, with increasing average exposure = 0.75, 0.83, 1.46, 1.52.
Case–Control Study	
Gerin et al. 1998	Ever exposed: OR = 0.3 (0.0-2.6), n = 1 Adjusted for age, family, income, ethnic group, cigarette smoking, and respondent status.

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; CI, confidence interval; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

Esophageal Cancer

The committee judged there to be credible but limited evidence that high exposure to styrene in workers is associated with esophageal cancer on the basis of observations from Kogevinas et al. (1994), Wong et al. (1994), and Ruder et al. (2004) (see Table 3-7). For esophageal cancer, as for pancreatic cancer, mortality is a relatively reliable index of incidence because of the typically high mortality and short survival of patients.

An internal analysis in the Kogevinas et al. (1994) mortality study found exposure–response patterns among the higher-exposed subjects and an SMR of 5.8 (1.0–34) after 20 years following the first exposure on the basis of six cases. The Kolstad et al. (1994) study did not find any association in the full cohort and did not report on highly exposed subgroups. Wong et al. (1994) observed an SMR of 1.92 (95% CI 1.05–3.22) that was based on 14 cases, but subgroup analyses, all with small numbers, did not show clear patterns. The updated publication of Collins et al. (2013) did not include data on esophageal cancer; its unpublished background report (Collins et al. 2012) indicated that no increased SMRs were found for esophageal cancer. No proportional hazard analyses like those reported for pancreatic cancer and kidney cancer were included. The Ruder et al. (2004) study found an SMR of 2.30 (95% CI 1.19–4.02) with 12 cases in the full cohort (the highest-exposure subgroup had a similar SMR but only two cases). The committee did not identify any large case–control studies of esophageal cancer that included an assessment of styrene exposure, but it did identify a Canadian population-based case–control study that reported 99 cases of esophageal cancer (Gerin et al. 1998). The study authors did not report an association between esophageal cancer and styrene exposure.

Overall, while the epidemiologic evidence is weaker for esophageal cancer than for kidney or pancreatic cancer, there are nevertheless repeated observations of mortality associations with styrene among two independent U.S. cohorts, each with relative risk estimates of approximately double what was expected in the comparison group. Therefore, the evidence fulfills NTP's listing criteria of limited evidence, not sufficient evidence, for an association between exposure to styrene and esophageal cancer.

Lung and Breast Cancers

The committee does not consider there to be credible epidemiologic evidence of an association of styrene exposure and lung or breast cancer (Table 3-8). However, it decided to summarize the data from the most informative studies because lung cancers and breast cancers have been observed in experimental animals after treatment with styrene (see review below). For lung cancer (that is, cancer of the lung, bronchus, or trachea), the Wong et al. (1994) and Collins et al. (2013) studies found statistically significant increases in their combined

TABLE 3-7 Summary of Observations for Esophageal Cancer

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 0.82 (0.47–1.31), n = 1 ≥20 years after first exposure: SMR = 5.82 (1.0-33.91), n = 6 Industrial process SMRs: Laminators: 1.81 (0.87–3.34), n = 0 Unspecified task: 0.83 (0.27–1.93), n = 5 Other exposed jobs: no = 0 Unexposed: 0.82 (0.47–1.31), n = 17 Cumulative exposure (ppm–years): <75 reference: n = 5 100–199: MRR = 1.01 (0.20–5.23), n = 2 200–499: MRR = 1.67 (0.39–7.18), n = 3 ≥500: MRR = 1.76 (0.42–7.30), n = 4 Test for trend p = 0.31
Wong et al. 1994	Full study cohort: SMR = 1.92 (1.05–3.22), n = 14 SMRs by latency (years) (CIs not reported): <10: 1.43, n = 2 10–19: 2.66, n = 8 ≥20: 1.38, n = 4 SMRs by duration of exposure to styrene (years) (CIs not reported): <1: 1.55, n = 2 1–1.9: 2.37, n = 3 2–4.9: 2.41, n = 4 5–9.9: 0.73, n = 1 ≥10: 2.34, n = 4 SMRs by cumulative styrene exposure (ppm–years) (CIs not reported): <10: 2.51, n = 4

	10–29.9: 1.24, n = 2 30–99.9: 2.95, n = 6, p < 0.05 ≥100: 0.97, n = 2 SMRs employed ≥2 years in processing categories (CIs not reported): Open-mold processing: 3.57, n = 2 Mixing and closed-mold processing: n = 0 Finish and assembly: 3.01, n = 4 Plant office support: 0.98, n = 1 Maintenance and preparation: 2.30, n = 3 Supervisory and professional: 1.99, n = 1 Proportional-hazard models, cumulative exposure, duration of exposure to styrene were not significant.
Kolstad et al. 1995	Full study cohort: SIR = 0.92 (0.50–1.57), n = 13
Ruder et al. 2004	Full study cohort: SMR = 2.30 (1.19–4.02), n = 12 High exposure: SMR = 1.85 (0.22–6.67), n = 2 Low exposure: SMR = 2.42 (1.16–4.44), n = 10 SMRs for those employed >1 year: Total: 1.27 (0.26–3.72), n = 3 High exposure: 2.71 (0.07–15.0), n = 1 Low exposure: 1.01 (0.12–3.64), n = 2 SMRs for duration of exposure in high-exposure department (years) <1: 1.18 (0.02–9.61), n = 1 >1: 2.74 (0.04–22.3), n = 1
Case–Control Study	
Gerin et al. 1998	Ever exposed: OR = 1.0 (0.0–3.5), n = 3 Adjusted for age, family, income, ethnic group, cigarette smoking, and respondent status.

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; CI, confidence interval; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

cohort on the basis of 162 and 556 cases, respectively. In the analysis by Wong et al. (1994), the most highly exposed subgroup, which consisted of people who worked in open-mold processing for at least 2 years, did not have an excess SMR (eight cases). A previous nested case–control analysis by Wong (1990) that was based on the same cohort found a strong association with smoking (Mantel-Haenszel Relative Risk = 7.33, chi-square = 4.27, p = 0.04) but no association with styrene exposure (Mantel-Haenszel Relative Risk = 0.63, chi-square = 1.11, p = 0.29). Collins et al. (2013) observed an increased SMR for lung cancer (SMR = 1.34, 95% CI 1.23–1.46) but reported inverse linear trends for cumulative exposure ($p < 0.001$). The proportional hazard ratio was below 1.0, and the 95% CI included 1.0. Ruder et al. (2004) found marginally increased lung-cancer SMRs (see Table 3-8). The Danish and European cohort studies found no evidence of an association between styrene exposure and lung cancer. Scélo et al. (2004) conducted a case–control study of industrial exposures and lung cancer in which there were no increased ORs associated with having been ever exposed to styrene or with the highest category of exposure duration, duration weighted by frequency, or cumulative ppm–years.

For breast cancer, no increase in the frequency of disease was observed in the reinforced-plastics industry; all three cohorts that included women (Kogevinas et al. 1994; Wong et al. 1994; Ruder et al. 2004; Collins et al. 2013) found lower than expected SMRs for breast cancer. When high-exposure subsets were analyzed for breast cancer, no indication of an exposure–response relationship was observed. The paucity of exposed women in the cohorts limits the conclusions that can be drawn. No pertinent case–control studies have been identified. In addition, the mortality data used in those studies comprise a less reliable index of breast cancer compared to incidence data.

Conclusion on Epidemiologic Literature

After identifying the most informative epidemiologic studies and evaluating their results, the committee found that there is limited evidence for the carcinogenicity of styrene on the basis of epidemiologic studies. A causal interpretation is credible, but alternative explanations—such as chance, bias, and confounding factors—cannot adequately be excluded.

CANCER STUDIES IN EXPERIMENTAL ANIMALS

Several studies have been published in which the tumor response to styrene-exposed animals has been measured, but as described in Appendix D, no relevant studies were identified after publication of the RoC. In general, the committee considered studies to be more informative when they included more than one dose, well-matched controls, chronic exposure, treatment groups of

TABLE 3-8 Summary of Observations for Lung, Bronchial, and Tracheal Cancers (Unless Otherwise Indicated)

Reference	Observations (95% CI)
Reinforced-Plastics Industry Cohorts	
Kogevinas et al. 1994	Full study cohort: SMR = 0.99 (0.87–1.13), n = 235 Industrial process SMRs: Laminators: 1.06 (0.81–1.36), n = 60 Unspecified task: 0.99 (0.78–1.24), n = 78 Other exposed jobs: 0.89 (0.65–1.21), n = 42 Unexposed: 0.84 (0.58–1.16), n = 37 Cumulative exposure (ppm–years): <75 reference: n = 73 100–199: MRR = 0.75 (0.47–1.19), n = 25 200–499: MRR = 0.74 (0.47–1.16), n = 26 ≥500: MRR = 0.90 (0.58–1.38), n = 37 Test for trend p < 0.43 (sic)
Wong et al. 1994	Full study cohort: SMR = 1.41 (1.20–1.64), n = 162 SMRs by latency (years) (CIs not reported): <10: 1.07, n = 23 10–19: 1.46, n = 70, p < 0.01 ≥20: 1.51, n = 69, p < 0.01 SMRs by duration of exposure to styrene (years) (CIs not reported): <1: 1.83, n = 37, p < 0.01 1–1.9: 1.25, n = 25 2–4.9: 1.68, n = 44, p < 0.01 5–9.9: 1.37, n = 30 ≥10: 0.97, n = 26 SMRs by cumulative styrene exposure (ppm-years) (CIs not reported): <10: 1.50, n = 37, p < 0.05

(Continued)

TABLE 3-8 Continued

Reference	Observations (95% CI)
	10–29.9: 1.88, n = 48, p < 0.01 30–99.9: 1.33, n = 43 ≥100: 1.04, n = 34 SMRs employed ≥2 years in processing categories (CIs not reported): Open-mold processing: 0.90, n = 8 Mixing and closed-mold processing: 1.24, n = 10 Finish and assembly: 1.43, n = 31 Plant office support: 1.07, n = 17 Maintenance and preparation: 1.49, n = 30, p < 0.05 Supervisory and professional: 0.66, n = 5 Proportional-hazard models, cumulative exposure, duration of exposure to styrene were not significant. Nested case control from same cohort at earlier period (Wong 1990): Direct exposure to styrene: Mantel-Haenszel Relative Risk = 0.63, n exposed cases = 15, p = 0.29 Smoking: Mantel-Haenszel Relative Risk = 7.33, n exposed cases = 30, p = 0.04
Kolstad et al. 1995	Full study cohort: SIR = 1.12 (0.98–1.26), n = 248 Incidence Rate Ratio by exposure probability: Unexposed controls: n = 123 Low probability: 0.9 (0.7–1.1), n = 176 High probability: 1.0 (0.7–1.3), n = 72 All reinforced-plastics workers: 0.9 (0.7–1.1), n = 248
Ruder et al. 2004	Full study cohort: SMR = 1.14 (0.90–1.43), n = 76 High exposure: SMR = 1.29 (0.76–2.04), n = 18 Low exposure: SMR = 1.10 (0.84–1.43), n = 58 SMRs for those employed >1 year: Total: 0.99 (0.67–1.41), n = 31 High exposure: 1.11 (0.40–2.41), n = 6 Low exposure: 0.97 (0.62–1.43), n = 25

	SMRs for duration of exposure in high-exposure department (years): <1: 1.40 (0.77–2.39), n = 14 >1: 0.73 (0.20–2.03), n = 4
Collins et al. 2013	Total cohort: SMR = 1.34 (1.23–1.46), n = 556 ≥15 years latency: SMR= 1.35 (1.24–1.48), n = 501 Cumulative exposure SMRs (ppm–months): 0.0–149.9: 1.60 (1.36–1.87), n = 157 150–399.9: 1.41 (1.18–1.67), n = 131 400–1,199.9: 1.31 (1.10–1.55), n = 138 ≥1,200: 1.10 (0.92–1.31), n = 130 Test for trend: p = 0.003 for inverse trend Proportional-hazard model: hazard ratio for styrene exposure = 0.997 (0.993–1.002) SMR (number of days with ≥15 min of styrene at >100 ppm): None: 1.32 (1.18–1.47), n = 314 1–719*: 1.50 (1.28–1.76), n = 154 720–1799: 1.34 (1.00–1.77), n = 49 ≥1800: 1.06 (0.76–1.46), n = 39 Test for trend: p = 0.201 for inverse trend *Corrected from publication, which printed "1–179".
Case–Control Study	
Gerin et al. 1998	Low exposure to styrene: OR = 0.3 (0.1–1.9), n = 5 Medium / high exposure to styrene: OR = 0.9 (0.2–3.3), n = 5 Adjusted for age, family income, ethnic group, cigarette smoking, respondent status, arsenic, asbestos, chromium VI, nickel, crystalline silica, beryllium, cadmium, and polycyclic aromatic hydrocarbons.
Scélo et al. 2004	Ever exposed to styrene: OR = 0.70 (0.42–1.18), n = 51 Exposure duration OR (years): 1–6: 0.98 (0.37–2.61), n = 13 7–14: 0.72 (0.33–1.59), n = 19 ≥14: 0.59 (0.26–1.34), n = 19

(Continued)

TABLE 3-8 Continued

Reference	Observations (95% CI)
	Duration x frequency of exposure OR (years): 0.01–0.50: 0.67 (0.28–1.56), n = 13 0.51–3.00: 1.19 (0.52–2.73), n = 21 ≥3.00: 0.38 (0.13–1.03), n = 17 Cumulative exposure (years x frequency x ppm) OR: 0.01–2.75: 1.15 (0.55–2.41), n = 22 2.76–12.50: 0.37 (0.13–1.08), n = 9 ≥12.50: 0.53 (0.20–1.43), n = 20 All ORs adjusted for center, sex, age, tobacco consumption, vinyl chloride, acrylonitrile, formaldehyde, and inorganic pigment dust.

Abbreviations: MRR, mortality rate ratio; n, number of cases or deaths in cohort studies or number of exposed cases in case–control studies; CI, confidence interval; OR, odds ratio; SMR, standardized mortality ratio; SIR, standardized incidence ratio. Source: committee-generated.

adequate size, the use of well-characterized test material of high purity, thorough necropsy and pathologic evaluation of tissues according to established criteria, and statistical evaluation of tumor data with accepted methods. The quality of the studies varied considerably; the value of some of them is limited by the numbers of animals treated, exposure duration, observation period, dose selection, or incomplete reporting of methods or results. Studies were considered less informative if any of those attributes were missing or could not be verified from the study description.

Studies of Styrene

Despite weaknesses in some individual studies, the overall body of evidence is sufficient to permit an evaluation of evidence on carcinogenicity. The strongest evidence of a tumorigenic response to styrene is in the mouse lung. Inhalation exposure to styrene has been observed to produce significant increases in alveolar and bronchiolar tumors in both male and female CD-1 mice (Cruzan et al. 2001; Cohen et al. 2002), including a significant increase in malignant tumors in females at the highest dose. CD-1 mice (70 males and females) were exposed to styrene vapor 5 days/week for 6 hours/day. Two groups of 10 mice were sacrificed after 52 and 78 weeks, and the remaining 50 were exposed for 104 weeks (males) or 98 weeks (females). Styrene concentrations were 0, 20, 40, 80, or 160 ppm. Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues in the control and high-dose groups were examined histopathologically. Selected organs and grossly abnormal tissues were examined histopathologically in intermediate-dose groups. After 24 months, the incidence of bronchiolar and alveolar adenomas was increased significantly in males exposed to styrene at 40 ppm or higher and in females exposed at 20, 40, or 160 ppm (see Table 3-9). The incidence of carcinomas alone was significantly increased only in females exposed at 160 ppm. The lung tumors occurred late in the study, and no increases were observed in subgroups of animals terminated after 52 and 78 weeks of exposure.

After oral exposure by gavage, a significant increase in alveolar and bronchiolar tumors combined was observed in male mice at the highest dose, and there was a significant dose-related trend (NCI 1979a). B6C3F1 mice (50 males and 50 females) were given styrene in a corn-oil vehicle by oral gavage 5 days/week for 78 weeks in doses of 150 or 300 mg/kg. Mice (20 males and 20 females) given corn-oil vehicle alone served as controls. Mice in all treatment groups were euthanized 13 weeks after the last dose. Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues were examined histopathologically in all groups with the exception of some

TABLE 3-9 Lung-Tumor Incidence in CD-1 Mice Exposed to Styrene by Inhalation[1]

		Tumor Incidence by Styrene Concentration				
Sex	**Lung Tumor Type**	**0 ppm**	**20 ppm**	**40 ppm**	**80 ppm**	**160 ppm**
Males	Bronchioalveolar adenoma	15/50 (30%)	21/50 (42%)	35/50 (70%)[2]	30/50 (60%)[2]	33/50 (66%)[2]
	Bronchioloalveolar carcinoma	4/50 (8%)	5/50 (10%)	3/50 (6%)	6/50 (12%)	7/50 (14%)
Females	Bronchioalveolar adenoma	6/50 (12%)	16/50 (32%)[2]	16/50 (32%)[2]	11/50 (22%)	24/50 (48%)[2]
	Bronchioloalveolar carcinoma	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	7/50 (14%)[2]

[1]Source: Data from Tables 5 and 6 in NCI (1979a). Observations are expressed as number of animals with the indicated tumor over the number of animals examined.
[2]$p < 0.05$.
Source: Data from Table 4 in Cruzan et al. 2001.

moribund animals. A significant increase in combined adenoma and carcinoma of the lung was observed in male mice at the higher styrene dose compared with controls (Table 3-10). The authors of this National Cancer Institute (NCI 1979a) study also compared their results with those in historical controls that were treated differently (dietary controls rather than mice treated with corn-oil vehicle) and found that the lung-tumor incidences were similar (an average of 12%—incidences were as high as 20% in two studies). That appears to have led the authors to discount to some extent the male mouse lung-tumor findings. The authors concluded that "the findings of an increased incidence of a combination of adenomas and carcinomas of the lung provided suggestive evidence for the carcinogenicity of styrene in male B6C3F1 mice" but also stated, "However, it is concluded that, under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in Fischer 344 rats or B6C3F1 mice of either sex" (p. VIII).

With respect to the male mouse lung-tumor findings in the NCI (1979a) study, the committee considers the use of the historical controls to be inappropriate in that they were not well matched to the treatment conditions of the study. As discussed in Chapter 2, many factors are related to the genetic makeup of the animals and husbandry practices that can influence tumor incidences in control and treated animals. They can include such details as the strain and substrain of experimental animal, the specific supplier, and even the subpopulation within the colony from which the animals were derived. Caging conditions, ventilation, diet, drinking water, and treatment vehicle are also important (Haseman et al. 1984; Festing and Altman 2002; Keenan et al. 2009). For those reasons, controls must be carefully matched, and the best opportunity to do this is almost always with concurrent controls. Although attempts were made by both NCI

TABLE 3-10 Lung-Tumor Incidence in B6C3F1 Mice Exposed to Styrene by Gavage[1]

	Alveolar and Bronchiolar Tumor Incidence			
Sex		**Vehicle Control**	**150 mg/kg**	**300 mg/kg**
Male	Adenoma	0/20 (0%)	3/44 (7%)	4/43 (9%)
	Carcinoma	0/20 (0%)	3/44 (7%)	5/43 (11%)
	Combined	0/20 (0%), p = 0.023	6/44 (14%)	9/43 (20%), p = 0.024
Female	Adenoma	0/20 (0%)	1/43 (2%)	3/43 (7%)
	Carcinoma	0/20 (0%)	0/43 (0)	0/43 (0)
	Combined	0/20 (0%)	1/43 (2%)	3/43 (7%)

[1]Source: Data from Tables 5 and 6 in NCI (1979a). Control data from concurrent controls. Initial numbers of mice: 20 controls and 50 in each dose group. Statistical comparison results in each treatment group were from comparison with controls by one-tailed Fischer's exact test. Results were not significant unless otherwise stated. Probability value for trend from Cochran Armitage test is shown below vehicle control results. No results for trend were presented for adenomas alone.

(1979a) and NTP (2008) to compare results with historical controls, neither documented the extent to which experimental conditions of the historical controls varied from those of the treated groups in the NCI study. The NTP historical-control group included studies in different laboratories, and this raises the possibility that genetic and husbandry factors were not well matched; the NCI historical control comparison included animals treated differently (that is, without corn-oil vehicle treatment). The committee views concurrent controls as an appropriate basis of comparison and interprets the findings of the NCI study, with respect to lung tumors after oral exposure in male mice, as being positive. No significant increases in lung tumors were observed in females, and tumors at other sites were not significantly increased in male or female mice.

In view of the importance of the observation of increased lung tumors in mice following oral exposure in the NCI (1979a) study, the committee took the additional step of confirming that the increases would be statistically significant if contemporary statistic tests were applied. With input from a statistical consultant, the committee applied an age-adjusted analysis of alveolar and bronchiolar tumors for male and female mice using information provided in the NCI report. Tumor incidences in styrene-treated mice were compared with concurrent study controls. On the basis of data included in the report, it was difficult to determine the time of death for animals that did not reach the end of the study, which precluded the use of the poly-3 trend test. However, given that all tumor-bearing mice were identified at terminal sacrifice, the Peto test could be applied using survival curve data and estimates of the numbers of missing or unexamined animals in each treatment group. For some groups, it was possible to infer the number of animals examined in specific treatment groups on the basis

of the data presented in the publication. In other groups, there were several possibilities for the number of animals examined in specific treatment groups, and each possibility was considered. As a result, comparisons between groups resulted in a range of p-values rather than a single value. The p-values obtained from the Peto test are summarized in Table 3-11. Alveolar and bronchiolar adenomas or carcinomas were significantly increased in the high-dose group, and the trend with dose was also statistically significant. Those results are consistent with the results obtained using the statistical test in the original NCI (1979a) report and indicate that the same conclusion is reached using a more modern statistical approach.

Studies of short-term exposure of pregnant mice and their progeny to high doses of styrene (Ponomarkov and Tomatis 1978) are not as well suited as the longer-term studies discussed above to assess tumor induction caused by chronic exposure. However, the short-term studies do provide some limited support for a tumorigenic effect of styrene on the lung. O20 and C57BL pregnant mice were orally exposed to styrene, and carcinogenic and developmental toxic effects were investigated (Ponomarkov and Tomatis 1978). O20 (29 treated) and C57BL (15 treated) mice were exposed at 1,350 mg/kg and 300 mg/kg, respectively, on gestation day 17. After weaning, progeny were exposed once a week at the same doses as dams for each strain; O20 mice were exposed for 16 weeks (discontinued because of toxicity), and C57BL for 120 weeks or until death. The authors indicate that all surviving animals were necropsied with histopathologic examination of all major organs, but the specific organs that were included in the examination were not stated. The preweaning mortality of the O20 mice (43%) was significantly increased compared with controls in which an olive-oil vehicle was used (22%); no difference in postweaning mortality was observed. The O20 strain had a significantly increased total lung-tumor incidence in both sexes compared with vehicle controls ($p < 0.01$). There was no significant increase in preweaning or postweaning mortality or tumor incidence in the C57BL mice in either dams or male and female progeny.

TABLE 3-11 Statistical Comparison of Mouse Lung Tumor Data from the 1979 NCI Study Using the Peto Test

	Tumor	Comparison	P-value
Males	Alveolar/Bronchiolar Carcinoma	Low Dose vs. Control	0.296 – 0.318
		High Dose vs. Control	0.110 – 0.115
		Trend	0.057 – 0.067
	Alveolar/Bronchiolar Adenoma or Carcinoma	Low Dose vs. Control	0.081 – 0.094
		High Dose vs. Control	0.015 – 0.017
		Trend	0.011 – 0.014
Females	Alveolar/Bronchiolar Adenoma	Low Dose vs. Control	0.684 – 0.690
		High Dose vs. Control	0.304 – 0.304
		Trend	0.124 – 0.126
	Alveolar/Bronchiolar Carcinoma	—[1]	—[1]

[1]No alveolar/bronchiolar carcinomas observed. Source: committee generated.

