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NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Styrene

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PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction and development caused by agents to which humans may be exposed.

Styrene was selected for expert panel evaluation because of public concern for the possible health effects of human exposures and exposure data available recently. Styrene (ethenylbenzene; CAS RN: 100-42-5) is a high production volume chemical used in the production of polystyrene resins and as a co-polymer with acrylonitrile and 1,3-butadiene. Styrene is found in items such as foam cups, dental fillings, matrices for ion exchange filters, construction materials, and boats. It is also used in protective coatings, reinforced glass fiber, agricultural products, and as a food additive. In addition to occupational exposures, the general public can be exposed to styrene by ingesting food or drink that has been in contact with styrene polymers or through inhalation of polluted air or cigarette smoke.

To obtain information about styrene for the CERHR evaluation, the PubMed (Medline) and Toxline databases were searched through March, 2005, with CAS RNs for styrene (100-42-5), styrene oxide (96-09-3), and relevant keywords. References were also identified from databases such as REPROTOX®, HSDB, IRIS, and DART and from the bibliographies of reports being reviewed.

This evaluation results from the effort of a 10-member panel of government and non-government scientists that culminated in a public Expert Panel meeting held June 1–3, 2005. This report is a product of the Expert Panel and is intended to (1) interpret the strength of scientific evidence that styrene is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk. This report has been reviewed by CERHR staff scientists and by members of the Styrene Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies.

This Expert Panel Report will be a central part of the subsequent NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Styrene. This monograph will include the NTP-CERHR Brief, the Expert Panel Report, and all public comments on the Expert Panel Report. The NTP-CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

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1.0 CHEMISTRY USE AND HUMAN EXPOSURE

Section 1 is initially based on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if the studies were released subsequent to the reviews.

1.1 Chemistry

1.1.1 Nomenclature. The CAS RN for styrene is 100-42-5. Synonyms for styrene include (ChemIDplus, 2004): benzene, vinyl-; cinnamene; ethenylbenzene; ethylene, phenyl-; phenethylene; phenylethene; phenylethylene; styrene monomer; styrole; styrolene; styropol SO; vinyl benzene; vinylbenzene; and vinylbenzol.

1.1.2 Formulae and molecular mass. Styrene has a molecular mass of 104.16 and a molecular formula of C_8H_8 (ATSDR, 1992). The structure for styrene is shown in Figure 1.

1.1.3 Chemical and physical properties. Styrene is a colorless to yellowish-colored liquid with a sweet, sharp odor (ATSDR, 1992). Physicochemical properties are listed in Table 1. In air, 1 mg/m^3 = 0.23 ppm; 1 ppm = 4.33 mg/m^3 (ATSDR, 1992).

1.1.4 Technical products and impurities. According to the European Union (EU, 2002), suppliers list purity of styrene at 99.7 to <99.9% (w/w). Impurities vary by plant and production method and can include ethylbenzene (<0.1%), isopropylbenzene (cumene, <0.1%), 2-phenylpropene (<0.1%), water (<0.025%), phenyl acetate (<0.02%), *p*-xylene (<0.06%), and *m*-xylene (<0.001%). 4-*tert*-Butylpyrocatechol (4-*tert*-butylbenzene-1,2-diol) is often added at <0.006 to 0.01% (w/w) to inhibit polymerization. Additional impurities that have been detected in styrene include benzaldehyde, hydrogen peroxides, benzene, sulfur, chlorides, α-methylstyrene, vinyltoluene, phenylacetylene, and *p*-divinylbenzene IARC, 2002).

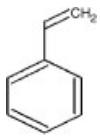

Fig. 1. Structure of styrene.

Table 1
Physicochemical Properties of Styrene[a]

Property	Value
Odor threshold	0.011–0.73 mg/L (water), 1.36 mg/m^3
Boiling point	145.2°C
Melting point	−30.6°C
Flammability	Flammable[b]
Specific gravity	0.906
Solubility in water	300 mg/L
Vapor pressure	5 mm Hg
Stability/reactivity	Can polymerize or oxidizes in presence of light and air[b]
Log K_{ow}	2.95

[a]ATSDR (1992).
[b]EU (2002).

No information on trade names for styrene was located.

1.2 Use and Human Exposure

1.2.1 Production information. Styrene is manufactured from ethylbenzene using one of two methods (ATSDR, 1992; Miller et al., 1994; EU, 2002). In the most common method, ethylbenzene is dehydrogenated using steam and an iron/zinc/magnesium oxide catalyst and the resulting styrene is purified under vacuum distillation. The second method involves oxidation of ethylbenzene to ethylbenzene hydroperoxide, which is reacted with propylene to yield propylene oxide and methyl phenyl carbinol. Using an acid catalyst, the carbinol is dehydrated to produce styrene.

Past or current manufacturers of styrene include BP Amoco Corp., Chevron Chemical Corp., Cos-Mar, Inc., Dow Chemical USA, Huntsman Chemical Corp., Lyondell Chemical Co., NOVA Chemicals, Inc., Sterling Chemicals, Inc., and Westlake Styrene Corp. (HSDB, 2002).

Styrene production in the U.S. was reported at 10.58 billion pounds in 1999 and 10.79 billion pounds in 2000 (HSDB, 2002). Volume of styrene imported to the U.S. was 1.038 million pounds in 1999 and 1.265 million pounds in 2000. The amount exported from the U.S. was 2.552 billion pounds in 1999 and 2.730 billion pounds in 2000.

1.2.2 Use. Styrene is used in the manufacture of polystyrene or copolymers, which can contain trace levels of the monomer (Cohen et al., 2002; EU, 2002). Table 2 lists the types of products manufactured from styrene, percent of total resin production, and products manufactured from each type of polymer. A search of the NLM household products database (NLM, 2004) showed that styrene is an ingredient of some putties and wood fillers used in hobbies or home maintenance.

In addition to the uses outlined in Table 2, styrene is approved for use as a direct food additive at an unspecified concentration (21 CFR 172.515) (FDA, 2004). Styrene is also approved for use as an indirect food additive. A list of styrene-based polymers approved for use in food contact materials is included in 21 CFR

A report of the CERHR Expert Panel with the support of CERHR Staff: NTP/NIEHS, Michael Shelby, Ph.D. (Director, CERHR), Christopher Portier, Ph.D. (Associate Director, National Toxicology Program); Sciences International, Inc., Anthony Scialli, M.D. (Principal Scientist), Annette Iannucci, M.S. (Toxicologist), Gloria Jahnke, D.V.M. (Toxicologist), Jessie Poulin, (Analyst).

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site (http:/cerhr.niehs.nih.gov/). The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. Bold, square brackets are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by the Panel.

The findings and conclusions of this report are those of the expert panel and should not be construed to represent the views of the National Toxicology Program.

Table 2
Use of Styrene in Resins[a]

Resin type	Estimated resin production (%)	Typical products produced from resins
Polystyrene	50	Construction materials, cups, plates, egg cartons, audio-visual equipment (e.g., cassettes), packaging, dairy containers, toys, furniture, industrial moldings (e.g., medical dental), insulation
Styrene-butadiene rubber	15	Tires, automobile parts (e.g., hoses, belts, seals, wire insulation) **[The Expert Panel notes that styrene-butadiene rubber is also used in other general rubber goods.]**
Unsaturated polyester resins (glass reinforced)	12	Boats, tubs, shower stalls, spas, hot tubs, cultured marble products, building panels, trucks
Styrene-butadiene latexes	11	Backing for carpets and upholstery, paper coatings, floor tile adhesives
Acrylonitrile butadiene styrene	10	Appliances, automobile parts, business equipment, construction materials, drains, ventilation pipes, hobby equipment, casings
Styrene acrylonitrile	1	Appliances, automobile parts, housewares, battery casings, packaging
Unsaturated polyester resins (not reinforced)	Not reported	Liners, seals, putty, adhesives

[a]Based on information reviewed by the EU (2002) and Cohen et al., (2002).

