



**NTP**  
National Toxicology Program

# Report on Carcinogens Draft Substance Profile for Styrene

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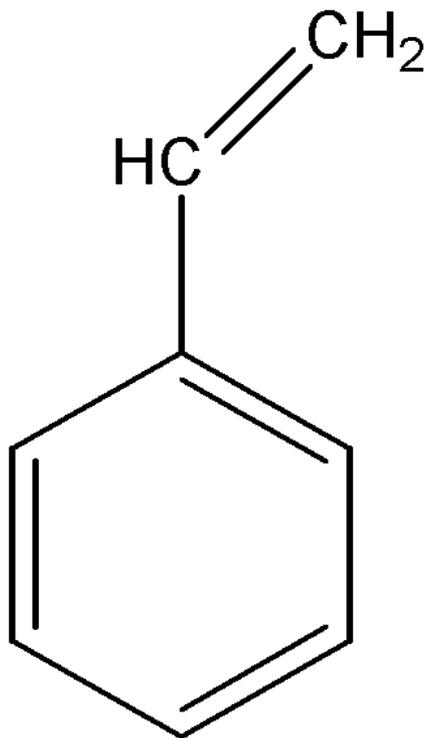
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# Styrene

CAS No. 100-42-5





## Use and Production

- Used worldwide in the production of polymers
  - Rubber
  - Reinforced plastics
  - Polystyrene food containers
- U.S. production: 2006 - 11.4 billion pounds



## Significant U.S. Exposure

- Occupational exposure
  - Reinforced plastics industry
  - Styrene-butadiene rubber (SBR) industry
  - Styrene monomer and polymer industry
- General public
  - Much lower exposure than workers
  - Inhalation of outdoor air contaminated by emissions from industrial processes
  - Tobacco smoke
  - Ingestion of food and water



## Proposed Styrene Listing

### *Reasonably Anticipated to be a Human Carcinogen*

- Limited evidence from studies in humans
- Sufficient evidence from studies in experimental animals
- Supporting mechanistic evidence



## Studies in Humans

- Lymphohematopoietic cancer (LHC) among subgroups of styrene-exposed workers
- DNA adducts and genetic damage in lymphocytes from exposed workers
- Together support classification *limited evidence of carcinogenicity from studies in humans*



## Outline for Human Studies

- Informative industries
- Informative studies
- Summary of findings from informative studies
- Genetic damage



## Informative Industries

### Reinforced Plastics Industry

- Strengths
  - Highest levels of styrene
  - Few other potentially carcinogenic exposures
- Limitations
  - Short-term workers (< 1 yr)
  - Short follow-up

### SBR Industry

- Strengths
  - Long-term workers
  - Detailed analyses of LHC
  - Greater numbers of exposed cases of lymphomas and leukemia
- Limitations
  - Lower levels of styrene
  - Co-exposure to butadiene



