A Summary of Scientific Research

SIRC's Continuing Commitment to Science & Stewardship

SIRC Styrene Information & Research Center

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SIRC Styrene Information & Research Center

1750 K Street NW, Suite 700
Washington, DC 20006
202.787.5996
sirc@styrene.org
www.styrene.org
www.youknowstyrene.org

A Continuing Commitment to Science and Stewardship

Summary of Scientific Investigations to Understand Styrene's Potential Health Effects Supported by the Styrene Information & Research Center

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Summary of Scientific Investigations to Understand Styrene's Potential Health Effects Supported by the Styrene Information & Research Center

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INTRODUCTION

SIRC-Sponsored Scientific Investigations to Understand Styrene's Potential Health Effects

Styrene is the base material for thousands of everyday products for home, school, work and play. Styrene is used to make food containers and packaging materials, cars, boats, computers and video games among countless products. Styrene helps create remarkably strong, flexible and lightweight products that represent a vital part of our economy and contribute to improved quality of life.

Although styrene occurs naturally in certain foods such as cinnamon, coffee and strawberries, styrene derived from petroleum and natural gas by-products is so widely used that questions have arisen about whether manufacturing styrene and styrenic products or using styrene-based consumer goods have the potential to affect human health or the environment.

In 1987, styrene manufacturers established the Styrene Information & Research Center (SIRC) to find answers to those questions. Since its inception, SIRC has undertaken a comprehensive research program to better understand the potential, if any, for styrene to affect human health, as well as to understand the fate and effects of styrene in the environment. SIRC's approach has been – and continues to be – to sponsor new research in areas where gaps, deficiencies and limitations in the existing data exist. SIRC also commissions independent reviews of existing health effects studies. Scientific research and literature reviews underwritten by SIRC are carried out with the intention that final reports will be published in appropriate peer-reviewed journals. To date, 70 such papers have been published regarding styrene research.

SIRC has worked closely over the years with the U.S. Environmental Protection Agency (EPA) Office of Research and Development (ORD), the U.S. Food and Drug Administration (FDA) and other regulatory agencies in developing protocols for research it has sponsored.

SIRC's commitment to science has produced a robust, credible, reliable and still growing information resource for regulatory decision-making. The science that has been sponsored by SIRC has followed a step-wise approach to ensure that each research study builds on findings from past studies, starting with an understanding of styrene's environmental fate and leading up to a human risk assessment using the wealth of data that has become available since SIRC was founded in 1987. The total cost of SIRC-sponsored research program and activities exceeds $25 million.

Ethylbenzene is the basic starting material used in the production of commercial styrene. In 2013, SIRC assumed responsibility for ethylbenzene. Although it is not included in this summary, SIRC has also supported scientific research on this substance.

The following pages contain summaries of each of the published* studies that SIRC has sponsored to better understand styrene's potential human health and environmental effects.

A. Styrene Fate and Natural Occurrence

1. Biodegradation of Styrene in Samples of Natural Environments by Min-Hong Fu and Martin Alexander, Ph.D., Laboratory of Soil Microbiology, Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, NY, published in *Environmental Science & Technology,* 26(*8*), pp. 1540-1544, 1992.
   - Purpose – Studied the fate of styrene in selected environmental samples and assessed the possible significance of volatilization, biodegradation and sorption (the process of being taken up and held), particularly when styrene entered water and soils.
   - Key Conclusions – Styrene was rapidly destroyed by biodegradation in most environments having oxygen, but the rate might be slow at low concentrations in aquifers and lake waters and in environments at low pH.

2. Desorption and Biodegradation of Sorbed Styrene in Soil and Aquifer Solids by Min-Hong Fu, and two other authors, Laboratory of Soil Microbiology, Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, NY, published in *Environmental Toxicology and Chemistry,* 13(*5*), pp. 749-753, 1994.
   - Purpose – This study examined the sorption and biodegradation of styrene in environmental samples, including loam and muck. It is understood that the solid surfaces of environments into which styrene is introduced might influence its fate. The compound might affix to a particle-type material, which would affect its mobility and the extent to which it might be of possible concern to public health or to animals or plants.
   - Key Conclusions – Being broken down by microbes is a major mechanism by which styrene is destroyed in soils. The biodegradation of styrene that has been in natural environments for some time might be different from the transformations that occurred with the freshly added compound.

3. Determination of Styrene in Selected Foods by David H. Steel, Ph.D., Midwest Research Institute, Kansas City, MO, and six other authors, published in the *Journal of Agricultural and Food Chemistry,* 42(*8*), pp.1661-1665, 1994.
   - Purpose - Determined styrene levels that occur naturally in various foodstuffs. Foods tested were wheat, oats, peanuts, pecans, coffee beans, tomatoes, peaches, strawberries, cinnamon, beef, chicken and milk. Food samples were collected in a manner to ensure that they did not contact styrene-based plastics, thereby excluding the possibility of migration of styrene into the samples from processing or packaging.
   - Key Conclusions – Styrene occurred as a natural constituent of foods in the human diet. The highest measured concentrations of styrene were found in cinnamon. Lower concentrations were found in beef and coffee beans. Very low levels of styrene were found in wheat, pecans, oats, strawberries and peaches.

4. Biodegradation of Styrene in Waterlogged Soils and Aquifer Solids by Min Hong Fu, Ph.D and Martin Alexander, Ph.D, Department of Soil, Crop, and Atmospheric Sciences, Cornell University, Ithaca, NY, published in *Soil Science,* 161(*12*), pp. 846-851, 1996.
   - Purpose – Determined the fate of styrene in waterlogged samples of aquifer solids and loam, with particular attention paid to investigate the possible formation of styrene oxide.
   - Key Conclusions – Biodegradation of styrene in waterlogged soils and aquifer solids was initially rapid, but then the rate declined. Significant amounts of styrene persisted under waterlogged conditions. Styrene oxide was not detected. Because many waste sites are oxygen deficient, the persistence of styrene under waterlogged conditions might be of environmental importance. Further research is required to assess whether such accumulations of styrene occur in nature, as well as under laboratory conditions.

5. Ecotoxicity Hazard Assessment of Styrene by Janette R. Cushman, Ph.D., Chevron Research & Technology Co., Environmental Health Center, Richmond, CA, and 11 other authors, published in *Ecotoxicology and Environmental Safety,* 37(*2*), pp. 173-180, 1997.
   - Purpose – This study aimed to better characterize styrene's potential environmental hazards. Aquatic and soil toxicity studies were conducted on fathead minnows, daphnids, amphipods, freshwater green algae and earthworms.

- Key Conclusions – Styrene was shown to be moderately toxic to the minnows, daphnids and amphipods. It was shown to be highly toxic to green algae, and slightly toxic to earthworms. There was no indication of a concern for chronic toxicity based on these studies. Styrene's potential impact on aquatic and soil environments was significantly mitigated by the rapid rate at which it evaporated and biodegraded in the environment.

6. Environmental Fate and Effects of Styrene by Martin Alexander, Ph.D., Department of Soil, Crop and Atmospheric Sciences, Cornell University, Ithaca, NY, published in *Critical Reviews in Environmental Science and Technology,* 27(4), pp. 383-410, 1997.
   - Purpose – Reviewed and evaluated research and monitoring data on the fate of styrene in water, soil and the atmosphere to determine the potential for these media to be sources of human exposure to styrene.
   - Key Conclusions – Transport of styrene in nature was "very limited" because of its volatility from soils and surface waters, its rapid destruction in air, and its biodegradation in soils and surface and ground waters. The most probable source for human exposure was the atmosphere. However, because styrene was highly reactive and dissipated rapidly in air, it was unlikely to be transported or to be found in water or soils to any significant extent. There was little possibility of styrene occurring in drinking water, and it was found in very minor amounts in the food chain.

B. Health Effects Studies in Styrene-exposed Workers

7. An Updated Cohort Mortality Study of Workers Exposed to Styrene in the Reinforced Plastics and Composites Industry by Otto Wong, Sc.D., Applied Health Sciences, San Mateo, CA; and three other authors, published in *Occupational and Environmental Medicine,* 51(6), pp. 386-396, 1994.
   - Purpose – Updated cause-of-death data for a cohort of workers in the reinforced plastics and composites industry with exposures to styrene monomer and other chemicals. The cohort consisted of 15,826 male and female employees who were exposed to styrene for at least six months between 1948 and 1977 at 30 participating U.S. manufacturing plants. Follow-up studies were published in 1999 and 2013.
   - Key Conclusions – There was no evidence among the findings for a link between styrene exposure or styrene processes and increased mortality from any cause,

including cancer. In particular, the study found fewer-than-anticipated cases of lymphoma and leukemia.

