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

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Introduction

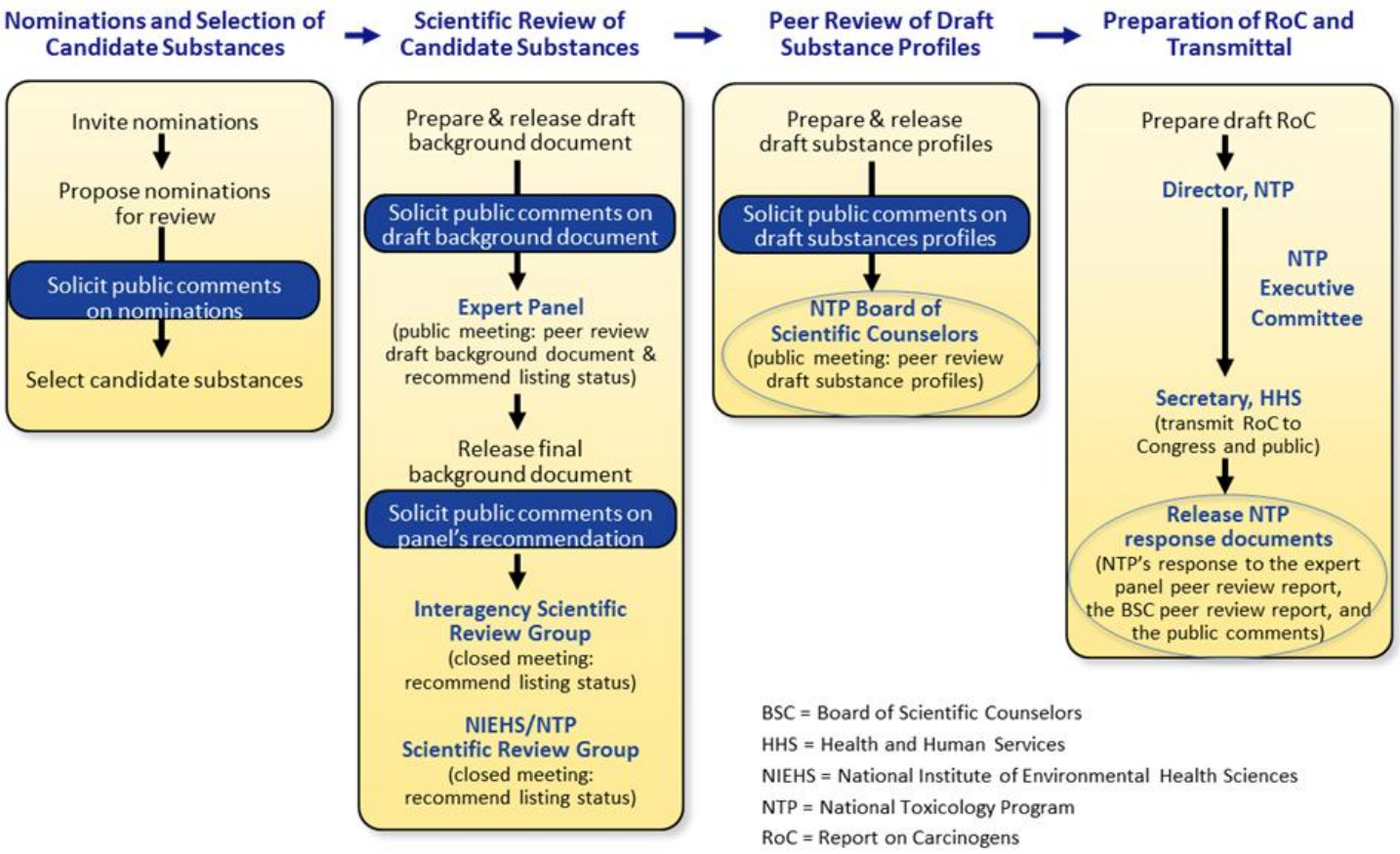
The National Toxicology Program (NTP) followed a formal process for the review of candidate substances for the *Twelfth Report on Carcinogens* (*12th RoC*) (see page 2 for a schematic of the review process) that included the peer review of the draft substance profiles for each candidate substance by the Board of Scientific Counselors (BSC) and opportunity for public comment (see part 3 of the review process). The peer review for five candidate substances took place at a public meeting on February 24, 2009 (see page 3 for the attending BSC members). Three other candidate substances were reviewed at a second meeting on June 21–22, 2010.