177.1010. Styrene-based polymers are also approved for use in ion exchange membranes (21 CFR 173.20 and 173.25), in adhesives and coatings for food packaging (21 CFR 175.105 and 175.300), and in paper and paperboard (21 CFR 176.170 and 176.180).

The FDA requires that residual styrene monomer levels (w/w) not exceed 1% (10,000 ppm) in basic polystyrene polymers, 0.5% (5000 ppm) in basic styrene polymers intended for contact with fatty foods, and 0.5% in rubber-modified polystyrene (21 CFR 177.1640) (FDA, 2004). Residual styrene monomer levels in food containers were measured at 16–1300 mg/kg in the U.K. before 1983 (EU, 2002) and 809–3019 mg/kg in Canada in 1978 (Health Canada, 1993). The EU (2002) reported residual styrene levels present in styrene-based polymers in 1980, and those values are summarized in Table 3. The values may not represent residual levels of styrene in polymers currently manufactured in the U.S. Regulations dictating allowable levels of monomer may differ in the U.S. versus Europe and may have changed since the 1980s. In addition, residual monomer levels can be decreased by improved production methods. The EU (2002) stated that polystyrene currently contains 300–600 ppm styrene monomer, with typical levels of ~400 ppm. The typical level of styrene monomer in expandable polystyrene is currently 800 ppm. Current monomer levels in acrylonitrile butadiene styrene polymers are typically 400 ppm.

Table 3
Residual Styrene Levels in Polymers in 1980[a]

	Residual styrene levels (ppm)	
Polymer	Typical	Maximum
Polystyrene	300–1000	2500
Acrylonitrile butadiene styrene (food uses)	200–300	600
Acrylonitrile butadiene styrene (other uses)	300–1000	2000
Styrene acrylonitrile	600–1200	2000
Methyl methacrylate butadiene styrene	Not detected to 10	30
Glass-reinforced plastic	20–200	1000
Styrene acrylic copolymers	60 in latex	Not reported
Styrene butadiene (raw polymer)	10–30	Not reported

[a]EU (2002).

1.2.3 Occurrence. Styrene is potentially present in food, drinking water, indoor air, or the environment as a result of anthropogenic or natural processes.

Styrene may be present in the environment as a result of direct releases or leaching of residual monomer from polymers. According to the Toxics Release Inventory database, total environmental release of styrene in 2002 was ~47.7 million pounds, with releases of ~47.3 million pounds to air, 6000 pounds to water, 160,000 pounds to underground injection, and 207,000 pounds to land (USEPA, 2002). In addition to industrial releases, exhaust from gasoline-powered motor vehicles is a significant source of styrene in ambient air (Cohen et al., 2002; EU, 2002). It is estimated that 30% of ambient styrene originates from motor vehicle exhaust, 40% from composites and boat building industries, and 30% from all other sources (Cohen et al., 2002).

Styrene released to the atmosphere is rapidly degraded by hydroxy radicals and tropospheric ozone (ATSDR, 1992; EU, 2002; Health Canada, 1993). Estimated atmospheric half-lives for styrene range from 0.5–17 hr. Styrene is not likely to be removed from air by rain or photolysis.

Due to its high vapor pressure and low to moderate solubility in water, styrene released to surface water is rapidly lost through volatilization (ATSDR, 1992; Health Canada, 1993; EU, 2002). Extent of volatilization depends on water depth and turbulence with no volatilization occurring in stagnant deep water. Styrene biodegrades rapidly in water under aerobic conditions but biodegrades slowly in ground water under anaerobic conditions. Limited data suggest the possibility of styrene persistence under anaerobic conditions, such as in anoxic

aquifers, but more data are needed (Alexander, 1997). Estimated half-lives of styrene in surface waters range from 1 hr in a shallow body of water to 13 days in a lake. Half-life of styrene in ground water is estimated at 4–30 weeks (ATSDR, 1992; EU, 2002).

A carbon/water partition coefficient (K_{oc}) has not been measured for styrene, but values of 260–370 were estimated (ATSDR, 1992; Health Canada, 1993; EU, 2002). Based on the estimated K_{oc}, styrene is expected to be moderately mobile in soil. Adsorption of styrene is expected to be greater in surface soils with a high organic content and less in deeper soils with a low organic content (ATSDR, 1992). One review cited studies demonstrating downward and lateral movement of styrene through soil (Alexander, 1997). Some reviews reported that volatilization of styrene from soil surfaces is rapid, with an estimated half-life of 1 min (Health Canada, 1993; EU, 2002). Another review reported that volatilization from soils is slower than from surface waters and cited a study reporting volatilization of 26% styrene in 31 days when added at 2 mg/kg to a 1.5-cm deep sample of loamy soil (Alexander, 1997). All reviews agreed that volatilization slows with increasing soil depth. Microorganisms capable of utilizing styrene as a sole carbon source were isolated from soils, thus suggesting that styrene may be biodegraded in soils (ATSDR, 1992; EU, 2002). Biodegradation products were identified as phenylethanol and phenylacetic acid. Other biodegradation products identified in laboratory studies are styrene oxide, acetophenone, phenylethanediol, mandelic acid, phenylacetaldehyde, 2-hydroxyphenylacetic acid, ethylbenzene, and 1,2-dihydroxy-3-ethyenyl-3-cyclohexene (Alexander, 1997). It was noted that there is no evidence indicating presence of these compounds at detectable levels in the environment.

ATSDR (1992) and the EU (2002) noted that the K_{ow} for styrene ($\sim$3) suggests partitioning to fat tissues. However, one study reported a bioconcentration factor for goldfish of 13.5, a value lower than expected based on the styrene K_{ow}. The EU observed that the study reporting the bioconcentration factor did not provide sufficient experimental details. Both ATSDR and the EU noted that the lower than expected bioconcentration factor could be due to rapid metabolism and excretion. Based on K_{ow}, the EU estimated a worst case bioconcentration factor of 74 for styrene. ATSDR reported a bioconcentration factor of 25 that was derived from an empirical regression. Based on the observation that toluene, xylene, and ethylbenzene do not accumulate substantially in aquatic organisms, the EU concluded that styrene will not accumulate in aquatic organisms.

Styrene is present in cigarette smoke; therefore, smoking is a potential source of styrene in indoor air. Styrene emissions from cigarette smoke have been estimated at 0.002–147 μg/cigarette (Cohen et al., 2002; EU, 2002). Styrene levels were $\sim$0.5 μg/m^3 **[0.12 ppb]** higher in homes of smokers compared to non-smokers (Cohen et al., 2002). Off-gassing of residual styrene from household products such as carpet glues, construction adhesives, and polyester-containing flooring materials are other potential sources of styrene in indoor air (Miller et al., 1994). In a review of studies published between 1986 and 1988, ATSDR (1992) reported indoor air levels of styrene at 0.8–8.9 μg/m^3 **[0.18–2.0 ppb]**. A U.S. survey of residential indoor air conducted in the early 1980s and expanded in 1986 obtained 2125 data points and reported a mean styrene level of 6.12 μg/m^3 (1.41 ppb) (Miller et al., 1994). In a 1991 Canadian survey of 757 single family homes and apartments, 24-hr styrene concentrations were measured at $<$0.48–128.93 μg/m^3 **[$<$0.11–30.0 ppb]** and averaged 0.28 μg/m^3 **[0.064 ppb]** (Health Canada, 1993). In a study of volatile organic compound exposure through consumer products in the U.S., seven volunteers performed 25 common activities during a 3-day period (Miller et al., 1994). Elevated styrene exposures **[assumed to be through inhalation]** were not related to most activities **[values not reported]**, but 26 μg/m^3 **[6.0 ppb]** styrene was detected in one personal sample of a volunteer who cleaned carburetors.

Ambient measurements of styrene typically show airborne concentrations of $\sim$1 ppb, although concentrations exceeding 5 ppb have been recorded in some urban areas (Cohen et al., 2002). The EU (2002) review reported styrene levels in outdoor air, and the U.S. and Canadian data are summarized in Table 4.