A/J mice (25 females) were given an intraperitoneal injection three times a week for a total of 20 doses, which equaled a total exposure of 200 µmol of styrene (about 100 mg/kg); 4-(*N*-Nitrosomethylamino-)-1-(3-pyridyl)-1-butanone was used as a positive control (Brunnemann et al. 1992). Mice were sacrificed 20 weeks after their last injection. Complete necropsies were performed on all animals. Some of the major organs and sites (head, lung, heart, liver, spleen, pancreas, kidney, and adrenal) and grossly abnormal tissues were preserved and examined histopathologically in all groups. Lung adenomas were observed in three styrene-treated mice and one control; the difference was not statistically significant.

In contrast with the positive findings of lung tumors in mice, styrene exposure of rats by both oral and inhalation routes has had consistently negative results except for mammary tumors. Sprague-Dawley rats (30 males and 30 females in each dose group and 60 male and 60 female controls) were exposed to styrene vapor by inhalation (at 25, 50, 100, 200, and 300 ppm) 4 hours/day, 5 days/week for 52 weeks and then observed until death (Conti et al. 1988). Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues were examined histopathologically in all groups. There was no significant difference in mortality or body weight between any group and controls. A nonsignificant increase in total malignant tumors was observed in both males and females at 100 ppm but not at the higher concentrations tested. There was a significant increase in malignant mammary tumors in females in all exposure groups.

Cruzan et al. (1998) exposed Sprague-Dawley rats (70 males and 70 females) to styrene vapor at 0, 50, 200, 500, and 1,000 ppm 6 hours/day, 5 days/week for 104 weeks. Rats were sacrificed at 105 and 107 weeks. Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues were examined histopathologically in the control and high-dose groups. Selected organs and grossly abnormal tissues were examined histopathologically in the intermediate-dose groups. Body-weight gains were lower in the male 500- and 1,000-ppm groups and in the female 200-, 500-, and 1,000-ppm groups compared with controls. There were no significant increases in any tumor type in males or females.

Ponomarkov and Tomatis (1978) gave 21 pregnant BD IV rats an oral gavage with 1,350 mg/kg of styrene in olive oil on gestation day 17. After weaning, progeny (73 males and 71 females) were given styrene at 500 mg/kg via a gastric tube once a week throughout their lifespan or until 120 weeks, when they were sacrificed. The authors indicated that all surviving animals were necropsied with histopathologic examination of all major organs, but the specific organs that were examined were not stated. There was no difference in litter size, body weight, or tumor incidence between the treated and control groups.

NCI (1979a) gave F344 rats (50 males and 50 females) an oral gavage 5 days/week for 103 weeks at 500 mg/kg or for 78 weeks at 1,000 or 2,000 mg/kg. Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues were examined histopathologically in all groups except for some moribund animals. The 53-week survival of the males in the high-dose group was six of 50, and the 70-week survival of the females in the high-dose group was seven of 50. The 90-week survival in the medium-dose group was 44 of 50 in both males and females and in the low-dose group 47 of 50 in both sexes. No differences in tumor incidence were observed in either sex at any of the doses compared with the corn-oil vehicle controls.

Conti et al. (1988) used Sprague-Dawley rats (40 males and 40 females) to test oral exposure to styrene at 50 and 250 mg/kg via a gastric tube. Rats were exposed 4 or 5 days/week for 52 weeks and then observed until death. Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues were examined histopathologically in all groups. Increased mortality was observed in the females in the high-dose group compared with the olive-oil vehicle controls; no significant difference was observed in males. There was no significant difference in body weight or tumor incidences in either sex.

Beliles et al. (1985) used Sprague-Dawley rats (50 treated males, 70 treated females, 76 control males, and 106 control females) to study drinking-water exposure to styrene. Rats were exposed via drinking water for 2 years at 125 or 250 ppm. In males, the daily doses were estimated to be 7.7 and 14.0 mg/kg for the low and high doses, respectively. In females, the daily doses were estimated to be 12.0 and 21.0 mg/kg for the low and high doses, respectively. Complete necropsies were performed on all animals. The major organs in all organ systems (respiratory, gastrointestinal, cardiovascular, urinary, reproductive, nervous, lymphohematopoietic, endocrine, musculoskeletal, and cutaneous) and grossly abnormal tissues were examined histopathologically in the control and high-dose groups, including moribund animals. No observed effects on mortality, tumor rates, or type of tumors were reported. A separate analysis of the data (Huff 1984) found a dose-related increase in combined mammary tumors in females that was significant in terms of trend ($p = 0.032$) and when the high-dose group was compared to controls ($p = 0.039$).

Conti et al. (1988) gave Sprague-Dawley rats (40 males and 40 females) four intraperitoneal injections at 2-month intervals (200 mg total); no significant differences in tumor incidence were observed when the exposed rats were compared with the olive-oil vehicle controls. In the same study, the researchers gave

Sprague-Dawley rats (40 males and 40 females) a single subcutaneous injection of 50 mg of styrene at the age of 13 weeks and observed them until death; no significant differences were observed when the exposed rats were compared with the olive-oil vehicle controls.

As evident from the study descriptions above, a styrene effect on mammary tumors in rats is contradictory. Rats exposed to styrene by inhalation were reported to have a significant increase in malignant mammary tumors (Conti et al.1988) although inconsistencies in reporting of the data render this observation inconclusive (IARC 1994a). Huff (1984) analyzed data from oral exposure of Sprague-Dawley rats to styrene in the same study and found a significant increase in combined mammary tumors in high-dose females and a significant trend with dose. In contrast with those suggestive findings, no increase in mammary tumors was observed in the NCI (1979a) oral study of styrene-exposed rats, and the inhalation study by Cruzan et al. (1998) found a significant inverse trend between dose and mammary gland carcinoma after styrene inhalation in Sprague-Dawley rats (that is, the incidence decreased with increasing styrene concentration in air). There is no evidence from mouse and rat bioassays of increased cancer incidence at other sites.

Studies of Styrene-7,8-oxide and Styrene Mixtures

A bioassay was conducted with a mixture of 70% styrene and 30% beta-nitrostyrene in B6C3F1 mice and F344 rats (NCI 1979b). The mixture was administered to male rats by gavage in corn oil at 150 or 300 mg/kg and to female rats at 75 or150 mg/kg. The mixture was administered to mice by gavage in corn oil at 87.5 and 175 mg/kg for mice of both sexes. Each dose group consisted of 50 males and 50 females, and the controls were 20 males and 20 females that were given only corn oil. Rats were exposed for 79 weeks and observed for an additional 29 weeks, and mice were exposed for 78 weeks and observed for an additional 14 weeks. No significant increases in tumors at any site were observed in rats. Significant increases in alveolar or bronchiolar carcinoma and alveolar and bronchiolar adenoma were observed in the low-dose male mice but not the high-dose male mice, which experienced high mortality. Because the bioassay involved a combination of styrene with another substance (beta-nitrostyrene), the interpretation of findings with respect to potential styrene carcinogenicity is confounded, and the committee did not include it in its evaluation.

Styrene-7,8-oxide, a metabolite of styrene, was evaluated in a cancer bioassay. Styrene-7,8-oxide is the principal metabolite of styrene in rodents and humans, and its carcinogenicity is potentially relevant as supporting information in a determination of the carcinogenicity of styrene in humans. Four studies

evaluated tumor response to administration by gavage. In one, B6C3F1 mice and F344 rats (52 males and 52 females per treatment group per species) were given styrene-7,8-oxide by gavage in corn oil 3 days/week for 104 weeks (Lijinsky 1986). Doses were 0, 375, and 750 mg/kg for mice and 0, 275, and 550 mg/kg for rats. Significant increases in forestomach tumors were observed after both high and low doses in both species and sexes. Similarly, significant increases in forestomach tumors were observed in male and female Sprague-Dawley rats (40 per treatment group per sex) that were given styrene-7,8-oxide by gavage in corn oil at 50 or 250 mg/kg for 52 weeks (Conti et al. 1988). Significant increases in forestomach tumors were also observed in BD IV rats given styrene-7,8-oxide by gavage in olive oil at 200 mg/kg (Ponomarkov et al. 1984). The only other tumor response observed was a significant increase in hepatocellular neoplasms in B6C3F1 mice given styrene-7,8-oxide by gavage at 375 mg/kg (but not 750 mg/kg) (Lijinsky 1986).

Styrene-7,8-oxide is a reactive compound, so it is not surprising that tumors occurred primarily in tissues proximal to the site of administration—in the case of gavage, the forestomach. If administered by a different route, or when styrene-7,8-oxide is formed from metabolism of styrene, the distribution of styrene-7,8-oxide in the body would probably be substantially different and would plausibly lead to different sites of tumorigenesis. In view of that, despite the discordant sites of tumors between styrene (lung) and styrene-7,8-oxide (forestomach), positive findings with styrene-7,8-oxide are considered supporting evidence of the carcinogenicity of styrene.

Summary of Evidence from Studies in Animals

In summary, the committee identified studies that showed positive findings of lung tumors in mice after both inhalation and oral administration of styrene in well-conducted chronic bioassays (NCI 1979a; Cruzan et al. 2001). Results of another study that is more limited in value for assessing carcinogenicity (Ponomarkov and Tomatis 1978) are also reasonably consistent with the production of lung tumors in mice after styrene exposure. Contradictory findings on mammary tumors have been observed in rats. For other tumor sites, rats exposed to styrene by both oral and inhalation routes have been consistently negative (Jersey et al. 1978; NCI 1979a; Beliles et al. 1985; Conti et al. 1988; Cruzan et al. 1998); the tumorigenic response appears to be species-specific.

MECHANISTIC AND OTHER RELEVANT DATA

Genotoxicity

Styrene is a highly reactive chemical whose potential for genotoxicity has been investigated for 3 decades. Many studies have been designed to determine

whether styrene or styrene-7,8-oxide—its reactive epoxide metabolic product—elicits DNA damage that leads to mutagenic and clastogenic events in animal and human cells or in animals and humans exposed to styrene or styrene-7,8-oxide. A comprehensive review of data with respect to carcinogenicity of styrene-7,8-oxide was conducted by the International Agency for Research on Cancer (IARC 1994b). The formal evaluation at that time was that there was sufficient evidence of the carcinogenicity of styrene-7,8-oxide in experimental animals. A later review by IARC (2002) concluded that exposure of humans to styrene leads to the generation of styrene-7,8-oxide-induced DNA adducts and other forms of DNA damage. The overall evaluation by IARC, which attached heavy weight to the evidence of genotoxicity, was that styrene is possibly carcinogenic in humans. NTP also reviewed styrene-7,8-oxide and in 2002 listed it as "reasonably anticipated to be a human carcinogen" (NTP 2002).

DNA adducts are considered mechanism-based biomarkers of exposure to chemical carcinogens and have been used to identify people and populations at risk for cancer and to set exposure limits for occupational carcinogens (Swenberg et al. 2008; Jarabek et al. 2009). The presence of DNA adducts in target tissues reflects the formation of reactive metabolites, such as styrene-7,8-oxide, that bind covalently to DNA and to proteins (Poirier 2012). The presence of structurally modified DNA bases substantially increases the probability that polymerase errors during DNA synthesis will create mutations in genes that may lead to cancer (Knobel and Marti 2011).

There are also reports that oxidative DNA damage, mediated by reactive oxygen species, is caused by exposure of tissues to styrene-7,8-oxide and contributes to its genotoxic effects. A study by Laffon et al. (2002a) that suggested exposure to styrene may result in oxidative DNA damage was cited in the background document for styrene; however, Gamer et al. (2004), using 8-oxoguanine as a biomarker, found no evidence of oxidative stress. In a comparative study of styrene-exposed workers and unexposed clerks (Manini et al. 2009), exposed workers showed lower concentrations of 8-oxoguanine adducts in white blood cell DNA but higher concentrations of 8-oxoguanine in urine. Similar results were obtained by Wongvijitsuk et al. (2011). Considering that 8-oxoguanine is a weak mutagen and is efficiently repaired by base-excision repair, it seems unlikely that oxidative DNA damage plays a strong role in styrene-associated genotoxicity.

The mutagenic and carcinogenic effects of DNA adducts are affected by the efficiency of DNA repair (both base-excision repair, as in the case of oxidative DNA damage, and nucleotide-excision repair of adducts derived from styrene). Thus, susceptibility to styrene genotoxicity may be affected at the individual level by DNA-repair capacity, some aspects of which may be inducible (Vodicka et al. 2004a, 2006).The effects of single-nucleotide polymorphisms on styrene genotoxicity in vivo have been comprehensively reviewed by Vodicka et al. (2006).

Evaluation of Genotoxicity Evidence

The committee's charge was to "integrate the level-of-evidence conclusions, and consider…all relevant information in accordance with RoC listing criteria" (see Appendix B). The RoC includes "studies on genotoxicity (ability to damage genes) and biological mechanisms" for each substance listed (NTP 2011c, p. 3). That information is evaluated with other relevant evidence to address the RoC listing criteria. Specifically, "data derived from studies of tissues or cells obtained from humans exposed to the substance in question, which is particularly valuable in evaluating whether a relevant cancer mechanism is operating in humans," constitute one of several lines of evidence used to establish whether there is sufficient or limited evidence of carcinogenicity from studies in humans (NTP 2011c).

The committee reviewed the relevant literature, including all recently published studies, with the goal of determining whether it is biologically plausible for styrene to act as a carcinogen through a genotoxic mechanism. The committee's comprehensive review of scientific peer-reviewed literature on the genotoxicity and mutagenicity of styrene and the dates covered by the search are described in Appendix D. As noted in the Environmental Protection Agency Cancer Guidelines (EPA 2005), one must go beyond simply counting the numbers of studies that report statistically significant results or statistically nonsignificant results on carcinogenesis and related modes of action to reach credible conclusions about the relative strength of the evidence and the likelihood of causality. Accordingly, the committee first categorized evidence pertaining to styrene and styrene-7,8-oxide genotoxic and clastogenic mechanistic events into tables on DNA damage (Table 3-12), sister-chromatid exchanges (Table 3-13), micronuclei (Table 3-14), and chromosomal aberrations (Table 3-15). Studies in each table were categorized as positive if a statistically significant effect was observed. Studies were categorized as negative if they reported an absence of a particular effect (that is, no statistically significant difference from the appropriate control group). Committee members exercised their scientific judgment in categorizing studies, but they did not perform a formal quality assessment of each individual study or make critical judgments regarding study design or methodology, recognizing that all studies cited have been subjected to some form of peer review. Table 3-16 summarizes the evidence. For each mechanistic event (Tables 3-13 to 3-15), the committee used a set of causal criteria (EPA 2005) as general guidance to determine the strength of the overall evidence of causality.

TABLE 3-12 Studies of DNA Damage Associated with Styrene or Styrene-7,8-oxide (Including Adducts and Strand Breaks)[a]

		Styrene		Styrene-7,8-oxide	
		Positive	Negative	Positive	Negative
Human	*In vitro*	—	—	Bastlová et al. 1995 Vodicka et al. 1996 Marczynski et al. 1997b Pauwels and Veulemans 1998 Laffon et al. 2001b Laffon et al. 2002b Vodicka et al. 2002a Laffon et al. 2003b Cemeli et al. 2009[c] Fabiani et al. 2012[c]	—
	In vivo[b]	Brenner et al. 1991 Maki-Paakkanen et al. 1991 Vodicka et al. 1993 Vodicka et al. 1994 Vodicka et al. 1995 Marczynski et al. 1997a Somorovska et al. 1999 Vodicka et al. 1999 Laffon et al. 2002a Migliore et al. 2002 Shamy et al. 2002 Buschini et al. 2003 Fracasso et al. 2009[c] Manini et al. 2009[c] Mikes et al. 2010[c] Wongvijitsuk et al. 2011[c] Costa et al. 2012[c]	Holz et al. 1995 Vodicka et al. 2004a Godderis et al. 2004 Hanova et al. 2010[c] Teixeira et al. 2010[c] Hanova et al. 2011[c]	—	—

(Continued)

TABLE 3-12 Continued

		Styrene		Styrene-7,8-oxide	
		Positive	Negative	Positive	Negative
Rodent	In vitro	Sina et al. 1983	—	Sina et al. 1983 Liu et al. 1988a Dypbukt et al. 1992 Bjørge et al. 1996 Herrero et al. 1997	—
	In vivo[b]	Walles and Orsen 1983[d] Byfält -Nordqvist et al. 1985[d] Cantoreggi and Lutz 1993 Pauwels et al. 1996[d] Vaghef and Hellman 1998[d] Boogaard et al. 2000b Vodicka et al. 2001b Otteneder et al. 2002 Mikes et al. 2009[c] Gate et al. 2012[c]	Kligerman et al. 1993	Walles and Orsen 1983[d] Byfalt-Nordqvist et al. 1985[d] Lutz et al. 1993[e] Sasaki et al. 1997[d] Vaghef and Hellman 1998[d] Tsuda et al. 2000[d]	Gate et al. 2012[c]

[a]Studies were categorized as positive if a statistically significant effect was observed. Studies were categorized as negative if there was an absence of a particular effect (that is, no statistically significant change from the appropriate control group); [b]Route of administration is inhalation unless noted otherwise; [c]Identified through committee's literature search; [d]Denotes chemical administration through intraperitoneal injection; [e]Denotes chemical administration through oral gavage.

TABLE 3-13 Studies of Sister-Chromatid Exchanges Associated with Styrene or Styrene-7,8-oxide[a]

		Styrene		Styrene-7,8-oxide	
		Positive	Negative	Positive	Negative
Human	In vitro	Norppa et al. 1980a Norrpa et al. 1983a Norppa and Vainio 1983 Norppa and Tursi 1984 Chakrabarti et al. 1993 Lee and Norppa 1995	—	Norppa et al. 1980a Norppa et al. 1983a Pohlova et al. 1984 Pohlova and Sram 1985 Zhang et al. 1993 Lee and Norppa 1995 Uüskula et al. 1995 Chakrabarti et al. 1997 Ollikainen et al. 1998 Laffon et al. 2001b	—
	In vivo[b]	Andersson et al. 1980 Camurri et al. 1983 Camurri et al. 1984 Yager et al. 1993 Hallier et al. 1994 Tates et al. 1994 Artuso et al. 1995 Karakaya et al. 1997 Biro et al. 2002 Laffon et al. 2002a Teixeira et al. 2004 Teixeira et al. 2010[e] Costa et al. 2012[e]	Meretoja et al. 1978a Watanabe et al. 1981 Watanabe et al. 1983 Hansteen et al. 1984 Maki-Paakkanen 1987 Kelsey et al. 1990 Brenner et al. 1991 Maki-Paakkanen et al. 1991 Sorsa et al. 1991 Van Hummelen et al. 1994 Holz et al. 1995 Rappaport et al. 1996	—	—
Rodent	In vitro	De Raat 1978 Norppa and Tursi 1984 Norppa et al. 1983b	—	De Raat 1978 Nishi et al. 1984 Von der Hude et al. 1991	—

(Continued)

TABLE 3-13 Continued

		Styrene		Styrene-7,8-oxide	
		Positive	Negative	Positive	Negative
Rodent	In vivo[b]	Conner et al. 1979 Conner et al. 1980 Sharief et al. 1986[f] Kligerman et al. 1992	Preston and Abernethy 1993[d]	Conner et al. 1982[c] Sinsheimer et al. 1993[c,f]	Norppa et al. 1979[c] Conner et al. 1982[c]

[a]Studies were categorized as positive if a statistically significant effect was observed. Studies were categorized as negative if there was an absence of a particular effect (that is, no statistically significant change from the appropriate control group); [b]Route of administration is inhalation unless noted otherwise; [c]Identified from IARC (1994b); [d]Identified from IARC (2002); [e]Identified through committee's literature search; [f]Denotes chemical administration through intraperitoneal injection.

TABLE 3-14 Studies of Micronuclei Associated with Styrene or Styrene-7,8-oxide[a]

		Styrene		Styrene-7,8-oxide	
		Positive	Negative	Positive	Negative
Human	In vitro	Linnainmaa et al. 1978b	—	Linnainmaa et al. 1978a Linnainmaa et al. 1978b Laffon et al. 2001b Speit et al. 2012[d]	—
	In vivo[b]	Meretoja et al. 1977 Hogstedt et al. 1983 Nordenson and Beckman 1984 Brenner et al. 1991 Tates et al. 1994 Holz et al. 1995 Laffon et al. 2002a Godderis et al. 2004 Teixeira et al. 2004 Vodicka et al. 2004a Migliore et al. 2006a	Maki-Paakkanen 1987 Hagmar et al. 1989 Maki-Paakkanen et al. 1991 Sorsa et al. 1991 Tomanin et al. 1992 Yager et al. 1993 Van Hummelen et al. 1994 Anwar and Shamy 1995 Karakaya et al. 1997 Hanova et al. 2010[d] Teixeira et al. 2010[d] Costa et al. 2012[d]	—	—
Rodent	In vitro	—	—	Turchi et al. 1981	—
	In vivo[b]	Penttila et al. 1980[c] Norppa 1981[c,e]	Kligerman et al. 1992 Gate et al. 2012[d]	—	Fabry et al. 1978[c] Penttila et al. 1980[c] Gate et al. 2012[d]

[a]Studies were categorized as positive if a statistically significant effect was observed. Studies were categorized as negative if there was an absence of a particular effect (that is, no statistically significant change from the appropriate control group); [b]Route of administration is inhalation unless noted otherwise; [c]Identified from Scott and Preston (1994a); [d]Identified through committee's literature search; [e]Denotes chemical administration through intraperitoneal injection.

TABLE 3-15 Studies of Chromosomal Aberrations Associated with Styrene or Styrene-7,8-oxide[a]

		Styrene		Styrene-7,8-oxide	
		Positive	Negative	Positive	Negative
Human	In vitro	Linnainmaa et al. 1978a Linnainmaa et al. 1978b Pohlova et al. 1984[g] Pohlova and Sram et al. 1985 Jantunen et al. 1986	—	Linnainmaa et al. 1978a Linnainmaa et al. 1978b Fabry et al. 1978 Norppa et al. 1981 Pohlova et al. 1984[g] Pohlova and Sram 1985	—
	In vivo[b]	Meretoja et al. 1977 Meretoja et al. 1978a Fleig and Thiess 1978 Hogstedt et al. 1979 Andersson et al. 1980 Dolmierski et al. 1983 Camurri et al. 1983 Camurri et al. 1984 Hansteen at al. 1984 Forni et al. 1988 Tomanin et al. 1992 Tates et al. 1994 Artuso et al. 1995 Anwar and Shamy 1995 Lazutka et al. 1999 Somorovska et al. 1999 Helal and Elshafy 2013[f]	Thiess et al. 1980 Watanabe et al. 1981 Watanabe et al. 1983 Nordenson and Beckman 1984 Pohlova and Sram 1985 Maki-Paakkanen 1987 Jablonicka et al. 1988 Hagmar et al. 1989 Maki-Paakkanen et al. 1991 Sorsa et al. 1991 Oberheitmann et al. 2001 Biro et al. 2002 Vodicka et al. 2004a Vodicka et al. 2004c Migliore et al. 2006b	—	—
Rodent	In vitro	Matsuoka et al. 1979 Ishidate andYoshikawa 1980	Matsuoka et al. 1979	Turchi et al. 1981	—

Rodent	In vivo[b]	Meretoja et al. 1978b[e]	Loprieno et al. 1978[e,h] Norppa et al. 1980b[e] Sbrana et al. 1983[e,h] Sinha et al. 1983[e] Sharief et al. 1986[e] Kligerman et al. 1992 Preston and Abernethy 1993[d]	Loprieno et al. 1978[e,h] Sinsheimer et al. 1993[c,e]	Fabry et al. 1978[e] Norppa et al. 1979[e]

[a]Studies were categorized as positive if a statistically significant effect was observed. Studies were categorized as negative if there was an absence of a particular effect (that is, no statistically significant change from the appropriate control group); [b]Route of administration is inhalation unless noted otherwise; [c]Identified from IARC (1994a,b); [d]Identified from IARC (2002); [e]Identified from Scott and Preston (1994a); [f]Identified through committee's literature search; [g]Denotes chemical administration through intraperitoneal injection; [h]Denotes chemical administration through oral gavage.