8. Styrene Exposure and Color Vision by James E. Sheedy, O.D., Ph.D., Walnut Creek, CA, published in *The SIRC Review,** 5(*1*), pp. 7-30, 1997.

   - Purpose – Reviewed and evaluated reports that styrene exposure caused decreases in color discrimination.
   - Key Conclusions – Exposure to greater than 30 parts per million (ppm) styrene for at least several months resulted in decreased color discrimination. The degree of styrene-induced color vision deficiency was so mild that it was only found using sensitive tests and was not noticeable by those affected. Exposures at lower concentrations did not appear to affect color discrimination.

9. Mortality from Nonmalignant Diseases of the Respiratory, Genitourinary and Nervous Systems among Workers Exposed to Styrene in the Reinforced Plastics and Composites Industry in the United States by Otto Wong, Sc.D., Applied Health Sciences, San Mateo, CA, and Lisa Trent, M.S., Department of Epidemiology, School of Public Health, Tulane University Medical Center, New Orleans, LA, published in *Scandinavian Journal of Work Environment and Health,* 24(*4*), pp. 317-325, 1999.

   - Purpose – This was an update of an earlier study (see item 7) to examine causes of death from nonmalignant genitourinary diseases, nonmalignant respiratory diseases, and diseases of the nervous system among 15,826 U.S. workers exposed to styrene in 30 plants in the reinforced plastics industry.
   - Ken Conclusions – No relationship was found between causes of death from any of the diseases examined and styrene exposures. (Another update was published in 2013.)

10. Olfactory Function in Workers Exposed to Styrene in the Reinforced-Plastics Industry by Pamela Dalton, Ph.D., Monell Chemical Center, Philadelphia, PA, and six other authors, published in the *American Journal of Industrial Medicine,* 44(*1*), pp. 1-11, 2003.

    - Purpose – Impairment of olfactory function in humans has frequently been associated with occupational exposure to volatile chemicals. To investigate whether occupational exposure to styrene was associated with olfactory impairment, the researchers examined olfactory function in a group of workers with a minimum of

four years exposure to styrene in the reinforced plastics industry and in a group of age- and gender-matched, unexposed controls.

- Key Conclusions – The study found no evidence among reinforced plastics workers that current or historical exposure to styrene was associated with either self-reported or objective impairment of olfactory function.

11. Exposure Assessment for Study of Olfactory Function in Workers Exposed to Styrene in the Reinforced-Plastics Industry by Peter S. J. Lees, Ph.D., C.I.H, Johns Hopkins University School of Hygiene and Public Health, and three other authors, published in the *American Journal of Industrial Medicine,* 44(*1*), pp. 12-23, 2003.
    - Purpose – Provided quantitative styrene acute and chronic exposure information for workers who were the subjects of a simultaneous study examining the possible olfactory effects of such exposures (see study number 10 in this summary) in order to test the scientific validity of the companion study. Acute exposures were estimated through measurement of the concentration of styrene in air in the workers' breathing zones. Chronic exposures over the subjects' employment histories were reconstructed from job histories and historic exposure monitoring records.
    - Key Conclusions – The authors concluded that the data used in the companion study was valid. The data showed that current and historic measures of exposure presented a comprehensive and consistent characterization of the styrene exposure of the study population crucial to the interpretation and understanding of the simultaneously conducted olfactory function evaluation. It also provided a well-characterized population, with documented exposure histories stable over time and in the range suitable for the purposes of the associated study of olfactory function.

12. Occupational Styrene Exposure And Hearing Loss: A Cohort Study With Repeated Measurements by Gerhard Triebig, M.D., S.Sc., Institute and Outpatient Clinic for Occupational and Social Medicine, University Hospital of Heidelberg, Heidelberg, Germany and two other authors, published in *International Archives of Occupational and Environmental Health*, 82(*4*), pp. 463-480, 2009.
    - Purpose – Investigated associations between occupational styrene exposures and hearing loss and sought answers to three questions: 1) Were there hearing losses as measured by high frequency and standard audiometric tests? 2) Were there dose-response relationships and measureable thresholds of effects? And, 3) Were there signs of reversability of possible effects during an exposure-free period?

- Key Conclusions – Average inhaled styrene levels of about 30–50 ppm per work day over a period of about 15 years with higher exposure levels above 50 ppm in the past were associated with an elevated risk for impaired hearing thresholds (the formerly published results on ototoxic effects below 20 ppm could not be confirmed). In addition, no dose–response relationship between threshold and exposure data was found except for at frequencies of 1,000 and 1,500 Hz. Finally, improvements of hearing thresholds during work- and exposure-free periods were possible.

13. Occupational Styrene Exposure, Colour Vision and Contrast Sensitivity: A Cohort Study with Repeated Measurements by Andreas Seeber, Psy.D and M.D. Institute of Occupational Physiology, University of Dortmund, Dortmund, Germany and two other authors, published in *International Archives of Occupational and Environmental Health*, 82(*6*), pp. 757-770, 2009.
    - Purpose – Investigated associations between occupational styrene exposures and impairment of visual functions and sought answers to three questions: 1) Were previous findings of colour vision deficiencies reproducible? 2) If such effects existed, were they related to current or chronic exposures? And, 3) If such effects existed, were they reduced during an exposure-free period?
    - Key Conclusions – No cause and effect relationship was identified between either acute or chronic styrene occupational exposure and contrast senstitvity or colour vision. This contradicted the published results for styrene-related colour vision effects.

14. Occupational Styrene Exposure and Neurobehavioural Functions: A Cohort Study with Repeated Measurements by Andreas Seeber, Psy.D and M.D. Institute of Occupational Physiology, University of Dortmund, Dortmund, Germany and two other authors, published in *International Archives of Occupational and Environmental Health*, 82(*8*), pp. 969-984, 2009.
    - Purpose – Investigated associations between occupational styrene exposures and cognitive as well as psychomotor functions and sought answers to three questions: 1) Were previous findings of colour vision deficiencies reproducible? 2) If such effects existed, were they related to current or chronic exposures? And, 3) If such effects existed, were they reduced during an exposure-free period?
    - Key Conclusions – With the exception of subject performances in the Benton test and in a finger dexterity test – which were associated with parameters of long-term exposure but not with current exposures – neither acute nor long-term styrene

exposures were found to be associated with an elevated risk of developing impaired cognitive and psychomotor functions or increased symptom levels.

15. Cancer Mortality of Workers Exposed to Styrene in the U.S. Reinforced Plastics and Composite Industry by James J. Collins, Department of Epidemiology, The Dow Chemical Company, Midland, MI, and two other authors, published in *Epidemiology,* 24(*2*), pp. 195-203, 2013.
    - Purpose – Examined human mortality rates associated with cumulative exposure, duration of exposure, peak exposures, average exposure, and time since first exposure to styrene.
    - Key Conclusions – There was no trend with either cumulative exposure to styrene or number of peaks. No coherent evidence was found to support that styrene exposure increased risk from cancers of the lymphatic and hematopoietic tissue, pancreas or lung.

C. Styrene Toxicity, Metabolism and Mode of Action

16. Review of the Metabolic Fate of Styrene by Susan Jenkins Sumner, Ph.D., and Timothy R. Fennell, Ph.D., Chemical Industry Institute of Toxicology, Research Triangle Park, NC, published in *Critical Reviews in Toxicology,* 24(*S1*), pp. S11-S33, 1994.
    - Purpose – This literature review was undertaken to investigate the metabolism of styrene in laboratory animals, and to compare this data with similar information obtained in humans in order to enhance understanding of the risk to humans associated with styrene exposure.
    - Key Conclusions – The metabolism of styrene has been studied extensively and species differences in metabolism have been demonstrated.

17. Correlating Styrene Metabolism and Distribution with Hepatotoxicity by Susan C. Sumner, Ph.D., Chemical Industry Institute of Toxicology, Research Triangle Park, NC, and three other authors, published in the *Toxicologist* 15(*1*), Abstract 20, p. 4, 1995.
    - Purpose – This study examined biochemical mechanisms of styrene toxicity by comparing its metabolism, distribution and liver toxicity in one strain of laboratory rats and two different strains of laboratory mice following single and repeated exposures.
    - Key Conclusions – The study showed that a large percentage of the metabolites excreted by rats and mice following styrene exposure occurred following conversion of styrene to styrene oxide and further metabolism. The study demonstrated

differences among strains of mice and between rats and mice. And, therefore, metabolic differences among species might be important in explaining toxicity differences.