A draft substance profile provides the preliminary listing recommendation for a substance in the *12th RoC* (i.e., *known to be a human carcinogen* or *reasonably anticipated to be a human carcinogen*, or not to list); the carcinogenicity studies that support the recommendation; information on human exposure including data on use, production and occupational and environmental exposure; and current Federal regulations to limit exposure. The charge to the BSC was to determine whether the scientific information cited in the draft substance profile for a candidate substance is technically correct, clearly stated, and supports the NTP's preliminary policy decision regarding its listing in the *12th RoC*. The BSC's peer-review comments on the draft substance profiles are captured in the minutes for these meetings.[1]

The NTP carefully reviewed and considered the BSC peer-review comments in revising and finalizing the substance profiles, which were approved by the Secretary of the Department of Health and Human Services and are now part of the *12th RoC*.[2] As noted in the RoC review process (see part 4 of the review process), the NTP releases a report responding to the BSC peer-review comments at the time the *12th RoC* is published. The BSC's major scientific and technical comments and the NTP's response to those comments are provided in this report for each candidate substance.

[1] http://ntp.niehs.nih.gov/go/9741, choose meeting date and select meeting minutes.
[2] http://ntp.niehs.nih.gov/go/roc12

NTP Report on Carcinogens Review Process



NTP Board of Counselors Meetings: Roster of Attending Members

February 24, 2009 Meeting

Draft substance profiles for the following candidate substances were reviewed: aristolochic acids, captafol, *ortho*-nitrotoluene, riddelliine, and styrene.

Members

Tracie E. Bunton, D.V.M., Ph.D., DACVP, Eicarte LLC
Edward W. Carney, Ph.D., The Dow Chemical Company
Russell C. Cattley, V.M.D., Ph.D., Amgen
David A. Eastmond, Ph.D., University of California, Riverside
George Friedman-Jiménez, M.D., New York University School of Medicine
William P. Janzen, University of North Carolina at Chapel Hill
Mitzi Nagarkatti, Ph.D., University of South Carolina School of Medicine
Raymond F. Novak, Ph.D., Wayne State University School of Medicine (chair)
Michael V. Pino, D.V.M., Ph.D., Sanofi-Aventis Recherche & Développement
Kenneth M. Portier, Ph.D. (Chair), American Cancer Society
Jim E. Riviere, D.V.M., Ph.D., ATS, North Carolina State University
Diane Robins, Ph.D., University of Michigan School of Medicine
Ruthann A. Rudel, M.S., Silent Spring Institute
James L. Sherley, M.D., Ph.D., Boston Biomedical Research Institute
Gina M. Solomon, M.D., M.P.H., Natural Resources Defense Council

Pending Members

Elaine M. Faustman, Ph.D., University of Washington
Stephen W. Looney, Ph.D., Medical College of Georgia
Justin G. Teeguarden, Ph.D., Pacific Northwest Laboratory

Ad Hoc *Members*

Ronald Hines, Ph.D., Medical College of Wisconsin
Dana Loomis, Ph.D., University of Nevada, Reno

Aristolochic Acids

The draft substance profile on aristolochic acids was peer-reviewed by the BSC at the meeting held February 24, 2009[3] (see page 3 for a roster of attending members). The NTP's preliminary policy decision was that aristolochic acids should be listed in the *12th RoC* as *known to be human carcinogens* based on sufficient evidence of carcinogenicity from studies in humans and supporting data from studies on mechanisms of carcinogenesis. The NTP reviewed the BSC comments, revised the substance profile, and finalized its recommendation on the listing status of aristolochic acids in the *12th RoC*, which was approved by the Secretary of the Department of Health and Human Services. Aristolochic acids are listed as *known to be human carcinogens* in the *12th RoC*.