TABLE 3-16 Summary of Genotoxic Effects of Styrene in Humans and Rodents

		DNA Damage		Sister-Chromatid Exchanges		Micronuclei		Chromosomal Abberations	
		Styrene	SO	Styrene	SO	Styrene	SO	Styrene	SO
Human	In vitro	N/A	+ (10/0)	+ (6/0)	+ (10/0)	+ (1/0)	+ (4/0)	+ (5/0)	+ (6/0)
	In vivo	+/- (17/6)	N/A	+/- (13/12)	N/A	-/+ (11/12)	N/A	+/- (17/15)	N/A
Rodent	In vitro	+ (1/0)	+ (5/0)	+ (3/0)	+ (3/0)	N/A	+ (1/0)	+/- (2/1)	+ (1/0)
	In vivo	+ (10/1)	+ (6/1)	+ (4/1)	+/- (2/2)	+/- (2/2)	- (0/3)	- (1/7)	+/- (2/2)

"+" All or most of the studies indicate the effect.
"+/-" Most of the studies indicate the effect, although many studies show lack thereof.
"-/+" Most of the studies indicate lack of the effect, although many positive studies have been published.
"-" All or most of the studies indicate lack of the effect.
Parentheses indicate total number of studies demonstrating the effect or lack thereof.
N/A, no studies identified.
Abbreviations: SO, styrene-7,8-oxide.

Genotoxic and Clastogenic Effects of Styrene and Styrene-7,8-Oxide on in Vitro Human and Rodent Cells

The evidence available on all forms of DNA and genetic damage shows clearly that DNA damage (Table 3-12) and clastogenic effects (Tables 3-13 through 3-15) are observed when human or rodent cells are incubated in the presence of styrene or styrene-7,8-oxide. Studies conducted in various in vitro model systems, including freshly isolated human blood cells and whole blood, were consistently strong (one negative study identified among dozens of positive studies). Furthermore, positive effects were observed in connection with many types of mechanistic events that pertain to genotoxicity.

DNA adducts initiate carcinogenesis; however, those adducts may be undetectable in target tissues in later stages of the carcinogenesis process. DNA adducts also form in non-target tissues and bioactivation may occur at sites other than the target organ (Swenberg et al. 2008). Additionally, mutations induced by a specific adduct may require additional mutations to produce cancer (Vogelstein et al. 2013). The committee considered this mechanistic information in evaluating whether styrene,7-8,oxide–DNA adducts contribute to the development of human cancer.

The committee made several observations about evidence in the in vitro studies that used human and rodent cells. All studies that were evaluated in this category used purified styrene or styrene-7,8-oxide; thus, chemical specificity was firmly established. Most studies used positive and negative controls, and this strengthens the chemical specificity of the associations between exposure and genotoxicity. Temporality of the observed associations between styrene, styrene-7,8-oxide, and genotoxicity was clearly established. And concentration–response relationships between genotoxic effects and styrene or styrene-7,8-oxide were observed in studies that were designed to measure such effects.

The committee concludes that the evidence of genotoxicity and clastogenicity of styrene and styrene-7,8-oxide in human and rodent cells in vitro is consistent, strong, and specific with respect to exposure to styrene or styrene-7,8-oxide. Temporal and exposure–response relationships have been established. These mechanistic events have been studied extensively in human cells, and the results are consistent with those found in cells obtained from rodents.

Genotoxic and Clastogenic Effects in Animals or Humans Exposed to Styrene and in Rodents Exposed to Styrene-7,8-Oxide

Many studies have attempted to evaluate the genotoxicity and clastogenicity of styrene in humans exposed in an occupational setting. Styrene-induced DNA damage was found in many of the studies (Table 3-12). Most studies in rodents demonstrate sister-chromatid exchanges after exposure to styrene or styrene-7,8-oxide, but only about half of the human studies that examined styrene exposure demonstrated the same effects (Table 3-13) . With respect to micronuclei, studies of humans or rodents exposed to styrene are also equally divided; however, formation of micronuclei was not observed in three studies of rodents exposed to styrene-7,8-oxide (Table 3-14). For chromosomal aberrations (Table 3-15), about half of the studies of humans exposed to styrene show a significant effect. In exposed rodents, formation of chromosomal aberrations was not found in most of the studies of styrene exposure or in half studies of styrene-7,8-oxide exposure.

In rodent studies of styrene and styrene-7,8-oxide, evidence was less strong but mostly positive for DNA damage, sister-chromatid exchanges, and micronuclei; however, the total number of rodent studies was less than the number of studies of exposed humans. Effects of styrene or styrene-7,8-oxide were well documented, and this helps to establish that exposure to styrene or styrene-7,8-oxide was associated with the positive and negative observations. Studies of rodents provided evidence of a temporal relationship for the observed association in that effects were observed only after exposure to the agents in question. Studies of rodents also provided strong evidence of a genotoxic concentration–response relationship.

Evidence of genotoxicity and clastogenicity of styrene in exposed humans is generally strong, although some studies reported no effects. The strongest positive observations involved DNA-damage end points found in studies of diverse cohorts of subjects exposed to styrene in various occupations. Various assays were used to evaluate the mechanistic events, and the statistical significance of the effects was firmly established in the positive studies. Some investigators established exposure to styrene through biomonitoring, but many of their studies were of occupational groups and the contributions from other toxic agents cannot be excluded. An exposure–response association for several biomarkers of genotoxicity and clastogenicity was demonstrated through workplace dosimetry (Fracasso et al. 2009) or the use of urinary biomarkers of exposure (Teixeira et al. 2010).

The committee concludes that the biologic plausibility of genotoxic and clastogenic effects of styrene, as observed in exposed animals and humans, is supported by solid and extensive observational evidence. Several negative studies notwithstanding, the evidence is generally strong and specific with respect to styrene or styrene-7,8-oxide exposure. Both temporal and exposure–response relationships have been clearly established by diverse studies, including studies of exposed humans. That most of the observational evidence used in this evaluation is derived from studies of humans exposed to styrene substantially strengthens the relevance of the mechanistic evidence to the epidemiologic findings.

Summary of Evidence on Genotoxicity of Styrene

Styrene requires metabolic activation to electrophilic intermediates (for example, styrene-7,8-oxide) for it to be able to form covalent adducts with DNA. DNA damage, reflected by the presence of styrene-7,8-oxide-derived DNA adducts in human tissues, is highly likely to generate mutations, some of which may occur in genes that lead to cancer in susceptible people. The presence of DNA adducts—occurring predominantly at the N7, N2, and O6 positions of guanine—has been amply demonstrated in cell culture, experimental animals, and, most important, lymphocytes of workers occupationally exposed to styrene (Table 3-12). These findings have been reproduced in many laboratories and provide strong evidence of the genotoxic effects of styrene.

Unless removed by nucleotide excision repair, styrene-7,8-oxide DNA adducts invariably serve as a substrate for DNA polymerases, including specialized lesion-bypass polymerases that may either block DNA synthesis at the lesion site or catalyze the introduction of nucleotides that lead to mutational changes. Evidence of the mutagenicity of styrene and styrene-7,8-oxide was established early on in studies of bacteria and other nonmammalian systems (IARC 1994a, 2002). Consistently positive results, with or without metabolic activation, have been reported for gene-mutation end points in bacteria and other model organisms exposed to styrene-7,8-oxide. In studies with styrene, results were less consistent in the absence of metabolic activation; positive results were reported in *Salmonella typhimurium* strains TA1530 or TA1535 with the addition of an exogenous metabolic activation system (IARC 2002). Additional evidence of the mutagenic potential of styrene or styrene-7,8-oxide includes studies of Chinese hamster (V79) cells, mouse lymphoma (L5178Y), and human T lymphocytes (HPRT locus) (Vodicka et al. 2006). Moreover, many studies of occupationally exposed workers report a positive association between styrene exposure and frequency of sister-chromatid exchanges, micronuclei, and chromosomal aberrations (Tables 3-13 to 3-15). Although the evidence for and against an association of clastogenic effects with styrene exposure in humans is nearly equally divided, the diversity of studies, exposure scenarios, and methodology support the biologic plausibility of the genotoxicity of styrene in exposed humans. Even low-concentration occupational exposure to styrene was shown to result in an increase in various genotoxic effects (Wongvijitsuk et al. 2011).

Genotoxic effects have been explored in comparisons with structurally related epoxides, many of which are classified as human carcinogens or as likely to be human carcinogens (Fabiani et al. 2012).

The committee concludes that the genotoxicity and mutagenicity of styrene has been thoroughly and comprehensively investigated. The evidence reviewed by the committee also indicates that styrene-7,8-oxide, a major reactive metabolite of styrene that is produced in exposed humans, reacts with DNA to form covalent adducts and other premutagenic forms of DNA damage, which result in genotoxic effects. The committee recognizes that styrene-7,8-oxide may not be the only genotoxic metabolite of styrene. For example, styrene-3,4-oxide may also be mutagenic (Watabe et al. 1982). However, to the committee's knowledge, the potential contribution of styrene-3,4-oxide to the carcinogenic response to styrene and the potential contribution of other aromatic-ring metabolites of styrene in addition to styrene-7,8-oxide have not been investigated.

Overall, the observations in various studies performed over the last 3 decades have been consistent. Temporal and exposure–response relationships have been established. Not only is the experimental evidence extensive, it is likely to be relevant to all target tissues that have been associated with cancer after exposure to styrene. Causality is strengthened by the large amount of evidence obtained from studies of exposed humans.

Immunosuppression

The human immune system plays a critical role in defending the body against external pathogens and in being on perpetual alert against internally transforming (premalignant) or transformed malignant cells (cancers). The concept of "immune surveillance" describes those functions specifically and relies on the involvement of a network of white blood cells, also known as leukocytes. There are two basic types of leukocytes: phagocytes (including neutrophils, monocytes, and macrophages, which are important in innate immunity) and lymphocytes (including T, B, and natural killer [NK] cells that allow the immune system to recognize, memorize, and specifically respond to previous invaders). NK lymphocytes are critical for the innate and adaptive immune systems because they can destroy virus-infected or malignantly transformed cells. Therefore, they are extremely important in immunosurvillance. When deficiency is present in one or more immune components as a result of congenital or acquired conditions, immunodeficiency or immunosuppression might occur and the incidence of malignancies might increase. For example, Kaposi sarcoma, NHL, and cervical cancers occur at a higher rate in people who have acquired immunodeficiency syndrome (AIDS) as a result of infection by human immunodeficiency virus (HIV) (Labarga 2013). In addition, all de novo neoplasms have a greater incidence in renal-transplantation patients because of antilymphocytic treatment (Andrés 2005).

As discussed in Chapter 2, NTP identified immunosuppression as a possible mechanism by which styrene exposure could lead to malignancies, but the

background document and the substance profile lack strong evidence to support this mechanism. Therefore, the committee undertook an independent literature search to identify research that would inform the topic (see Appendix D). The committee considered studies relevant if they reported data on changes in basic hematologic measures, such as white blood cell (WBC) count and WBC differential count.[2] The committee also included studies that reported such measures as weight of lymphoid organs and expression of functional markers. Efforts were made to identify and include studies that reported effects on local, systemic, innate, and adaptive immunity in exposed animals or humans. Studies that reported genotoxic measures were excluded from this section because they are discussed in more detail in the genotoxicity sections of Chapter 2 and the present chapter. The committee's literature search yielded 233 results, 19 of which were relevant articles that were not already cited in the background document for styrene. Eight of the studies documented hematologic effects in experimental animal models or in animal cells (Table 3-17), and 11 described hematologic effects in humans or human cells (Table 3-18). Those studies were reviewed in detail by the committee and are discussed below.

Animal Studies

Leukocytopenia and Lymphocytopenia

Two studies described hematologic effects in peripheral blood that were consistent with leukocytopenia and lymphocytopenia. Brondeau et al. (1990) observed a transient decrease in WBCs (leukopenia) in rats exposed to styrene for 4 hours. Seidel et al. (1990) observed decreased lymphocyte counts (lymphocytopenia) in peripheral blood of female C57BL/6 x DBA/2 hybrid mice after exposure to styrene.

Systemic vs Localized Lymphoid Organs

In animal models, the response of lymphocytic organs to styrene exposure can vary at different locations. For example, the weight of the spleen was significantly lower in mice exposed to styrene than in controls, whereas the weights of peripheral lymph nodes were higher in exposed mice than in controls (Dogra et al. 1989). Lymphocytic proliferation in the spleen was significantly lower in styrene-7,8-oxide-exposed C57BL/6 mice than in styrene-exposed mice; this indicates that an active intermediate form of styrene may be needed for systemic inhibition to occur (Grayson and Grill 1986). In contrast, allergic responses

[2]A differential blood count gives the relative percentage of white blood cell types, such as neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

TABLE 3-17 Immune Effects of Inhalation or Intraperitoneal Exposure to Styrene in Animals

Cell Type	Hematologic Effects	Reference
RBC	↑ erythroid by inhalation	Nano et al. 2000
	No change by intraperitoneal administration	Nano et al. 2000
WBC	↓ by 4h exposure, but innate rather than specific effects cannot be ruled out	Brondeau et al. 1990
Neutrophils	↓ numbers of promyelocytes and myelocytes temporarily by inhalation (chronic) but unchanged by intraperitoneal administration (acute)	Nano et al. 2000
Monocytes	↓ nitro-blue tetrazolium, monocyte attachment, phagocytic activity	Dogra et al. 1989
NK cells	↓ activity by styrene, styrene-7,8-oxide	Grayson and Gill 1986
Lymphocyte	↓ numbers	Seidel et al. 1990
	↓ spleen weight	Dogra et al. 1989
	↓ splenic lymphocyte counts vs no change in lymphocyte counts in regional or peripheral lymph nodes or bone marrow	Dogra et al. 1989
T cells	↑ interferon-gamma in local lymph nodes by inhalation	Ban et al. 2003
	↑ T-helper lymphocyte cytokine and interleukin level	Ban et al. 2006
	↑mitogen-stimulated proliferation	Sharma et al. 1981; Dogra et al. 1989
	↑ delayed-type hypersensitivity	Dogra et al. 1989
B cells	↓ immunoglobulin M plaque-forming unit	Dogra et al. 1989
	↑ mitogen-stimulated proliferation at lowest and middle doses	Dogra et al. 1989
Stem cells	Unaffected in CFU-S and CFU-C but lower in BFU-E and CFU-E although statistical difference could not be reached	Seidel et al. 1990

Abbreviation: BFU-E, burst-forming unit-erythroid; CFU-C, colony-forming unit in culture; CFU-E, colony-forming unit-erythrocyte; CFU-S, colony-forming unit-spleen; NK, natural killer cell; RBC, red blood cell; WBC, white blood cell.

TABLE 3-18 Immune Effects of Inhalation Exposure to Styrene in Humans

Cell type	Hematologic Effects	Reference
RBC	↓ RBC and hermatocrit	Checkoway and Williams 1982
WBC	↓ absolute neutrophils	Checkoway and Williams 1982
	No difference in CBC	Hagmar et al. 1989; Tulinska et al. 2000; Biro et al. 2002; Jahnova et al. 2002
	↑ WBC	Somorovska et al. 1999
Monocytes	↑ adherent molecule expression	Somorovska et al. 1999
	↑ percentage	Hagmar et al. 1989; Stengel et al. 1990; Tulinska et al. 2000; Jahnova et al. 2002
	↓ percentage	Khristeva 1986
	↑ necrosis, apoptosis, increased bcl-2 and raf-1 proteins	Diodovich et al. 2004
Lymphocyte	↓ lymphocytes	Tulinska et al. 2000
	No change	Biro et al. 2002
	↑ lymphocytes	Khristeva 1986
T cells	↓ numbers	Tulinska et al. 2000
	↑ CD4+ T (Th) cells	Mutti et al. 1992; Bergamaschi et al. 1995; Biro et al. 2002
	No difference in mitogen-induced proliferation of lymphocytes	Hagmar et al. 1989; Somorovska et al. 1999
	↓ mitogen-induced proliferation of lymphocytes	Somorovska et al. 1999; Tulinska et al. 2000; Jahnova et al. 2002
	↓ large-granule lymphocytes	Somorovska et al. 1999
NK cells	↑ NK cells	Mutti et al. 1992; Bergamaschi et al. 1995
	↓ NK function (K562 cell lysis)	Bergamaschi et al. 1995
B cells	No change in numbers	Mutti et al. 1992; Tulinska et al. 2000
	↑ CD25+ expression in B cells	Bergamaschi et al. 1995
	No change in mitogen-induced proliferation of lymphocytes	Hagmar et al. 1989; Tulinska et al. 2000; Jahnova et al. 2002

Abbreviation: CBC, complete blood count; CD4, cluster of differentiation 4; NK, natural killer cell; RBC, red blood cell; WBC, white blood cell.

through such mechanisms as increased interferon-gamma, interleukin (specifically IL-4, IL-5, and IL-13), and immunoglobulin E (IgE) production were observed more in lung and lymph nodes than in those produced from lymphocytes in the spleen of female BALB/c mice (Ban et al. 2003, 2006).

Innate vs Adaptive Immunity

Immune responses are typically divided into two categories—innate (nonspecific) responses and adaptive (antigen-specific) responses. Monocytes, macrophages, neutrophils, and NK cells are the main effector cells in innate immunity, and T and B lymphocytes are part of adaptive immunity. In the studies reviewed by the committee, styrene generally had more suppressive effects than stimulatory effects on innate immunity. For example, a substantial impairment was observed in macrophage and monocyte functional studies and resulted in a reduction in nitroblue tetrazolium, changes in surface attachment, and changes in phagocytic indexes in mice exposed to styrene (Dogra et al 1989). In addition, in a dose-dependent manner, styrene and styrene-7,8-oxide were strong suppressors of NK-cell activity in exposed mice, whereas cytotoxic T-cell activity was not affected (Grayson and Grill 1986).

In contrast, styrene exerted more stimulatory effects on adaptive cellular immunity in mice by enhancing delayed hypersensitivity (also known as type IV hypersensitivity) (Dogra et al. 1989), mitogen-stimulated lymphoblastic transformation (Sharma et al. 1981), increased production of interferon-gamma and cytokines, and increased production of interleukins by T-helper type 2 lymphocytes (Sharma et al. 1981; Dogra et al. 1989; Ban et al. 2003, 2006). For B lymphocytes, Dogra et al. (1989) observed reduced IgM plaque-forming colonies but increased liposaccharide-stimulated proliferation.

Hematopoietic Malignancy

The committee found two animal studies that provided information on hematopoietic measures of malignancies (Seidel et al. 1990; Nano et al. 2000). Animals exposed to styrene had reduced erythroid lineage colony-forming function (specifically, burst-forming unit erythroid [BFU-E] and colony-forming unit erythroid [CFU-E]), but normal colony-forming unit function in the spleen (CFU-S) and colony-forming unit function in culture (CFU-C) (Seidel et al. 1990). Nano et al. (2000) aimed to determine whether there was a higher frequency of malignancies in hematopoietic tissues of rats treated with styrene by either injection or inhalation; they did not observe an increase in the frequency of preleukemic or leukemic disorders in rats exposed to styrene, although decreased promyelocytes and myelocytes were observed after exposure by inhalation (Nano et al. 2000).

Human Studies

Leukocytopenia and Lymphocytopenia

The studies of humans exposed to styrene reported inconsistent results for WBC and lymphocyte counts. Abnormal WBC differential was identified in two studies (Somorovska et al. 1999; Jahnova et al. 2002), but more results showed either normal complete blood counts or increased WBCs in exposed people (Hagmar et al. 1989; Somorovska et al. 1999; Tulinska et al. 2000; Biro et al. 2002; Jahnova et al. 2002).

Systemic vs Localized Lymphoid Organs

The committee did not identify any studies of humans that reported systemic vs localized effects on the immune system after exposure to styrene.

Innate vs Adaptive Immunity

The committee identified studies that reported effects on the innate immune system following exposure to styrene. Increases in monocytes or monocytosis were reported in five of seven studies (Hagmar et al. 1989; Stengel et al. 1990; Somorovska et al. 1999; Tulinska et al. 2000; Jahnova et al. 2002). Only one study found a decrease in monocytes (Khristeva 1986) and another found that necrosis and apoptosis were increased in monocytes (Diodovich et al. 2004). For NK cells, two studies found that the number of NK cells were increased in workers exposed to styrene (Mutti et al. 1992; Bergamaschi et al. 1995). Bergamaschi et al. (1995) also performed a functional study of NK cells (that is, in vitro lysis of leukemia cell lines) and demonstrated that workers exposed to styrene had significantly lower cytotoxic activity toward leukemia cells than the control group. On the basis of those results, styrene might have suppressive effects on NK cells, but more studies are needed before a stronger conclusion can be reached.

Among the studies that investigated effects of styrene on adaptive immunity, inconsistent results were observed between the number of lymphocytes in workers exposed to styrene and the number in controls. For example, Khristeva (1986) reported an increase in the number of lymphocytes, but Tulinska et al. (2000) observed a decrease in the number of lymphocytes and Biro et al. (2002) found no change. Three studies found a decrease in mitogen-induced T-cell proliferation (Somorovska et al. 1999; Tulinska et al. 2000; Jahnova et al. 2002), and two found no difference between exposed and non-exposed groups (Hagmar et al. 1989; Somorovska et al. 1999). Most of the studies found no change in the number of B lymphocytes and no change in mitogen-induced proliferation of B lymphocytes in workers exposed to styrene (Hagmar et al. 1989; Mutti et al. 1992; Bergamaschi et al. 1995; Tulinska et al. 2000; Jahnova et al. 2002).

Conclusions on Immunosuppression Evidence

As mentioned in Chapter 2 and above, the background document and the substance profile lack strong evidence to support immunosuppression as a potential mechanism of carcinogenesis, so the committee undertook a literature search (see Appendix D). No relevant studies of the immunosuppressive effects of styrene were identified that were not available to NTP (that is, all relevant articles were published by June 10, 2011). After reviewing the relevant studies, the committee determined that the evidence on immune effects after exposure to styrene varies and is inconsistent. In animals, inhibitory effects were observed mainly on the innate immune system, including decreases in lymphocyte counts and weights in the spleen, suppressed monocyte and macrophage activity, and suppressed NK-cell activity. In adaptive immunity, stimulatory effects were observed in cellular immunity, including increased type IV hypersensitivity and increased production of cytokines, interferon-gamma, and interleukins. In contrast, effects on humoral immunity in styrene-exposed animals varied. For example, IgM plaque-forming cells were decreased, but lipopolysaccharide-induced proliferation was increased. In humans exposed to styrene, effects were more varied and both suppressive and stimulatory effects were observed. Additional research is needed to understand the effects of styrene on the immune system and to explore whether immunosuppression is a possible mechanism for styrene-induced carcinogenesis.

Cytotoxicity

The use of cytotoxic responses to investigate the mode of action by which exposure to metabolically activated compounds, such as styrene, produces tumors depends on a clear definition of the conditions that render a specific organ or tissue susceptible to injury. As outlined in the section "Metabolism and Toxicokinetics" above, many factors contribute to those conditions. Recent literature regarding cytotoxicity of styrene addresses three general questions: Which isozymes of the cytochrome P450 mono-oxygenase system are involved in metabolic activation? What are the chemical nature and reactivity of the metabolites? Which antioxidants and phase II enzymes interact with the reactive metabolites to modulate toxicity?

Pulmonary and Hepatic Toxicity

Recent studies of the mechanisms by which styrene produces cytotoxic injury have relied on the cytotoxic response in the lungs, and occasionally the liver, of one species: the mouse. The relevance of bioactivation (by cytochrome P450 mono-oxygenases) and detoxification (by glutathione S-transferases and epoxide hydrolases) pathways has been evaluated by using relatively nonspecific assays (Carlson 2010b, 2011a,b, 2012; Meszka-Jordan et al. 2009; Shen et al.