Styrene was not detected in drinking water samples from 102 surface water sources and 12 groundwater sources in the U.S. from 1977–1981 (EU, 2002). Studies reviewed by ATSDR (1992) and Miller et al. (1994) reported that styrene was not detected in more than 1000 U.S. drinking water samples analyzed in three federal surveys between 1975–1981. ATSDR reported that styrene is occasionally detected in drinking water samples from several states. No quantitative data were available in the studies reviewed by ATSDR. Miller et al. (1994) also noted that styrene was not detected in drinking water obtained from 272 sites in Kansas, Missouri, and Nebraska in 1982 or in a study of 945 drinking water samples obtained from groundwater sources. Cohen et al. (2002) stated that extensive studies of U.S. drinking water supplies suggest that styrene is not present or is present at concentrations $<$1 μg/L. The U.S. EPA Maximum Contaminant Level for styrene in drinking water is 0.1 mg/L **[100 μg/L; 0.1 ppm]** (USEPA, 2004).

Information on styrene levels in environmental water sources is limited. Styrene was detected at $\leq$100 μg/L in a small number of industrial effluents in the U.S. before 1980 (Alexander, 1997). A review by Alexander (1997) reported that investigation of 617 private wells and 1174 community wells in Wisconsin before 1986 found styrene in only one well near a municipal wastewater system. The level of styrene in the well was 0.0047 μg/L, whereas the styrene level in influent water was 0.61 μg/L; the review author questioned the sensitivity of analytical methods. One study reported a maximum styrene level of 1.7 μg/L and mean levels ranging from $<$0.1–0.5 μg/L in samples from the Canadian Great Lakes before 1993 (EU, 2002). Styrene levels in water samples from Germany and Japan before 1991 were $\leq$0.5 μg/L. Based on a review of U.S., Canadian, and European data, Alexander (1997) concluded that, with the exception of areas adjacent to industrial discharge sources, styrene is not present or is found at low concentrations in surface or groundwater.

Styrene may be present in food as a result of natural processes or leaching from food packaging or contact materials. Styrene occurs naturally in some animal- and plant-based foods and is a metabolite generated by microorganisms during production of foods such as

Table 4
Styrene Levels in Ambient Air Samples from the U.S. and Canada

Location	Concentration ($\mu g/m^3$) **[ppb]**	Year
Point sources		
Houston: industrial complex, near major transport routes[a]	Mean = 2.165 **[0.50]**	1987–1988
U.S.: 135 samples	Interquartile range = 0.17–7.2 **[0.039–1.66]** Median = 2.3 **[0.53]**	Not reported (study published in 1983)
Tunnel in Pennsylvania Turnpike[b]	Range = 1.1–6.6 **[0.25–1.5]**	Not reported (study published in 1983)
Contaminated sites (locations not reported)[a]	Maximum = 67.1 **[15.4]**	Not reported (study published in 1994)
Urban locations		
California[a]	Range = 8–63 **[1.8–14]** Mean = 21	1965
California Air Resources Board: 20 test stations in areas representing the greatest portion of California population[c]	Mean = 0.9 **[0.20]** over 6 years Highest measurement = 12.4 **[2.9]**	1989–1995
Canada: 586 samples from 18 urban sites[a]	24-hr Mean = 0.09–2.35 **[0.02–0.54]** Overall mean = 0.59 **[0.14]** Highest daily maximum = 32.4 **[7.45]**	1988–1990
New Jersey and California: six sets of samples from residential areas[a]	Median = 0.28–4.2 **[0.06–1.0]** Maximum = 1.0–11 **[0.23–2.5]**	Not reported (study published in 1986)
Three cities in New Jersey	Means = 0.30, 0.47, 0.55 **[0.069, 0.11, 0.13]** in summer Means = 0.64, 0.60, and 1.0 **[0.15, 0.15, 0.23]** in winter	Not reported (study published in 1984)
Los Angeles[a]	Range = 2.2–13 **[0.51–3.0]**	1981
Los Angeles[b]	Range = 0.4–2.3 **[0.09–0.53]**	Not reported (study published in 1984)
U.S.: four states, TEAM study[a]	Range = non-detect to 3.8 **[0.87]**	1981–1984
Rural locations		
Canada[a,b]	Maximum = 3.2 **[0.74]** Means = 0.09–0.5 **[0.02–0.12]**	1988–90

[a]EU (2002).
[b]Alexander (1997).
[c]IARC (2002).

wine, beer, grains, and cheese (Alexander, 1997; Cohen et al., 2002). Naturally occurring styrene levels measured in foods assumed not to be contaminated by plastic materials are summarized in Table 5. With the exception of cinnamon, styrene levels in most foods were well below 10 ppb. **[The Expert Panel notes that the cinnamon values were obtained from raw cinnamon. It is not known what effect processing and storage would have on the styrene content of cinnamon as it is consumed in foods, or to what extent cinnamon in foods adds to styrene exposure.]**

Migration of residual styrene from food containers and packaging materials is another possible source of styrene in foods. ATSDR (1992) reported that migration of styrene is mainly determined by its diffusion coefficient and the lipophilicity of the food. For example, percentage of available styrene monomer migrating from polystyrene packaging within 10 days was 4–6% in corn or sunflower oil but 0.3–0.6% in milk, beef, or water. Migration of styrene from foam cups was $\sim 8\,ng/cm^2$ in water, tea, or coffee but $36\,ng/cm^2$ in 8% ethanol. **[The units of measurement are questionable. Although ATSDR reported units of ng/cm^2, units of ng/cm^3 were reported by the EU (2002).]**

Table 5
Levels of Naturally Occurring Styrene in Foods

Food[a]	Styrene level in $\mu g/kg$ (ppb)
Black currants	2–6
Wheat	0.4–2
Apples, cauliflower, onions, tomatoes	<1
Cinnamon	170–39,000[b]
Peanuts	1–2.2
Coffee beans	1.6–6.4
Strawberries	0.37–3.1
Beef	5.3–6.4
Oats	<0.65–1.6
Peaches	<0.18–0.3
Tomatoes, peaches, raw milk, chicken, pecans	<2

[a]Contact with packaging materials was avoided for these foods. Values were obtained from two or three samples of each food, measured in duplicate.
[b]Reported figures were rounded in the secondary source. The individual rounded values were 170, 180, 2300, 2700, 37,000, and 39,000 ppb in the paired samples. From EU (2002) and Cohen et al. (2002).

Table 6
Surveys of Styrene Levels in Packaged Foods

Foods surveyed	Styrene level in μg/kg (ppb)	Country	Year of survey	Reference
Yogurts, creams, salads, coleslaw, soft cheeses, margarines, hot and cold beverages from vending machines, spreads, fresh and cooked meats, candied fruits, fresh strawberries, and fast foods	1–200; <10 in 77% of foods and <1 in 26% of foods	U.K.	Before 1983	EU (2002)
Yogurt	26	U.K.	1981	EU (2002)
Dessert products	22			
Soft cheese	16			
Cream	11			
Spreads	10			
Low fat spread samples	20–100	U.K.	1994	EU (2002)
Milk and cream products	23–223; mean = 134			
Other food types	Mean = <30			
Beer	10–200	Not reported	1965–1991	EU (2002); Miller et al. (1994)
Coffee	20–360			
Bilberries	25			
Black currants	60			
Yogurt	5.5–34.6	Canada	Before 1976	Miller et al. (1994)
Yogurt	Trace to 13.0	Canada	1978	Health Canada (1993)
Sour cream	143.3–245.9			
Survey of 34 food groups that approximate Canadian diet	<1.0 μg/L in liquids; <0.005 μg/g **[5 ppb]** in solids	Canada	1992	Health Canada (1993)
Must (“mosti-vino rossi”) and wine	Trace to 0.05 ppm **[50 ppb]**	Italy	1986	EU (2002)

Table 7
Summary of Styrene Levels in Food as Reported by an FDA Market Basket Survey[a]