## Informative Studies

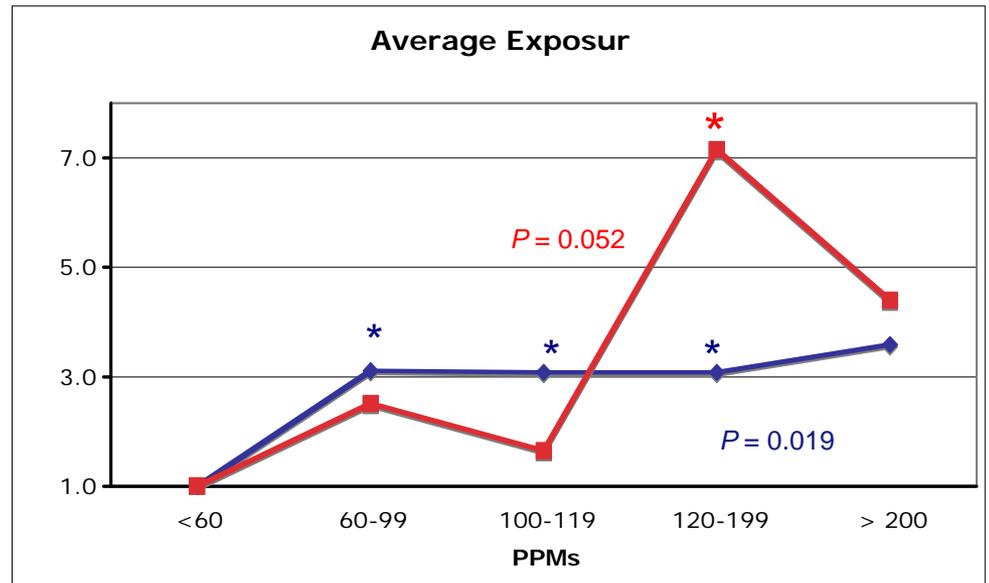
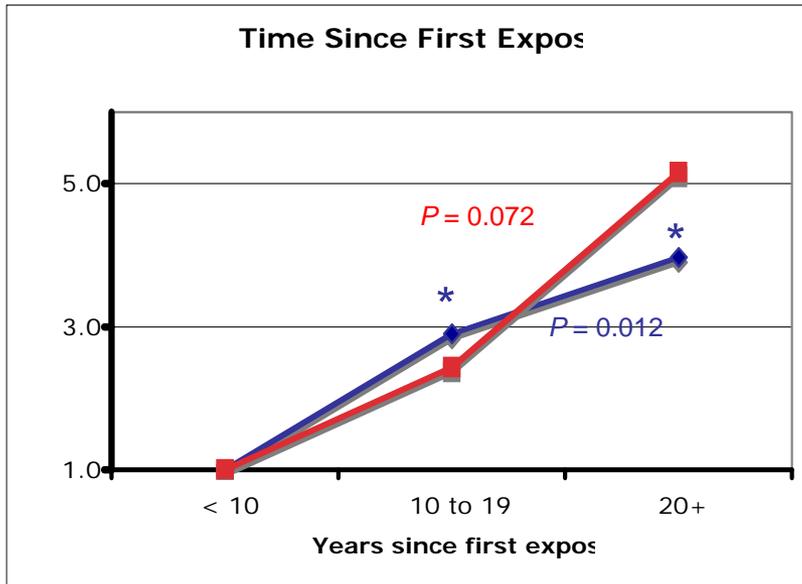
- All studies do not contribute equally to evaluation of carcinogenicity
  - Low statistical power in many cohorts
- Characteristics of most informative studies
  - Greater number of exposed cases
  - Adequate length of follow-up
  - Internal analyses on exposure-response relationships
    - Internal analyses help control for confounding
- Heterogeneity of exposure within a cohort
  - Subgroup and internal analyses most informative
  - Risk estimates (SMR) of the entire cohort less useful



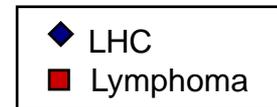
## Reinforced Plastics Industry

- Multi-national cohort: Kogevinas *et al.* 1994
  - 40,688 workers, 13 year follow-up, 60% < 2 years
  - 47 LHC deaths for internal analyses
  - 3 metrics used for internal analyses of LHC, lymphoma, and leukemia
    - Average exposure
    - Cumulative exposure
    - Time since first employment (latency)

# LHC and Lymphomas



LHC = lymphohematopoietic cancer, \* 95% CI does not include 1



- Risk of LHC and lymphomas increased with increasing time since first exposure and average exposure
- Cumulative exposure:
  - LHC: no relationship
  - Lymphoma: NS increased relative risks for all exposure levels, but no significant trend



## Reinforced Plastics Industry: Danish Workers

- Incidence cohort study: Kolstad *et al.* 1994\*
  - 36, 610 workers, 60% < 1 yr, average follow-up 11 yrs
  - 112 cases LHC, 42 of which were leukemia
  - Standard Incidence Ratio (SIR) and internal analyses
    - Years since first employment (< 10 yrs, and > 10 yrs)
    - Short-term vs. long-term workers
    - Date of first employment (3 categories, test for trend)
      - Indirect measure of exposure intensity: exposure levels decreased 4-fold over time
  - Limitation: exposure assessment

\* Overlap with multi-national cohort (Kogevinas *et al.* 1994)