2010). For substances released into the airway space, bronchoalveolar-lavage fluid has been analyzed for protein, cells, and succinic dehydrogenase. For the liver, serum has been analyzed for sorbitol dehydrogenase. Although those non-specific approaches appear to provide reliable screening tools and reflect the overall responses of the organs, they lack sufficient specificity to define the response in the presumed target cells for styrene, the Clara cells. That is especially true in the mouse lung, in which cells that have high metabolic potential are not restricted to the terminal bronchioles but are distributed throughout the airway tree, with some activity in the gas-exchange area (Buckpitt et al. 1995). Studies with another CYP450-activated cytotoxic aromatic hydrocarbon, naphthalene, have also documented that acute Clara cell injury increases in terminal bronchioles as the intraperitoneal dose is elevated and that injury extends about as far as lobar bronchi at higher doses (Plopper et al. 1992). When naphthalene is administered via inhalation, acute injury is equal to or greater in proximal bronchi than that produced in terminal bronchioles (West et al. 2001).

Other studies of styrene have assessed toxicity by examining proliferation in the presumed target area (terminal bronchioles). Toxicity was determined on the basis of differential counts of cells that have undergone proliferation and that have incorporated and expressed a DNA precursor (5-bromo-2'-deoxyuridine, BrdU) (Cruzan et al. 2012, 2013). The committee notes that restricting the histopathologic and quantitative analysis to terminal bronchioles may not accurately characterize the full response, because the cells that are most likely to be undergoing replication, and most of their daughter cells, are also the cells that are most likely to be damaged by bioactivated cytotoxicants. In addition, at higher doses, the level of cell death may be so high that repopulation of distal airways is principally by progenitor cells that are found in more proximal airways and at airway bifurcations (Stripp et al. 1995; Lawson et al. 2002).

Kaufmann et al. (2005) reported high levels of labeled cells in proximal bronchi following 3 days of exposure to styrene and two of its metabolites, styrene-7,8-oxide and 4-vinylphenol. Further complicating the interpretation of BrdU–incorporation studies of styrene cytotoxicity is the fact that epithelial cells injured by initial exposure to a cytotoxic agent undergo a cycle of necrosis and exfoliation of injured cells and squamation and proliferation of surviving cells, followed by migration and differentiation of newly produced cells to repopulate injured sites. The first phase is usually complete by 2 to 3 days following a single exposure and the second phase by 5 to 7 days following exposure. The timing depends on the route and concentration of the toxicant exposure. This process has been well documented for the oxidant gas ozone (Paige and Plopper 1999; Plopper et al. 2001) and for naphthalene (Van Winkle et al. 1995; Lawson et al. 2002), and seems to be the case for styrene based on observations by Kaufmann et al. (2005). When this injury–repair cycle occurs in the presence of elevated levels of the cytotoxicant, as are produced by repeated daily exposures, the repaired population becomes tolerant to further injury, obviating the need for proliferation and repair, even at doses approaching the LD50 (the dose that is lethal to 50% of the test organisms). The production of tolerance has been doc-

umented for ozone (Paige and Plopper 1999; Plopper et al. 2001), nitrogen dioxide (Kubota et al. 1987), naphthalene (O'Brien et al. 1989; West et al. 2003), 4-ipomeanol (Boyd et al. 1981), and coumarin (Born et al. 1999). Assessments of metabolite reactivity and cellular antioxidant responses have relied on direct assays of relevant cellular chemicals either in isolated target cells or in organ homogenates or serum (Carlson 2010a; Harvilchuck and Carlson 2009; Harvilchuck et al. 2008, 2009).

Bioactivation by Cytochrome P450 Mono-oxygenases

A review cited in the background document for styrene listed a substantial number of CYP450 isozymes that have been identified in the lungs and liver of mice, rats, and humans as having the ability to metabolize styrene to styrene-7,8-oxide and other metabolites (Vodicka et al. 2006). A more recent review summarizes the large number of isozymes found in human lung (Carlson 2008). Although the specific CYP450 mono-oxygenases capable of catalyzing the metabolism of styrene have been reported, the committee found almost no available information on the kinetics of the process or an evaluation of the catalytic efficiencies of the enzymes involved (that is, K_{cat}/K_m). Thus, it is still not known which isozymes are critical for the generation of metabolites that result in cytotoxicity. Of further concern is the inadequate characterization of possible compensatory changes in gene expression in the CYP2F2–null animals. No data were identified that demonstrated how the null animals differed from the wild-type in terms of disposition kinetics of styrene or styrene-7,8-oxide.

A recent in vitro study that used lung and liver microsomes, principally from mice, has identified additional phenolic metabolites whose production appears to be based primarily on the activity of two CYP450 isozymes, CYP2F2 and CYP2E1, on the basis of modestly selective CYP450 inhibitors, and on the basis of studies that used very high substrate concentrations (500 μM) (Shen et al. 2010). The study also strongly emphasized that there is active metabolism of styrene in both the liver and the lung. However, the toxicity of the metabolites was tested only in the mouse lung and only at concentrations that did not generate toxicity by the parent compounds, styrene and styrene-7,8-oxide, or by any of the phenols except 4-vinylphenol. It is not clear how the liver would respond to those metabolites or to what degree production of the metabolites by the liver might contribute to toxic responses in the lung.

Three studies that used the same strain of CYP2F2 knockout mice demonstrated that metabolism by the 2F2 isozyme is critical for the production of high levels of distal bronchiolar cytotoxicity in lungs of mice, whether styrene is administered via intraperitoneal injection (Carlson 2012; Cruzan et al. 2012, 2013) or orally (Cruzan et al. 2012). Liver toxicity was also present in both deficient and wild-type mice (Carlson 2012). Metabolism of styrene to the R- and S-styrene oxide enantiomers was identical in the liver of wild-type and knockout mice but markedly reduced in the lungs of knockout mice (Carlson 2012). Although CYP2F2 knockout mice appear to be insensitive to styrene exposure,

leading the authors to conclude that this is a key enzyme associated with the metabolic activation of styrene, no data were presented to demonstrate a change in the rates of metabolism in airways of knockout mice compared with wild-type mice. Furthermore, although the knockout animals were characterized to determine whether there were compensatory changes in the concentration of CYP450s, no attempts were made to evaluate alterations in proteins associated with detoxification. It is not clear what the full metabolic potential of the lungs and liver of the knockout animals are for metabolizing styrene to other metabolites.

On the basis of an assay of bronchiolar epithelial proliferation, bronchiolar toxicity, produced by both styrene-7,8-oxide enantiomers, was markedly lower in knockout than in wild-type mice and is equal to that of carrier-treated controls (Cruzan et al. 2012). Whether this was true for epithelium in other airways that were more proximal was not assessed. The study also found that the portion of bronchiolar epithelial cells, which contained BrdU labelling, actually decreased at higher doses of styrene, and this suggests that cells that have the potential for replication may be lost as part of the toxic response at higher doses. In CYP2F2-/- mice in which a transgene for three human CYP450 mono-oxygenases (CYP2F1, 2A13, and 2B6) was inserted, lung toxicity, on the basis of the same proliferation assay, was observed with 4-vinylphenol but not styrene or the R- or S-styrene oxides (Cruzan et al. 2013). A major deficiency of these studies is that there were no quantitative measurements of the differences in metabolic capacity of the airways in wild-type and transgenic mice. How the presence of the CYP450 isozymes in the liver and other organs affected their response was not addressed. In knockout mice deficient in hepatic CYP450 reductase, which is critical for CYP450 function, lavage and serum markers of lung and liver toxicity were higher than in carrier-treated controls (Carlson 2012). Although the metabolism of styrene to the R- or S-styrene oxides was markedly reduced in the liver, production of the R-styrene oxide in the lungs doubled, and S-styrene oxide production was unchanged compared with controls.

The literature suggests that more than one CYP450 isozyme is involved in generating cytotoxic metabolites from styrene. More organs than the lung appear to serve as sites for both metabolic activation and cytotoxic injury, and the responses in different organs are unique to the organ. However, a clear understanding of the roles of CYP450s in the cytotoxicity of styrene will require further studies with a more comprehensive approach, including comparisons of not only liver and lung but other organs.

Cellular Oxidative Stress Response

Styrene and its principle metabolites, R- and S-styrene oxide and 4-vinylphenol, have been used to define markers of oxidative stress and the cellular stress response only in Clara cells in mice. Expression of Clara cell secretory protein (CC10) mRNA was affected differently when exposure to those compounds was in vitro (expression was increased by R- and S-styrene oxide and

decreased by 4-vinylphenol) as opposed to in vivo by intraperitoneal injection (expression was decreased by racemic and R-styrene oxide over a time course) (Harvilchuck et al. 2008). Expression of CC10 protein followed the same pattern. Reactive oxygen species were increased in Clara cells by both short-term in vitro and in vivo exposure to styrene, racemic styrene oxide, R-styrene oxide, and S-styrene oxide, but not 4-vinylphenol (Harvilchuck et al. 2009). Cellular markers of oxidative stress (8-hydroxydeoxyguanosine and superoxide dismutase) and indicators of apoptosis (bax/bcl-2 and caspase 3) were also increased by styrene or R-styrene oxide. Expression of all four markers returned to control values over an extended postexposure period after R-styrene oxide treatment but not in all cases for styrene. Repeated exposures to styrene and R-styrene oxide produced different short-term responses for the expression of CC10 mRNA and bax/bcl-2 mRNA (Harvilchuck and Carlson 2009). None of those studies addressed oxidative stress in other organs created by the presence of circulating styrene and its metabolites. To define mechanisms by which the metabolites of styrene react with potential target cells more clearly, future studies will need to address oxidative stress in other cell populations that have different levels of susceptibility in the lungs and in other organs, such as the liver.

Cellular Antioxidants

Extracellular pools of the antioxidant glutathione were markedly altered in mice by exposure to a single intraperitoneal dose of styrene or R-styrene oxide (Carlson 2010a). Depletion in both bronchoalveolar lavage fluid and plasma ranged from 30% up to 90%. Replenishment to steady-state concentrations after exposure to either compound required about 24 hours. Systemic pretreatment with known antioxidants to increase cellular antioxidant pools modulated styrene toxicity differently in two of the principal target organs (lung and liver) (Meszka-Jordan et al. 2009). On the basis of exposure to the most toxic metabolite, R-styrene oxide, glutathione pretreatment reduced liver toxicity without altering toxicity in the lungs. *N*-Acetylcysteine pretreatment had the same effect on the liver and a partial reductive effect on lung toxicity. Administration of a synthetic tetrapeptide analogue of glutathione, 4-methoxy-L-tyrosinyl-g-L-glutamyl-L-cysteinyl-glycine (UPF1), for a week before R-styrene oxide treatment enhanced toxicity in both lung and liver. Glutathione appears to play a role in modulating cellular toxicity produced by styrene metabolites, but a clear definition of the roles of the cellular and extracellular pools requires further studies that compare responses in a number of cell populations and organs that have different degrees of susceptibility.

Detoxification Pathways

The role of enzyme systems responsible for the detoxification of styrene and its metabolites has not been clearly defined for most potential target organs.

Recent work that focused on the glutathione conjugation pathway used mice that were deficient in glutathione *S*-transferase pi (GST P1P2-/-), one of the three classes of glutathione *S*-transferases (Carlson 2011b). The results demonstrated that absence of this form did not alter the liver or lung toxicity of styrene (Carlson 2011b). The toxicity of racemic styrene oxide was not altered in the liver but was increased in the lungs of deficient mice. That was also the case for 4-vinylphenol. Depletion of glutathione in the lung and liver by styrene or 4-vinylphenol was unaltered in deficient mice. Expression of peroxiredoxin VI (a bifunctional enzyme) in the liver of GST P1P2-/- mice was substantially increased in comparison to wild-type mice (Kitteringham et al. 2003), and the effect of this change on metabolism and cytotoxicity, and possibly other alterations in protein concentration, is unclear.

Microsomal epoxide hydrolase is thought to play a critical role in the detoxification of styrene by the hydrolysis of styrene-7,8-oxide to styrene glycol. Mice deficient in microsomal epoxide hydrolase do not exhibit a difference in the metabolism of styrene to the R- or S-styrene oxides in either the liver or the lung (Carlson 2010b). Metabolism of styrene-7,8-oxide to glycol is reduced for R- and S-styrene oxide in the liver and for R-styrene oxide in the lung. The toxicity of styrene is substantially higher in both the lung and liver of deficient mice than in wild-type mice. However, the toxic response to racemic styrene-7,8-oxide did not differ in the liver and lung between deficient and wild-type mice. Depletion of glutathione by styrene was increased in the liver of deficient mice but not in the lung. There was a difference between the toxic responses to R- and S-styrene oxide in microsomal epoxide hydrolase–deficient mice (Carlson 2011a). Neither enantiomer produced liver toxicity, but S-styrene oxide produced greater lung toxicity. R-styrene oxide substantially depleted liver glutathione, but S-styrene oxide did not.

The glutathione S-transferases and epoxide hydrolase pathways appear to play a role in the detoxification of reactive styrene metabolites. Their contributions to cellular injury will require more comprehensive studies that compare activity and responses in multiple organs that have different levels of susceptibility.

Summary of Cytotoxicity Evidence

In summary, the studies cited above, in combination with those included in the background document for styrene (NTP 2008), suggest that the mode of action by which styrene produces toxicity is highly complex. The final cellular outcome associated with exposure to a metabolically activated chemical, particularly with chemicals present at relatively low concentrations in the environment, is highly dependent on both the catalytic efficiency of the enzymes involved in the activation and detoxification processes and the amount of protein present in target cells. Establishing the mode of action for styrene on the basis of cytotoxicity and later proliferation at injured sites will depend on a comprehensive approach to identify

the cellular, metabolic, and chemical processes involved in different organs and to define rigorously how their interactions modulate the toxic response. Although research points to the importance of CYP2F2 in biomarker alterations (that is, BrdU labeling indices) that have been observed in styrene-exposed mice, the committee judged the studies to generally lack the scientific rigor necessary to ensure the validity of the conclusions. All the studies noted above relied on the intraperitoneal injection of styrene and its metabolites into the model species, the mouse, with the exception of Cruzan et al. (2012) study, which exposed mice via gavage. Consequently, the response of the candidate target organ, the lung, is based on the concentration of the compound delivered to it by the circulation. In none of the studies were the circulating concentrations determined. Other organ systems in the animal were exposed at the same time. When the response in another organ, the liver, was compared with that in the lung, it became clear that at least two organs are targets for cytotoxicity produced by styrene and its circulating metabolites. Studies of workers in the styrene industry found styrene or its metabolites in both blood and urine and identified a number of additional target organs in at least three other systems—the lymphohematopoietic system (bone marrow, lymph nodes, and spleen), gastrointestinal system (esophagus and pancreas), and urinary system (kidney and bladder)—that should be included in mechanistic studies that use animal models. The need to study other organs in addition to the lungs is especially true for studies in which metabolic capabilities of the model are altered by eliminating the genes for specific activation and detoxification enzymes in the animal as a whole. Additional studies that compare the kinetics of styrene metabolism using a range of recombinant P450 proteins, including CYP2F1 (the human orthologue of CYP2F2), are needed to establish the catalytic efficiency of these proteins with styrene. When both liver and lung were assessed in the studies evaluated in this section, the metabolic function and toxic response in both organs were altered. When gene manipulation was restricted to one organ, the liver, the toxic response in the other, the lung, was altered. Circulating concentrations of key compounds in the toxic response (when evaluated) were also altered. Taken as a whole, this evidence suggests that the activities and toxic responses of multiple organs may play a role in modulating the circulating concentrations of styrene, its metabolites, and other key compounds, such as glutathione, and in affecting the toxic response of other organs in the same individual.

SUMMARY OF EVIDENCE AND CONCLUSIONS

The statement of task (Appendix B) directed the committee to "integrate the level-of-evidence conclusions, and considering all relevant information in accordance with the RoC listing criteria, make an independent listing recommendation for styrene and provide scientific justification for its recommendation." As discussed throughout this report, a substance can be categorized as reasonably anticipated to be a human carcinogen on the basis of sufficient evidence in animals or limited evidence in humans and a substance can be catego-

rized as known to be a human carcinogen on the basis of sufficient evidence in humans (see Box 1-2). Guided by the RoC listing criteria, the committee integrated data from individual studies to determine whether the evidence in experimental animals reached the level of limited or sufficient and to determine whether the evidence in humans reached the level of limited or sufficient. Supporting information was provided from mechanistic studies. The RoC listing criteria do not provide guidance on the integration of information across data streams (that is, across human, experimental animal, and mechanistic information) or the reconciliation of cross-data inconsistencies, so the committee only integrated information within data streams to derive a listing recommendation.

The committee identified evidence of styrene exposure that would potentially lead to carcinogenicity through genotoxic and mutagenic mechanisms, and that evidence is considered strong, inasmuch as it has been found in vivo and in vitro in both humans and rodents. The genotoxic mechanism is probably relevant for all target tissues associated with cancer after exposure to styrene. Identification of styrene metabolites, such as styrene-7,8-oxide, strongly supports the production of reactive intermediates in a variety of tissues in both humans and animals. The reactive metabolites, which may be produced in one organ and transported to produce toxicity in other sites, have been identified in the blood of humans exposed to styrene. Animal toxicology and carcinogenesis studies clearly support the possibility that multiple organs can be affected regardless of their capacity for metabolic activation. In humans, evidence of carcinogenicity in multiple organs is credible but limited. Those findings were based on large occupational cohort studies in the reinforced-plastics industry and on case–control studies.

In sum, the committee finds that compelling evidence exists to support a listing of styrene as, at a minimum, *reasonably anticipated to be a human carcinogen*. That conclusion is based on credible but limited evidence of carcinogenicity in traditional epidemiologic studies, on sufficient evidence of carcinogenicity in animals, and on convincing evidence that styrene is genotoxic in exposed humans.

The listing criteria state that a substance should be classified as known to be a human carcinogen if "there is sufficient evidence of carcinogenicity from studies in humans". The footnote associated with that sentence states that "this evidence can include data derived from the study of tissues or cells from humans exposed to [styrene] that can be useful for evaluating whether a relevant cancer mechanism is operating in people". The evidence of styrene genotoxicity in exposed humans is convincing, so a strong argument could be made to support the listing of styrene as a *known human carcinogen* if data derived from the study of tissues or cells from humans in and of themselves are considered sufficient for making such a determination. The committee notes that there is ambiguity with respect to weighing the mechanistic evidence in applying the listing criteria.

The types of evidence that are available to determine the listing and classification of substances in the RoC continue to evolve. In the future, there will

probably be more powerful mechanistic evidence in exposed humans to use for cancer hazard evaluation. Similarly, improvements in exposure-assessment methods may be developed to improve the identification and characterization of exposed persons. This is true not only for styrene and styrene-7,8-oxide, but for all substances in the RoC. Thus, the committee finds that further clarification and expanded guidance by NTP regarding the types and strength of mechanistic evidence and the use of that evidence in the context of the RoC listing criteria are warranted.

REFERENCES

Andersson, H.C., E.A. Tranberg, A.H. Uggla, and G. Zetterberg. 1980. Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory. Mutat. Res. 73(2):387-401.

Andrés, A. 2005. Cancer incidence after immunosuppressive treatment following kidney transplantation. Crit. Rev. Oncol. Hematol. 56(1):71-85.

Anwar, W.A., and M.Y. Shamy. 1995. Chromosomal aberrations and micronuclei in reinforced plastics workers exposed to styrene. Mutat. Res. 327(1-2):41-47.

Artuso, M., G. Angotzi, S. Bonassi, S. Bonatti, M. De Ferrari, D. Gargano, L. Lastrucci, L. Miligi, C. Sbrana, and A. Abbondandolo. 1995. Cytogenetic biomonitoring of styrene-exposed plastic boat builders. Arch. Environ. Contam. Toxicol. 29(2):270-274.

Ban, M., D. Hettich, and P. Bonnet. 2003. Effect of inhaled industrial chemicals on systemic and local immune response. Toxicology 184(1):41-50.

Ban, M., I. Langonne, N. Huguet, E. Pepin, and G. Morel. 2006. Inhaled chemicals may enhance allergic airway inflammation in ovalbumin-sensitised mice. Toxicology 226(2-3):161-171.

Bastlová, T., P. Vodicka, K. Peterková, K. Hemminki, and B. Lambert. 1995. Styrene oxide-induced HPRT mutations, DNA adducts and DNA strand breaks in cultured human lymphocytes. Carcinogenesis 16(10):2357-2362.

Becker, N., E. Deeq, and A. Nieters. 2004. Population-based study of lymphoma in Germany: Rationale, study design and first results. Leuk. Res. 28(7):713-724.

Beliles, R.P., J.H. Butala, C.R. Stack, and S. Makris. 1985. Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats. Fundam. Appl. Toxicol. 5(5):855-868.

Bergamaschi, E., A. Smargiassi, A. Mutti, I. Franchini, and R. Lucchini. 1995. Immunological changes among workers occupationally exposed to styrene. Int. Arch. Occup. Environ. Health 67(3):165-171.

Biro, A., E. Pallinger, J. Major, M.G. Jakab, T. Klupp, A. Falus, and A. Tompa. 2002. Lymphocyte phenotype analysis and chromosome aberration frequency of workers occupationally exposed to styrene, benzene, polycyclic aromatic hydrocarbons or mixed solvents. Immunol. Lett. 81(2):133-140.

Bjørge, C., G. Brunborg, R. Wiger, J.A. Holme, T. Scholz, E. Dybing, and E.J. Søderlund. 1996. A comparative study of chemically induced DNA damage in isolated human and rat testicular cells. Reprod. Toxicol. 10(6):509-519.

Boogaard, P.J., K.P. de Kloe, S.C. Sumner, P.A. van Elburg, and B.A. Wong. 2000a. Disposition of [ring-U-14C] styrene in rats and mice exposed by recirculating nose-only inhalation. Toxicol. Sci. 58(1):161-172.

Boogaard, P.J., K.P. de Kloe, B.A. Wong, S.C. Sumner, W.P. Watson, and N.J. van Sittert. 2000b. Quantification of DNA adducts formed in liver, lungs, and isolated lung cells of rats and mice exposed to 14C-styrene by nose-only inhalation. Toxicol. Sci. 57(2):203-216.

Born, S.L., A.S. Fix, D. Caudill, and L.D. Lehman-McKeeman. 1999. Development of tolerance to Clara cell necrosis with repeat administration of coumarin. Toxicol. Sci. 51(2):300-309.

Boyd, M.R., L.T. Burka, B.J. Wilson, and B.V. Sastry. 1981. Development of tolerance to the pulmonary toxin, 4-ipomeanol. Toxicology 19(2):85-100.

Brenner, D.D., A.M. Jeffrey, L. Latriano, L. Wazneh, D. Warburton, M. Toor, R.W. Pero, L.R. Andrews, S. Walles, and F.P. Perera. 1991. Biomarkers in styrene-exposed boatbuilders. Mutat. Res. 261(3):225-236.

Brondeau, M.T., P. Bonnet, J.P. Guenier, P. Simon, and J. de Ceaurriz. 1990. Adrenal-dependent leucopenia after short-term exposure to various airborne irritants in rats. J. Appl. Toxicol. 10(2):83-86.

Brunnemann, K.D., A. Rivenson, S.C. Cheng, V. Saa, and D. Hoffmann. 1992. A study of tobacco carcinogenesis. XLVII. Bioassays of vinylpyridines for genotoxicity and for tumorigenicity in A/J mice. Cancer Lett. 65(2):107-113.

Bucher, J.R. 2013. Follow-up Questions Submitted by the NAS Committee on Review of the Formaldehyde Assessment in the NTP 12th RoC and the NAS Committee on Review of the Styrene Assessment in the NTP 12th RoC, April 2, 2013.