Food	Range of styrene values, ppm **[ppb]**
Dairy (milk, cheese, ice cream, sour cream)	0.0020–0.1960 **[2.0–196]**
Meats (unprocessed: beef, pork, poultry, lamb; processed: sausage, bacon, frankfurters, lunch meat, meatloaf)	0.0020–0.0850 **[2.0–85]**
Fish (canned tuna or fish sticks)	0.0020–0.014 **[2.0–14]**
Eggs, scrambled	0.0020–0.0160 **[2.0–16.0]**
Nuts and nut butters (peanut, mixed nuts)	0.0110–0.1040 **[11.0–104]**
Starchy foods (popcorn, bread, muffins, corn chips, potato chips, cereals, crackers, teething biscuits)	0.0020–0.5100 **[2.0–510]**
Fruits/vegetables and juices (oranges and orange juice, bananas, strawberries, raisins, avocados, tomatoes, carrots, apple juice)	0.0020–1.3800 **[2.0–1380]**
Salads (coleslaw with dressing)	0.0020–0.0060 **[2.0–6.0]**
Fast or take out foods (hamburgers, chicken, french fries, fish, frankfurters, tacos, pizza, Chinese food)	0.0020–0.0940 **[2.0–94.0]**
Fats and oils (margarine, butter, olive/safflower oil)	0.0030–0.0540 **[3.0–54.0]**
Desserts (cake, sweet rolls, cookies, pies, doughnuts, brownies)	0.0020–0.1990 **[2.0–199]**
Candy (chocolate, caramel)	0.0020–0.0760 **[2.0–76.0]**
Soy infant formula	0.0020 **[2.0]**
Popsicles	0.004–0.0110 **[4.0–11.0]**

[a]FDA (2003).

Numerous surveys were conducted to measure styrene levels in packaged foods, and the survey results are summarized in Table 6. The EU (2002) noted that in general, styrene levels in foods were directly related to fat content and inversely related to container size. **[CERHR notes that higher levels of styrene were also detected in coffee and beer.]**

More recent measurements of styrene levels in U.S. food are available from a 2003 FDA report of a market basket survey conducted to determine levels of pesticides and organic compounds in foods (FDA, 2003). Styrene was detected in 72 different types of foods and only the positive results were reported. **[No other details about the study were provided, such as analytical methods and detection limits.]** The FDA findings are summarized in Table 7. The lowest styrene level was reported to be 0.002 ppm. The highest styrene value reported was for strawberries (1380 ppb), which greatly exceeded styrene

levels reported for strawberries in other surveys ($\leq$3.1 ppb; Table 5). The next highest values for styrene were reported in muffins (510 ppb), cheese (196 ppb), and cookies (199 ppb). Levels of styrene in cheese were similar to those reported in previous surveys conducted in Europe or Canada (Table 6).

Whole body styrene was measured at 15–100 mg/kg in walleye and splake, a cross of brook and lake trout, and detected but not quantified in several other species of fish captured from the St. Clair River in 1981 (Health Canada, 1993; EU, 2002). Styrene levels were $<$10.0 μg/kg in edible shellfish from the Canadian Atlantic. In neither report was it indicated if results were expressed as dry or wet weight basis. In Japanese surveys conducted in 1986, styrene was measured at 350 μg/kg in mussels and at 0.5–2.3 μg/kg in 28 of 131 fish samples (EU, 2002). **[Although not specified, it is assumed that fish was not packaged.]**

Styrene levels have also been measured in human tissues. According to three studies published in the 1980s and reviewed by ATSDR (1992), styrene was detected in adipose tissue (8–350 ng/g), blood (mean = 0.4 μg/L), and exhaled breath (mean = 0.7–1.6 μg/m^3).

1.2.4 Human exposure

1.2.4.1 General population exposure: A number of agencies have estimated human exposure to styrene.

ATSDR (1992) reported an exposure estimate conducted by the U.S. EPA in 1988. Worst case exposures **[adjusted for a 70-kg body weight]** were estimated at 0–0.5 μg/day from drinking water **[0.007 μg/kg bw/day]**, 30 μg/day from food **[0.4 μg/kg bw/day]**, and 65,000 μg/day **[930 μg/kg bw/day]** from air.

In an exposure estimate conducted by ATSDR (1992), inhalation of indoor air was considered the most significant styrene exposure source for the general population. Assumptions used in the calculation are that the average person spends 20.4 hr/day indoors, inhales 20 m^3 of air/day, and that styrene levels in air range from 0.8–8.9 μg/m^3. The typical indoor air exposure to styrene was estimated at 14–151 μg/day **[0.2–2 μg/kg bw/day]**. ATSDR did not estimate exposure resulting from outdoor air or food. Exposure through drinking water from municipal supplies was considered insignificant.

Health Canada (1993) estimated styrene exposures to adults and children and a summary of their exposure estimates is included in Table 8. Health Canada concluded that substantial exposure to styrene occurs through indoor air. Food may also provide a substantial exposure, but the food survey data for Health Canada were limited by insufficient detection limits. Exposures through drinking water and soil were expected to be negligible. Although food intake values represented upper limits, higher levels of styrene were reported in U.S. and Canadian surveys of indoor air. Exposure estimates ranged from $<$0.20–$<$0.79 μg/kg bw/day in the nonsmoking population and 2.86–3.51 μg/kg bw/day in teen and adult smokers.

Using modeled data on styrene levels in biota resulting from various release scenarios and estimated intakes from air and drinking water, the EU (2002) estimated human exposure levels ranging from 1.6×10^{-5} to 0.11 mg/kg bw/day **[0.016–110 μg/kg bw/day]**. An alternate exposure estimate provided a value of 0.019 mg/kg bw/day **[19 μg/kg bw/day]** by assuming styrene levels of 30–100 μg/kg in food, 80 μg/m^3 in air downwind from a reinforced plastics processing facility, and 10 μg/L in water **[not specified if drinking water]**.

A review by the an Expert Panel of the Harvard Center for Risk Analysis (Cohen et al., 2002) concluded that exposure to styrene through ingestion of or dermal contact with water is apparently negligible. Based on styrene values similar to the upper ranges reported in Table 5 and a food consumption rate of 3 kg/day, the Harvard Panel estimated that styrene intake through naturally-occurring sources is 0.6 μg/day. The Harvard Panel based their estimate of dietary styrene exposure resulting from residual styrene in food packaging on a study by Lickly et al. (1995). As explained by Lickly et al. (1995), levels of styrene in packaged foods were estimated by considering migration rates and the percentage of foods that contact polystyrene packaging or containers. Based on those calculations, it was estimated that a mean 1.95 μg styrene/kg food is contributed from polystyrene food packaging, and another 1.05 μg styrene/kg food is contributed to diet through disposable polystyrene materials. Food consumption rate was assumed to be 3 kg/day. The upper bound exposure to styrene through food packaging or containers was therefore estimated at 9 μg/day **[3 μg styrene/kg food $\times$ 3 kg food/day = 9 μg styrene/day]**. The estimate was considered conservative because there were no considerations of reduced leaching associated with aging of polystyrene, rapid volatilization of styrene from liquid materials in open

Table 8
Health Canada Exposure Estimates[a]

Medium	Styrene levels	Estimated intakes (μg/kg bw/day)[b] by age				
		0–6 months	7 months–4 years	5–11 years	12–19 years	20–70 years
Ambient air	0.09–2.35 μg/m^3	0.004–0.11	0.006–0.15	0.007–0.17	0.006–0.14	0.005–0.13
Indoor air	0.28 μg/m^3	0.07	0.09	0.10	0.09	0.08
Drinking water	$<$0.05–0.250 μg/L	$<$0.005–0.03	$<$0.003–0.02	$<$0.002–0.008	$<$0.001–0.006	$<$0.001–0.005
Food	[b]	$<$0.58	$<$0.53	$<$0.30	$<$0.15	$<$0.11
Total intake						
Nonsmokers	[b]	$<$0.66–$<$0.79	$<$0.63–$<$0.79	$<$0.41–$<$0.58	$<$0.25–$<$0.39	$<$0.20–$<$0.33
Smokers	10 μg/cigarette, 20 cigarettes/day	N/A	N/A	N/A	3.51	2.86

[a]Health Canada (1993).
[b]See Health Canada document for explanation of assumptions used in calculations.

containers such as cups, or losses of styrene during cooking. The Harvard Panel identified 0.6 μg/day as a reasonable upper-bound estimate for exposure to naturally occurring styrene in food. Therefore, 10 μg/day **[9 μg/day from packaging+0.6 μg/day from natural sources]** or 0.2 μg/kg bw/day, based on a 70-kg body weight and rounded to one significant figure, was considered a reasonable estimate for upper bound dietary exposure.