BSC Comments and NTP Responses: Scientific and Technical Issues

BSC Comments:

1. Discuss the potential for aristolochic acid contamination of grains worldwide.
2. Add information about the potential exposure to aristolochic acids from the use of extracts as flavorings, such as in alcoholic beverages.
3. Add information regarding the doses of aristolochic acid reported in the Belgian weight loss epidemic.
4. The recent, in-depth review (Debelle *et al.* 2008) of the role of aristolochic acids in both Chinese-herb and Balkan-endemic nephropathies should be cited.
5. Add information about the incidence and prevalence of transitional cell carcinoma from studies of Chinese populations who have renal disease or received a renal transplant and who did not consume products containing aristolochic acid. These studies (not referenced in the draft profile) report a prevalence of 0.55 to 1.76% for urothelial cancers.
6. The section on potential mechanisms includes information on the specific tissues in which adducts are found. Clarify whether aristolochic acids are also direct acting mutagens because the metabolites are identified as the active agents in the draft substance profile.

NTP Response: The NTP concurs with these suggestions and incorporated the requested information into the appropriate sections of the final substance profile for aristolochic acids. Additional information was added as recommended including comments 1 to 3 to the "Exposure" section and comments 4 to 6 to the "Carcinogenicity" section.

7. Include the chemical structures of key compounds.

NTP Response: Aristolochic acids encompass a large family of nitrophenanthrene carboxylic acids that occur naturally in plants in the family Aristolochiaceae. Although the predominant aristolochic acids are I and II, the listing in the substance profile includes many other compounds found in these plants, such as aristolochic acids III, IIIa, IV, IVa, aristolactams, and dioxoaporphines (NTP 2008). It is not the

[3] For the complete minutes from the NTP BSC meeting, see http://ntp.niehs.nih.gov/go/9741, February 24, 2009 meeting, and select meeting minutes.

convention of the RoC to provide structures for individual chemicals when a large class, such as aristolochic acids, is listed.

8. Discuss the relevancy of dermal exposure and transdermal absorption, noting gardeners could be at risk.

NTP Response: No additional information on absorption after dermal exposure was identified.

References

Debelle FD, Vanherweghem JL, Nortier JL. 2008. Aristolochic acid nephropathy: a worldwide problem. *Kidney Int* 74(2): 158-169.

NTP. 2008. *Report on Carcinogens Background Document for Aristolochic Acids*. Research Triangle Park, NC: National Toxicology Program, 274 pp. http://ntp.niehs.nih.gov/files/Aristolochic_Acids_(FINAL-02Sep08)_Redo2%5B3%5D.pdf

Captafol

The draft substance profile on captafol was peer-reviewed by the BSC at the meeting held February 24, 2009[4] (see page 3 for a roster of attending members). The NTP's preliminary policy decision was that captafol should be listed in the *12th RoC* as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting data from studies on mechanisms of carcinogenesis. The NTP reviewed the BSC comments, revised the substance profile, and finalized its listing recommendation for captafol in the *12th RoC*, which was approved by the Secretary of the Department of Health and Human Services. Captafol is listed as *reasonably anticipated to be a human carcinogen* in the *12th RoC*.

BSC Comments and NTP Responses: Scientific and Technical Issues

BSC Comments:

1. Delete the definitions of neoplasm and lymphosarcoma.

   NTP Response: The NTP changed the term "neoplasm" to "tumors" in the final substance profile. The definition of lymphosarcoma was not deleted because the RoC is used by non-scientific audiences in addition to scientific audiences.

2. It is unclear from the profile which of the potential mechanisms is the most relevant to captafol's carcinogenicity, alteration of the side-chain or formation of tetrahydrophthalimide (THPI). Presentation of the mechanistic data should be clearer and more focused.

   NTP Response: THPI is formed rapidly in the presence of sulfhydryl groups but has not been tested in carcinogenicity assays. The metabolic products of the side-chain, although not as prominent as THPI, have been shown to be genotoxic and carcinogenic. Both mechanisms are now emphasized in the substance profile.