Buckpitt, A., A.M. Chang, A. Weir, L. Van Winkle, Z. Duan, R. Philpot, and C. Plopper. 1995. Relationship of cytochrome P450 activity to Clara cell cytotoxicity. IV. Metabolism of naphthalene and naphthalene oxide in microdissected airways from mice, rats, and hamsters. Mol. Pharmacol. 47(1):74-81.

Bui, P.H., and O. Hankinson. 2009. Functional characterization of human cytochrome P450 2S1 using a synthetic gene-expressed protein in *Escherichia coli.* Mol. Pharmacol. 76(5):1031-1043.

Buschini, A., G. De Palma, P. Poli, A. Martino, C. Rossi, P. Mozzoni, E. Scotti, L. Buzio, E. Bergamaschi, and A. Mutti. 2003. Genetic polymorphism of drug-metabolizing enzymes and styrene-induced DNA damage. Environ. Mol. Mutagen. 41(4):243-252.

Byfält Nordqvist, M., A. Löf, S. Osterman-Golkar, and S.A. Walles. 1985. Covalent binding of styrene and styrene-7,8-oxide to plasma proteins, hemoglobin and DNA in the mouse. Chem. Biol. Interact. 55(1-2):63-73.

Camurri, L., S. Codeluppi, C. Pedroni, and L. Scarduelli. 1983. Chromosomal aberrations and sister-chromatid exchanges in workers exposed to styrene. Mutat. Res. 119(3):361-369.

Camurri, L., S. Codeluppi, L. Scarduelli, and S. Candela. 1984. Sister chromatid exchanges in workers exposed to low doses of styrene. Basic Life Sci. 29(Pt. B):957-963.

Cantoreggi, S., and W.K. Lutz. 1993. Covalent binding of styrene to DNA in rat and mouse. Carcinogenesis 14(3):355-360.

Carlson, G.P. 2002. Effect of the inhibition of the metabolism of 4-vinylphenol on its hepatotoxicity and pneumotoxicity in rats and mice. Toxicology 179(1-2):129-136.

Carlson, G. 2004. Influence of selected inhibitors on the metabolism of the styrene metabolite 4-vinylphenol in wild-type and CYP2E1 knockout mice. J. Toxicol. Environ. Health A 67(12):905-909.

Carlson, G.P. 2008. Critical appraisal of the expression of cytochrome P450 enzymes in human lung and evaluation of the possibility that such expression provides evidence of potential styrene tumorigenicity in humans. Toxicology 254(1-2):1-10.

Carlson, G.P. 2010a. Depletion by styrene of glutathione in plasma and bronchioalveolar lavage fluid of non-Swiss albino (NSA) mice. J. Toxicol. Environ. Health A 73(11):766-772.

Carlson, G.P. 2010b. Metabolism and toxicity of styrene in microsomal epoxide hydrolase-deficient mice. J. Toxicol. Environ. Health A 73(24):1689-1699.

Carlson, G.P. 2011a. Comparison of styrene oxide enantiomers for hepatotoxic and pneumotoxic effects in microsomal epoxide hydrolase-deficient mice. J. Toxicol. Environ. Health A 74(6):347-350.

Carlson, G.P. 2011b. Hepatotoxicity and pneumotoxicity of styrene and its metabolites in glutathione S-transferase-deficient mice. Drug Chem. Toxicol. 34(4):440-444.

Carlson, G.P. 2012. Modification of the metabolism and toxicity of styrene and styrene oxide in hepatic cytochrome P450 reductase deficient mice and CYP2F2 deficient mice. Toxicology 294(2-3):104-108.

Carlson, G.P., A.A. Perez Rivera, and N.A. Mantick. 2001. Metabolism of the styrene metabolite 4-vinylphenol by rat and mouse liver and lung. J. Toxicol. Environ. Health A 63(7):541-551.

Cemeli, E., E. Mirkova, G. Chiuchiarelli, E. Alexandrova, and D. Anderson. 2009. Investigation on the mechanisms of genotoxicity of butadiene, styrene and their combination in human lymphocytes using the Comet assay. Mutat. Res. 664(1-2):69-76.

Chakrabarti, S., M.A. Duhr, M. Senécal-Quevillon, and C.L. Richer. 1993. Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects. Environ. Mol. Mutagen. 22(2):85-92.

Chakrabarti, S., X.X. Zhang, and C.L. Richer. 1997. Influence of duration of exposure to styrene oxide on sister chromatid exchanges and cell-cycle kinetics in cultured human blood lymphocytes in vitro. Mutat. Res. 395(1):37-45.

Checkoway, H., and T.M. Williams. 1982. A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. Am. Ind. Hyg. Assoc. J. 43(3):164-169.

Chen, C.C., M.C. Shih, K.Y. Wu, and P.K. Sen. 2008. Exterior exposure estimation using a one-compartment toxicokinetic model with blood sample measurements. J. Math. Biol. 56(5):611-633.

Cocco, P., A. t'Mannetje, D. Fadda, M. Melis, N. Becker, N., S. de Sanjose, L. Foretova, J. Mareckova, A. Staines, S. Kleefeld, M. Maynadie, A. Nieters, P. Brennan, and P. Boffetta. 2010. Occupational exposure to solvents and risk of lymphoma subtypes: Results from the Epilymph case-control study. Occup. Environ. Med. 67(5):341-347.

Cohen, J.T., G. Carlson, G. Charnley, D. Coggon, E. Delzell, J.D. Graham, H. Greim, D. Krewski, M. Medinsky, R. Monson, D. Paustenbach, B. Petersen, S. Rappaport, L. Rhomberg, P.B. Ryan, and K. Thompson. 2002. A comprehensive evaluation of the potential health risks associated with occupational and environmental exposure to styrene. J. Toxicol. Environ. Health B Crit. Rev. 5(1-2):1-265.

Collins, J.J., K.M. Bodner, and J. Bus. 2012. Cancer Mortality of Workers Exposed to Styrene in the U.S. Reinforced Plastics and Composite Industry. Final Report, April 3, 2012.

Collins, J.J., K.M. Bodner, and J.S. Bus. 2013. Cancer mortality of workers exposed to styrene in the U.S. Reinforced plastics and composite industry. Epidemiology 24(2):195-203.

Conner, M.K., Y. Alarie, R.L. Dombroske. 1979. Sister chromatid exchange in regenerating liver and bone marrow cells of mice exposed to styrene. Toxicol. Appl. Pharmacol. 50(2):365-367.

Conner, M.K., Y. Alarie, R.L. Dombroske. 1980. Sister chromatid exchange in murine alveolar macrophages, bone marrow and regenerating liver cell induced by styrene inhalation. Toxicol. Appl. Pharmacol. 55(1):37-42.

Conner, M.K., Y. Alarie, and R.L. Dombroske, 1982. Multiple tissue comparisons of sister chromatid exchanges induced by inhaled styrene. Environ. Sci. Res. 25:433-441.

Conti, B., P. Maltoni, G. Perino, and A. Ciliberti. 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. Ann. NY Acad. Sci. 534:203-234.

Costa, C., S. Costa, S. Silva, P. Coelho, M. Botelho, J. Gaspar, J. Rueff, B. Laffon, and J.P. Taixeira. 2012. DNA damage and susceptibility assessment in industrial workers exposed to styrene. J. Toxicol. Environ. Health A 75(13-15):735-746.

Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C.J. Hardy, D.W. Coombs, P.A. Mullins, and W.R. Brown. 1998. Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks. Toxicol. Sci. 46(2):266-281.

Cruzan, G., J.R. Cushman, L.S. Andrews, G.C. Granville, K.A. Johnson, C. Bevan, C.J. Hardy, D.W. Coombs, P.A. Mullins, and W.R. Brown. 2001. Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks. J. Appl. Toxicol. 21(3):185-198.

Cruzan, G., G.P. Carlson, M. Turner, and W. Mellert. 2005. Ring-oxidized metabolites of styrene contribute to styrene-induced Clara-cell toxicity in mice. J. Toxicol. Environ. Health A 68(3):229-237.

Cruzan, G., J. Bus, M. Banton, R. Gingell, and G. Carlson. 2009. Mouse specific lung tumors from CYP2F2-mediated cytotoxic metabolism: An endpoint/toxic response where data from multiple chemicals converge to support a mode of action. Regul. Toxicol. Pharmacol. 55(2):205-218.

Cruzan, G., J. Bus, J. Hotchkiss, J. Harkema, M. Banton, and S. Sarang. 2012. CYP2F2-generated metabolites, not styrene oxide, are a key event mediating the mode of action of styrene-induced mouse lung tumors. Regul. Toxicol. Pharmacol. 62(1):214-220.

Cruzan, G., J. Bus, J. Hotchkiss, R. Sura, C. Moore, G. Yost, M. Banton, and S. Sarang. 2013. Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors. Regul. Toxicol. Pharmacol. 66(1):24-29.

de Raat, W.K. 1978. Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate. Chem. Biol. Interact. 20(2):163-170.

Diodovich, C., M.G. Bianchi, G. Bowe, F. Acquati, R. Taramelli, D. Parent-Massin, and L. Gribaldo. 2004. Response of human cord blood cells to styrene exposure: Evaluation of its effects on apoptosis and gene expression by genomic technology. Toxicology 200(2-3):145-157.

Dogra, R.K., S. Khanna, S.N. Srivastava, L.J. Shukla, and R. Shanker. 1989. Styrene-induced immunomodulation in mice. Int. J. Immunopharmacol. 11(5):577-586.

Dolmierski, R., M. Szczepanik, G. Danielewicz-Garbalinska, D. Kunikowska, W. Mickiewicz, M. Chomicz, and R. Glosnicka. 1983. Mutagenic action of styrene and its metabolites. 1. Chromosome aberration in persons exposed to the action of styrene. Introductory investigations. Bull. Inst. Marit. Trop. Med. Gdynia 34(1-2):89-93.

Dypbukt, J.M., L.G. Costa, L. Manzo, S. Orrenius, and P. Nicotera. 1992. Cytotoxic and genotoxic effects of styrene-7,8-oxide in neuroadrenergic Pc 12 cells. Carcinogenesis 13(3):417-424.

EPA (U.S. Environmental Protection Agency). 2005. Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, DC [online]. Available: http://www.epa.gov/raf/publications/pdfs/Cancer_Guidelines_Final_3-25-05.pdf [accessed Jan. 23, 2014].

Fabiani, R., P. Rosignoli, A. De Bartolomeo, R. Fuccelli, and G. Morozzi. 2012. Genotoxicity of alkene epoxides in human peripheral blood mononuclear cells and HL60 leukemia cells evaluated with the comet assay. Mutat. Res. 47(1):1-6.

Fabry, L., A. Léonard, and M. Roberfroid. 1978. Mutagenicity tests with styrene oxide in mammals. Mutat. Res. 51(3):377-381.

Festing, M.F., and D.G. Altman. 2002. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 43(4):244-258.

Filser, J.G., W. Kessler, and G.A. Csanády. 2002. Estimation of a possible tumorigenic risk of styrene from daily intake via food and ambient air. Toxicol. Lett. 126(1):1-18.

Fleig, I., and A.M. Thiess. 1978. Mutagenicity study of workers employed in the styrene and polystyrene processing and manufacturing industry. Scand. J. Work Environ. Health 4(suppl. 2):254-258.

Forni, A., E. Goggi, E. Ortisi, R. Cacchetti, G. Cortona, G. Sesana, and L. Alessio. 1988. Cytogenic findings in styrene workers in relation to exposure. Pp. 159-162 in Enviromental Hygiene, N.H. Seemayer, and W. Hadnagy, eds. Berlin, Germany: Springer.

Fracasso, M.E., D. Doria, M. Carrieri, G.B. Bartolucci, S. Quantavalle, and E. De Rosa. 2009. DNA single and double-strand breaks by alkaline- and immune-comet assay in lymphocytes of workers exposed to styrene. Toxicol. Lett. 185(1):9-15.

Fukami, T., M. Katoh, H. Yamazaki, T. Yokoi, and M. Nakajima. 2008. Human cytochrome P450 2A13 efficiently metabolizes chemicals in air pollutants: Naphthalene, styrene, and toluene. Chem. Res. Toxicol. 21(3):720-725.

Fustinoni, S., L. Campo, A. Manini, M. Buratt, S. Waidyanatha, G. De Palma, A. Mutti, V. Foa, A. Colombi, and S.M. Rappaport. 2008. An integrated approach to biomonitoring exposure to styrene and styrene-(7,8)-oxide using a repeated measurements sampling design. Biomarkers 13(6):560-578.

Gamer, A.O., E. Leibold, K. Deckardt, B. Kittel, W. Kaufmann, H.A. Tennekes, and B. van Ravenzwaay. 2004. The effects of styrene on lung cells in female mice and rats. Food Chem. Toxicol. 42(10):1655-1667.

Gate, L., J.C. Micillino, S. Sebillaud, C. Langlais, F. Cosnier, H. Nunge, C. Darne, Y. Guichard, and S. Binet. 2012. Genotoxicity of styrene-7,8-oxide and styrene in Fisher 344 rats: A 4-week inhalation study. Toxicol. Lett. 211(3):211-219.

Gerin, M., J. Siemiatychi, M. Desy, and D. Krewski. 1998. Associations between several sites of cancer and occupational exposure to benzene, toluene, xylene, and styrene: Results of a case-control study in Montreal. Am. J. Ind. Med. 34(2):144-156.

Godderis, L., M. De Boeck, V. Haufroid, M. Emmery, R. Mateuca, S. Gardinal, M. Kirsch-Volders, H. Veulemans, and D. Lison. 2004. Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. Environ. Mol. Mutagen. 44(4):293-303.

Grayson, M.H., and S.S. Gill. 1986. Effect of in vitro exposure to styrene, styrene oxide, and other structurally related compounds on murine cell-mediated immunity. Immunopharmacology 11(3):165-173.

Hagmar, L., B. Hogstedt, H. Welinder, A. Karlsson, and F. Rassner. 1989. Cytogenetic and hematological effects in plastics workers exposed to styrene. Scand. J. Work Environ. Health 15(2):136-141.

Hallier, E., H.W. Goergens, K. Hallier, and H.M. Bolt. 1994. Intervention study on the influence of reduction of occupational exposure to styrene on sister chromatid exchanges in lymphocytes. Int. Arch. Occup. Environ. Health 66(3):167-172.

Hanova, M., R. Stetina, L. Vodickova, R. Vaclavikova, P. Hlavac, Z. Smerhovsky, A. Naccarati, V. Polakova, P. Soucek, M. Kuricova, P. Manini, R. Kumar, K. Hemminki, and P. Vodicka. 2010. Modulation of DNA repair capacity and mRNA expression levels of XRCC1, hoGG1, and XPC genes in styrene-exposed workers. Toxicol. Appl. Pharmacol. 248(3):194-200.

Hanova, M. L. Vodickova, R. Vaclavikova, Z. Smerhovsky, R. Stetina, P. Hlavac, A. Naccarati, J. Slyskova, V. Polakova, P. Soucek, R. Kumar, K. Hemminki, and P. Vodicka. 2011. DNA damage, DNA repair rates and mRNA expression levels of cell cycle genes (TP53, p21(CDKN1A), BCL2 and BAX) with respect to occupational exposure to styrene. Carcinogenesis 32(1):74-79.

Hansteen, I.L., O. Jelmert, T. Torgrimsen, and B. Forsund. 1984. Low human exposure to styrene in relation to chromosome breaks, gaps and sister chromatid exchanges. Hereditas 100(1):87-91.

Harvilchuck, J.A., and G.P. Carlson. 2009. Effect of multiple doses of styrene and R-styrene oxide on CC10, bax, and bcl-2 expression in isolated Clara cells of CD-1 mice. Toxicology 259(3):149-152.

Harvilchuck, J.A., R.J. Zurbrugg, and G.P. Carlson. 2008. CC10 mRNA and protein expression in Clara cells of CD-1 mice following exposure to styrene or its metabolites styrene oxide or 4-vinylphenol. Toxicol. Lett. 183(1-3):28-35.

Harvilchuck, J.A., X. Pu, J.E. Klaunig, and G.P. Carlson. 2009. Indicators of oxidative stress and apoptosis in mouse whole lung and Clara cells following exposure to styrene and its metabolites. Toxicology 264(3):171-178.

Haseman, J.K., J. Huff, and G.A. Boorman. 1984. Use of historical control data in carcinogenicity students in rodents. Toxicol. Pathol. 12(2):126-135.

Haufroid, V., M. Jakubowski, B. Janasik, D. Ligocka, J.P. Buchet, E. Bergamaschi, P. Manini, A. Mutti, S. Ghittori, M. Arand, N. Hangen, F. Oesch, A. Hirvonen, and D. Lison. 2002. Interest of genotyping and phenotyping of drug-metabolizing enzymes for the interpretation of biological monitoring of exposure to styrene. Pharmacogenetics 12(9):691-702.

Helal, S.F., and W.S. Elshafy. 2013. Health hazards among workers in plastic industry. Toxicol. Ind. Health 29(9):812-819.

Herrero, M.E., M. Arand, J.G. Hengstler, and F. Oesch. 1997. Recombinant expression of human microsomal epoxide hydrolase protects V79 Chinese hamster cells from styrene oxide- but not from ethylene oxide-induced DNA strand breaks. Environ. Mol. Mutagen. 30(4):429-439.

Hogstedt, B., K. Hedner, E. Mark-Vendel, F. Mitelman, A. Schutz, and S. Skerfving. 1979. Increased frequency of chromosome aberrations in workers exposed to styrene. Scand. J. Work Environ. Health 5(4):333-335.

Hogstedt, B., B. Akesson, K. Axell, B. Gullberg, F. Mitelman, R.W. Pero, S. Skerfving, and H. Welinder. 1983. Increased frequency of lymphocyte micronuclei in workers producing reinforced polyester resin with low exposure to styrene. Scand. J. Work Environ. Health 9(3):241-246.

Holz, O., G. Scherer, S. Brodtmeier, F. Koops, K. Warncke, T. Krause, A. Austen, J. Angerer, A.R. Tricker, F. Adlkofer, and H.F. Rüdiger. 1995. Determination of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers at a styrene plant. Occup. Environ. Med. 52(6):420-428.

Huff, J.E. 1984. Styrene, styrene oxide, polystyrene, and β-nitrostyrene/styrene carcinogenicity in rodents. Pp. 227-238 in Industrial Hazards of Plastics and Synthetic Elastomers, J. Järvisalo, P. Pfäffli, and H. Vainio, eds. New York: Alan R. Liss.

IARC (International Agency for Research on Cancer). 1994a. Styrene. Pp. 233-320 in Some Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 60. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol60/mono60.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 1994b. Styrene-7,8-oxide. Pp. 321-346 in Some Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 60. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol60/mono60.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 2002. Styrene. Pp. 437-550 in Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans Vol. 82. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf [accessed Feb. 12, 2014].

Ishidate, M., Jr., and K. Yoshikawa. 1980. Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation--a comparative study on mutagens and carcinogens. Arch. Toxicol. Suppl. 4:41-44.

Jablonicka, A., J. Karelova, H. Polakova, and M. Vargova. 1988. Analysis of chromosomes in peripheral blood lymphocytes of styrene-exposed workers. Mutat. Res. 206(2):167-169.

Jahnova, E., J. Tulinska, S. Weissova, M. Dusinska, and L. Fuortes. 2002. Effects of occupational exposure to styrene on expression of adhesion molecule on leukocytes. Hum. Exp. Toxicol. 21(5):235-240.

Jantunen, K., J. Mäki-Paakkanen, and H. Norppa. 1986. Induction of chromosome aberrations by styrene and vinylacetate in cultured human lymphocytes: Dependence on erythrocytes. Mutat. Res. 159(1-2): 109-116.

Jarabek, A.M., L.H. Pottenger, L.S. Andrews, D. Casciano, M.R. Embry, J.H. Kim, R.J. Preston, M.V. Reddy, R. Schoeny, D. Shuker, J. Skare, J. Swenberg, G.M. Williams, and E. Zeiger. 2009. Creating context for the use of DNA adduct data in cancer risk assessment. Crit. Rev. Toxicol. 39(8):659-678.

Jensen, A.A., N.O. Breum, J. Bacher, and E. Lynge. 1990. Occupational exposures to styrene in Denmark 1955-88. Am. J. Ind. Med. 17(5):593-606.

Jersey, G.C., M.F. Balmer, J.F. Quast, C.N. Park, D.J. Schuetz, J.E. Beyer, K.J. Olson, S.B. McCollister, and L.W. Rampy. 1978. Two-Year Chronic Inhalation Toxicity

and Carcinogenicity Study on Monomeric Styrene in Rats. Final Report. The Dow Chemical Company, Midland, MI.

Karakaya, A.E., B. Karahalil, M. Yilmazer, N. Aygün, S. Sardas, and S. Burgaz. 1997. Evaluation of genotoxic potential of styrene in furniture workers using unsaturated polyester resins. Mutat. Res. 392(3): 261-268.

Karami, S., P. Boffetta, P. Brennan, P.A. Stewart, D. Zaridze, V. Matveev, V. Janout, H. Kollarova, V. Bencko, M. Navratilova, N. Szeszenia-Dabrowska, D. Mates, J.P. Gromiec, R. Sobotka, W.H. Chow, N. Rothman, and L.E. Moore. 2011. Renal cancer risk and occupational exposure to polycyclic aromatic hydrocarbons and plastics. J. Occup. Environ. Med. 53(2):218-223.

Karami, S. 2013. National Cancer Institute at the National Institutes of Health. Unpublished material. Request for information to Dr. Karami from the Committee to Review the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens. Received on July 29, 2013.

Kaufmann, W., W. Mellert, B. van Ravenzwaay, R. Landsiedel, and A. Poole. 2005. Effects of styrene and its metabolites on different compartments of the mouse: Cell proliferation and histomorphology. Regul. Toxicol. Pharmacol. 42(1): 24-36.

Keenan, C., S. Elmore, S. Francke-Carroll, R. Kemp, R. Kerlin, S. Peddada, J. Pletcher, M. Rinke, S.P. Schmidt, I. Taylor, and D.C. Wolf. 2009. Best practices for use of historical control data of proliferative rodent lesions. Toxicol. Pathol. 37(5):679-693.

Kelsey, K.T., T.J. Smith, S.K. Hammond, R. Letz, and J.B. Little. 1990. Sister-chromatid exchanges in lymphocytes from styrene-exposed boat builders. Mutat. Res. 241(2):215-221.

Khristeva, V. 1986. Changes in the peripheral blood of workers engaged in ethylbenzene-styrene and synthetic rubber and latex manufacture [in Bulgarian]. Probl. Khig. 11:90-95.

Kitteringham, N.R., H. Powell, R.E. Jenkins, J. Hamlett, C. Lovatt, R. Elsby, C.J. Henderson, C.R. Wolf, S.R. Pennington, and B.K. Park. 2003. Protein expression profiling of glutathione S-transferase pi null mice as a strategy to identify potential markers of resistence to paracetamol-induced toxicity in the liver. Proteomics 3(2):191-207.

Kligerman, A.D., J.W. Allen, M.F. Bryant, J.A. Campbell, B.W. Collins, R.L. Doerr, G.L. Erexson, P. Kwanyuen, and D.L. Morgan. 1992. Cytogenetic studies of mice exposed to styrene by inhalation. Mutat. Res. 280(1):35-43.

Kligerman, A.D., J.W. Allen, G.L. Erexson, and D.L. Morgan. 1993. Cytogenetic studies of rodents exposed to styrene by inhalation. Pp. 217-224 in Butadiene and Styrene: Assessment of Health Hazards, M. Sorsa, K. Peltonen, H. Vainio, and K. Hemminki, eds. IARC Scientific Publications No. 127. Lyon, France: International Agency for Research on Cancer.

Knobel, P.A., and T.M. Marti. 2011. Translesion DNA synthesis in the context of cancer research. Cancer Cell Int. 11:39.