The Harvard Panel (Cohen et al., 2002) estimated styrene exposures occurring through ambient air, smoking, and living near industrial sources. Maximum annual and lifetime average exposure estimates are outlined in Table 9. Ambient exposure estimates are based on reporting of typical airborne concentrations ≤1 ppb **[4 μg/m^3]** (by volume) and occasional concentrations >5 ppb **[22 μg/m^3]**. The smoking estimates are based upon several studies demonstrating that styrene exposures in smokers are about six times higher than in nonsmokers. Annualized lifetime exposure to styrene through smoking is lower than annual exposure because individuals do not smoke their entire lives. Estimated exposure to individuals living near industrial sources is based upon analyses conducted in an unpublished 1999 study by Johnson. For the analyses, a group of hypothetical facilities representing 80% of facilities emitting more than 10,000 pounds styrene/year was developed. Ambient styrene concentrations in the vicinity of the hypothetical facilities were estimated using Version 3 of the Industrial Source Complex Short Term model, which complies with U.S. EPA requirements. Factors considered in the analyses were residential occupancy time, time spent at residence/day, hr/day spent outdoors and indoors, movement of contaminants from outdoor to indoor air, contaminant decay in indoor air, and air exchange rate at residence.

Tang (2000) estimated styrene exposures in the German population. Diet and ambient air were considered to be the main sources of styrene exposure. Dietary exposure was estimated 0.003–0.017 μg/kg bw/day using food consumption patterns for the German population, assuming styrene levels of 5–30 ppb in fatty foods and 1–3 ppb in wine, and assuming that 10% of foods are packaged in polystyrene. Inhalation exposure to styrene was estimated at 0.3–0.8 μg/kg bw/day assuming an average styrene level of 1–3 μg/m^3 in indoor or outdoor air and a pulmonary retention rate of 60%. Exposure in smokers was estimated at 100 μg/day **[1.4 μg/kg bw/day at a 70-kg bw]** assuming that each cigarette results in exposure to 5 μg styrene, and 20 cigarettes are smoked per day.

Fishbein (1992) estimated exposures in the general population according to various scenarios and expressed the results as nominal daily intake. Those estimates are outlined in Table 10, along with CERHR conversion to μg/kg bw/day values according to a 70-kg body weight. **[No details were provided about the data used to estimate styrene exposures.]**

[The Expert Panel notes that there is no exposure or use information on some automobile body fillers and fiberglass repair kits that contain styrene. This exposure source may apply to consumer and occupational scenarios.]

Fisher et al. (1997) developed a physiologically based pharmacokinetic (PBPK) model to estimate intakes of styrene and other chemicals by breast fed infants of occupationally exposed women. Blood/air and blood/milk partition coefficients were experimentally derived from blood and milk samples obtained from 6–10 women. Metabolic constants and information about milk production, typical nursing schedules, and milk intake by infants

Table 9
Styrene Air Exposures[a]

Scenario	Maximum annual average (ppb) **[μg/m^3]**	Lifetime average (ppb) **[μg/m^3]**
Typical ambient exposure	1 **[4]**	1 **[4]**
High-end ambient exposure	5 **[22]**	5 **[22]**
Exposure through smoking	6 **[26]**	<6 **[26]**
At residence 100 meters from a 100,000 pound/year emission facility (95th percentile individual)	12 **[52]**	2.8 **[12]**
Residence at greatest exposure point in vicinity of a 1 million pound/year emission facility (95th percentile individual)	700 **[3031]**	219 **[948]**

[a]Harvard Review (Cohen et al., 2002).

Table 10
Estimated Styrene Exposure in the General Population from Different Exposure Sources

Source	Estimated concentrations	Nominal daily intake[a] (μg)	Intake in μg/kg bw/day[b]
Within 1 km of a production unit	30 μg/m^3	600	9
Polluted urban atmosphere	20 μg/m^3	400	6
Urban atmosphere	0.3 μg/m^3	6	0.09
Indoor air	0.3–50 μg/m^3	6–1000	0.09–14
Polluted drinking water (2 L/day)	1 μg/L	2	0.03
Cigarette smoke (20 cigarettes/day)	20–48 μg/cigarette	400–960	6–14

[a]Calculated assuming a daily respiratory intake of 20 m^3 at home or in an urban atmosphere.
[b]Calculated by CERHR, assuming a 70-kg bw.

were obtained from the literature. For styrene, the blood/air partition coefficient was reported at 69.74 ± 38.2 (SD) and the milk/air partition coefficient was reported at 151.35 ± 69.19; therefore, the ratio of milk to blood was 2.17. Assuming that the mother was exposed to 50 ppm styrene, the threshold limit value (TLV), it was estimated that a nursing infant would consume 0.65 mg/day **[650 µg/day]** styrene. The study authors stated that more data on the toxicokinetics of lactational transfer of volatile organic compounds were needed to determine the significance of lactational exposure.

1.2.4.2 Occupational exposures: Occupational exposure to styrene could occur during manufacture of the monomer, production of polystyrene or other styrene-based polymers, processing of styrene-based polymers, and manufacture of glass-reinforced plastics. Inhalation is the primary route of exposure. One study using charcoal samplers attached to various body parts of reinforced plastics workers demonstrated dermal exposure to styrene, including on surfaces covered by clothing, but did not determine if styrene was absorbed through the skin (Eriksson and Wiklund, 2004). Studies reviewed by Harvard (Cohen et al., 2002) and IARC (2002) reported limited skin absorption of styrene in workers, but in exposure scenarios where there is prolonged and repeated contact with liquid styrene, dermal uptake could contribute to internal dose equivalent to the dose achieved by inhalation exposure in the lower range of occupational exposures (1–2 ppm; 4–8 mg/m^3 **[estimated by Expert Panel]**). Styrene and styrene-based polymers or copolymers are generally manufactured using closed processes that limit potential exposure to the monomer (Miller et al., 1994). Exposures to styrene are also reported to be low during the processing of polymers or copolymers, such as styrene-butadiene rubber. During processing of thermoplastics, heating of the plastics can release small amounts of fumes containing styrene in addition to other hydrocarbons. Styrene air levels measured in different types of processes are discussed below.

The highest styrene exposures occur during the production of glass-reinforced plastics, which often involve open processes. The types of open processes used to manufacture glass-reinforced plastic products include open mold, filament winding, pultrusion molding, and continuous lamination (Lemasters et al., 1985a). Although this paragraph provides a general description of each of these processes and comparative levels of styrene exposures, actual styrene exposure levels are discussed below. The open mold method is used to manufacture large items such as boats. In the process, resin is sprayed onto a wax-coated mold. A layer of fibrous glass is added and then coated with liquid polyester resin applied by spray gun, brush, or hand roller. Resins contain up to 40% styrene, and up to 10% of the styrene monomer can be released during application and curing. Filament winding is an open process used to manufacture tanks and pipes. In that process, strands of fibrous glass pass through resin and are wound on a rotating spool while the worker guides the product by hand and uses rollers to remove excess resin and air. Pultrusion molding is used to manufacture rods and tubes by drawing continuous filaments through a heated orifice. Continuous lamination methods are semi-automated and result in lower worker exposures. The method is used to manufacture large panels and involves passing a fibrous glass mat through a resin bath, pressing it between two sheets of cellophane, and compressing it between rollers. Press (closed) mold processes are also used to manufacture glass-reinforced products. Match-metal die is the most common press mold process (Lemasters et al., 1985a). The process involves placing fiberglass and resin between male and female dies. When the press opens, vapors containing unpolymerized styrene are released. Injection molding methods are used to manufacture glass-reinforced plastics but involve enclosed processes. Air preform methods that involve spraying chopped strands and resins on a rotating screen mold can be conducted using automated or hand-held methods. The manufacture of bulk mold compounds used in metal die operations involves mixing and pressing of fiberglass and resin through both manual and automated processes.