[4] For the complete minutes from the NTP BSC meeting, see http://ntp.niehs.nih.gov/go/9741, February 24, 2009 meeting, and select meeting minutes.

ortho-Nitrotoluene

The draft substance profile on *ortho*-nitrotoluene (also known as *o*-nitrotoluene) was peer-reviewed by the BSC at the meeting held February 24, 2009[5] (see page 3 for a roster of attending members). The NTP preliminary policy decision was that *ortho*-nitrotoluene should be listed in the *12th RoC* as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting evidence from studies on mechanisms of carcinogenesis. The NTP reviewed the BSC comments, revised the substance profile, and finalized its listing recommendation for *ortho*-nitrotoluene in the *12th RoC*, which was approved by the Secretary of the Department of Health and Human Services. *ortho*-Nitrotoluene is listed as *reasonably anticipated to be a human carcinogen* in the *12th RoC*.

BSC Comments and NTP Responses: Scientific and Technical Issues

BSC Comments:

1. Add information on the route of exposure for occupationally exposed workers.
2. Provide more detail about metabolism and metabolites and about differences between biliary excretion in the male and female rat.
3. Add specific definitions to explain mesothelial neoplasia and hyperplasia.
4. Add that microbial metabolism in the gut is critical to the mode of action.

NTP Response: The NTP incorporated the requested information into the final substance profile for *ortho*-nitrotoluene. Additional information was added as recommended including comment 1 to the "Exposure" section, and comments 2 and 4 to the "Carcinogenicity" section. The information for comment 3 was added to the "Glossary" rather than the profile.

5. Add that hemoglobin adducts in workers mirror those found in rodents.

NTP Response: No change was made because the substance profile states that the hemoglobin adducts are the same in both species.

[5] For the complete minutes from the NTP BSC meeting, see http://ntp.niehs.nih.gov/go/9741, February 24, 2009 meeting, and select meeting minutes.

Riddelliine

The draft substance profile on riddelliine was peer-reviewed by the BSC at the meeting held February 24, 2009[6] (see page 3 for a roster of attending members). The NTP's preliminary policy decision was that riddelliine should be listed in the *12th RoC* as *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals and supporting evidence from studies on mechanisms of carcinogenesis. The NTP reviewed the BSC comments, revised the substance profile, and finalized its listing recommendation for riddelliine, which was approved by the Secretary of the Department of Health and Human Services. Riddelliine is listed as *reasonably anticipated to be a human carcinogen* in the *12th RoC*.

BSC Comments and NTP Responses: Scientific and Technical Issues

BSC Comments:

1. Provide a table containing all the plant genera and species that contain riddelliine and their common names.
2. Emphasize the potential for cumulative effects resulting from low-level exposure.
3. Discuss inflammation and oxidative stress as causes of carcinogenicity.

NTP Response: The NTP concurs with these suggestions and incorporated the requested information into the appropriate sections of the final substance profile for riddelliine. Additional information was added as recommended including comment 1 to the "Exposure" section and comments 2 and 3 to the "Carcinogenicity" section.

[6] For the complete minutes from the NTP BSC meeting, see http://ntp.niehs.nih.gov/go/9741, February 24, 2009 meeting, and select meeting minutes.

Styrene

The draft substance profile on styrene was peer-reviewed by the BSC at the meeting held February 24, 2009[7] (see page 3 for a roster of attending members). The NTP's preliminary policy decision was that styrene should be listed in the *12th RoC* as *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity from studies in humans, sufficient evidence of carcinogenicity in animals, and supporting data from studies on mechanisms of carcinogenesis. The NTP reviewed the BSC comments, revised the substance profile, and finalized its listing recommendation for styrene in the *12th RoC*, which was approved by the Secretary of the Department of Health and Human Services. Styrene is listed as *reasonably anticipated to be a human carcinogen* in the *12th RoC*.