Kogevinas, M., G. Ferro, A. Andersen, T. Bellander, M. Biocca, O. Coggon, V. Gennaro, S. Hutchings, H. Kolstad, I. Lundberg, E. Lynge, T. Partanen, and R. Saracci. 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. Scand. J. Work Environ. Health 20(4):251-261.

Kolstad, H.A., E. Lynge, J. Olsen, and N. Breum. 1994. Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry. Scand. J. Work Environ. Health 20(4):272-278.

Kolstad, H.A., K. Juel, J. Olsen, and E. Lynge. 1995. Exposure to styrene and chronic health effects: Mortality and incidence of solid cancers in the Danish reinforced plastics industry. Occup. Environ. Med. 52(5):320-327.

Kubota, K., M. Murakami, S. Takenaka, K. Kawai, and H. Kyono. 1987. Effects of long-term nitrogen dioxide exposure on rat lung: Morphological observations. Environ. Health Perspect. 73:157-169.

Labarga, P. 2013. Cancer in HIV patients. AIDS Rev. 15(4):237-238.

Laffon, B., E. Pásaro, and J. Méndez. 2001b. Genotoxic effects of styrene-7,8-oxide in human white blood cells: Comet assay in relation to the induction of sister-chromatid exchanges and micronuclei. Mutat. Res. 491(1-2):163-172.

Laffon, B., E. Pásaro, and J. Méndez. 2002a. Evaluation of genotoxic effects in a group of workers exposed to low levels of styrene. Toxicology 171(2-3):175-186.

Laffon, B., E. Pásaro, and J. Méndez. 2002b. DNA damage and repair in human leukocytes exposed to styrene-7,8-oxide measured by the comet assay. Toxicol. Lett. 126(1):61-68.

Laffon, B., B. Pérez-Cadahia, E. Pásaro, and J. Méndez. 2003b. Effect of epoxide hydrolase and glutathione *S*-tranferase genotypes on the induction of micronuclei and DNA damage by styrene-7,8-oxide in vitro. Mutat. Res. 536(1-2):49-59.

Lawson, G.W., L.S. Van Winkle, E. Toskala, R.M. Senior, W.C. Parks, and C.G. Plopper. 2002. Mouse strain modulates the role of the ciliated cell in acute tracheobronchial airway injury-distal airways. Am. J. Pathol. 160(1):315-327.

Lazutka, J.R., R. Lekevicius, V. Dedonytë, L. Maciuleviciutë-Gervers, J. Mierauskienë, S. Rudaitienë, and G. Slapšytë. 1999. Chromosomal aberrations and sister-chromatid exchanges in Lithuanian populations: Effects of occupational and environmental exposures. Mutat. Res. 445(2):225-239.

Lee, S.H., and H. Norppa. 1995. Effects of indomethacin and arachidonic acid on sister chromatid exchange induction by styrene and styrene-7,8-oxide. Mutat. Res. 348(4):175-181.

Lijinsky, W. 1986. Rat and mouse forestomach tumors induced by chronic oral administration of styrene oxide. J. Natl. Cancer Inst. 77(2):471-476.

Linhart, I., J. Mraz, J. Scharff, J. Krouzelka, S. Duskova, H. Nohova, and L. Vodickova. 2010. New urinary metabolites formed from ring-oxidized metabolic intermediates of styrene. Chem. Res. Toxicol. 23(1):251-257.

Linhart, I., J. Mraz, L. Dabrowska, M. Malis, J. Krouzelka, and M. Korinek. 2012. Vinylphenylmercapturic acids in human urine as biomarkers of styrene ring oxidation. Toxicol. Lett. 213(2):260-265.

Linnainmaa, K., T. Meretoja, M. Sorsa, and H. Vainio. 1978a. Cytogenetic effects of styrene and styrene oxide on human lymphocytes and Allium cepa. Scand. J. Work Environ. Health 4 (suppl. 2):156-162.

Linnainmaa, K., T. Meretoja, M. Sorsa, and H. Vainio. 1978b. Cytogenetic effects of styrene and styrene oxide. Mutat. Res. 58(2-3):277-286.

Little, Inc. 1981. Industrial Hygiene Evaluation of Retrospective Mortality Study Plants. A.D. Little, Inc., Boston, MA

Liu, S.F., S.M. Rappaport, J. Rasmussen, and W.J. Bodell. 1988a. Detection of styrene oxide-DNA adducts by 32P-postlabeling. Carcinogenesis 9(8):1401-1404.

Loprieno, N., S. Presciuttini, I. Sbrana, G. Stretti, L. Zaccaro, A. Abbondandolo, S. Bonatti, R. Fiorio, and A. Mazzaccaro. 1978. Mutagenicity of industrial compounds. VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyses. Scand. J. Work Environ. Health 4(suppl. 2):169-178.

Lutz, W.K., S. Cantoreggi, and I. Velic. 1993. DNA binding and stimulation of cell division in the carcinogenicity of styrene 7,8-oxide. Pp. 245-252 in Butadiene and Styrene: Assessment of Health Hazards, M. Sorsa, K. Peltonen, H. Vainio, and K. Hemminki, eds. IARC Scientific Publications No. 127. Lyon, France: International Agency for Research on Cancer.

Maki-Paakkanen, J. 1987. Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene. Mutat. Res. 189(4):399-406.

Maki-Paakkanen, J., S. Walles, S. Osterman-Golkar, and H. Norppa. 1991. Single-strand breaks, chromosome aberrations, sister-chromatid exchanges, and micronuclei in blood lymphocytes of workers exposed to styrene during the production of reinforced plastics. Environ. Mol. Mutagen. 17(1):27-31.

Manini, P., G. De Palma, R. Andreoli, B. Marczynski, M. hanova, P. Mozzoni, A. Naccarati, L. Vodickova, P. Hlavac, A. Mutti, and P. Vodicka. 2009. Biomarkers of nucleic acid oxidation, polymorphism in, and expression of, hOGG1 gene in styrene-exposed workers. Toxicol. Let. 190(1):41-47.

Marczynski, B., P. Rozynek, H.J. Elliehausen, M. Korn, and X. Baur. 1997a. Detection of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in white blood cells of workers occupationally exposed to styrene. Arch. Toxicol. 71(8):496-500.

Marczynski, B., M. Peel, and X. Baur. 1997b. Changes in high molecular weight DNA fragmentation following human blood exposure to styrene-7,8-oxide. Toxicology 120(2):111-117.

Matsuoka, A., M. Hayashi, and M. Ishidate, Jr. 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. Mutat. Res. 66(3):277-290.

Meretoja, T., H. Vainio, M. Sorsa, and H. Harkonen. 1977. Occupational styrene exposure and chromosomal aberrations. Mutat. Res. 56(2):193-197.

Meretoja, T., H. Jarventaus, M. Sorsa, and H. Vainio. 1978a. Chromosome aberrations in lymphocytes of workers exposed to styrene. Scand. J. Work Environ. Health 4(suppl. 2):259-264.

Meretoja, T., H. Vainio, and H. Jarventaus. 1978b. Clastogenic effects of styrene exposure on bone marrow cells of rat. Toxicol. Lett. 1(5-6):315-318.

Meszka-Jordan, A., R. Mahlapuu, U. Soomets, and G.P. Carlson. 2009. Oxidative stress due to (R)-styrene oxide exposure and the role of antioxidants in non-Swiss albino (NSA) mice. J. Toxicol. Environ. Health A 72(10):642-650.

Migliore, L., A. Naccarati, A. Zanello, R. Scarpato, L. Bramanti, and M. Mariani. 2002. Assessment of sperm DNA integrity in workers exposed to styrene. Hum. Reprod. 17(11):2912-2918.

Migliore, L., A. Naccarati, F. Coppede, E. Bergamaschi, G. De Palma, A. Voho, P. Manini, H. Jarventaus, A. Mutti, H. Norppa, and A. Hirvonen. 2006a. Cytogenetic biomarkers, urinary metabolites and metabolic gene polymorphisms in workers exposed to styrene. Pharmacogenet. Genomics 16(2):87-99.

Migliore, L., R. Colognato, A. Naccarati, and E. Bergamaschi. 2006b. Relationship between genotoxicity biomarkers in somatic and germ cells: Findings from a biomonitoring study. Mutagenesis 21(2):149-152.

Mikes, P., M. Korinek, I. Linhart, J. Krouzelka, E. Frantik, L. Vodickova, and L. Neufussova. 2009. Excretion of urinary N7 guanine and N3 adenine DNA adducts in mice after inhalation of styrene. Toxicol. Lett. 184(1):33-37.

Mikes, P., M. Korínek, I. Linhart, J. Krouzelka, L. Dabrowská, V. Stránský, and J. Mráz. 2010. Urinary N3 adenine DNA adducts in humans occupationally exposed to styrene. Toxicol. Lett. 197(3):183-187.

Mutti, A., C. Buzio, F. Perazzoli, E. Bergamaschi, M.C. Bocchi, L. Selis, F. Mineo, and I. Franchini. 1992. Lymphocyte subpopulations in workers exposed occupationally to styrene [in Italian]. Med. Lav. 83(2):167-177.

Nakajima, T., R.S. Wang, E. Elovaara, F.J. Gonzalez, H.V. Gelboin, H. Vainio, and T. Aoyama. 1994. CYP2C11 and CYP2B1 are major cytochrome P450 forms involved in styrene oxidation in liver and lung microsomes from untreated rats, respectively. Biochem. Pharmacol. 48(4):637-642.

Nano, R., A. Rossi, C. Fenoglio, and P. de Piceis Polver. 2000. Evaluation of a possible styrene-induced damage to the haematopoietic tissues in the rat. Anticancer Res. 20(3A):1615-1619.

NCI (National Cancer Institute). 1979a. Bioassay of Styrene for Possible Carcinogenicity. Technical Report No. 185. NIH 79-1741. National Cancer Institute, National Institute of Health, Bethesda, MD.

NCI (National Cancer Institute). 1979b. Bioassay of a Solution of beta-Nitrostyrene and Styrene for Possible Carcinogenicity. Technical Report No. 170. NCI-CG-TR-170. National Cancer Institute, National Institute of Health, Bethesda, MD.

NCI (National Cancer Institute). 2014. Surveillance, Epidemiology, and End Results Program (SEERP) Stat Fact Sheets: Kidney and Renal Pelvis Cancer [online]. Available: http://seer.cancer.gov/statfacts/html/kidrp.html [accessed Mar. 4, 2014].

Nishi, Y., M.M. Hasegawa, M. Taketomi, Y. Ohkawa, and N. Inui. 1984. Comparison of 6-thioguanine-resistant mutation and sister chromatid exchanges in Chinese hamster V79 cells with forty chemical and physical agents. Cancer Res. 44(8):3270-3279.

Nordenson, I., and L. Beckman. 1984. Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene. Hum. Hered. 34(3):178-182.

Norppa, H. 1981. Styrene and vinyltoluene induce micronuclei in mouse bone marrow. Toxicol. Lett. 8(4-5):247-251.

Norppa, H., and F. Tursi. 1984. Erythrocyte-mediated metabolic activation detected by SCE. Pp. 547-559 in Sister Scromatid Exchange, Vol. 29B, R.R. Tice, and A. Hollander, eds. New York: Plenum Press.

Norppa, H., and H. Vainio. 1983. Induction of sister-chromatid exchanges by styrene analogues in cultured human lymphocytes. Mutat. Res. 116(3-4):379-387.

Norppa, H., E. Elovaara, K. Husgafvel-Pursiaiòen, M. Sorsa, and H. Vainio. 1979. Effects of styrene oxide on chromosome aberrations, sister chromatid exchange and hepatic drug biotransformation in Chinese hamsters in vivo. Chem. Biol. Interact. 26(3):305-315.

Norppa, H., M. Sorsa, P. Pfaeffli, and H. Vainio. 1980a. Styrene and styrene oxide Induce SCEs and are metabolized in human lymphocyte cultures. Carcinogenesis 1(4):357-361.

Norppa, H., M. Sorsa, and H. Vainio. 1980b. Chromosomal aberrations in bone marrow of Chinese hamsters exposed to styrene and ethanol. Toxicol. Lett. 5(3-4):241-244.

Norppa, H., K. Hemminki, M. Sorsa, and H. Vainio. 1981. Effect of monosubstituted epoxides on chromosome aberrations and SCE in cultured human lymphocytes. Mutat. Res. 91(3):243-250.

Norppa, H., H. Vainio, and M. Sorsa. 1983a. Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes. Cancer Res. 43(8):3579-3582.

Norppa, H., F. Tursi, and P. Einistö. 1983b. Erythrocytes as a metabolic activation system in mutagenicity studies. Pp. 35-50 in Mutagenesis and Genetic Toxicology, P. Janiaud, D. Averbeck, and E. Moustacchi, eds. Paris, France: INSERM.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington, DC: The National Academies Press.

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) Process. Washington, DC: The National Academies Press.

NRC USCG (National Response Center U.S. Coast Guard). 2008. Query results for styrene. NRC FOIA Data. National Response Center, U.S. Coast Guard [online]. Available: http://www.nrc.uscg.mil/apex/f?p=109:2:6333530711141909:pg_R_1810817102655439:NO&pg_min_row=1&pg_max_rows=20&pg_rows_fetched=20 [accessed May 13, 2008] (as cited in NTP 2011).

NTP (National Toxicology Program). 2002. Report on Carcinogens, 10th Ed. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC.

NTP (National Toxicology Program). 2008. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2011a. Styrene. Pp. 383-392 in Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Styrene.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2011b. Listing Criteria [online]. Available: http://ntp.niehs.nih.gov/?objectid=47B37760-F1F6-975E-7C15022B9C93B5A6 [accessed Feb. 24, 2014].

NTP (National Toxicology Program). 2011c. Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf [accessed Aug. 14, 2013].

Oberheitmann, B., R. Frentzel-Beyme, and W. Hoffmann. 2001. An application of the challenge assay in boat builders exposed to low levels of styrene - a feasibility study of a possible biomarker for acquired susceptibility. Int. J. Hyg. Environ. Health 204(1):23-29.

O'Brien, K.A., C. Suverkropp, S. Kanekal, C.G. Plipper, and A.R. Buckpitt. 1989. Tolerance to multiple doses of the pulmonary toxicant, naphthalene. Toxicol. Appl. Pharmacol. 99(3):487-500.

Okun, A.H., J.J. Beaumont, T.J. Meinhardt, and M.S. Crandall. 1985. Mortality patterns among styrene-exposed boat builders. Am. J. Ind. Med. 8(3):193-205.

Ollikainen, T., A. Hirvonen, and H. Norppa. 1998. Influence of GSTT1 genotype on sister chromatid exchange induction by styrene-7,8-oxide in cultured human lymphocytes. Environ. Mol. Mutagen. 31(4):311-315.

Otteneder, M., U. Lutz, and W.K. Lutz. 2002. DNA adducts of styrene-7,8-oxide in target and non-target organs for tumor induction in rat and mouse after repeated inhalation exposure to styrene. Mutat Res. 500(1-2):111-116.

Paige, R.C., and C.G. Plopper. 1999. Acute and chronic effects of ozone in animal models. Pp. 531-557 in Air Pollution and Health, S.T. Holgate, J.M. Samet, H.S. Koren, and R.L. Maynard, eds. London: Academic Press.

Pauwels, W., and H. Veulemans. 1998. Comparison of ethylene, propylene and styrene 7,8-oxide in vitro adduct formation on N-terminal valine in human haemoglobin and on N-7-guanine in human DNA. Mutat. Res. 418(1):21-33.

Pauwels, W., P. Vodicèka, M. Severi, K. Plná, H. Veulemans, and K. Hemminki. 1996. Adduct formation on DNA and haemoglobin in mice intraperitoneally administered with styrene. Carcinogenesis 17(12):2673-2680.

Penttila, M., M. Sorsa, and H. Vainio. 1980. Inability of styrene to induce nondisjunction in Drosophila or a positive micronucleus test in the Chinese hamster. Toxicol. Lett. 6(2):119-123.

Plopper, C.G., J. Macklin, S.J. Nishio, D.M. Hyde, and A.R. Buckpitt. 1992. Relationship of cytochrome P450 activity to Clara cell cytotoxicity. III. Morphometric comparison of changes in the epithelial populations of terminal bronchioles and lobar bronchi in mice, hamsters, and rats after parenteral administration of naphthalene. Lab. Invest. 67(5):553-565.

Plopper, C.G., R. Paige, E. Schelegle, A. Buckpitt, V. Wong, B. Tarkington, L. Putney, and D. Hyde. 2001. Time-response profiles: Implications for injury, repair, and adaptation to ozone. Pp. 23-37 in Relationship Between Acute and Chronic Effects of Air Pollution, U. Heinrich and U. Mohr, eds. Washington, DC: ILSI Press.

Pohlova, H., and R.J. Sram. 1985. Cytogenetic analysis of peripheral blood lymphocytes of workers occupationally exposed to styrene. J. Hyg. Epidemiol. Microbiol. Immunol. 29(2):155-161.

Pohlova, H., P. Rossner, and R.J. Sram. 1984. Cytogenetic analysis of human peripheral blood lymphocytes in culture exposed in vitro to styrene and styrene oxide. J. Hyg. Epidemiol. Microbiol. Immunol. 29(3):269-274.

Poirier, M.C. 2012. Chemical-induced DNA damage and human cancer risk. Discov. Med. 14(77):283-288.

Ponomarkov, V., and L. Tomatis. 1978. Effects of long-term oral administration of styrene to mice and rats. Scand. J. Work Environ. Health 4(suppl. 2):127-135.

Ponomarkov, V., J.R. Cabral, J. Wahrendorf, and D. Galendo. 1984. A carcinogenicity study of styrene-7,8-oxide in rats. Cancer Lett. 24(1):95-101.

Preston, R.J., and D.J. Abernethy. 1993. Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation. Pp. 225-233 in Butadiene and Styrene: Assessment of Health Hazards, M. Sorsa, K. Peltonen, H. Vainio, and K. Hemminki, eds. IARC Scientific Publications No. 127. Lyon: IARC Press.

Rappaport, S.M., K. Yeowell-O'Connell, W. Bodell, J.W. Yager, and E. Symanski. 1996. An investigation of multiple biomarkers among workers exposed to styrene and styrene-7,8-oxide. Cancer Res. 56(23): 5410-5416.

Ruder, A.M., E.M. Ward, M. Dong, A.H. Okun, and K. Davis-King. 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: An update. Am. J. Ind. Med. 45(2):165-176.

Sarangapani, R., J.G. Teeguarden, G. Cruzan, H.J. Clewell, and M.E. Andersen. 2002. Physiologically based pharmacokinetic modeling of styrene and styrene oxide respiratory-tract dosimetry in rodents and humans. Inhal. Toxicol. 14(8):789-834.

Sasaki, Y.F., F. Izumiyama, E. Nishidate, N. Matsusaka, and S. Tsuda. 1997. Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow). Mutat. Res. 391(3):201-214.

Sbrana, I., D. Lascialfari, A.M. Rossi, N. Loprieno, M. Bianchi, M. Tortoreto, and C. Pantarotto. 1983. Bone marrow cell chromosomal aberrations and styrene transformation in mice given styrene on repeated oral schedule. Chem. Biol. Interact. 45(3):349-357.

Scélo, G., V. Constantinescu, I. Csiki, D. Zaridze, N. Szeszenia-Dabrowska, P. Rudnai, J. Lissowska, E. Fabiánová, A. Cassidy, A. Slamova, L. Foretova, V. Janout, J. Fevotte, T. Fletcher, A. Mannetje, P. Brennan, and P. Boffetta. 2004. Occupational exposure to vinyl chloride, acrylonitrile and styrene and lung cancer risk (Europe). Cancer Causes Control 15(5):445-452.

Scott, D., and R.J. Preston. 1994a. A re-evaluation of the cytogenetic effects of styrene. Mutat. Res. 318(3):175-203.

Seidel, H.J., J. Herkommer, D. Seitz, L. Weber, and E. Barthel. 1990. Haemopoitic stem cells in mice chronically exposed to styrene vapour. Arch. Toxicol. 64(6):466-469.

Seidler, A., M. Mohner, J. Berger, B. Mester, E. Deeg, G. Elsner, A. Nieters, and N. Becker. 2007. Solvent exposure and malignant lymphoma: A population-based case-control study in Germany. J. Occup. Med. Toxicol. 2:2.

Shamy, M.Y., H.H. Osman, K.M. Kandeel, N.M. Abdel-Moneim, and K.F. El Said. 2002. DNA single strand breaks induced by low levels of occupational exposure to styrene: The gap between standards and reality. J. Environ. Pathol. Toxicol. Oncol. 21(1):57-61.

Sharief, Y., A.M. Brown, L.C. Backer, J.A. Campbell, B. Westbrook-Collins, A.G. Stead, and J.W. Allen. 1986. Sister chromatid exchange and chromosome aberration analysis in mice after in vivo exposure to acrylonitrile, styrene, or butadiene monoxide. Environ. Mutagen. 8(3):439-448.

Sharma, R.P., F.A. Smith, and P.J. Gehring. 1981. Styrene epoxide intermediate as a possible stimulant of lymphocytic function. J. Immunopharmacol. 3(1):67-78.

Shen, S., F. Zhang, L. Gao, S. Zeng, and J. Zheng. 2010. Detection of phenolic metabolites of styrene in mouse liver and lung microsomal incubations. Drug Metab. Dispos. 38(11):1934-1943.

Sina, J.F., C.L. Bean, G.R. Dysart, V.I. Taylor, and M.O. Bradley. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113(5): 357-391.

Sinha, A.K., G.C. Jersey, V.A. Linscombe, R.L. Adams, A.M. Mueller, and M.L. McClintock. 1983. Cytogenetic evaluation of bone marrow cells from rats exposed to styrene vapor for one year. Fundam. Appl. Toxicol. 3(2):95-98.

Sinsheimer, J.E., R. Chen, S.K. Das, B.H. Hooberman, S. Osorio, and Z. You. 1993. The genotoxicity of enantiomeric aliphatic epoxides. Mutat. Res. 298(3):197-206.

Somorovská, M., E. Jahnová, J. Tulinská, M. Zámecníková, J. Šarmanová, A. Terenová, L. Vodicková, A. Líšková, B. Vallová, P. Soucek, K. Hemminki, H. Norppa, A. Hirvonen, A.D. Tates, L. Fuortes, M. Dušinská, and P. Vodicka. 1999. Biomonitoring of occupational exposure to styrene in a plastics lamination plant. Mutat. Res. 428(1-2):255-269.

Sorsa, M., A. Anttila, H. Jarventaus, R. Kubiak, H. Norppa, L. Nylander, K. Pekari, P. Pfaffli, and H. Vainio. 1991. Styrene revisited - exposure assessment and risk estimation in reinforced plastics industry. Prog. Clin. Biol. Res. 372:187-195.

Speit, G., R. Linsenmeyer, P. Schutz, and S. Kuehner. 2012. Insensitivity of the in vitro cytokinesis-block miconucleaus assay with human lymphocutes for the detection of DNA damage present at the start of the cell culture. Mutagenesis 27(6):743-747.

Stengel, B., A. Touranchet, H.L. Boiteau, H. Harousseau, L. Mandereau, and D. Hemon. 1990. Hematological findings among styrene-exposed workers in the reinforced plastics industry. Int. Arch. Occup. Environ. Health 62(1):11-18.

Stripp, B.R., K. Maxson, R. Mera, and G. Singh. 1995. Plasticity of airway cell proliferation and gene expression after acute naphthalene injury. Am. J. Physiol. 269(6 Pt.1):791-799.

Sumner, S.J., and T.R. Fennell. 1994. Review of the metabolic fate of styrene. Crit. Rev. Toxicol. 24(suppl.):S11-S33.

Swenberg, J.A., E. Fryar-Tita, Y.C. Jeong, G. Boysen, T. Starr, V.E. Walker, and R.J. Albertini. 2008. Biomarkers in toxicology and risk assessment: Informing critical dose response relationships. Chem. Res. Toxicol. 21(1):253-265.