In the National Occupational Exposure Survey, it was estimated that 333,219 workers, 86,908 of them female, were exposed to styrene in 1981–1983 (NIOSH, 1981–1983).

The American Council of Governmental Industrial Hygienists (ACGIH) established a time weighted average (TWA) TLV of 20 ppm and short term exposure limit (STEL) of 40 ppm for styrene (ACGIH, 2003). The exposure limits were based upon neurotoxicity, irritation, and central nervous system (CNS) effects. ACGIH also developed biological exposure indices for styrene. Those end of shift indices are 400 mg mandelic acid plus phenylglyoxylic acids/g creatine in urine and 0.2 mg/L styrene in venous blood (see Section 2 for explanation of metabolism). ACGIH rated styrene as an A4 compound, *Not Classifiable as a Human Carcinogen*. The NIOSH TWA relative exposure limit (REL) is 50 ppm, and the STEL is 100 ppm (NIOSH, 2000). The OSHA TWA permissible exposure level (PEL) for styrene is 100 ppm; the ceiling limit of 200 ppm must not be exceeded, with the exception of exposures to 600 ppm occurring no longer than 5 min in any 3-hr period (OSHA, 1997). In 1989, OSHA granted an exemption to allow the use of respirators to maintain styrene exposures within acceptable levels at plants manufacturing large parts (Miller et al., 1994).

IARC (2002) reviewed studies discussing expected levels of biological exposure indices immediately after and the morning after a shift in which a worker inhaled 50 ppm styrene for 8 hr; the values are summarized in Table 11.

Harvard (Cohen et al., 2002) and IARC (2002) between them reviewed 10 exposure studies conducted between the 1960s through 1990s that reported air levels of styrene in styrene monomer, polymer, and copolymer manufacturing plants, many in the U.S. Mean air levels of styrene were reported at $\leq$35 ppm, with most values below 10 ppm. Higher values were usually associated with older studies. Levels occasionally peaked at up to 50 ppm. Peaks were reported to occur during filling of drums or during occasional bursts or leakage from equipment. IARC reviewed three studies measuring air styrene levels in industries processing polystyrene, acrylonitrile butadiene styrene, and styrene-butadiene rubber polymers in the 1970s and 1980s; measurements were obtained in at least one U.S. plant. With the exception of styrene levels of 17–285 mg/m^3 **[3.9–65.6 ppm]** measured in one U.S. plant in the late 1970s,

Table 11
Estimated Exposure Indices in Workers Inhaling Styrene

Exposure scenario	Biological exposure index		
	Urinary mandelic acid (mg/g creatinine)[a]	Urinary phenylglyoxylic acid (mg/g creatinine)[a]	Blood styrene level (mg/L)[a]
Inhalation of 50 ppm styrene for 8 hr (end of shift value)	800–900	200–300	0.5–1
Inhalation of 50 ppm styrene for 8 hr (morning after shift value)	300–400	100	0.02

[a]Expected values as reviewed by IARC (2002).

Table 12
Estimated Styrene Exposures in Glass-Reinforced Plastics Workers[a]

Process[b]/Exposure[c]	Product	Number		Estimated means (ppm)	Intervals (ppm)	
		Samples	Companies[d]		95% CI	1 SD
Open/direct	Boats	270	8	82	79–84	28–230
	Truck parts	23	2	61	40–92	21–171
	Tubs/showers	149	3	47	45–48	16–131
	Yachts	184	3	37	34–41	6–198
	Tanks/pipes	364	4	24	24–25	8–68
	Domes	40	1	13	11–14	4–36
	Fans	2	1	5	3–10	1–13
Press/direct	Auto/truck parts	214	2	26	22–31	8–70
	Electrical boxes	15	1	13	9–20	4–35
	Containers	55	2	13	12–14	4–35
	Trays	13	1	11	8–15	3–29
	Forms	21	1	11	8–14	3–29
Open/indirect	Boats	2	1	19	11–33	9–35
	Tanks/pipes	38	3	15	13–17	7–27
	Yachts	5	1	7	6–9	3–12
	Tubs	7	1	3	1–5	1–5
Press/indirect	Containers	12	1	9	7–12	4–16
	Trays	52	1	4	3–5	1–6
	Forms	20	1	5	4–8	2–9

[a]Lemasters et al. (1985a).
[b]See text for descriptions of open versus press methods of manufacture.
[c]Direct exposures defined as having contact with wet styrene-containing resins for $\geq$50% of the work day; indirect exposures involved $\leq$50% of the day spent in direct contact with styrene-containing resins.
[d]It seems that authors actually meant total number of exposure sources.

all other measurements were well below 1 ppm. In a study reviewed by Harvard, maintenance workers in nonproduction departments of international paper mills were exposed to a mean TWA of 9.9 ppm styrene; the maximum value reported was 100 ppm and 4 of 93 TWA values exceeded 50 ppm.

Macaluso et al. (2004) estimated historical exposure to styrene in six North American styrene-butadiene rubber plants using a model that considered job tasks and factors affecting exposure potential (e.g., work practices, equipment used, exposure reduction mechanisms). Across all job categories, mean estimated TWA styrene exposures were within 2 ppm since the 1940s and declined to 0.5 ppm in the 1980s. Estimated TWA exposures were about an order of magnitude higher for tank farm operators who monitor monomer transfer from a pump house, laboratory technicians who collect and test samples, and unskilled laborers involved in cleanup. In most cases, however, those exposures tended to decrease over time. Possible reasons for exposure reductions over time included improved pump designs that prevent styrene loss from equipment, smaller size and number of samples required for testing, and use of hydroblasting techniques for cleaning. The authors noted limitations in their study, such as potentially inaccurate estimates and lack of adequate industrial hygiene data to verify the estimates.

The most detailed study on exposures in the U.S. of glass-reinforced plastic workers was conducted by Lemasters et al. (1985a), who compiled 1500 occupational exposure values from 28 manufacturers of glass-reinforced plastic products. **[A value of 38 companies was listed in Table VI of the study. According to the text, two or more exposure sources were available at 15 companies. It seems that the number in the table defines total exposure sources that could include multiple plants and both open and press-mold processes at the same location.]** The exposures values were retrospectively obtained form industrial hygiene surveys conducted by companies, NIOSH, and OSHA from 1969–

1981. Means, 95% confidence intervals (CI), and standard deviations (SD) were estimated, and are summarized in Table 12. Mean exposures in workers directly exposed in open mold processes ranged from 5–82 ppm, with most values measured at 24–82 ppm. The values were generally higher than mean exposures observed in workers directly exposed to press mold processes, which were 11–26 ppm.

Additional exposure values for workers in the reinforced plastics industry were provided in the IARC review (IARC, 2002). Values were provided for two U.S. companies before 1985. Mean exposure values ($\leq$331 mg/m^3 **[$\leq$76 ppm]**) were within ranges reported by Lemasters et al. (1985a). Ranges of styrene exposures at the two locations were 0–511 mg/m^3 **[0–118 ppm]**. It has been reported that styrene concentrations are dropping over time due to improvements in work processes (Miller et al., 1994). Welp et al. (1996) reported that exposures in European glass-reinforced plastics workers from 6 countries dropped from 200 ppm in the 1960s to 20–40 ppm in the late 1980s. A review by Pfäffli and Säämänen (1993), summarized mean styrene exposures reported in 16 studies for reinforced-plastics lamination workers in nine European, North American, or Asian countries. Mean (ranges) of styrene exposures were reported at 91–171 ppm (5–292 ppm) from the 1950 s through 1970s and 23–110 ppm (2–203 ppm) from the 1980s through 1990s.