BSC Comments and NTP Responses: Scientific and Technical Issues

BSC Comments:

1. Add to the "Exposure" section, information about contamination of styrene with styrene oxide in exposure studies.
2. Emphasize that styrene exposure to the general public through food is orders of magnitude lower than occupational exposures.
3. Note that occupational exposure levels of styrene have decreased.
4. Add the values for the 95th percentile and maximum blood styrene levels found in the general population, as measured by the Centers for Disease Control and Prevention (CDC), to provide a context for the levels observed in occupationally exposed workers.
5. For completeness, add the findings from the study by Wong *et al.* (1994), which did not find an association between styrene exposure and lymphohematopoietic cancer risk.
6. Address the observation that, although different types of lymphomas and leukemias are usually considered as different diseases, a similar pattern of increases in different types of lymphohematopoietic cancers is found with other epoxides or substances metabolized to epoxides related to styrene, such as ethylene oxide and butadiene, which have presumably similar modes of action as styrene. Also, the key studies categorized the different lymphohematopoietic cancers differently, which limits the ability to discern consistent associations between studies. Address the fact that the studies had limited powder to detect lymphohematopoietic cancers.
7. Correct the profile to state that the Delzell *et al.* (2006) study was done only in men.
8. The draft substance profile incorrectly states that the Kogevinas *et al.* (1994) study adjusted for exposure duration in the cumulative exposure analyses.
9. Note that "the ability of styrene to induce cell proliferation in the terminal bronchioles of the lung after oral dosing as shown by Green *et al*. (2001) in mice

[7] For the complete minutes from the NTP BSC meeting, see http://ntp.niehs.nih.gov/go/9741, February 24, 2009 meeting, and select meeting minutes.

provides additional support for the revised assessment of the NCI study that oral administration of styrene can induce cancer-related changes in the lung."

10. Discuss the findings for mammary-gland tumors in studies in rats.
11. Add information regarding the presence of CYP2E1 in type II pneumocytes and Clara cells in the human lung, and in lymphocytes.
12. It was suggested that the terms genotoxic and non-genotoxic be used, rather than genotoxic and epigenetic, when describing mode of action.
13. Clarify that cytotoxicity alone does not result in a tumor, as a mutation must also occur.
14. Add a discussion of Johanson *et al.* (2000), which showed that styrene-7,8-oxide adducts were found under environmental conditions in which individuals were exposed to styrene, but were not exposed to styrene-7,8-oxide.
15. Add information related to potential immunosuppression effects from styrene exposure.
16. Add a discussion on the role of genetic polymorphisms in susceptibility and resistance to cancer.

NTP Response: The NTP concurs with these suggestions and incorporated the requested information into the appropriate sections of the final substance profile for styrene. Additional information was added as recommended including comments 1 to 4 to the "Exposure" section and comments 5 to 16 to the "Carcinogenicity" section.

17. One reviewer suggested adding information that Wong *et al.* (1994) reported statistically significant increases in risk for all lymphohematopoietic tumors with duration of exposure, but not with cumulative exposure, with insignificant increases for non-Hodgkin's lymphoma and leukemia.

NTP Response: The NTP was not able to locate this information (significant increases for all lymphohematopoietic cancers or insignificant risks for leukemia) in the Wong *et al.*1994 study. A statistically non-significant risk (approximately 2 fold) for lymphosarcoma and reticulosarcoma (combined) was observed among workers with the longest exposure (greater than 10 years); however, the effect estimate was based on small numbers of deaths (2), thus the NTP did not feel that this information should be included in the substance profile.

18. Include a discussion from the Delzell *et al.* 2006 study, on "the cross-classification analyses between butadiene and styrene, which suggests an effect modification with butadiene; however, there is not enough power to test for an interaction."

NTP Response: Although the cross-classification analyses between butadiene and styrene are interesting, the NTP does not believe it is clear that these analyses suggest an effect modification with butadiene. The cross-classification analysis calculated effect estimates for leukemia for three levels of cumulative exposure to styrene (low, medium, and high) stratified by three levels of cumulative exposure to butadiene (low, medium, and high). This analysis found that among individuals with high cumulative exposure to butadiene, no cases of leukemia were found among workers with low cumulative exposure to styrene, whereas significant effect estimates were found among individuals with medium and high cumulative exposures to styrene

suggesting that styrene exposure may increase the risk of butadiene-induced leukemia. However, the risks of leukemia did not increase with increasing cumulative exposure to styrene (i.e., effect estimates were similar for medium and high cumulative exposures to styrene), thus the findings are unclear. This type of discussion is beyond the scope of the substance profile.