18. Cell Proliferation in Rat Forestomach Following Oral Administration of Styrene Oxide by Walden E. Dalbey, Ph.D., Stonybrook Laboratories Inc., Princeton, NJ, and two other authors, published in *Fundamental and Applied Toxicology,* 30(*1*), pp. 67-74, 1996.
    - Purpose – Styrene oxide (SO) is a primary metabolite of styrene, and was known to cause tumors in the forestomach of rats and mice dosed orally. This study's primary purpose was to provide data on the effect of orally administered SO on cell proliferation in the forestomach of rats for potential use in human risk assessment.
    - Key Conclusions – The degree of increased cell proliferation in the forestomach of rats dosed orally with styrene oxide paralleled the degree of formation of stomach tumors. This suggested that cellular damage and repair might be important in tumor formation from oral exposure to styrene oxide.

19. Pneumotoxicity and Hepatotoxicity of Styrene and Styrene Oxide by Melinda G. Gadberry, Department of Pharmacology and Toxicology, Purdue University, West Lafayette, IN, and two other authors, published in the *Journal of Toxicology and Environmental Health,* 48(*3*), pp. 273-294, 1996.
    - Purpose – Investigated the toxicity of styrene and styrene oxide in the lung of the mouse in comparison to their toxicity in the liver.
    - Key Conclusions – Styrene and styrene oxide at high doses caused both liver and lung damage in mice. This study indicated that styrene exerts dose-dependent, consistent liver and lung toxicity, with the former appearing earlier. The study also concluded that the toxicity of styrene oxide, the primary metabolite of styrene, may be increased by inhibitors of its detoxification. These findings are useful for human risk assessment of styrene and styrene oxide.

20. Subchronic Inhalation Studies of Styrene in CD Rats and CD-1 Mice by George Cruzan, Ph.D., DABT, ToxWorks, Ringoes, NJ, and seven other authors, published in *Fundamental and Applied Toxicology,* 35(*2*), pp. 152-165, 1997.
    - Purpose – Evaluated styrene's potential to produce health effects in laboratory mice and rats exposed via inhalation and, thereby, develop scientific data that could be

used to help predict styrene's potential health effects on humans. More specifically, this work was conducted to establish parameters for chronic rat and mouse studies planned for later on (see items 25 and 32 in this summary).

- Key Conclusions – Styrene had no effect on survival or the health of rats at exposure concentrations up to 1,500 parts per million (ppm). Some mice exposed to styrene at 200 ppm died. In rats, the only toxicity found was damage to the cells in nasal tissue. In mice, however, the study not only indicated cellular damage in the nasal tissue, but also liver and lung toxicity, suggesting significantly different physiological responses between rats and mice. In the nasal tissue, mice were affected by a 10-fold lower styrene concentration than were rats.

21. Comparison of Mouse Strains for Susceptibility to Styrene-Induced Hepatotoxicity and Pneumotoxicity by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in the *Journal of Toxicology and Environmental Health,* 51(*2*), pp. 177-187, 1997.
    - Purpose – In determining the risk of chemicals to humans using data obtained from studies in animals, it is important to consider strain as well as species differences. These comparative studies were carried out to investigate differences in susceptibility to styrene toxicity among various strains of laboratory mice, including how they converted styrene to its more toxic metabolite, styrene oxide.
    - Key Conclusions – This research indicated that the difference in susceptibility of mouse strains to the toxicity of styrene did not appear to be related to styrene's metabolism to styrene oxide, and that there might be strain differences in the ability to detoxify styrene oxide. The authors concluded "[f]urther studies are needed to examine this possibility." These findings are relevant for human risk assessment involving styrene.

22. Evaluation of the Metabolism and Hepatotoxicity of Styrene in F344 rats, B6C3F1 Mice, and CD-1 Mice Following Single and Repeated Inhalation Exposures by Susan C. J. Sumner, Ph.D., Chemical Industry Institute of Toxicology, Research Triangle Park, NC, and four other authors, published in *Chemico-Biological Interactions* 106(*1*), pp. 47-65, 1997.
    - Purpose – The investigations focused on determining the metabolism of styrene in F344 rats, B6C3F1 mice and CD-1 mice following single and repeated inhalation exposures and correlating these parameters with measures of liver toxicity in mice.

- Key Conclusions – Striking differences in susceptibility to styrene between species (mice vs. rats, for example) and subtle differences between mouse strains (B6C3F1 vs. CD-1 mice, for example) were likely due to a variety of factors. Identification and evaluation of toxic metabolites and other factors warranted investigation for use in predicting inter- and intra-species differences in sensitivity to styrene. These findings are useful for human risk assessment involving styrene.

23. Effects of Inducers and Inhibitors on the Microsomal Metabolism of Styrene to Styrene Oxide in Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in the *Journal of Toxicology and Environmental Health,* 51(*5*), pp. 477-488, 1997.
    - Purpose – Correlated the effects of inducers (*e.g.*, phenobarbital, pyridine) on styrene toxicity as previously observed in mice (Gadberry *et al.*, 1996) with changes in styrene oxidation, compared mouse strains (CD-1 and Non-Swiss Albino) for responsiveness to the inducers, and compared the effects of induction on styrene metabolism in mice with results previously demonstrated in rats.
    - Key Conclusions – These induction studies validated earlier investigations indicating that the strain differences in susceptibility to styrene-induced toxicity might not be explainable on the basis of the conversion of styrene to styrene oxide in mice and rats. These findings are useful for human risk assessment involving styrene.

24. Effects of Inhibitors of CYP1A and CYP2B on Styrene Metabolism in Mouse Liver and Lung Microsomes by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, and two other authors, published in *Toxicology Letters,* 98(*3*) pp. 131-137.
    - Purpose – There was a general belief that the toxicity of styrene was associated with its conversion to styrene oxide. Both liver and lung had been shown to carry out this metabolic step, but there were differences reported as to how this occurred. The purpose of this study was to examine and illuminate how this conversion occurred in certain mouse liver and lung microsomes.
    - Key Conclusions – The data using selected inhibitors indicated that CYP1A played little or no role in the metabolism of styrene to styrene oxide in mouse hepatic or pulmonary cells and that CYP2B made only a minor contribution in animals not previously subjected to experiments. This was in contrast to the greater involvement of CYP2E1 and CYP2F2 (especially in the lung). The results aided in understanding the relevance for human risk assessment purposes of styrene's effects in mice.

25. Chronic Toxicity/Oncogenicity Study of Styrene in CD Rats by Inhalation Exposure for 104 Weeks by George Cruzan, Ph.D., DABT, ToxWorks, Ringoes, NJ, and eight other authors, published in *Toxicological Sciences,* 46(*2*), pp. 266-281, 1998.
    - Purpose – Evaluated styrene's potential to produce health effects in laboratory rats exposed via inhalation over a two-year period (essentially lifetime) and, thereby, develop data that might help predict styrene's potential health effects on humans.
    - Key Conclusions – Styrene had no effect on survival in males, but females exposed to 500 to 1,000 parts per million of styrene had a dose-related increase in survival. The only evidence of toxicity was decreased body weight and cellular damage in nasal tissue. There was no evidence that styrene exposure caused increases of any tumor type in males or females or in the number of tumor-bearing rats in the exposed groups compared to controls. Based on an overall evaluation of eight studies in rats (seven previously published), there was clear evidence that styrene does not induce cancer in rats.

26. Metabolism of Styrene by Mouse and Rat Isolated Lung Cells by Dawn E. Hynes, Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN, and two other authors, published in *Toxicological Sciences,* 51(*2*), pp. 195-201, 1999.
    - Purpose – This study had several objectives. One was to identify the primary cell types involved in respiratory system styrene metabolism. A second was to determine contributions of specific cell structures involved in the metabolism of styrene to its more toxic metabolites by isolated cells. A third was to compare mice with rats to identify metabolic differences that could account for the mouse being more susceptible to styrene toxicity. The final objective was to synthesize this information on cell and species differences regarding the toxicity of styrene and its metabolites.
    - Key Conclusions – The research indicated the importance of a certain type of lung cells (Clara cells[†]) in styrene metabolism, and suggested that differences in metabolism between mice and rats might be responsible for the greater susceptibility of the mouse to styrene-induced toxicity. As part of an ongoing research program, these conclusions contributed to a better understanding of the potential human risks associated with styrene exposure.

[†] Originally called "Clara Cells"; the editorial boards of most major respiratory journals renamed them "Club Cells" in 2013.

27. Uptake of Styrene in the Upper Respiratory Tract of the CD Mouse and Sprague-Dawley Rat by John B. Morris, Ph.D., Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT, published in *Toxicological Sciences,* 54(*1*), pp. 222-228, 2000.
    - Purpose – Quantitative inhalation risk assessment for styrene requires knowledge of the nasal dosimetry of this vapor. Styrene uptake in the upper respiratory tract of certain types of laboratory mice and rats was studied to provide insights into the metabolism of styrene in nasal tissues and to yield comparative information between the two species, both of which have been utilized in styrene inhalation toxicity testing.
    - Key Conclusions – The results showed that styrene vapor was absorbed from the air stream by cells in the nasal passages. Metabolism of styrene by these cells was largely responsible for the styrene absorption, *i.e.*, when metabolism was inhibited, very little styrene was removed from the air stream. As part of an ongoing research program, these conclusions contributed to a better understanding of the potential risks associated with styrene exposure in humans.