Styrene exposure data in glass-reinforced plastics workers are available in a 2003 study by Lees et al. (2003). Exposures were assessed at four U.S. sites that had stable manufacturing processes and control technologies during the past 10 years. TWA styrene exposures (8-hr) and pre- and post-shift urinary mandelic acid and phenylglyoxylic acid levels were measured. Because respirators were worn by some workers, respirator-corrected values were estimated by dividing actual exposure measurements by an arbitrary value of 5. The respirators used by the workers are commonly assigned a protection value of 10, but a value of 5 was used to account for possible poor fit and periods when the respirator was not worn. Historic exposures were estimated to verify that levels remained fairly consistent during the past 15 years, a requirement for a companion study to evaluate olfactory function. The main focus of this summary is the current worker exposures. Study details and results are presented in Table 13. Styrene exposures were fairly consistent with previously reported data and were highest in open processes. Adjustments for respiratory protection mostly affected workers at sites with the highest styrene concentrations because they were most likely to use respirators. The study authors noted that mean post-shift mandelic acid and phenylglyoxylic acid levels at Site 1 exceeded recommended **[by ACGIH]** biological exposure indices. Urinary metabolite levels in workers not wearing respiratory protection were highly correlated with styrene air concentrations ($r^2 = 0.78$ for mandelic acid and 0.51 for phenylglyoxylic acid). In four workers wearing respiratory protection, points fell below the regression line in two workers, indicating effective protection, and were on the line for the other two workers, indicating little-to-no protection.

Styrene exposure data in reinforced plastics workers are also available in a 2004 study by Luderer et al. (2004). Exposures were assessed at 17 U.S. workplaces from December, 1996 through July, 1999. One to five (average of three) TWA personal breathing zone full shift (approximately 8-hr) styrene measurements were obtained during a single work week, and post-shift blood styrene levels were obtained during one of those days. Data were collected on the use of personal protective equipment during the sampling shifts by trained observers. Study details and results are presented in Table 14. Styrene exposures were fairly consistent with the data reported for similar processes by Lees et al. (2003). Use of respiratory protection was associated with higher blood and air styrene concentrations, consistent with greater use of respiratory protection among the more highly exposed workers. The slope of the regression line of blood styrene as a function of air styrene for workers wearing respiratory protection was 0.011, which was lower than for workers not wearing respirators (0.014). Respirator use was a significant predictor of blood styrene in a regression model with air styrene (personal communication, U. Luderer, May 30, 2005).

Table 13
Details and Results of Styrene Exposure Study in Four U.S. Plants[a]

Parameter	Site 1	Site 2	Site 3	Site 4
Production details	Production of bathtub and shower enclosures using manual spray techniques	Production of bathtub and shower enclosures using manual spray techniques	Sheet production using press method in a highly automated, enclosed system	Hand lay-up or die-molding operations
No. air samples	22	31	24	22
No. samples from workers using respiratory protection	10	2	3	0
No. urinary samples	11	13	19	9
Styrene TWA, ppm[b]				
Mean	55.0 (24)	18.2 (13.9)	16.8 (14.8)	9.2 (9.2)
Range	11.6–140.3 (4.9–45.1)	0.1–92.7 (0.1–45.1)	3.4–50.9 (2.7–50.9)	3.9–28.1 (3.9–28.1)
Post-shift urinary values in mg/g creatinine, mean (range)				
Mandelic acid	1740 (200–6980)	320 (<10–1020)	260 (<10–1040)	190 (50–350)
Phenylglyoxylic acid	490 (80–2250)	90 (<10–290)	80 (<10–370)	90 (20–210)

[a]Lees et al. (2003).
[b]Values in parentheses divided by 5 in workers using respiratory protection.

Table 14
Styrene Exposures in 17 Reinforced Plastics Workplaces[a]

Parameter	Type of business				
	Production of bathtub and shower enclosures	Boat building	Pipe and tank manufacture	Truck and RV manufacture	Boat repair
No. air samples	10	103	43	109	23
No. samples from workers using respiratory protection	2	23	5	23	5
No. blood samples	9	99	34	101	22
Air styrene TWA in ppm					
Mean ± SEM	19.4 ± 7.5	14.0 ± 7.9	16.5 ± 2.0	27.3 ± 2.9	9.2 ± 1.4
Range	< 1.0–62.9	< 1.0–86.9	2.2–50.0	< 1.0–141.7	< 1.0–53.4
Blood styrene in mg/L					
Mean ± SEM	0.21 ± 0.08	0.21 ± 0.03	0.21 ± 0.05	0.33 ± 0.04	0.09 ± 0.02
Range	< 0.01–2.05	< 0.01–1.31	0.02–1.33	0.01–2.05	< 0.01–0.56

[a]Luderer et al. (2004) and personal communication from U. Luderer (May 30, 2005). These data are for the first study session per subject.

These results indicate that the respirators were somewhat protective. Although the regression line of blood styrene as a function of air styrene for workers wearing gloves had a slightly lower slope than that for workers not wearing gloves, glove use was not a significant predictor of blood styrene (personal communication, U. Luderer, May 30, 2005). Overall, blood styrene concentrations were highly correlated with air styrene concentrations ($r^2 = 0.77$).

NIOSH Health Hazard Evaluations (HHE) were searched for more recent data on occupational styrene exposures in the U.S. (NIOSH, 2004a). Data from companies apparently manufacturing or processing styrene-based materials from 1997–2001 are presented in Table 15. The limited amount of available data are consistent with older data showing that open processes result in the highest exposures (personal TWAs of 1.4–46 ppm), which sometimes exceed the ACGIH TLV or approach the NIOSH PEL. Respirators were used at two of the locations (HETA 96-0145-2684; HETA 94-0072-2648; August 1997).

Fishbein (1992) estimated occupational exposures to styrene. For the exposure estimates, it was assumed that reinforced plastics workers are exposed to 200,000 μg/m^3 **[46 ppm]** styrene and styrene polymerization workers are exposed to 10,000 μg/m^3 **[2.3 ppm]** styrene. Based on a daily respiratory rate of 10 m^3 at work, nominal daily intakes were estimated at 2 g **[29 mg/kg bw/day based on 70-kg body weight]** for reinforced plastics workers and 100 mg **[1.4 mg/kg bw/day based on 70-kg body weight]** for styrene polymerization workers.

Based on biological exposure indices measured in one study, protective clothing and gloves did not reduce styrene exposure in workers; the finding refuted earlier reports that dermal absorption is an important route of exposure (IARC, 2002). Exposures were reduced through proper use of respirators designed to filter out organic vapors.

1.3 Utility of Data

A limited amount of information reported styrene levels in environmental samples such as air and water. A market basket survey of styrene levels in U.S. foods was released by the FDA in 2003. Additional food surveys were conducted in the U.K. and Canada. Levels of styrene in workplace air were also reported. Human exposure to styrene was estimated by various agencies. The main limitation of exposure estimates is that many values were derived using non-U.S. data. However, some U.S. data, such as the market basket survey, are available for comparison.

1.4 Summary of Human Exposure Data

Styrene is used in the manufacture of polystyrene or styrene copolymers, which can contain trace levels of the monomer (Cohen et al., 2002; EU, 2002). Types of styrene polymers manufactured and their uses are outlined in Table 2. Styrene is also an ingredient of some putties and wood fillers used in hobbies or home maintenance (NLM, 2004). Styrene is approved for use as a direct and indirect food additive (FDA, 2004).