Tates, A.D., T. Grummt, F.J. van Dam, F. de Zwart, F.J. Kasper, R. Rothe, H. Stirn, A.H. Zwinderman, and A.T. Natarajan. 1994. Measurement of frequencies of HPRT mutants, chromosomal aberrations, micronuclei, sister-chromatid exchanges and cells with high frequencies of SCEs in styrene/dichloromethane-exposed workers. Mutat. Res. 313(2-3):249-262.

Teixeira, J.P., J. Gaspar, S. Silva, J. Torres, S.N. Silva, M.C. Azevedo, P. Neves, B. Laffon, J. Méndez, C. Gonçalves, O. Mayan, P.B. Farmer, and J. Rueff. 2004. Occupational exposure to styrene: Modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. Toxicology 195(2-3):231-242.

Teixeira, J.P., J. Gaspar, P. Coelho, C. Costa, S. Pinho-Silva, S. Costa, S. Da Silva, B. Laffon, E. Psaro, J. Rueff, and P. Farmer. 2010. Cytogenetic and DNA damage on workers exposed to styrene. Mutagenesis 25(6):617-621.

Thiess, A.M., H. Schwegler, and I. Fleig. 1980. Chromosome investigations in lymphocytes of workers employed in areas in which styrene-containing unsaturated polyester resins are manufactured. Am. J. Ind. Med. 1(2):205-210.

Tomanin, R., C. Ballarin, G.B. Bartolucci, E. De Rosa, G. Sessa, G. Iannini, A.R. Cupiraggi, and F. Sarto. 1992. Chromosome aberrations and micronuclei in lymphocytes of workers exposed to low and medium levels of styrene. Int. Arch. Occup. Environ. Health 64(3):209-215.

Tsuda, S., N. Matsusaka, H. Madarame, Y. Miyamae, K. Ishida, M. Satoh, K. Sekihashi, and Y.F. Sasaki. 2000. The alkaline single cell electrophoresis assay with eight mouse organs: Results with 22 mono-functional alkylating agents (including 9 dialkyl N-nitrosoamines) and 10 DNA crosslinkers. Mutat. Res. 467(1):83-98.

Tulinska, J., M. Dusinska, E. Jahnova, A. Liskova, M. Kuricova, P. Vodicka, I. Vodickova, M. Sulcova, and L. Fuortes. 2000. Changes in cellular immunity among workers occupationally exposed to styrene in a plastics lamination plant. Am. J. Ind. Med. 38(5):576-583.

Turchi, G., S. Bonatti, L. Citti, P.G. Gervasi, and A. Abbondandolo. 1981. Alkylating properties and genetic activity of 4-vinylcyclohexene metabolites and structurally related epoxides. Mutat. Res. 83(3): 419-430.

Tursi, F., M. Samaia, M. Salmona, and G. Belvedere. 1983. Styrene oxidation to styrene oxide in human erythrocytes is catalyzed by oxyhemoglobin. Experientia 39(6):593-594.

Uüskula, M., H. Järventaus, A. Hirvonen, M. Sorsa, and H. Norppa. 1995. Influence of GSTM1 genotype on sister chromatid exchange induction by styrene-7,8-oxide and 1,2-epoxy-3-butene in cultured human lymphocytes. Carcinogenesis 16(4):947-950.

Vaghef, H., and B. Hellman. 1998. Detection of styrene and styrene oxide-induced DNA damage in various organs of mice using the comet assay. Pharmacol. Toxicol. 83(2):69-74.

Van Hummelen, P., M. Severi, W. Pauwels, D. Roosels, H. Veulemans, and M. Kirsch-Volders. 1994. Cytogenetic analysis of lymphocytes from fiberglass-reinforced plastics workers occupationally exposed to styrene. Mutat. Res. 310(1):157-165.

Van Winkle, L.S., A.R. Buckpitt, S.J. Nishio, J.M. Isaac, and C.G. Plopper. 1995. Cellualar response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. Am. J. Physiol. 269(6 Pt 1):800-818.

Verner, M.A., R. McDougall, and G. Johanson. 2012. Using population physiologically based pharmacokinetic modeling to determine optimal sampling times and to interpret biological exposure markers: The example of occupational exposure to styrene. Toxicol. Lett. 213(2):299-304.

Vodicka, P., L. Vodickova, and K. Hemminki. 1993. 32P-postlabeling of DNA adducts of styrene-exposed lamination workers. Carcinogenesis 14(10):2059-2061.

Vodicka, P., L. Vodicková, K. Trejbalová, R.J. Srám, and K. Hemminki. 1994. Persistence of *O*6-guanine DNA adducts in styrene-exposed lamination workers determined by 32P-postlabelling. Carcinogenesis 15(9):1949-1953.

Vodicka, P., T. Bastlová, L. Vodicková, K. Peterková, B. Lambert, and K. Hemminki. 1995. Biomarkers of styrene exposure in lamination workers: Levels of O6-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes. Carcinogenesis 16(7):1473-1481.

Vodicka, P., R. Stetina, R. Kumar, K. Plna, and K. Hemminki. 1996. 7-Alkylguanine adducts of styrene oxide determined by 32P-postlabeling in DNA and human embryonal lung fibroblasts (HEL). Carcinogenesis 17(4):801-808.

Vodicka, P., T. Tvrdik, S. Osterman-Golkar, L. Vodicková, K. Peterková, P. Soucek, J. Šarmanová, P.B. Farmer, F. Granath, B. Lambert, and K. Hemminki. 1999. An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers. Mutat. Res. 445(2):205-224.

Vodicka, P., M. Koskinen, L. Vodicková, R. Štetina, P. Šmerák, I. Bárta, and K. Hemminki. 2001b. DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation. Chem. Biol. Interact. 137(3):213-227.

Vodicka, P., M. Koskinen, M. Arand, F. Oesch, and K. Hemminki. 2002a. Spectrum of styrene-induced DNA adducts: The relationship to other biomarkers and prospects in human biomonitoring. Mutat. Res. 511(3):239-254.

Vodicka, P., J. Tuimala, R. Stetina, R. Kumar, P. Manini, A. Naccarati, L. Maestri, L. Vodickova, M. Kuricova, H. Jarventaus, Z. Majvaldova, A. Hirvonen, M. Imbriani, A. Mutti, L. Migliore, H. Norppa, and K. Hemminki. 2004a. Cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in styrene-exposed lamination workers. Environ. Health Perspect. 112(8):867-871.

Vodicka, P., R. Kumar, R. Stetina, L. Musak, P. Soucek, V. Haufroid, M. Sasiadek, L. Vodickova, A. Naccarati, J. Sedikova, S. Sanyal, M. Kuricova, V. Brsiak, H. Norppa, J. Buchancova, and K. Hemminki. 2004c. Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant. Environ. Mol. Mutagen. 44(4):283-292.

Vodicka, P., M. Koskinen, A. Naccarati, B. Oesch-Bartlomowicz, L. Vodickova, K. Hemminki, and F. Oesch. 2006. Styrene metabolism, genotoxicity, and potential carcinogenicity. Drug Metab. Rev. 38(4):805-853.

Vogelstein, B., N. Papadopoulos, V.E. Velculescu, S. Zhou, L.A. Diaz, Jr., and K.W. Kinzler. 2013. Cancer genome landscapes. Science. 339(6127):1546-1558.

von der Hude, W., S. Carstensen, and G. Obe. 1991. Structure-activity relationships of epoxides: Induction of sister-chromatid exchanges in Chinese hamster V79 cells. Mutat. Res. 249(1):55-70.

Walles, S.A.S., and I. Orsen. 1983. Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide. Cancer Lett. 21(1):9-15.

Watabe, T., N. Ozawa, and K.Yoshikawa. 1982. Studies on metabolism and toxicity of styrene. V. The metabolism of styrene, racemic, (*R*)-(+)-, and (*S*)-(–)-phenyloxiranes in the rat. J. Pharmacobiodyn. 5(2):129–133.

Watanabe, T., A. Endo, K. Sato, T. Ohtsuki, M. Miyasaka, A. Koizumi, and M. Ikeda. 1981. Mutagenic potential of styrene in man. Ind. Health 19(1):37-45.

Watanabe, T., A. Endo, M. Kumai, and M. Ikeda. 1983. Chromosome aberrations and sister chromatid exchanges in styrene-exposed workers with reference to their smoking habits. Environ. Mutagen. 5(3): 299-309.

West, J.A., G. Pakenhham, D. Morin, C.A. Fleschner, A.R. Buckpitt, and C.G. Plopper. 2001. Inhaled naphthalene causes dose dependent Clara cell cytotoxicity in mice but nor rats. Toxicol. Appl. Pharmacol. 173(2):114-119.

West, J.A., L.S. Van Winkle, D. Morin, C.A. Fleschner, H.J. Forman, and C.G. Plopper. 2003. Repeated inhalation exposures to the bioactivated cytotoxicant naphthalene (NA) produce airway-specific Clara cell tolerance in mice. Toxicol. Sci. 75(1):161-168.

Wong, O. 1990. A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry. Br. J. Ind. Med. 47(11):753-762.

Wong, O., L.S. Trent, and M.D. Whortont. 1994. An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry. Occup. Environ. Med. 51(6):386-396.

Wongvijitsuk, S, P. Navasumrit, U. Vattanasit, V. Parniob, and M. Ruchirawat. 2011. Low level occupational exposure to styrene: Its effects on DNA damage and DNA repair. Int. J. Hyg. Environ. Health 214(2):127-137.

Yager, J.W., W.M. Paradisin, and S.M. Rappaport. 1993. Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene. Mutat. Res. 319(3):155-165.

Zhang, F., E.R. Lowe, D.L. Rick, X. Qiu, E. Leibold, G. Cruzan, and M.J. Bartels. 2011. In vitro metabolism, glutathione conjugation, and CYP isoform specificity of epoxidation of 4-vinylphenol. Xenobiotica 41(1):6-23.

Zhang, X.X., S. Chakrabarti, A.M. Malick, and C.L. Richer. 1993. Effects of different styrene metabolites on cytotoxicity, sister-chromatid exchanges and cell-cycle kinetics in human whole blood lymphocytes in vitro. Mutat. Res. 302(4):213-218.

Appendix A

Biographic Information on the Committee to Review the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens

Jane E. Henney *(Chair)* is the Home Secretary of the Institute of Medicine (IOM). She has held a series of senior health-policy leadership positions in the public sector. Beginning in 1980, Dr. Henney served for 5 years as the deputy director of the National Cancer Institute. She later joined the University of Kansas Medical Center as vice chancellor of health programs and, for 18 months, interim dean of the School of Medicine. She then served as deputy commissioner for operations of the US Food and Drug Administration (FDA), where she stayed until becoming the first vice president for health sciences at the University of New Mexico. In 1998, she was nominated by President Bill Clinton and confirmed by the Senate as the commissioner of the FDA. She served in that capacity until January 2001. After leaving FDA, she was appointed a senior scholar in residence at the Association of Academic Health Centers. From July 2003 until the beginning of 2008, she served as senior vice president and provost for health affairs at the University of Cincinnati. She continued her academic service to the university as a professor of medicine until 2013. Dr. Henney is a member of the Board of Directors of AmerisourceBergen Corporation and CIGNA in Philadelphia, Pennsylvania; Cubist Pharmaceuticals in Lexington, Massachusetts; the Monell Chemical Senses Center; the Commonwealth Fund; and the China Medical Board. She has received many honors and awards in her field, including election to the Society of Medical Administrators and honorary membership in the American College of Health Care Executives. She is a recipient of the Excellence in Women's Health Award from the Jacobs Institute, the Public Health Leadership Award from the National Organization of Rare Disorders, and, twice, the Public Health Service Commendation Medal. She is a member of IOM and has served as a member and chair of numerous National Academies committees. Dr. Henney earned an MD from Indiana University and

completed her subspecialty training in medical oncology at the M D Anderson Hospital and Tumor Institute and the National Cancer Institute.

John C. Bailar III is an emeritus professor of the University of Chicago. His research interests have included trends in cancer, assessment of such health risks as those posed by new chemicals, and misconduct in science. His expertise includes statistics, biostatistics, epidemiology, and environmental and occupational hazards. Dr. Bailar worked at the National Cancer Institute for 22 years, and he has held academic appointments at Harvard University, McGill University, and the University of Chicago. For 11 years, he was the statistical consultant and a member of the Editorial Board for *The New England Journal of Medicine*. He was a MacArthur Fellow from 1990 to 1995 and was elected to both the Institute of Medicine and the International Statistical Institute. Dr. Bailar has served as a member and as chair of many National Academies committees. His most recent committee work has included participation in the Committee on the Analysis of Cancer Risks in Populations near Nuclear Facilities—Phase I and the Committee to Review Studies of Possible Toxic Effects from Past Environmental Contamination at Fort Detrick. Dr. Bailar earned an MD from Yale and a PhD in statistics from American University.

Arthur P. Grollman is the Distinguished Professor of Pharmacological Sciences, the Evelyn G. Glick Professor of Experimental Medicine, and director of the Zickler Laboratory of Chemical Biology at the State University of New York at Stony Brook. His research interests focus on the biologic consequences of DNA damage as related to molecular mechanisms of replication, mutagenesis, and repair. Research in his laboratory has been instrumental in establishing the mechanism of action of the antitumor agent bleomycin and in defining the biochemical pathway that protects cells against mutations produced by oxidative DNA damage. He has received an American Cancer Society Scholarship Award and a MERIT award from the National Cancer Institute and has been elected to the Johns Hopkins Society of Scholars. Dr. Grollman earned an MD from the Johns Hopkins University School of Medicine.

Judith B. Klotz is an adjunct associate professor at the Drexel University School of Public Health and at the Rutgers School of Public Health. Previously, she was program manager of the cancer surveillance and environmental epidemiology programs of the New Jersey Department of Health. Her research interests are in epidemiologic studies of cancer incidence and reproductive outcomes, gene–environment interactions, evaluation of biologic exposures to environmental contaminants, and the application of health risk assessment and epidemiology to public policy. Dr. Klotz served as a member of the National Research Council Committee to Review Possible Toxic Effects from Past Environmental Contamination at Fort Detrick and Subcommittee on Fluoride in Drinking Water. She received her DrPH in environmental health sciences from Columbia University School of Public Health.

Xiaomei Ma is an associate professor in the Department of Chronic Disease Epidemiology of the Yale School of Public Health. Her research interests are in the epidemiology of hematologic malignancies, including leukemia, myelodysplastic syndromes, and lymphoma. Specifically, she is interested in environmental and genetic factors in the etiology of childhood leukemia, the outcomes of myelodysplastic syndromes, and methodologic issues in the design of various types of epidemiologic studies. Dr. Ma served as a member of the National Research Council Committee on Contaminated Drinking Water at Camp Lejeune. She received a PhD in epidemiology from the University of California, Berkeley.

John B. Morris is the Board of Trustees Distinguished Professor, a professor of pharmacology and toxicology in the Department of Pharmaceutical Sciences, and interim dean of the University of Connecticut School of Pharmacy. His research focuses on toxicity of inhaled irritant vapors, irritants and asthma, regional uptake and metabolism of inspired vapors, physiologically based pharmacokinetic modeling, and risk assessment. Dr. Morris has served on the editorial boards of *Toxicological Sciences* and *Inhalation Toxicology* and on advisory panels for the National Institutes of Health, the US Environmental Protection Agency, and the Department of Energy. He has also served as a member of the National Research Council Committee on Emergency and Continuous Exposure Guidance Levels for Selected Submarine Contaminants. Dr. Morris earned a PhD in toxicology from the University of Rochester.

Charles G. Plopper is professor emeritus of the School of Veterinary Medicine of the University of California, Davis. His research interests include pulmonary cellular and developmental biology, injury and repair responses of lung cells to inhaled and bioactivated toxicants, lung toxicology, lung cellular pathobiology, and cell biology and anatomy. Dr. Plopper has been an adviser to the National Institutes of Health, the US Environmental Protection Agency, and the California Environmental Protection Agency, and he has served as a visiting scientist at the National Cancer Institute. He has been recognized for career achievements by the Society of Toxicology and received the Pfizer Award for Research Excellence twice at the University of California, Davis. He has also received the Norden Distinguished Teaching Award from the University of California, Davis, School of Veterinary Medicine and the Meritorious Teaching Award from the University of Hawaii. Dr. Plopper earned a PhD in anatomy from the University of California, Davis.

Stephen M. Roberts is a professor and director of the Center for Environmental and Human Toxicology in the College of Veterinary Medicine of the University of Florida. He previously served on the faculties of the College of Pharmacy of the University of Cincinnati and the College of Medicine of the University of Arkansas. His research interests include mechanisms of drug and chemical toxicity, cell defense mechanisms against toxicity, toxicokinetics, bioavailability of environmental contaminants, risk assessment, and novel gene-therapy approach-

es for cancer. Dr. Roberts earned a PhD in pharmacology from the University of Utah College of Medicine.

Ivan Rusyn is a professor in the Department of Environmental Sciences and Engineering of the School of Public Health of the University of North Carolina (UNC) at Chapel Hill. He directs the Laboratory of Environmental Genomics and the Carolina Center for Computational Toxicology of the Gillings School of Global Public Health at UNC-Chapel Hill. He is a member of the Lineberger Comprehensive Cancer Center, the Center for Environmental Health and Susceptibility, the Bowles Center for Alcohol Studies, and the Carolina Center for Genome Sciences. Dr. Rusyn's laboratory focuses on the mechanisms of action of environmental toxicants, the genetic determinants of susceptibility to toxicant-induced injury, and computational toxicology. He has served on several National Research Council committees and is currently a member of the Committee on Use of Emerging Science for Environmental Health Decisions and the Committee on Toxicology. Dr. Rusyn received his MD from the Ukrainian State Medical University in Kiev and his PhD in toxicology from UNC-Chapel Hill.

Elaine Symanski is an associate professor and director of the Southwest Center for Occupational and Environmental Health of the University of Texas Health Science Center at Houston. Her research interests include development and application of quantitatively based approaches for evaluating occupational and environmental exposures, retrospective exposure assessment for workplace contaminants, and investigation of health effects associated with exposure to occupational and environmental contaminants. She served as a member of the National Research Council Committee on Contaminated Drinking Water at Camp Lejeune. Dr. Symanski received her PhD in environmental sciences and engineering from the University of North Carolina at Chapel Hill.

Weiqiang (John) Zhao is an assistant professor of pathology and director of the Pathology Core Facility at Ohio State University. His research interests include the investigation of molecular mechanisms of leukemogenesis, the determination of prognostic genetic abnormalities of leukemia and lymphoma cells, and the elucidation of host genetic determinates of treatment response and late effects. He is certified by the American Board of Pathology, the American Board of Hematopathology, and the American Board of Molecular Genetic Pathology. Dr. Zhao earned an MD from the Hunan Medical University in Changsha, China, and a PhD in molecular and cell biology from Tulane University.

Appendix B

Statement of Task of the Committee to Review the Styrene Assessment in the National Toxicology Program 12th Report on Carcinogens

A committee of the National Research Council (NRC) will conduct a scientific peer review of the styrene assessment presented in the National Toxicology Program (NTP) 12th Report on Carcinogens (RoC). The committee will identify and evaluate relevant, publicly available, peer-reviewed literature, with particular emphasis on literature published as of June 10, 2011, the release date of the 12th RoC. The committee will document its decisions for inclusion or exclusion of literature from its evaluation and will identify the set of information deemed most critical to the evaluation. The committee will apply independently the NTP's established RoC listing criteria to the scientific evidence from studies in humans, experimental animals, and other studies relevant to mechanisms of carcinogenesis and make independent level-of-evidence determinations with respect to the human and animal studies. The committee will integrate the level-of-evidence conclusions, and considering all relevant information in accordance with the RoC listing criteria, make an independent listing recommendation for styrene and provide scientific justification for its recommendation.

Note: The NRC has an agreement with the Department of Health and Human Services to undertake a scientific peer review of the determinations concerning formaldehyde and styrene in the National Toxicology Program's 12th Report on Carcinogens (RoC). The expert committees appointed by the Academy for this assignment will follow standard Academy practices in carrying out their independent scientific reviews, which may include consideration of any and all issues that the committees and the Academy decide are necessary to carry out credible, independent, scientific evaluations of the two determinations, potentially including the criteria for the determinations. The statements of task for these two peer reviews were recently modified to make it clear that the NRC's assignment does not also include a separate review of the National Toxicology Program's listing criteria.

Appendix C

Review of the Literature Search Used in the National Toxicology Program 12th Report on Carcinogens

In support of the assessment of styrene, the National Toxicology Program (NTP) searched PubMed, Scopus, and Web of Science by using substance-specific terms (the substance name, major synonyms, and major metabolites) and topic-specific terms (see Table C-1). The results underwent a first level of review, during which titles and abstracts were screened for relevance. In a second level of review, the full text of references were reviewed for their relevance and substance. In the second level of review, 986 references were considered. Seventy additional references were recommended to NTP by an expert panel (Phillips et al. 2008 a,b). In total, 551 references were cited in the final background document (NTP 2008). The date on which the searches were run and the specific search strings used for each database were not provided to the committee. The committee found that providing more detail on the search strategies and the inclusion and exclusion criteria would have improved transparency of the methods that NTP used to identify and evaluate relevant literature related to styrene exposure. Similar observations about clear and concise descriptions of literature searches have been made by previous committees of the National Academies (IOM 2011; NRC 2011, 2014), and approaches that ensure greater transparency in literature search and review are being initiated by the US Environmental Protection Agency's Integrated Risk Information System (EPA 2013) and NTP's Office of Health Assessment and Translation (NTP 2013).

The final background document for styrene (NTP 2008) summarizes the literature up to the date of the peer review of the background document (July 2008), and the substance profile (NTP 2011) summarizes literature up to the date of the peer review by the Board of Scientific Counselors (February 2009) (Bucher et al. 2013). (See Figure 1-1 for a schematic of the 12th Report on Carcinogens process.) After peer review, both the background document and the substance profile were updated to reflect peer-review and public comments. NTP periodically reviewed the scientific literature up to the release of the 12th Report on Carcinogens (June 2011) "for any new studies that would warrant a re-review of the NTP's preliminary recommendations to the [Health and Human Services] Secretary" (Bucher 2013).

TABLE C-1 Topic-Specific Search Terms Used in the National Toxicology Program's Literature Searches

Human Cancer	Animal Tumors	Genotoxicity	ADME/Mechanisms
MeSH terms Case reports Case–control studies Cohort studies Epidemiology Epidemiologic studies Mortality Neoplasms Occupational exposure Prospective studies Retrospective studies Manpower Text words Case-referent Cancer Carcinogenic Epidemiolog* Tumor Workers	MeSH terms Adenocarcinoma Adenoma Carcinogens Carcinoma Neoplasms Precancerous condition Sarcoma Animals Text words Cancer Foci Malignan* Mice Oncogenic* Rats Tumor Tumorigenic*	MeSH terms Aneuploidy Cell transformation, neoplastic Chromosome aberrations Cytogenic analysis DNA adducts DNA damage DNA repair Germ-line mutation Micronuclei Mutagens Mutagenesis Mutation Oncogenes Polyploidy Sister-chromatid exchange SOS response Text words Chromosom* Clastogen* Genetic toxicology Strand break Unscheduled DNA synthesis	MeSH terms Absorption Biotransformation Metabolism Pharmacokinetics Cytochrome P-450 enzyme system Text words Activation Bioactivation Clearance Detoxif* Distribution Excretion Kinetics Mechanism Metabolite

*The asterisk, sometimes referred to as a wild card, represents a truncation and is used to find all terms that begin with the given text string. Abbreviations: ADME, absorption, distribution, metabolism, and excretion; MeSH, medical subject headings. Source: Bucher 2013.

REFERENCES

Bucher, J.R. 2013. Follow-up Questions. Material submitted by the NAS Committee on Review of the Formaldehyde Assessment in the NTP 12th RoC and the NAS Committee on Review of the Styrene Assessment in the NTP 12th RoC, April 2, 2013.