28. Metabolism of Styrene by Human Liver and Lung by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, and two other authors, published in the *Journal of Toxicology and Environmental Health, Part* A, 59(*8*), pp. 591-595, 2000.
    - Purpose – In mice, styrene is toxic in the lungs and there was some evidence that it caused lung tumors. Some authors hypothesized this toxicity was related to styrene's bioactivation to styrene oxide in the lung. This study measured the metabolism of styrene to styrene oxide in human liver and lung cell preparations to determine if human tissues have the same capacity as mouse tissues.
    - Key Conclusions – Human liver cells metabolized styrene to styrene oxide, but lung cells had essentially no activity. The data suggested that human lung has low styrene metabolizing activity and might be much less of a target organ than in the mouse. As part of an ongoing research program, these conclusions contributed to a better understanding of the potential risks associated with styrene exposure in humans.

29. Styrene Oxide in Blood, Hemoglobin Adducts, and Urinary Metabolites in Human Volunteers Exposed to $^{13}C_8$–Styrene Vapors by Gunnar Johanson, Ph.D, D.D.S., Toxicology and Risk Assessment, National Institute for Working Life, Stockholm, Sweden, and six other authors, published in *Toxicology and Applied Pharmacology,* 168(*1*), pp. 36-49, 2000.
    - Purpose – This study aimed to clarify the toxic action of inhaled styrene in human volunteers, with particular emphasis on the quantification of styrene oxide (SO), a key styrene metabolite that exhibited higher toxicity than styrene. The study was undertaken to allow quantitative comparisons between rodents and man with respect to SO blood levels and metabolite profiles.
    - Key Conclusions – The styrene blood level in humans was about one-and-one-half to two times higher than in rats and four times higher than in mice. In contrast, the SO levels in human blood were approximately four-fold lower than in mice. This study demonstrated that humans detoxify styrene oxide to non-toxic end products. Based on urinary metabolites, humans metabolize styrene similar to rats, but not similar to mice.

30. Quantification of DNA Adducts Formed in Liver, Lungs, and Isolated Lung Cells of Rats and Mice Exposed to ^{14}C-Styrene by Nose-Only Inhalation by Peter J. Boogaard, Ph.D., Pharm.D., DABT, ERT, Shell Research and Technology Center, Amsterdam, The Netherlands, and five other authors, published in *Toxicological Sciences,* 57(*2*), pp. 203-216, 2000.
    - Purpose – Lung tumors were observed in mice exposed chronically to 160 parts per million (ppm) of styrene, whereas no tumors were seen in rats at concentrations of up to 1,000 ppm. The purpose of this study was to determine and quantify DNA adducts (point at which activity occurs) in specific tissues and cell types of rats and mice exposed to styrene through nose-only inhalation.
    - Key Conclusions – The overall results demonstrated that DNA adduct formation did not play a significant role in the formation of lung tumors that were observed in mice chronically exposed to styrene. It was more likely that a non-genotoxic or other mechanism was involved in this process. The results aided in understanding the causes of the sensitivity of the mouse to the effects of styrene and helped determine the relevance of the effects in mice for human risk assessment.

31. Disposition of [Ring-U-^{14}C]styrene in Rats and Mice Exposed by Recirculating Nose-Only Inhalation by Peter J. Boogaard, Ph.D., Pharm.D., DABT, ERT, Shell Research and Technology Center, Amsterdam, The Netherlands, and five other authors, published in *Toxicological Sciences,* 58(*1*), pp. 161-172, 2000.
    - Purpose – Lung tumors were observed in mice exposed chronically to 160 parts per million (ppm) of styrene, whereas no tumors were seen in rats at concentrations of up to 1,000 ppm. This study was one of a series of studies intended to obtain a more complete and detailed picture of styrene's disposition, metabolism and genotoxic potency in rats and mice. Specifically, this study employed a single inhalation exposure of 160 ppm radio-labeled styrene to study possible differences its effects on rats and mice.
    - Key Conclusions – The overall results demonstrated that, following inhalation exposure to styrene, mice exhaled significantly more radio-labeled styrene than did rats. This was thought to indicate that the aromatic ring is opened in mouse lung tissue to form reactive ring-opened metabolites, which might be related to the observed development of lung tumors and nasal effects in mice.

32. Chronic Toxicity/Oncogenicity Study of Styrene in CD-1 Mice by Inhalation Exposure for 104 Weeks by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and nine other authors, published in the *Journal of Applied Toxicology,* 21(*3*), pp. 185-198, 2001.
    - Purpose – Evaluated the potential of styrene to produce health effects in laboratory mice exposed via inhalation over a two-year period (essentially lifetime) and, thereby, develop data that could help predict styrene's potential health effects on humans.
    - Key Conclusions – Styrene had no effect on survival in males. Two high-dose females died during the first two weeks; the remaining exposed females had a slightly higher survival than control mice. Styrene caused extensive progressive lung damage, resulting in increased late-occurring tumors in a single organ (lung), which in the mouse had a high incidence of spontaneous tumors. Styrene also caused cellular damage in nasal tissues.

33. Styrene Metabolism in Rats, Mice, and Humans by Susan C. J. Sumner, Ph.D., CIIT Centers for Health Research, Research Triangle Park, NC, and three other authors, published in *CIIT Activities,* 21(*3-4*), pp. 1-8, 2001.
    - Purpose – A comparison of the metabolism of chemicals in rodents used in long-term toxicity testing with human data is a key element of risk assessment. This study was intended to help elucidate the metabolism of inhaled styrene in human volunteers and to compare these results with those obtained for rodents. Understanding the metabolism of chemicals is important in categorizing mode of action, and mode of action could be important in estimating the human risk from exposure to chemicals.
    - Key Conclusions – The greater toxicity of styrene observed in mice might be related to a number of metabolic factors: greater uptake of styrene and production of styrene oxide (SO); greater conjugation of SO with glutathione, resulting in glutathione depletion and oxidant stress; greater production of phenylacetaldehyde as a metabolic intermediate; and greater production of ring epoxide(s). Uptake and metabolism of styrene in humans was more like that observed in the rat than in the mouse. However, substantial differences in metabolism were apparent between rats and humans. The differences among rats, mice and humans in the metabolism of styrene might contribute to differences in toxicity of styrene, and thus species differences might be critical in assessing styrene's potential human health risks.

34. The Toxicity of Styrene to the Nasal Epithelium of Mice and Rats: Studies on the Mode of Action and Relevance to Humans by Trevor Green, Ph.D., Syngenta Central Toxicology Laboratory, Cheshire, UK, and five other authors, published in *Chemico-Biological Interactions,* 137(*2*), pp. 185-202, 2001.
    - Purpose – Inhaled styrene is known to be toxic to the nasal epithelium (membrane tissue) of both mice and rats, although mice are markedly more sensitive. This study sought to understand the role of styrene metabolism in the development of nasal toxicities seen in rats and mice exposed to this chemical, with the findings concerning metabolic differences being used to help predict potential human effects.
    - Key Conclusions – Both rats and mice metabolized styrene to styrene oxide (SO) in nasal tissue, but metabolism in human tissue was not detected. In mice, inhibition of styrene metabolism in nasal tissue prevented nasal toxicity, indicating that a metabolite, not styrene, caused the nasal toxicity. The rat was able to detoxify SO at higher rates than the mouse, thus leading to less toxicity. Human nasal tissues were able to metabolize SO efficiently. Thus, since metabolism of styrene to SO was not detected in human nasal tissue and human nasal tissue was able to readily detoxify any SO present, styrene was unlikely to be toxic to the human nasal epithelium.

35. Metabolism of the Styrene Metabolite 4-Vinylphenol by Rat and Mouse Liver and Lung, by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, and two other authors, published in *Journal of Toxicology and Environmental Health, Part A*, 63(7), pp. 541-551, 2001.
    - Purpose – Determined if the formation and metabolism of 4-Vinylphenol in rat and mouse liver and lung preparations could be measured and quantified.
    - Key Conclusions – The study successfully measured 4-Vinylphenol metabolizing activity in mouse and rat liver microsomes; this activity was three times greater in mouse liver microsomes than that in rat liver microsomes, and activity in mouse lung microsomes was eight times greater than that in rat lung microsomes. The differences among rats and mice in the metabolism of styrene might contribute to differences in toxicity of styrene, and thus species differences might be critical in assessing styrene's potential human health risks.