## Danish Workers: Findings

- Significant SIRs for leukemia\*
  - Time since first employment (> 10 years)
    - SIR 1.57 (1.07 - 2.22); 32 cases
  - Earlier start date (indirect measure of exposure level)
    - SIR 1.69 (1.09 - 2.49); 25 cases
    - Trend analyses:  $P = 0.02$  (internal analyses)
  - Short term workers (> 10 years since first employment)
    - SIR 2.34 (1.43 - 3.61); 20 cases
    - Short term workers may have been exposed to high levels of styrene
  - No clear exposure-response patterns with NHL or all LHC

\* Similar results obtained from internal analysis



## SBR Industry

- Multi-plant cohort of U.S. and Canadian workers (Delzell *et al.* 2006)
  - Includes most workers from two earlier cohorts (Meinhardt and Matanoski)
  - 16, 579 workers (for internal analysis), median employment duration 11 years, average follow-up 30 years
  - 81 leukemia and 58 non-Hodgkin's lymphoma (NHL) cases
  - Internal analyses on two types of exposure metrics
    - Peak exposure: leukemia only
    - Cumulative exposure: leukemia and lymphoma

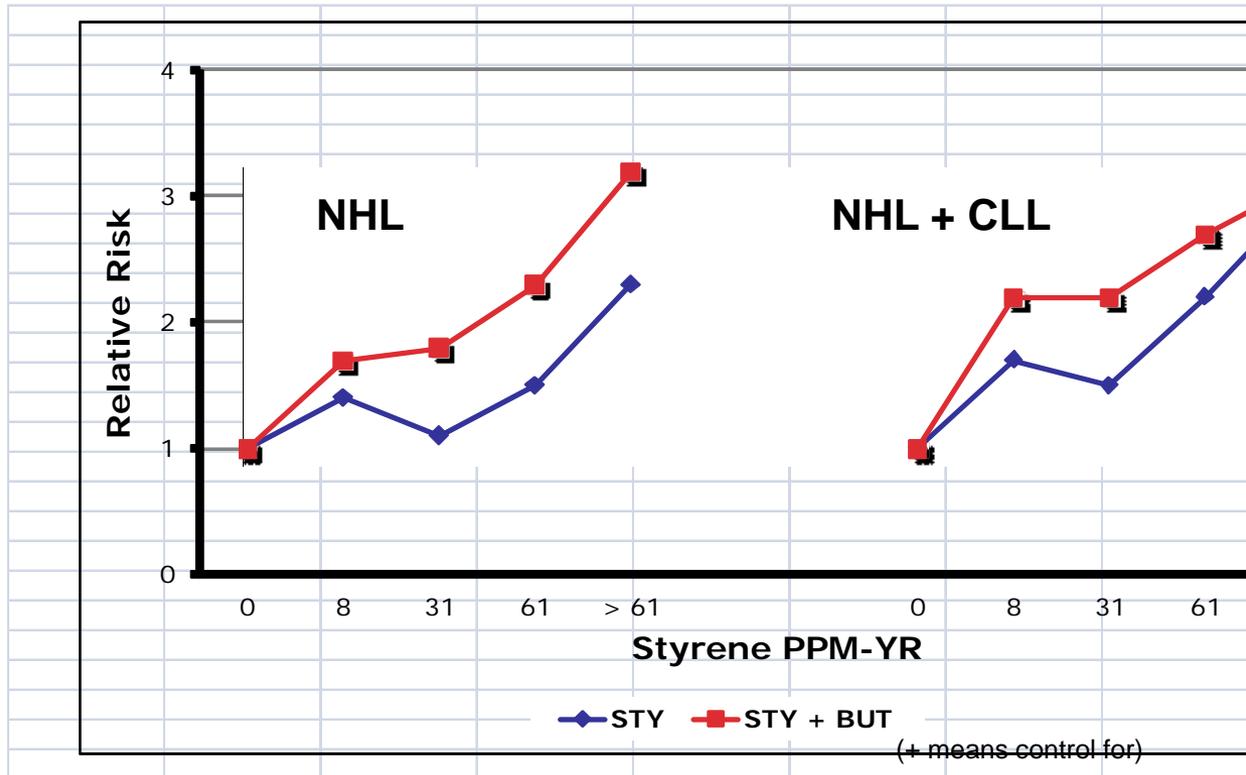


## Standard Mortality Ratio Analyses

- Significantly elevated SMRs for subgroups of workers
  - Long duration of employment (>10 yrs) and long latency (>20 yrs since first employment)
    - Leukemia: SMR = 2.24 (1.49 - 3.23); 28 deaths
    - NHL + CLL: SMR = 1.90 (1.01 - 3.25); 13 deaths
  - Highest levels of cumulative exposure to styrene
    - Leukemia: SMR = 1.91 (1.09 - 3.10); 16 deaths
    - NHL + CLL: SMR = 2.29 (1.36 - 3.62); 18 deaths