EPA (U.S. Environmental Protection Agency). 2013. Part 1. Status of Implementation of Recommendations. Materials Submitted to the National Research Council, by Integrated Risk Information System Program, U.S. Environmental Protection Agency, January 30, 2013 [online]. Available: http://www.epa.gov/iris/pdfs/IRIS%20Program%20Materials%20to%20NRC_Part%201.pdf [accessed Jan. 13, 2014].

IOM (Institute of Medicine). 2011. Finding What Works in Health Care: Standards for Systematic Reviews. Washington, DC: National Academies Press.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington, DC: National Academies Press.

NRC (National Research Council). 2014. Review of EPA's Integrated Risk Information System (IRIS) Process. Washington, DC: National Academies Press.

NTP (National Toxicology Program). 2008. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2011. Styrene. Pp. 383-392 in Report on Carcinogens, 12th Ed. U.S. Department of Health and Human Services, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Styrene.pdf [accessed Aug. 14, 2013].

NTP (National Toxicology Program). 2013. Draft OHAT Approach for Systematic Review and Evidence Integration for Literature-Based Health Assessments. U.S. Department of Health and Human Services, National Institute of Health, National Institute of Environmental Health Sciences, Division of National Toxicology Program, Office of Hazard Assessment and Translation, February 2013 [online]. Available: http://ntp.niehs.nih.gov/NTP/OHAT/EvaluationProcess/DraftOHATApproach_February2013.pdf [accessed Jan. 13, 2014].

Phillips, D., S. Snedeker, S. Eustis, M. Stone, P. Infante, E.M. Ward, G. Matanoski, G.S. Yost, S.S. Que Hee, L. Zeise, and T.J. Smith. 2008. Part A – Peer Review of the Draft Background Document on Styrene. Styrene Expert Panel Report [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2008/ExpertPanelMtgs/Styrene_PanelReportPartA_508.pdf [accessed July 17, 2013].

Phillips, D., S. Snedeker, S. Eustis, M. Stone, P. Infante, E.M. Ward, G. Matanoski, G.S. Yost, S.S. Que Hee, L. Zeise, and T.J. Smith. 2008. Part B – Recommendation for Listing Status for Styrene and Scientific Justification for the Recommendation. Styrene Expert Panel Report [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2008/ExpertPanelMtgs/Styrene_PanelReportPartB_508.pdf [accessed July 17, 2013].

Appendix D

Literature Search Strategies Used in Support of the Committee's Independent Assessment of Styrene

The committee undertook several literature searches to inform its independent assessment of styrene. The general topics of the searches included metabolism, human epidemiology, experimental-animal studies, and mechanisms of carcinogenicity (specifically, genotoxicity, mutagenicity, immunosuppression, and cytotoxicity). Each search was originally run on May 28, 2013, and updated on November 13, 2013.[1] The search strategies, exclusion criteria, and number of resulting studies are described below. The committee completed the searches with guidance from NRC (2011), and its work was informed by a recently published case study of applying the principles of systematic review to identify and present mechanistic evidence in human health assessments (Kushman et al. 2013).

METABOLISM

The search strategy used for studies relevant to the metabolism of styrene is presented in Box D-1. The search resulted in 229 papers. National Research Council staff reviewed the titles and abstracts of all 229 results and excluded 184 as not relevant (see Figure D-1). Studies were excluded on the basis of the criteria described in Box D-1. The 45 remaining studies were identified as likely to be relevant or possibly relevant. A committee member reviewed the titles and abstracts and found 27 that could be excluded because they were not focused on styrene; they were focused on urinary biomarkers or on reversible physiologic effects; or they were more pertinent to the consideration of cytotoxicity than of metabolism of styrene. The remaining 18 studies were examined in detail to determine whether they contained information or analysis that should be included as part of the committee's independent assessment.

[1]The cutoff date for the literature search was chosen to allow the committee time to review the literature within the time constraints of the project schedule.

BOX D-1 Exclusion Criteria and Search Strategy for Studies of the Metabolism of Styrene

Exclusion Criteria:

- The study was not focused on styrene metabolism or was focused on biomarkers.
- The publication was already cited in the substance profile of styrene in the NTP 12th RoC.
- The publication did not contain primary data.
- The publication did not include information sufficient to determine what experimental methods were used.

Search Strategy:

PubMed: [("Styrene"[Title/Abstract]) AND ("Cytochrome P-450 Enzyme System"[Mesh] OR "Cytochrome P-450 Enzyme System" OR "Biotransformation"[Mesh] OR "Biotransformation " OR "Metabolism"[Mesh] OR "Metabolism " OR "Pharmacokinetics"[Mesh] OR "Pharmacokinetics") AND (activation OR bioactivation OR clearance OR detoxif* OR distribution OR excretion OR kinetics OR mechanism OR metabolite)]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Medline and Embase: [(styrene.ab. or styrene.ti.) and (Cytochrome P-450 Enzyme System/ or Cytochrome P-450 Enzyme System.mp. or Biotransformation/ or Biotransformation .mp. or Metabolism/ or Metabolism.mp. or Pharmacokinetics or Pharmacokinetics.mp.) AND (activation.mp. OR bioactivation.mp. OR clearance.mp. OR detoxif*.mp. OR distribution.mp. OR excretion.mp. OR kinetics.mp. OR mechanism.mp. OR metabolite.mp.)]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Scopus: [("styrene") AND ("Cytochrome P-450 Enzyme System" OR "Biotransformation" OR "Metabolism" OR "Pharmacokinetics") AND ("activation" OR "bioactivation" OR "clearance" OR "detoxif*" OR "distribution" OR "excretion" OR "kinetics" OR "mechanism" OR "metabolite")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Web of Science: [("styrene") AND ("Cytochrome P-450 Enzyme System" OR "Biotransformation" OR "Metabolism" OR "Pharmacokinetics") AND ("activation" OR "bioactivation" OR "clearance" OR "detoxif*" OR "distribution" OR "excretion" OR "kinetics" OR "mechanism" OR "metabolite")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

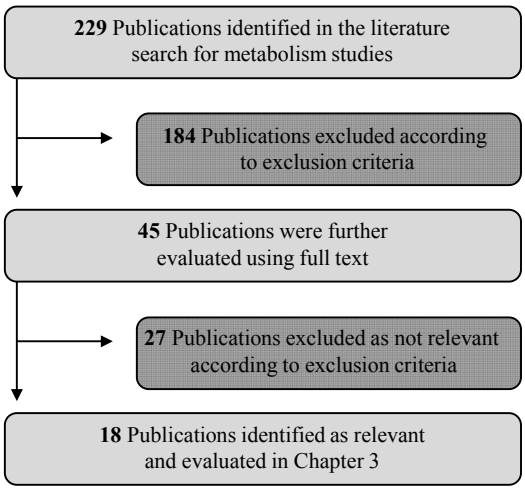

FIGURE D-1 Literature tree for search of studies relevant to styrene metabolism.

CANCER STUDIES IN HUMANS

The search algorithms and exclusion criteria used to identify human epidemiology studies are in Box D-2. The initial search yielded 46 articles (see Figure D-2). National Research Council staff reviewed the titles and abstracts of all 46 and excluded 29 as not relevant. The 17 remaining studies were identified as likely to be relevant or possibly relevant. Two committee members reviewed the titles and abstracts and found 13 that could be excluded. The remaining four studies were examined in detail to determine whether they contained information or analysis that should be included as part of the committee's independent assessment.

EXPERIMENTAL ANIMAL STUDIES

The literature search for publications of animal carcinogenicity bioassays published from January 2008 to November 13, 2013, yielded 118 results. The search terms are described in Box D-3 and a search tree representing the results is depicted in Figure D-3. National Research Council staff reviewed the titles and abstracts of the remaining 118 results and excluded 115 as not relevant. A committee member reviewed the titles and abstracts and found that all three could be excluded. No studies that exposed experimental animals to styrene and evaluated them for the presence of tumors were identified. Thus, the committee's independent evaluation of the evidence of styrene carcinogenicity in experimental animals relies on studies available to NTP when it conducted its review in 2011.

BOX D-2 Exclusion Criteria and Search Strategy for Human Studies

Exclusion Criteria:

- The publication did not evaluate ambient or occupational exposures of humans to styrene.
- The publication did not evaluate health effects related to carcinogenesis or genetic damage.
- The publication was already cited in the substance profile of styrene in the NTP 12th RoC.
- The publication did not include primary data.

Search Strategy:

PubMed: [("Styrene"[Title/Abstract]) AND ("Neoplasms"[Mesh] OR neoplasms OR cancer OR carcinogenic or tumor) AND ("Epidemiology"[Mesh] OR "Epidemiologic Studies"[Mesh] OR epidemiolog* OR case-referent OR "Occupational Exposure"[Mesh] OR workers OR cohort)]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Medline and Embase:[(styrene.ab. or styrene.ti.) and (Neoplasms/ or neoplasms.mp. or cancer.mp. or carcinogenic.mp. or tumor.mp.) and (Epidemiology/ or Epidemiologic Studies/ or epidemiolog*.mp. or case-referent.mp. or Occupational Studies/ or coworkers.mp. or cohort.mp.)]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Scopus: [("styrene") AND ("neoplasms" OR "cancer" OR "carcinogenic" OR "tumor") AND ("epidemiology" "epidemiologic studies" OR "epidemiolog*" OR "case-referent" OR "occupational exposure" OR "workers" OR "cohort")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Web of Science: [("styrene") AND ("neoplasms" OR "cancer" OR "carcinogenic" OR "tumor") AND ("epidemiology" OR "epidemiologic studies" OR "epidemiolog*" OR "case-referent" OR "occupational exposure" OR "worker" OR "cohort")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

STUDIES OF MECHANISMS OF CARCINOGENESIS

Genotoxicity and Mutagenicity

The committee identified and evaluated relevant, publicly available, peer-reviewed literature on the genotoxicity and associated mechanistic events that have been associated with exposure to styrene or styrene-7,8-oxide. Exclusion criteria for study relevance were defined and applied to the retrieved literature by reviewing the abstracts of the retrieved publications and then reviewing full

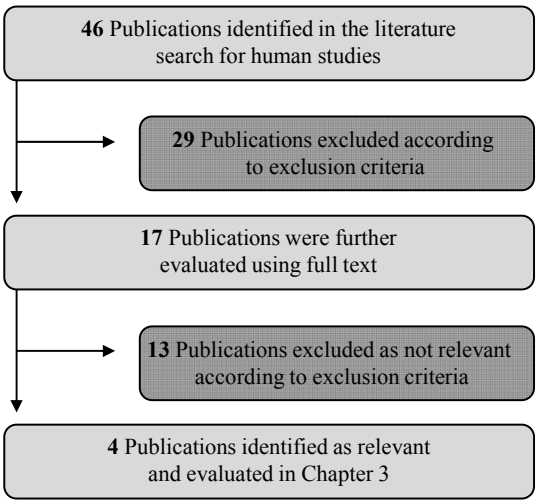

FIGURE D-2 Literature tree for search of studies relevant to humans.

text of selected papers. The committee's exclusion criteria and detailed search terms for studies related to genotoxicity and mutagenicity are presented in Box D-4. Literature trees were used to document identification and selection of the literature evidence (Figure D-4). The selected studies were summarized in evidence tables (see Tables 3-12 to 3-16).

Immunosuppression

The committee undertook a literature search to identify relevant publications on styrene exposure and immunosuppression. Because there was not a specific section in the background document for styrene (NTP 2008) that discussed immunosuppression in detail, the committee undertook a broad search without any date limits (see Box D-5 and Figure D-5). It considered studies relevant if they reported data on changes in basic hematologic measures, such as white blood cell (WBC) count and WBC differential count. The committee included studies that reported such measures as weight of lymphoid organs and expression of functional markers. Efforts were made to identify and include studies that reported effects on local, systemic, innate, and specific immunity in exposed animals or humans. Studies that reported genotoxic measures were excluded from discussion in this section because they are discussed in more detail in the genotoxicity sections of Chapters 2 and 3. The literature search yielded 233 results, 19 of which were relevant articles that were not already cited in the background document for styrene.

BOX D-3 Exclusion Criteria and Search Strategy for Experimental Animal Studies

Exclusion Criteria:

- The publication did not evaluate styrene exposures in animal models.
- The publication did not evaluate the incidence of tumors.
- The publication was already cited in the substance profile of styrene in the NTP 12th RoC.
- The publication did not include primary data.

Search Strategy:

Pubmed: [("Styrene"[Title/Abstract]) AND ("Neoplasms"[Mesh] OR "Carcinogen"[Mesh] OR cancer OR Foci OR Malignant* OR Oncogenic* OR Tumor OR Tumorigenic*) AND ("Animals"[Mesh] OR mice OR rats)]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Medline and Embase: [(styrene.ab. or styrene.ti.) AND (neoplasms/ or carcinogens/ or cancer.mp. or foci.mp. or malignan*.mp. or oncongenic.mp. or tumor.mp. or tumorgenic*.mp.) AND (animals/ or mice.mp. or rats.mp.)]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Scopus: [("Styrene") AND ("neoplasms" OR "carcinogens" OR "cancer" OR "foci" OR "malignan*" OR "oncogenic*" OR "tumor" OR "tumorigenic*") AND ("animals" OR "mice" OR "rats")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Web of Science: [("Styrene") AND ("neoplasms" OR "carcinogens" OR "cancer" OR "foci" OR "malignan*" OR "oncogenic*" OR "tumor" OR "tumorigenic*") AND ("animals" OR "mice" OR "rats")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Cytotoxicity

The committee and National Research Council staff developed a search algorithm to identify relevant cytotoxicity studies that were published in PubMed (see Box D-6 and Figure D-6). The first literature search was run by using selected metabolites as keywords, and a total of 259 references were identified, 12 of which were animal toxicity studies. A second search was undertaken by using *styrene* in the title or abstract in combination with keywords related to lung toxicity. This search identified 37 references, 14 of which dealt with original research on animal toxicity studies and two of which were reviews. Of the 14, 12 were duplicates of the previous search. On August 8, 2013, a third search was performed by refining the terms used in the first search. In this third search, each metabolite was searched in combination with keywords related to lung toxicity. A total of 12 references were identified, all of which had been identified in

the previous search. An additional PubMed search was performed on November 22, 2013. This search identified a total of 217 references, 16 of which addressed animal studies of toxicity of styrene or its metabolites, including two reviews, and all of which had been identified in the previous searches.

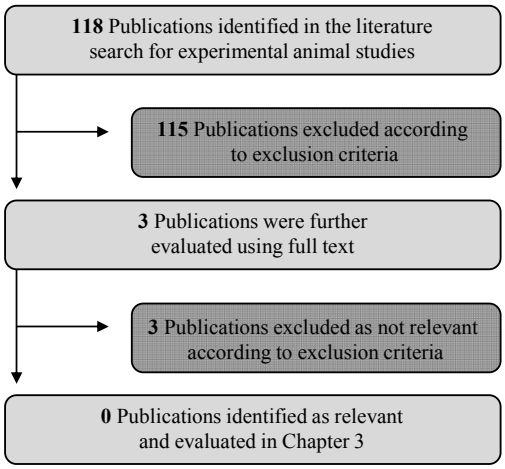

FIGURE D-3 Literature tree for search of studies relevant to experimental animals.

BOX D-4 Exclusion Criteria and Search Strategy for Studies of Genotoxicity and Related Mechanisms of Styrene

Exclusion Criteria

- The publication did not evaluate health effects of styrene or its metabolites known to be formed in humans.
- The study evaluated cellular, biochemical, or molecular effects not relevant to the carcinogenesis or the mechanistic event under consideration.
- The publication did not contain primary data.
- The publication did not include information sufficient to determine what species were studied or what experimental methods were used.

Search Strategy

PubMed: [("Styrene"[Title/Abstract]) AND ("Mutation"[Mesh] OR "Cell Transformation, Neoplastic"[Mesh] OR "Cytogenetic Analysis"[Mesh] OR "Mutagens"[Mesh] OR "Oncogenes"[Mesh] OR "Genetic Processes"[Mesh] OR chromosom* OR clastogen* OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

(Continued)

BOX D-4 Continued

Medline and Embase: [(styrene.ab. or styrene.ti.) and (1 or 2 or 3 or 4 or 5 or 6 or chromosom*.mp. or clastogen*.mp. or genetic toxicology.mp. or strand break.mp. or unscheduled DNA synthesis.mp. or DNA damage.mp. or DNA adducts.mp.), where the following keywords are: 1) Mutation, 2) Cell Transformation, Neoplastic, 3) Cytogenetic Analysis, 4) Mutagens, 5) Oncogenes, 6) Genetic Processes]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Scopus: [(("Styrene") AND ("mutation" OR "cell transformation, neoplastic" OR "cytogenetic analysis" OR "mutagens" OR "oncogenes" OR "genetic processes" OR "chromosom*" OR "clastogen* OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

Web of Science: [(("Styrene") AND ("mutation" OR "cell transformation, neoplastic" OR "cytogenetic analysis" OR "mutagens" OR "oncogenes" OR "genetic processes" OR "chromosom*" OR "clastogen*" OR "genetic toxicology" OR "strand break" OR "unscheduled DNA synthesis" OR "DNA damage" OR "DNA adducts")]. Search run on 05-28-2013; updated on 11-13-2013; and limited to 01-01-2008 to 11-13-2013.

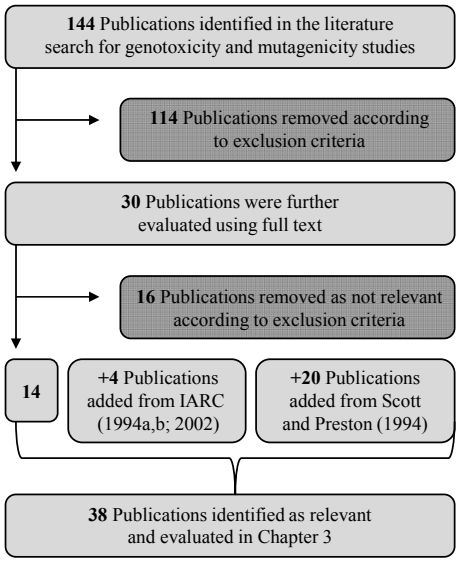

FIGURE D-4 Literature tree for search of studies relevant to genotoxicity and mutagenicity.

BOX D-5 Exclusion Criteria and Search Strategy for Immunosuppression Studies

Exclusion Criteria:

- The publication did not report data on changes in basic hematologic measures after exposure to styrene.
- The publication did not report measures of lymphoid organs.
- The publication did not contain primary data.
- The publication was already cited in the substance profile for styrene in the NTP 12th RoC.
- The publication did not include information sufficient to determine what species were studied or what experimental methods were used.

Search Strategy:

PubMed: ("styrene"[Title/Abstract]) AND ("lymph node" OR leukopenia OR lymphocyt* OR immunotoxicity OR immunosuppression OR hematologic OR hematopoietic). Search run on 05-28-2013 and updated on 11-26-2013.

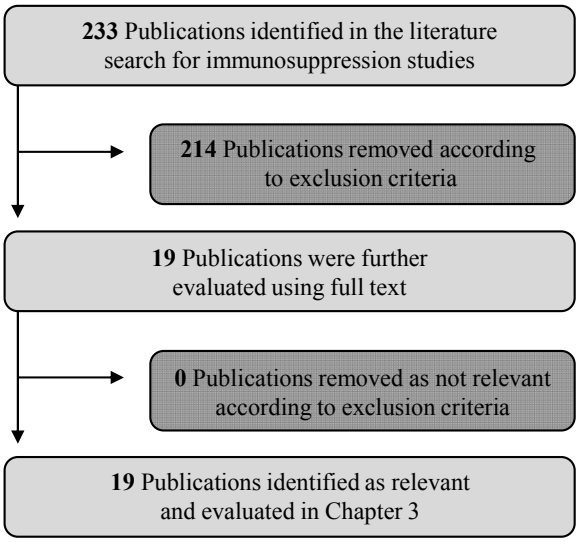

FIGURE D-5 Literature tree for search of studies relevant to styrene immunosuppression.

BOX D-6 Exclusion Criteria and Search Strategy for Cytotoxicity Studies

Exclusion Criteria:

- The publication did not contain information on lung cytotoxicity or damage to the lung, liver, or kidney in relation to exposure to styrene or to a metabolite of styrene.
- The publication did not contain primary data.
- The publication was already cited in the substance profile for styrene in the NTP 12th RoC.
- The publication did not include information sufficient to determine what species were studied or what experimental methods were used.

Search Strategies:

PubMed: [(styrene oxide OR vinylphenol OR 4-vinylphenol OR styrene-3,4-epoxide OR styrene-7,8-oxide OR epoxyethylbenzene OR mandelic acid OR hydroxymandelic acid OR poly(2-hydroxyethylmethacrylate)]. Search run on 05-09-2013 and limited from 01-01-2010 to 05-09-2013.

PubMed: [("Styrene"[Title/Abstract]) AND (lung OR pulmonary OR bronchiole OR bronchiolar OR "Clara cell" OR "alveolar type 2 cell")]. Search run on 05-28-2013 and limited from 01-01-2008 to 05-28-2013.

PubMed: [(lung OR pulmonary OR bronchiole OR bronchiolar OR "Clara cell" OR "alveolar type 2 cell") AND (styrene oxide OR vinylphenol OR 4-vinylphenol OR styrene-3,4-epoxide OR styrene-7,8-oxide OR epoxyethylbenzene OR mandelic acid OR hydroxymandelic acid OR poly(2-hydroxyethylmethacrylate)]. Search run on 08-08-2013 and limited from 01-01-2010 to 08-08-2013.

Pubmed: [(styrene) AND (lung OR liver OR kidney)]. Search run on 11-22-2013 and limited from 01-01-2008 to 11-22-2013.

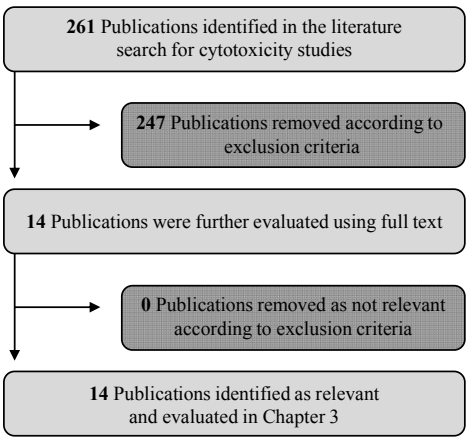

FIGURE D-6 Literature tree for search of studies relevant to styrene cytotoxicity.

REFERENCES

IARC (International Agency for Research on Cancer). 1994a. Styrene. Pp. 233-320 in Some Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 60. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol60/mono60.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 1994b. Styrene-7,8-oxide. Pp. 321-346 in Some Industrial Chemicals. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans, Vol. 60. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol60/mono60.pdf [accessed Feb. 12, 2014].

IARC (International Agency for Research on Cancer). 2002. Styrene. Pp. 437-550 in Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans Vol. 82. Lyon, France: IARC Press [online]. Available: http://monographs.iarc.fr/ENG/Monographs/vol82/mono82.pdf [accessed Feb. 12, 2014].

Kushman, M.E., A.D. Kraft, K.Z. Guyton, W.A. Chiu, S.L. Makris, and I. Rusyn. 2013. A systematic approach for identifying and presenting mechanistic evidence in human health assessments. Regul. Toxicol. Pharmacol. 67(2):266-277.

NRC (National Research Council). 2011. Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde. Washington, DC: The National Academies Press.

NTP (National Toxicology Program). 2008. Report on Carcinogens Background Document for Styrene, September 29, 2008. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Research Triangle Park, NC [online]. Available: http://ntp.niehs.nih.gov/NTP/roc/twelfth/2010/FinalBDs/Styrene_Final_508.pdf [accessed Aug. 14, 2013].

Scott, D., and R.J. Preston. 1994. A re-evaluation of the cytogenetic effects of styrene. Mutat. Res. 318(3):175-203.