36. The Role of Cytochromes P-450 in Styrene Induced Pulmonary Toxicity and Carcinogenicity by Trevor Green, Ph.D., Syngenta Central Toxicology Laboratory, Cheshire, UK, and two other authors, published in *Toxicology,* 169(2), pp. 107-117, 2001.
    - Purpose – Investigated the role of cytochromes P450 in the marked differences between mice and rats in their responses to acute exposures to styrene, and assessed the relevance of this phenomenon for human risk assessment and styrene.
    - Key Conclusions – Inhibition of cytochrome P4502F2 in mice prevented lung toxicity from styrene exposure. The metabolism of styrene by cytochromes P450 thus has been shown to be a critical event resulting in changes in the mouse lung that were consistent with the subsequent development of cancer. The authors proposed that a plausible mode of action for styrene-induced mouse lung cancer was that exceptionally high metabolic rates in a certain type of mouse lung cells (Clara cells) made the mouse lung more susceptible to potentially mutational effects. The human lung, on the other hand, had far fewer of this certain type of cell and practically no metabolism of styrene in human lung cells. Thus, it seemed unlikely, based on the known metabolic capacities of the human lung relative to the mouse lung, that styrene would be either toxic or carcinogenic in the human lung.

37. Styrene Respiratory Tract Toxicity and Mouse Lung Tumors Are Mediated by CYP2F-Generated Metabolites by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and six other authors, published in *Regulatory Toxicology and Pharmacology,* 35(*3*), pp. 308-319, 2002.
    - Purpose – Numerous studies demonstrated marked differences in toxicity between rats and mice to styrene exposure, especially by inhalation. Mice were particularly sensitive to respiratory tract and liver toxicity from styrene. Both species had a qualitatively similar response of the nasal olfactory mucosa, with the mouse being much more sensitive. The toxicologic and metabolic data that explained the nasal and lung differences and their relevance for human risk assessment were provided.
    - Key Conclusions – Styrene respiratory tract toxicity in mice and rats, including mouse lung tumors, was mediated by metabolites. The physiologically based pharmacokinetic (PBPK) model used in this study predicted that humans did not generate sufficient levels of these metabolites in the terminal bronchioles to reach a toxic level. Therefore, the postulated mode of action for these effects indicated that respiratory tract effects in rodents were not relevant for human risk assessment.

38. Physiologically Based Pharmacokinetic Modeling of Styrene and Styrene Oxide Respiratory-Tract Dosimetry in Rodents and Humans by Ramesh Sarangapani, Ph.D., The K. S. Crump Group, Inc., Research Triangle Park, NC, and four other authors, published in *Inhalation Toxicology,* 14(*8*), pp. 789-834, 2002.
    - Purpose – There were apparent differences in susceptibility to styrene toxicity among different species (*e.g.*, mouse, rat and human). This study's primary purpose was to develop a physiologically based pharmacokinetic (PBPK) model for inhaled styrene that was capable of describing the relationship between exposure and target-tissue dose of styrene and a reactive intermediate, styrene oxide (SO), in rodents and humans. An additional objective was to use the PBPK model in evaluating the extent to which there was a pharmacokinetic basis for the species-specific carcinogenic response following chronic inhalation exposure to styrene.
    - Key Conclusions – The authors developed and validated a PBPK model to describe the relationship between exposure and target-tissue dose of styrene and SO in rodents and humans. Predictions developed from the model supported a pharmacokinetic basis for species sensitivity in rodents and indicated that humans would be approximately 100-fold less sensitive to the induction of lung tumors following styrene exposure than were mice.

39. Comparing Respiratory-Tract and Hepatic Exposure-Dose Relationships for Metabolized Inhaled Vapors: A Pharmacokinetic Analysis by Ramesh Sarangapani, Ph.D., The K. S. Crump Group, Inc., Research Triangle Park, NC, and three other authors, published in *Inhalation Toxicology,* 14(*8*), pp. 835-854, 2002.
    - Purpose – Assessed differences in expected exposure-dose curves for metabolism in the portal of entry versus remote site (*i.e.*, respiratory tract tissue versus the liver) for inhaled styrene in rodents using a physiologically based pharmacokinetic (PBPK) model. This assessment relied on a PBPK model that was based on two earlier models, but extended to include nasal cavity and conducting airways.
    - Key Conclusions – Using a more complete lung model, the analysis showed significant differences in the shape of curves for rate of metabolism versus inhaled concentrations in airway epithelial (membrane) tissues compared to the liver. The predictions of this model were consistent with observations of nasal/lung toxicity and toxicity of these compounds in systemic tissues. Previous differences in dose-response relationships for inhalation toxicity in lung and liver/kidney became clarified for styrene and for other volatile compounds, such as chloroform, by elaborating the description of the respiratory tract.

40. Effect of the Inhibition of the Metabolism of 4-Vinylphenol on its Hepatotoxicity and Pneumotoxicity in Rats and Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology,* 179(*1-2*), pp. 129-136, 2002.
    - Purpose – Styrene is known to be toxic to the liver and pulmonary system in rodents. 4-vinylphenol had been shown to be a minor metabolite of styrene in some studies and had been shown to be a more potent toxicant in mice than either styrene or styrene oxide. This study addressed the question of whether the parent compound (4-vinylphenol) or a metabolite was responsible for the 4-vinylphenol-induced toxicity.
    - Key Conclusions – Rats as well as mice were found to be susceptible to the toxicity of 4-vinylphenol. Prior treatment of rats and mice with inhibitors prevented or greatly decreased 4-vinylphenol's toxicity to the liver and pulmonary system. Thus, the toxicity of 4-vinylphenol to the liver and pulmonary system was due to the metabolite(s) and not the parent compound. The data indicated that metabolism by CYP2F2 and/or CYP2E1 were key in this process.

41. 4-Vinylphenol-Induced Pneumotoxicity and Hepatotoxicity in Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, and three other authors, published in *Toxicologic Pathology,* 30(*5*), pp. 1-5, 2002.
    - Purpose – 4-vinylphenol has been reported to be a minor metabolite of styrene in rats and humans; it also occurs naturally in some foods and has been used as a flavoring agent in food products. The purpose of this study was to characterize the possible toxicity to the liver and pulmonary system associated with 4-vinylphenol in mice. These endpoints and this species were selected because the liver and pulmonary system were known targets of styrene toxicity in mice, whereas in rats the pulmonary toxicity was not observed.
    - Key Conclusions – The administration of 4-vinylphenol caused both liver and pulmonary damage in mice. As part of an ongoing research program, these findings contributed to a better understanding of the possible effects of styrene metabolites.

42. *In Vitro* Metabolism of Styrene to Styrene Oxide in Liver and Lung of CYP2E1 Knockout Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in the *Journal of Toxicology and Environmental Health, Part A*, 66(*9*), pp. 861-869, 2003.
    - Purpose – Knockout mice contain the same, artificially introduced mutation in every cell, removing the activity of a pre-selected gene. The resulting mutant's appearance, biochemical characteristics, behavior, etc., might provide some indication of the gene's normal role in the mouse, and by extrapolation, in people. Knockout mouse models are widely used to study human diseases caused by the loss of gene function. *In vivo* studies demonstrated that CYP2E1 knockout mice can metabolize styrene to a similar extent as wild-type mice. This study's purpose was to compare the ability of CYP2E1 knockout mice to metabolize styrene to styrene oxide with that of wild-type mice. A second goal was to determine the cytochromes (iron-containing proteins) P450 involved in styrene's metabolism by the knockout mouse.
    - Key Conclusions – When the metabolism of styrene to *R*- and *S*-styrene oxide was determined using hepatic microsomes from wild-type and knockout mice, there were no statistically significant differences between the two mouse strains. However, when pulmonary microsomes were used, the styrene-metabolizing activity in the knockout mice was about one-half that of the wild-type mice. This showed that at least in these knockout mice, other enzymes must have contributed to the metabolism of styrene (as chemical inhibitors were used, we could not clearly identify them). These results contributed to our understanding of styrene's metabolism and its relevance.

43. Metabolism and Toxicity of the Styrene Metabolite 4-Vinylphenol in CYP2E1 Knockout Mice by Kelly M. Vogie, and two other authors, School of Health Sciences, Purdue University, West Lafayette, IN, published in the *Journal of Toxicology and Environmental Health, Part A*, 67(*2*), pp. 145-152, 2004.
    - Purpose – 4-Vinylphenol (4-VP), a minor metabolite of styrene, is several times more potent as a liver and lung toxicant than is either the parent compound or the major metabolite of styrene, styrene oxide. 4-VP is metabolized primarily by CYP2E1 and CYP2F2. The liver and lung toxicity were compared in wild-type and CYP2E1 knockout mice (see study #42 in this summary for more information about knockout mice) in order to further elucidate the possible role of 4-VP in styrene-induced toxicity and the importance of its metabolism by CYP2E1. Knockout mice were used widely to study human diseases caused by the loss of gene function.
    - Key Conclusions – There were no marked differences between the wild-type and knockout mice in the rates of microsomal metabolism of 4-VP in either liver or lung. This unexpected result mimicked previous findings with styrene metabolism of 4-VP in wild-type and knockout mice. When mice were administered 100 mg/kg 4-VP, the knockout mice were more susceptible to liver toxicity than were the wild-type mice. There was no significant difference in the lung toxicity between the two strains. The data suggested that, as for styrene, additional cytochromes (iron-containing proteins) P450 were involved in the metabolism of 4-VP. These results contributed to our understanding of the metabolism of styrene and its relevance to human risk assessment.