CLL = chronic lymphocytic leukemia

# Lymphomas: Internal Analyses

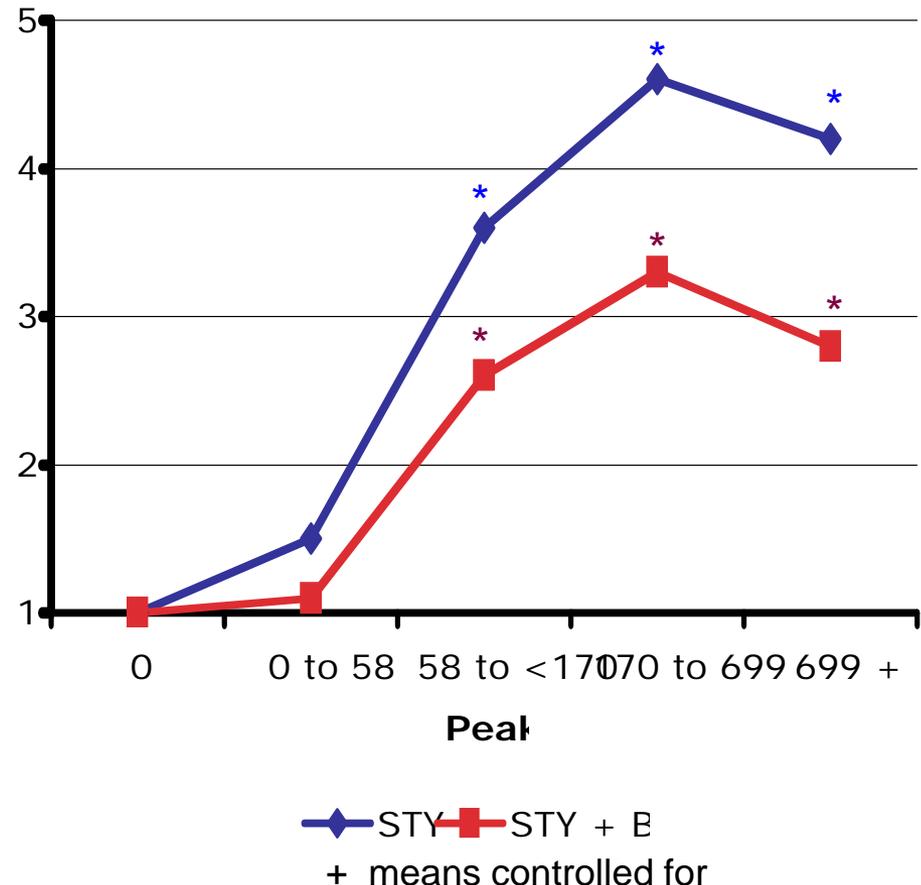


- RR increased with higher levels of exposure to styrene
- Butadiene not independently associated with risk of NHL
- Exposure-response pattern similar in models controlling for DMDTC and butadiene
- Trend tests not reported for any analyses

STY = Styrene, BUT = Butadiene, DMDTC = Dimethyldithiocarbamate, NHL = non-Hodgkins Lymphoma, CLL = Chronic lymphocytic leukemia \* 95% CI does not include 1

# Leukemia: Internal Analyses

- RR increased with increasing exposure to styrene peaks in all 3 models
- Significant RR for highest levels of exposure to styrene peaks
- Exposure-response pattern similar in models controlling for DMDTC and butadiene
- Cumulative exposure: Increased risks for styrene only, attenuated after control for butadiene



STY = Styrene, BUT = Butadiene, DMDTC = Dimethyldithiocarbamate, NHL = non-Hodgkins Lymphoma, CLL = Chronic lymphocytic leukemia \* 95% CI does not include 1, Tests for trend not reported