19. One reviewer suggested that a discussion of the findings for breast cancer should be added to the profile, noting that only a limited number of women were included in the occupational cohort studies. Specifically, the profile should state that breast cancer risk has not been adequately evaluated, and it should include the findings from three studies where internal comparisons showed an increased risk for breast cancer among subgroups of women exposed to styrene (Kogevinas *et al.* 1993, Wong *et al.* 1994, and Cantor *et al*. 1995).

NTP Response: The profile is a concise summary of the scientific evidence that supports the listing and is not a comprehensive review of the literature of all studies. While the findings on human breast cancer are interesting, the evidence at present is inadequate to evaluate the relationship between exposure specifically to styrene and breast cancer, and no additional information was added to the substance profile. In addition, Wong *et al.* 1994 and Kogevinas *et al.* 1993 did not report findings for breast cancer in internal analyses.

20. One reviewer thought that the evidence from the gavage bioassay studies in mice was suggestive but not conclusive. There were concerns about the use of historical controls in the NCI study from a different laboratory and about the high toxicity in the study by Ponomarkov and Tomatis (1978).

NTP Response: The NTP believes that the NCI study shows evidence that styrene causes lung tumors in male mice after oral exposure based on a positive dose-response trend and a significant increase in the incidences of lung tumors at the highest dose. The NTP believes the concurrent controls are generally the most appropriate ones to use in statistical analyses. With respect to historical controls, the NTP notes that the incidences of the high-dose males (21%) were outside of the historical control range for both the vehicle historical controls and the untreated historical controls from the same laboratory (Litton) as the NCI study and from studies at a concurrent laboratory (Hazleton) with similar study duration, and from the same supplier as used in the NCI study. Although the study by Ponomarkov and Tomatis was limited by high toxicity, it provides supporting evidence for the results reported in the NCI study.

21. Add more information on similarities and differences among styrene and three epoxide-forming carcinogens (vinyl chloride, 1,3-butadiene, and ethylene oxide) that are metabolized through similar metabolic pathways.

NTP Response: The substance profile is not a comprehensive review of the literature of all studies published on a specific issue; however, language was added to the substance profile for styrene to refer the reader to the substance profiles in the RoC for the three epoxide-forming carcinogens.

22. Recommended that the quantitative data on the formation of styrene-7,8-oxide in pulmonary tissue from mouse, rat, and human tissues be included in the profile because of the limited human carcinogenicity data, as this information would help in interpreting the mechanistic data.

NTP Response: The RoC does not present quantitative assessment of the risks of cancer, thus the requested information was not added to the substance profile because it is outside the scope of this document.

References

Cantor KP, Stewart PA, Brinton LA, Dosemeci M. 1995. Occupational exposures and female breast-cancer mortality in the United States. *J Occup Environ Med* 37(3): 336-348.

Delzell E, Sathiakumar N, Graff J, Macaluso M, Maldonado G, Matthews R. 2006. An updated study of mortality among North American synthetic rubber industry workers. *Res Rep Health Eff Inst* (132): 1-63, 65-74.

Green T, Toghill A, Foster JR. 2001. The role of cytochromes P-450 in styrene induced pulmonary toxicity and carcinogenicity. *Toxicology* 169(2): 107-117.

Johanson G, Ernstgard L, Gullstrand E, Lof A, Osterman-Golkar S, Williams CC, Sumner SC. 2000. Styrene oxide in blood, hemoglobin adducts, and urinary metabolites in human volunteers exposed to $^{13}C_8$-styrene vapors. *Toxicol Appl Pharmacol* 168(1): 36-49.

Kogevinas M, Ferro G, Saracci R, Andersen A, Biocca M, Coggon D, *et al.* 1993. Cancer mortality in an international cohort of workers exposed to styrene. In *Butadiene and Styrene: Assessment of Health Hazards*, IARC Scientific Publications No. 127. Sorsa M, Peltonen K, Vainio H, Hemminki K, eds. Lyon, France: International Agency for Cancer Research. pp. 289-300.

Kogevinas M, Ferro G, Andersen A, Bellander T, Biocca M, Coggon D, *et al.* 1994. Cancer mortality in a historical cohort study of workers exposed to styrene. *Scand J Work Environ Health* 20(4): 251-261.

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

Wong O, Trent LS, 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.