44. Comparison of the Susceptibility of Wild-Type and CYP2E1 Knockout Mice to the Hepatotoxic and Pneumotoxic Effects of Styrene and Styrene Oxide by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology Letters,* 150(*3*), pp. 335-339, 2004.
    - Purpose – Styrene causes both liver and lung damage in various strains of wild-type mice. This was thought to be due to the bioactivation of styrene to styrene oxide by cytochromes P450, principally CYP2E1 and CYP2F2. If so, one would expect CYP2E1 knockout mice to be less susceptible to styrene-induced toxicity than wild-type mice. However, previous *in vitro* and *in vivo* studies demonstrated little difference in the metabolism of styrene to styrene oxide between wild-type and CYP2E1 knockout mice. These findings suggested that there should be no difference in the toxic responses to styrene oxide between these two strains. This research was undertaken to determine which of these two possibilities was correct.

- Key Conclusions – The difference in styrene-induced liver toxicity between wild-type and knockout mice demonstrated the importance of CYP2E1 to the bioactivation of styrene in that tissue in the intact animal, whereas the lack of a strain difference in response to the lung effects appeared to reflect the importance of CYP2F2 in the tissue of both strains. These results contributed to our understanding of the metabolism of styrene and its relevance to human risk assessment.

45. Influence of Selected Inhibitors on the Metabolism of the Styrene Metabolite 4-Vinylphenol in Wild-Type and CYP2E1 Knockout Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in the *Journal of Toxicology and Environmental Health, Part A*, 67(*12*), pp. 905-909, 2004.
    - Purpose – 4-Vinylphenol (4-VP), a minor metabolite of styrene, is a more potent liver and lung toxicant than either styrene or styrene oxide. In CD-1 mice 4-VP is metabolized primarily by cytochrome (iron-containing protein) P450 CYP2E1 and CYP2F2. However, there was no difference in the rate of metabolism of 4-VP between wild-type and CYP2E1 knockout mice (see study #42 in this summary for more information about knockout mice), indicating that other cytochromes P450 played an important role. The purpose of this research was to use various inhibitors to study the possible role of cytochromes P450 in 4-VP metabolism in wild-type and CYP2E1 knockout mice. Knockout mice were used widely to study human diseases caused by the loss of gene function.
    - Key Conclusions – When chemical inhibitors of cytochromes P450 were added to microsomal incubations from livers of wild-type and CYP2E1 knockout mice, there were varying degrees of inhibition. Imipramine (CYP2C), MBA (CYP2B), and ANF (CYP1A) all produced significant decreases in both the wild-type and CYP2E1 knockout mice. 4-VP metabolizing activity was decreased significantly by more than 50% when 5PIP (CYP2F2) was added. However, in both wild-type and knockout mice, the greatest quantitative inhibition was observed with the CYP2E1 inhibitor DDTC. For most of the inhibitors, the observed enzymatic effects were less with pulmonary microsomes. In summary, multiple cytochromes P450 contributed to 4-VP metabolism with CYP2F2 playing an important role, especially in the lung. These results contributed to our understanding of the metabolism of styrene and its relevance to human risk assessment.

46. Comparison of the Depletion of Glutathione in Mouse Liver and Lung Following Administration of Styrene and its Metabolites Styrene Oxide and 4-Vinylphenol by Meredith Turner, School of Health Sciences, Purdue University, West Lafayette, Ind., and two other authors, published in *Toxicology,* 206(*3*), pp. 383-388, 2005.
    - Purpose – Styrene's major metabolite is styrene oxide and its minor, but potent, metabolite 4-vinylphenol caused similar toxicities. The purpose of this study was to determine the dose dependence and time course for the depletion of reduced glutathione by styrene in mouse liver and lung and compare it to the effects of the metabolites styrene oxide and 4-vinylphenol.
    - Key Conclusions – The decreases in GSH (glutathione) levels suggested the possibility that the toxicity of styrene in lung and liver might be related to a profound, but reversible oxidative stress in these tissues. Further studies are needed to characterize this oxidative stress in both tissues including an evaluation of the enzymes, which control GSH levels in these tissues. These results contributed to our understanding of styrene's metabolism and its relevance to human risk assessment.

47. Ring-Oxidized Metabolites of Styrene Contribute to Styrene-Induced Clara-Cell Toxicity in Mice by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and three other authors, published in the *Journal of Toxicology and Environmental Health, Part A*, 68(*3*), pp. 229-237, 2005.
    - Purpose – Assessed the role that ring-oxidized styrene metabolites (4-vinylphenol or its metabolites) played in the pneumotoxicity induced by styrene in mice versus rats.
    - Key Conclusions – The results added further support to previous studies on the role of ring-oxidized metabolites in the pneumotoxicity induced by styrene in mice and the lack thereof in rats. In total, the results of this and other studies were useful in helping to understand and predict human metabolism of and response to styrene.

48. Two Generation Reproduction Study of Styrene by Inhalation in Crl-CD Rats by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and seven other authors, published in *Birth Defects Research, Part B: Developmental and Reproductive Toxicology*, 74(*3*), pp. 211-220, 2005.
    - Purpose – The study was undertaken to refine understanding of the potential toxicological effects of exposure to styrene on reproduction of children of exposed parents. The evaluation covered potential effects on reproductive capability from whole body inhalation exposure of parental animals. Assessments included gonadal function, estrous cyclicity, mating, behavior, conception rate, gestation, parturition, lactation and weaning.

- Key Conclusions – The results of this study confirmed previous observations of slight body-weight effects of styrene exposure at 500 parts per million (ppm) or greater in rats and degeneration of nasal olfactory epithelium. It further demonstrated a lack of styrene effects on gonadal function, reproductive performance and offspring survival, enhancing conclusions of a previous three-generation study of styrene in drinking water. In addition, it countered one previous study suggesting testicular pathology and decreased sperm counts and supported the lack of effects on testes and ovaries as reported in several previous other studies of styrene. Based on the results, an exposure level of 50 ppm was considered to be the no observable adverse effects level (NOAEL) for parental systemic toxicity; the NOAEL for reproductive toxicity was 500 ppm or greater.

49. Developmental Neurotoxicity Study of Styrene by Inhalation in Crl-CD Rats by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and eight other authors, published in *Birth Defects Research, Part B: Developmental and Reproductive Toxicology*, 74(3), pp. 221-232, 2005.
    - Purpose – The study was undertaken to refine understanding of the potential neurotoxicity effects of exposure to styrene on children of exposed parents.
    - Key Conclusions – No specific effect on nervous system development was observed at exposures up to 500 parts per million (ppm) of styrene. Based on the results of this study, an exposure level of 50 ppm was considered to be the no observable adverse effects level (NOAEL) for growth of offspring; an exposure level of 500 parts per million was considered to be the NOAEL for developmental neurotoxicity.

50. Comparison of Styrene and its Metabolites Styrene Oxide and 4-Vinylphenol on Cytotoxicity and Glutathione Depletion in Clara Cells of Mice and Rats by Jill A. Harvilchuck and Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology,* 227(*1-2*), pp. 165-172, 2006.
    - Purpose – Since the Clara cell has the highest activity in the lung for metabolizing styrene, this study focused on the direct cytotoxicity of styrene and its metabolites in Clara cells, and the changes in glutathione levels due to treatment with these agents.
    - Key Conclusions – Clara cells in the rat were four-fold less susceptible to the cyto-toxicity produced by styrene and its metabolites than in the mouse. The cytotoxicity of styrene *in vitro* was greater than that of its metabolites; the opposite of what had been observed *in vivo*. *R*-styrene oxide caused a greater decrease in glutathione levels, supporting other data indicating it was the more toxic of the two enantiomers.

51. Effects of Styrene and Styrene Oxide on Glutathione-Related Antioxidant Enzymes by Gary P. Carlson, Ph.D., and two other authors, School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology,* 227(*3*), pp. 217-226, 2006.
    - Purpose – This study sought to determine if the more toxic *R*-styrene oxide had a greater effect on reduced glutathione levels than the less toxic *S*-styrene oxide and if the ratio of reduced to oxidized forms of glutathione was altered by either of these styrene forms.
    - Key Conclusions – The results of this study suggested that while styrene and its metabolite styrene oxide caused significant decreases in glutathione levels, they had little effect on the enzymes glutathione reductase and peroxidase and that, in response to decreased glutathione levels, there was an increase in its synthesis.