## Summary of Informative Studies

- Latency
  - Increased risks for LHC, lymphoma, and leukemia with time since first exposure in both industries
- High intensity exposure
  - Increased risk for leukemia in SBR (peak) and reinforced plastics industries (earlier start dates)
- Average exposure
  - Increased risks for lymphoma and LHC in both industries
    - SBR findings: nested case-control study by Matanoski *et al.* 1997
- Cumulative exposure
  - Increased risks for lymphoma in SBR industry but not reinforced plastics industry



## IARC Evaluation

NTP conclusions for LHC are consistent with the IARC evaluation (2002) that there is *limited evidence in humans for the carcinogenicity of styrene.*



## Studies of Lymphocytes in Styrene-Exposed Workers

Evidence for a genotoxic effect of styrene in humans

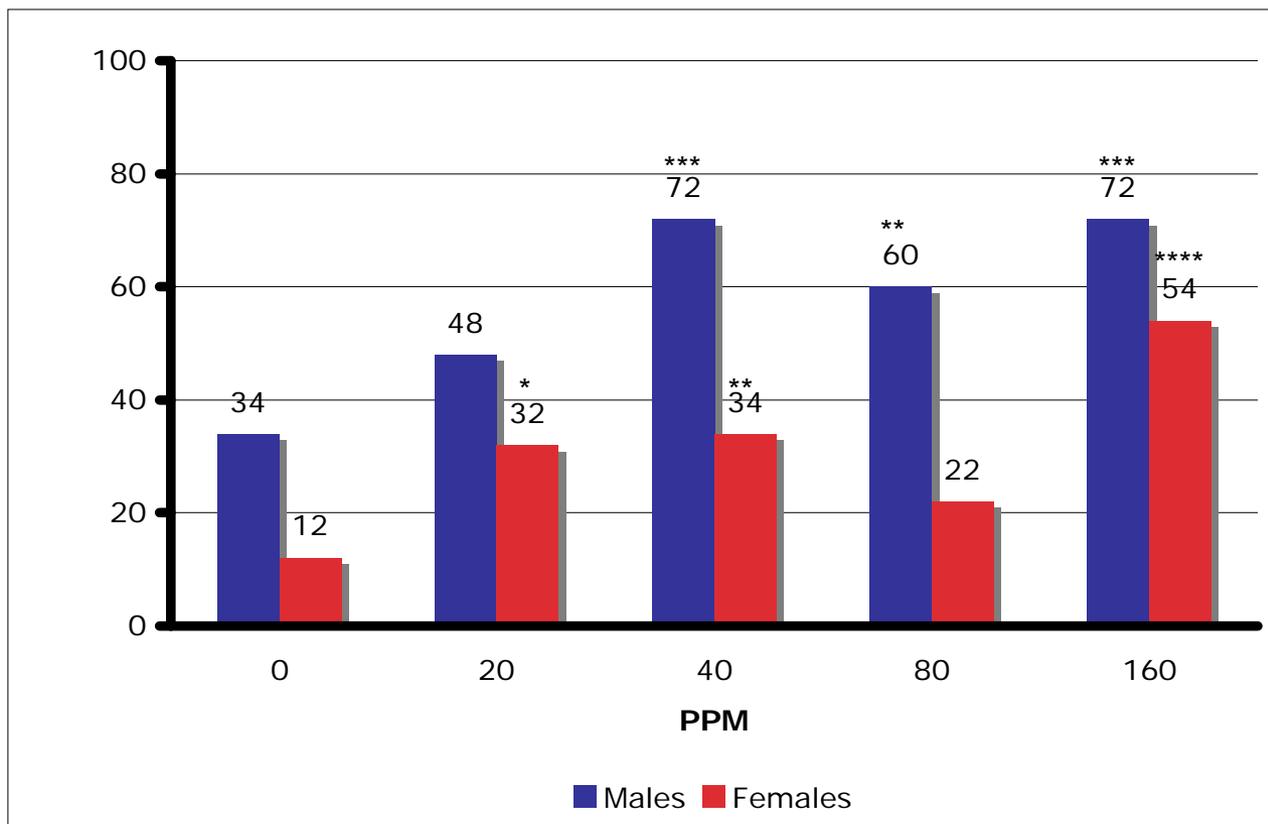
- Styrene-7,8-oxide adducts at base-pairing sites
  - O<sup>6</sup>-guanine, β-N<sup>1</sup>-adenine, N<sup>2</sup>-guanine
- Single strand breaks
- Chromosomal aberrations
  - Meta-analysis of 22 studies (Bonassi *et al.* 1996)
    - Weighted frequency ratio > 30 ppm TWA
    - 2.18, 95% CI = 1.52 to 3.13

## ***Sufficient Evidence in Experimental Animals: Multiple Routes of Exposure***

| <b>Strain, Authors</b>                  | <b>Methodology: Route, Dose, Duration</b>   | <b>Lung Tumors</b>  |
|---|---|---|
| CD-1 mice<br>Cruzan <i>et al.</i> 2001  | Inhalation, 4 doses<br>M: 104 weeks<br>F: 98 weeks                                  | Significant ↑ and D/R ↑<br>M: adenoma, and adenoma and carcinoma combined<br>F: adenoma, carcinoma and adenoma and carcinoma combined |
| B6C3F <sub>1</sub> mice<br>NCI 1979     | Gavage, 150 or 300 mg/kg<br>78 weeks, held for 13 weeks                             | M: Significant ↑ and D/R ↑<br>Adenoma and carcinoma combined  |
| O20 mice<br>Ponomarkov and Tomatis 1978 | Gavage, single dose, <i>in utero</i> (day 17) and weekly for 16 weeks after weaning | M & F offspring:<br>Significant ↑ in lung tumors  |



## Lung Tumors: Inhalation Study CD Mice



\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , \*\*\*\*  $P < 0.0001$

Cruzan *et al.* 2001



## Lung Tumors: Gavage study B6C3F<sub>1</sub> Mice

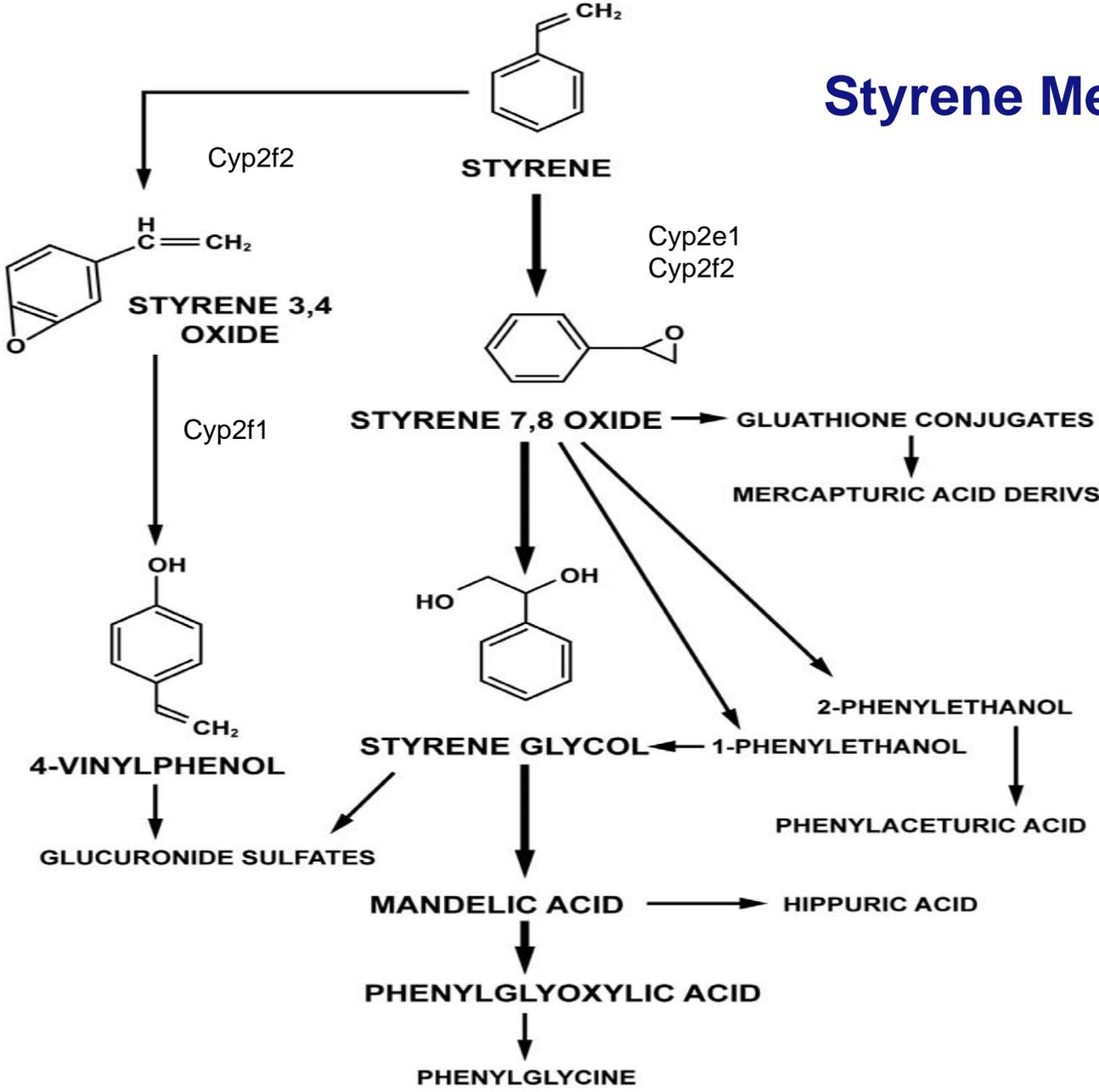
- Corn-oil gavage bioassay (NCI)
  - Conducted at Litton Laboratory
- 78-week exposure, 13-week observation
- Statistical analysis for animals surviving at least 52 weeks
  - Due to significant early mortality in high-dose male mice
- Findings
  - Significantly increased incidence of lung tumors in high-dose male mice (9/43, 21%) relative to controls (0/20)
  - Significant dose-response relationship