52. Critical Appraisal of the Expression of Cytochrome P450 Enzymes in Human Lung and Evaluation of the Possibility that Such Expression Provides Evidence sf Potential Styrene Tumorigenicity in Humans by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology,* 254(*1-2*), pp. 1-10, 2008.
    - Purpose – The purpose of this study was to determine how the tumorigenic effect in the mouse lung might relate to humans by examining metabolic activation rates between liver and lung, and the difference between rodent and human lungs. Emphasis was placed on the specific cytochromes P450 present in human lungs and what role they might play in bioactivation of styrene and other compounds.
    - Key Conclusions – Due to the low rate of metabolism in the human lung compared to the mouse lung and the very large differences in the metabolism of several chemicals in the human lung and liver, as well as the human lung and mouse lung, it could be deduced that it was doubtful significant amounts of styrene oxide would be generated in human lung to cause damage or subsequent tumor formation.

53. CC10 mRNA and Protein Expression in Clara Cells of CD-1 Mice Following Exposure to Styrene or its Metabolites Styrene Oxide or 4-Vinylphenol by Jill A. Harvilchuck, School of Health Sciences, Purdue University, West Lafayette, IN, and two other authors, published in *Toxicology Letters,* 183(*1-3*), pp. 28-35, 2008.
    - Purpose – The primary site for styrene metabolism and its effects in mouse lung is the Clara cell, which secretes Clara cell CC10 and surfactant protein A (SPA). The mode of action for styrene-induced lung tumor formation has yet to be elicited, but

this study explored one possibility that related to oxidative stress and decreased CC10 levels.

- Key Conclusions – This study demonstrated that acute changes in lung CC10 protein and mRNA expression did occur following treatment with styrene and its metabolites. These changes might be early indicators for a potential mechanism for lung tumor formation in mice, as it related to oxidative stress; this might deserve further study.

54. Oxidative Stress Due to *R*-Styrene Oxide Exposure and the Role of Antioxidants in Non-Swiss Albino (NSA) Mice by Anna Meszka-Jordan, School of Health Sciences, Purdue University, West Lafayette, IN, and three other authors, published in the *Journal of Toxicology and Environmental Health, Part A,* 72(*10*), pp. 642-650, 2009.
    - Purpose – The purpose of this study was to investigate the toxicity of *R*-styrene oxide, the more active enantiomeric metabolite of styrene, and the protective properties of certain antioxidants against *R*-styrene oxide-induced toxicity in non-Swiss Albino mice.
    - Key Conclusions – This study's results demonstrated significant protection against *R*-styrene oxide toxicity in the liver, but not the lung, by the oral administration of two antioxidants, glutathione and N-acetylcysteine. Treatment with UPF1, a synthetic antioxidant, for preconditioning did not result in any protection against liver and lung toxicity, and instead enhanced the toxicity when administered prior to the *R*-styrene oxide.

55. 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 by Jill A. Harvilchuck, and Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology.* 259(*3*), pp. 149-152, 2009.
    - Purpose – The acute effects following multiple doses of styrene or styrene oxide for five consecutive days simulated multiple exposures seen in workers. This study looked at early changes in indicators of oxidative stress, as well as the ratio of indicators regarding susceptibility to programmed cell death in Clara cells, the main target for styrene toxicity in the lung and the site of lung tumor formation in the mouse.
    - Key Conclusions – Oxidative stress was likely to be involved in the toxicity caused by styrene and styrene oxide. CC10 might be an early indicator of susceptibility to

carcinogenesis from styrene. Chronic decreases in CC10 might lead to increases in oxidative stress in the Clara cell, the main target for styrene-induced lung tumors in mice.

56. Indicators of Oxidative Stress and Apoptosis in Mouse Whole Lung and Clara Cells Following Exposure to Styrene and its Metabolites by Jill A. Harvilchuck, and three other authors, School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology.* 264(*3*), pp. 171-178, 2009.
    - Purpose – This study sought to determine what effects styrene and its active metabolites, primarily styrene oxide (SO), had on indicators of oxidative stress and attendant apoptosis to better understand the mechanism of styrene-induced toxicity.
    - Key Conclusions – The results of the study demonstrated oxidative stress occurs shortly (3 hours) after styrene or styrene oxide intraperitoneal administration as evidenced by increased reactive oxygen species (ROS), superoxide dismutase (SOD) and 8-hydroxydeoxyguanosine (8-OHdG) formation. The findings of limited apoptosis (cell death that occurs during normal growth and development) in Clara cells following acute exposure to styrene or SO might reflect the minimal extent to which apoptosis plays a role in acute styrene toxicity. In addition, the results indicated oxidative stress and oxidative effects on DNA were increased following exposure to styrene or SO, and these effects might play a role in the lung tumorigenesis in mice.

57. 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 by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and four other authors, published in *Regulatory Toxicology and Pharmacology,* 55(*2*), pp. 205-218, 2009.
    - Purpose – The purpose of this study was to integrate the results of evaluations of the metabolism of several structurally-related compounds by CYP2F isoforms of the cytochromes P450 family in mice. The authors proposed that a cytotoxicity-driven mode of action of these compounds in organs high in CYP2F (namely, CYP2F2 in nasal and lung tissue in mice and CYP2F4 in nasal tissues in rats) resulted in cytotoxicity and subsequent regenerative hyperplasia and drove in mice, but not in rats, an increase in lung tumors that were mostly benign and were not life shortening.

- Key Conclusions – While lung tumors from bronchiolar cell cytotoxicity are theoretically possible in humans, it was unlikely that metabolism by CYP2F1 would produce levels of cytotoxic metabolites in human lungs sufficient to result in lung cytotoxic responses and thus tumors. Therefore, it was unlikely any of the compounds that caused mouse lung tumors via CYP2F2 metabolism (including styrene) would cause lung tumors in humans. Additionally, the CYP2F1 isozyme expressed in humans appeared to have a low capacity to metabolize these compounds.

58. Depletion by Styrene of Glutathione in Plasma and Bronchioalveolar Lavage Fluid of Non-Swiss Albino (NSA) Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in the *Journal of Toxicology and Environmental Health, Part A*, 73(*11*), pp. 766-772, 2010.
    - Purpose – The purpose of this study was to investigate the effect of styrene and its primary metabolites, *R*-styrene oxide and *S*-styrene oxide, on glutathione (GSH) levels in the lung, as determined by amounts in bronchioalveolar lavage fluid and plasma, two indicators used to determine and diagnose lung disease.
    - Key Conclusions – When Non-Swiss Albino mice were administered styrene in this study, there was a significant fall in GSH levels in both indicator substances within three hours; however, these levels returned to normal by hour 12. Therefore, since GSH was the primary antioxidant in lung lining fluid, this styrene-induced fall might significantly impact the lung's ability to buffer oxidative damage.

59. Detection of Phenolic Metabolites of Styrene in Mouse Liver and Lung Microsomal Incubations by Shuijie Shen, Center for Developmental Therapeutics, Seattle Children's Research Institute, Seattle, WA, and four other authors, published in *Drug Metabolism and Disposition,* 38(*11*), pp. 1934-1943, 2010.
    - Purpose – Explored the metabolic vinyl epoxidation and aromatic hydroxylation of styrene in mouse liver microsomes, which had been reported to be mutagenic and carcinogenic, in order to draw connection between metabolites of styrene and the presence detected in workers and experimental animals after styrene exposure.
    - Key Conclusions – New styrene metabolites were identified in mouse liver microsomal incubations. CYP2F2 and CYP2E1 were found to catalyze formations from styrene in mouse liver and lung microsomes.

60. *In Vitro* Metabolism, Glutathione Conjugation, and CYP Isoform Specificity of Epoxidation of 4-Vinylphenol, by Fagen Zhang, Ph.D., Senior Research Scientist, Toxicology, Environmental Research & Consulting, The Dow Chemical Company, Midland, MI, and six other authors, published in *Xenobiotica*, 41(*1*), pp. 6-23, 2011.
    - Purpose – In support of research into the mode of action of mouse lung tumors, this study explored the possible toxicity mechanism of 4-vinylphenol (4VP) by investigating the metabolism of 4VP, the glutathione (GSH) conjugation of the metabolites of 4VP and its cytochrome P450 (CYP) specificity in epoxidation in different microsomes *in vitro.*
    - Key Conclusions – Two major metabolites of 4VP were identified, namely 4-(2-oxiranyl)-phenol of 4VP (4VPO) and 4VP catechol. 4VPO was found to react with GSH to form GSH conjugate and 4VP catechol was found to further be metabolized to electrophilic species, which reacted with GSH to form the corresponding 4VP catechol GSH conjugates. These findings provided better insight on the lung toxicity seen with 4VP, the toxic metabolite of commercial styrene.