## NCI Study: Controls

- Concurrent vehicle controls are the most relevant control group
- Contemporary historical controls for 44 studies
  - 2 treatment groups (untreated and corn-oil gavage controls) at 2 laboratories (Litton and Hazleton)
  - Low incidence (0 to 4%) of lung tumors found in 3 of the 4 groups
    - Significantly different than the 4th group (Litton untreated controls)
- Lung tumor incidence in high dose male mice (21%)
  - Exceeds historical control range for all 44 studies (untreated and corn-oil gavage vehicle controls) from both laboratories (0 to 20%)

# Styrene Metabolism





## Mechanistic Data

- Metabolized to styrene-7,8-oxide
  - *Reasonably anticipated to be a human carcinogen*
- Lung tumors in mice not rats
  - Similar to finding with other epoxides
  - Higher levels of ring-oxidized metabolites
- Potential mechanisms for carcinogenicity (not mutually exclusive)
  - Genotoxicity
  - Cytotoxicity



## Mechanism: Genotoxicity

- Metabolic conversion of styrene to styrene-7,8-oxide and subsequent induction of DNA damage in the target tissue
  - Styrene-7,8-oxide detected in blood of styrene-exposed workers
  - Genotoxic effects in animals: styrene-7,8-oxide adducts, single strand breaks, and sister chromatid exchange
  - Genotoxic effect in humans: styrene-7,8-oxide adducts, single strand breaks, and chromosomal aberrations



## Mechanism: Cytotoxicity

- Repeated exposure to styrene causes hyperplasia
- Interspecies differences explained by Cyp2f2 ring-oxidized metabolites in Clara cells in the mouse lung
  - Metabolites formed from ring oxidation, including 4-vinylphenol (4-VP), are ~6-fold higher in mice than rats
- 4-VP may be more potent than styrene-7,8-oxide in inducing cytotoxicity in the lung
- Hypothesis has not been tested in long-term cancer studies in Cyp2f2 knock-out mice



## **Styrene**

### **Proposed Listing: *Reasonably Anticipated to be a Human Carcinogen***

- Limited evidence in humans
  - Increased mortality or incidence of LHC in exposed workers
  - Increased DNA adducts and genetic damage in lymphocytes
- Sufficient evidence in experimental animals
  - Lung tumors in mice by two routes of exposure
- Supporting mechanistic evidence
  - Metabolized to styrene-7,8-oxide, which is reasonably anticipated to be a human carcinogen
  - Genotoxic effects in humans