61. CYP2F2-Generated Metabolites, not Styrene Oxide, are a Key Event Mediating the Mode of Action of Styrene-Induced Mouse Lung Tumors by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and five other authors, published in *Regulatory Toxicology and Pharmacology,* 62(*1*), pp. 214-220, 2012.
    - Purpose – It had been postulated that styrene and/or styrene oxide (SO) metabolism by mouse lung Clara cell-localized CYP2F2 was a key metabolic gateway responsible for both lung toxicity and possible tumorigenicity. To test this hypothesis, this study tested the lung toxicity of styrene and SO in mice using different types of styrene or SO in varying amounts for five days.
    - Key Conclusions – Since the human form of CYP2F (2F1) was not believed to catalyze significant styrene metabolism, this study clearly indicated that styrene-induced lung toxicity in mice was not relevant to potential styrene-induced lung toxicity in humans.

62. Modification of the Metabolism and Toxicity of Styrene and Styrene Oxide in Hepatic Cytochrome P450 Reductase Deficient Mice and CYP2F2 Deficient Mice by Gary P. Carlson, Ph.D., School of Health Sciences, Purdue University, West Lafayette, IN, published in *Toxicology,* 294(*2-3*), pp. 104-108, 2012.

- Purpose – This study looked at the relationship between styrene toxicity in the liver and lung of mice and the metabolism of styrene, particularly regarding mice deficient in hepatic cytochrome P450 reductase.
- Key Conclusions – Mice deficient in hepatic cytochrome P450 reductase had a poor metabolism of styrene and low toxicity in the liver, but had some protection against pneumotoxicity. For CYP2F2 deficient mice, there was a decreased metabolism by the lung, and this resulted in decreased toxicity in the lung, but not in the liver.

63. 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 by George Cruzan, Ph.D., DABT, ToxWorks, Bridgeton, NJ, and seven other authors, published in *Regulatory Toxicology and Pharmacology,* 66(*1*), pp. 24-29, 2013.
    - Purpose – This study further explored the role of *in vivo* CYP2F1 (human) metabolism of styrene or styrene oxide (both *S*- and *R*-enantiomers) in mediating mouse lung cytotoxicity.
    - Key Conclusions – The absence of styrene oxide toxicity in two mouse strains significantly challenged the hypothesis that styrene oxide, as a styrene metabolite common to both rodents and humans, represented a toxicologically plausible mode of action of lung toxicity and cancer in humans.

D. Assessing Styrene's Risk to the General Population

64. Reproductive and Developmental Toxicity of Styrene by Nigel A. Brown, Ph.D., Medical Research Council, St. George's Hospital Medical School, University of London (UK), published in Reproductive Toxicology, 5(1), pp. 3-29, 1991.
    - Purpose – Reviewed existing animal and human scientific data (including a large number of studies from Russia) to assess styrene's potential developmental toxicity.
    - Key Conclusions – Dr. Brown found little indication that styrene can exert any specific developmental or reproductive toxicity. Any putative effects on female reproduction noted in the studies could have a central nervous system site of action, which was compatible with the known neurotoxic actions of styrene. The author noted that while initial human studies linked styrene exposure with congenital malformation and spontaneous abortion, these findings were later disproved by

subsequent, more extensive investigations by the same researchers. An update was published in 2000.

65. The Neuroepidemiology of Styrene: A Critical Review of Representative Literature by Charles S. Rebert, Ph.D., SRI International, Menlo Park, CA, and Thomas A. Hall, Ph.D., Sciences International, Alexandria, VA, published in *Critical Reviews in Toxicology.* 24(*S1*), pp. S57-S106, 1994.
    - Purpose – Reviewed and evaluated 38 scientific papers concerning the potential neurotoxicity of styrene in order to draw some conclusions about styrene's potential (primarily through inhalation) to affect the human nervous system and the brain (particularly of workers exposed to styrene in the reinforced plastics industry).
    - Key Conclusions – The authors concluded that, like many solvents, high styrene concentrations that might be encountered in the reinforced plastics industry could produce mild intoxication; however, there was no evidence of permanent damage to the nervous system.

66. A Review of the Developmental and Reproductive Toxicity of Styrene by Nigel A. Brown, Ph.D., MRC Experimental Embryology and Teratology Unit, St. George's Hospital Medical School, University of London, UK; and three other authors, published in *Regulatory Toxicology and Pharmacology,* 32, pp. 228-247, 2000.
    - Purpose – This was an update of an earlier comprehensive review (see study #64) by Dr. Brown of the potential reproductive and developmental toxicity of styrene. This report reviewed all major papers published on the subject, emphasizing studies published following Dr. Brown's 1991 publication.
    - Key Conclusions – As with the earlier conclusions, there was little indication that styrene could exert any specific developmental or reproductive or endocrine toxicity. Suggested effects on female reproduction and neurobehavioral development could be the result of styrene effects on the central nervous system, which was compatible with the known neurotoxic actions of styrene at high exposure levels.

67. A Comprehensive Evaluation of the Potential Health Risks Associated with Occupational and Environmental Exposure to Styrene by Joshua T. Cohen, Ph.D., Harvard Center for Risk Analysis, Boston, MA, and 15 other authors, published in the *Journal of Toxicology and Environmental Health*, *Part B: Critical Reviews*, 5(*1-2*), pp. 1-263, 2002.

- Purpose – Assembled and examined all of the relevant scientific data on styrene to provide a real-world assessment of its potential risks in order to help inform industry and regulatory decision-making on styrene. A "blue ribbon" panel of experts considered toxicity, epidemiology (human effects) and exposure data on styrene to assess the potential human carcinogenic and non-carcinogenic risks from styrene.
- Key Conclusions – No cause for concern among the general public from exposure to styrene in the environment except possibly for persons who may live adjacent to hypothetical high-emitting reinforced plastic facilities. No cause for concern for the general public from exposure to styrene through foods (styrene occurs naturally in many foods, being detected at the growing source) or styrenic materials used in food-contact applications (*e.g.*, packaging and serving containers). No cause for concern for workers in styrene-based industries except possibly for those workers in the reinforced plastics industry who may be employed in jobs that result in high exposure levels.

68. Styrene and Breast Cancer Incidence in Texas: A Comment on an Ecological Association by Carol J. Burns, M.D., Department of Epidemiology, The Dow Chemical Company, Midland, MI, and two other authors, published in *Breast Cancer Research and Treatment,* 97(*3*), pp. 339-340, 2006.
    - Purpose – Letter to the Editor commented on and clarified a conclusion regarding breast cancer incidence in Texas as reported by Coyle, *et al.*
    - Key Conclusions – The finding of the Coyle, *et al*. study was likely incidental, and was very unlikely to be related to styrene emissions. Although Texas ranked first amont all US states for styrene emissions, the rates of breast cancer in Texas were the lowest among all US states.

69. Epidemiologic Studies of Styrene and Cancer: A Review of the Literature by Paolo Boffetta, M.D., MPH, International Prevention Research Institute, Lyon, France, and four other authors, published in the *Journal of Occupational & Environmental Medicine,* 51(*11*), pp. 1275-1287, 2009.
    - Purpose – Reviewed the epidemiologic literature on styrene and cancer.
    - Key Conclusions – This review of the existing scientific evidence found that the results of extensive peer-reviewed studies of workers in styrene-related industries collectively showed that exposure to styrene did not increase the risk of cancer. Specifically, the paper stated: "The evidence for human carcinogenicity of styrene is

inconsistent and weak. On the basis of the available evidence, one cannot conclude that there is a causal association between styrene and any form of cancer."

70. The Weight of Evidence Does Not Support the Listing of Styrene as "Reasonably Anticipated to be a Human Carcinogen" in NTP's 12th Report on Carcinogens by Lorenz R. Rhomberg, Ph.D., and two other authors, published in *Human and Ecological Risk Assessment: An International Journal,* 19(*1*), pp. 4-27, 2013.
    - Purpose – Presented scientific evidence and analysis regarding whether or not styrene met the National Toxicology Program's (NTP's) standard for "reasonably anticipated to be a human carcinogen" and if styrene should be listed in the *Report on Carcinogens*.
    - Key Conclusions – The effects of styrene observed in experimental animals did not meet the standard of "sufficient evidence in animals" and the underlying mode of action was species-specific and not applicable to humans. Human data did not consistently show increased incidence of or mortality from cancer. There was no concordance among the human, experimental animal and mode of action data. Thus, styrene should not be listed in the NTP *Report on Carcinogens*.

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