Styrene is released to the atmosphere by industry, and it is estimated that 47.7 million pounds of styrene were released to the environment in 2002. In addition to industrial releases, exhaust from gasoline-powered motor vehicles is a significant source of styrene in ambient air (Cohen et al., 2002; EU, 2002). It is estimated that 30% of ambient styrene originates from motor vehicle exhaust, 40% from composites and boat building industries, and 30% from all other sources. Cigarette smoke and off-gassing of residual styrene from household products such as carpet glues, construction adhesives, and polyester-containing flooring materials are potential sources of styrene in indoor air (Miller et al., 1994). Styrene emissions from cigarette smoke have been estimated from 0.002–147 μg/cigarette (Cohen et al., 2002; EU, 2002).

Styrene released to the atmosphere is rapidly degraded by hydroxy radicals and tropospheric ozone (ATSDR, 1992; Health Canada, 1993; EU, 2002). Volatilization of styrene is rapid from surface water but not deep, stagnant water. Biodegradation of styrene in water and soil is expected to occur under aerobic but not anaerobic conditions. Styrene is estimated to be moderately mobile in soil. There is uncertainty regarding reported K_{ow}s for styrene. Based on observations that toluene, xylene, and ethylbenzene do not accumulate substantially in aquatic organisms, the EU (2002) concluded that styrene is not

Table 15
Styrene Air Levels as Reported in NIOSH Health Hazard Evaluations 1997–2001[a]

Process description	Styrene in TWA personal samples in ppm (*n*)	Styrene in personal short term samples in ppm (*n*)	Styrene in TWA area samples in ppm (*n*)	Reference
Brush application of resin to fiberglass strip and application of strip to interior seems of tank.	2.5–43.9 (5) [mean = 15.9]	21.0–92.1 (2)	Not reported	HETA 2001-0189-2842 May 2001
Manufacture of circuit breaker cases by compression molding of styrene-vinyltoluene resin.	0.37–8.1 (7) [mean = 3.3]	Not reported	0.34–6.6 (3) [mean = 3.3]	HETA 97-0154-2693 June 1998
Assembly of doors using hot melt adhesive process; adhesive consisted of acrylates/meth-methylene bisphenyl diisocyanate.	0.46 (1) **[not a TWA exposure]**	Not reported	0.24–0.43 (4) **[mean = 0.31; not a TWA exposure]**	HETA 97-0217-2667 January 1998
Home decorative items manufactured by blending polyester resin, pouring it into preformed molds and curing; workers mixing and pouring significant amounts of resin wore respiratory protection.	1.4–46[b] (8) [mean = 18.8]	18–111[b] (4) [mean = 45.8]	25–179[b] (4) [mean = 77.3]	HETA 96-0145-2684 April 1998
Processes included a mix house where resin/fiberglass was used to make a sheet molding compound; some employees chose to wear respirators.	<4.0–19.3 **[includes personal and area samples; it is not clear if values are TWA exposures]**	Not reported	See personal samples column	HETA 94-0072-2648 August 1997

[a]NIOSH (2004a).
[b]Data reported in text of report seem to differ from data reported in table; values obtained from tables are presented.

likely to bioaccumulate. Limited surveys suggest that away from point sources of release, styrene levels are relatively low in ambient air (≤14 ppb), indoor air (≤30 ppb), and environmental water samples (<1.7 μg/L); styrene is rarely detected in drinking water.

Styrene may be present in food as a result of natural processes or leaching from food packaging or contact materials. With the exception of raw cinnamon, for which styrene values ranged from 170–39,000 ppb in three samples, naturally occurring styrene levels are low in foods such as meats, produce, and grains (≤6 ppb) (Cohen et al., 2002; EU, 2002). **[The Expert Panel does not know the relevance of cinnamon intake to human styrene burden.]** One survey reported 1380 ppb styrene in strawberries, but it is not known if the strawberries contacted food packaging materials (FDA, 2003). Surveys to determine levels of styrene in foods as a result of natural occurrence or leaching from packaging materials were conducted in the U.K. and Canada from the 1960s through 1990s (Health Canada, 1993; Miller et al., 1994; EU, 2002), and a U.S. market basket survey was released in 2003 (FDA, 2003). The surveys included various types of foods such as dairy products, meats, produce, salads, desserts, nuts, starchy foods, fast foods, fats and oils, candies, and beverages. With the exception of the single high level in strawberries mentioned above, muffins contained the highest level of styrene (510 ppb). In other foods, styrene levels were reported at ≤360 ppb, with the highest levels reported in fatty foods (e.g., dairy products), coffee, beer, and cookies. Limited surveys of styrene levels in fish generally report whole body levels below 350 ppb, but a value of 100,000 ppb was also reported (Health Canada, 1993; EU, 2002).

Styrene exposure in the general population can occur through oral intake or inhalation. Human exposure to styrene was estimated by various agencies. The best documented exposure estimate using actual styrene levels was conducted by Health Canada (1993). In nonsmokers, styrene exposure was estimated from <0.25–<0.39 μg/kg bw/day (ages 12–19 years) and from <0.20–<0.33 μg/kg bw/day (ages 20–70 years). Styrene in air represented the greatest exposure source. For smokers, Health Canada estimated styrene exposure at 3.51 μg/kg bw/day (ages 12–19 years) and 2.86 μg/kg bw/day (ages 20–70 years). In children, excluding adolescent smokers, the highest styrene exposures (<0.63–<0.79 μg/kg bw/day) were estimated for those 4 years or younger. ATSDR (1992) considered styrene in air as the only significant source of exposure, and their estimated styrene exposure of 0.2–2 μg/kg bw/day was similar to the Health Canada estimate for adults. A much wider range of styrene exposures (0.016–110 μg/kg bw/day) was estimated by the EU (2002) using modeled values of various release scenarios or point source exposures.

Inhalation is the major route of styrene exposure in occupational settings because skin absorption is believed to be minimal in most exposure scenarios. For ease of presentation, occupational exposures are expressed in units of ppm instead of the ppb units that were used to describe general population exposures above. The highest levels of styrene in air were measured in facilities

using open processes to manufacture glass-reinforced polyester materials. In contrast, styrene monomers and polymers are manufactured using closed processes and generally result in lower styrene levels in air. Information about levels of styrene in workplace air is available from reviews (Cohen et al., 2002; IARC, 2002), three surveys of U.S. workplaces manufacturing glass-reinforced plastic products (Lemasters et al., 1985a; Lees et al., 2003; Luderer et al., 2004), and a small number of NIOSH HHEs (NIOSH, 2004a). In monomer and polymer production facilities, styrene levels in air were usually measured below 10 ppm, but peaks up to 65 ppm were sometimes reported. Reported mean 8-hr TWA levels of styrene in air of glass-reinforced plastic facilities ranged from 3–82 ppm with individual 8-hr TWA levels up to ~140 ppm. Some studies or reviews reported data indicating that generally styrene exposures have tended to decrease over time (Pfäffli and Säämänen, 1993; Welp et al., 1996; Macaluso et al., 2004). However, styrene exposures in the reinforced plastic facilities as reported by Lemasters et al. (1985a) were essentially similar to the exposure levels reported by Lees et al. (2003) and Luderer et al. (2004). Based on the reported exposure range (3–82 ppm), a daily respiratory rate of 10 m^3 at work, and a 70-kg body weight, nominal daily styrene intake in the reinforced plastic industry is estimated at 2–51 mg/kg bw/day. Nominal daily intake in styrene polymerization workers based on air levels of around 2 ppm styrene would be 1.4 mg/kg bw/day.

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

2.1 Toxicokinetics and Metabolism

Section 2 is initially based on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant to a CERHR evaluation of developmental or reproductive toxicity or if important studies were released subsequent to the reviews.

2.1.1 Humans. The most thorough reviews on human toxicokinetics and metabolism were conducted by IARC (2002) and ATSDR (1992) and they were thus the basis for the summary presented below. Additional sources were occasionally used and are referenced below.

2.1.1.1 Absorption: There are no known studies on absorption of styrene by humans after oral exposure. Styrene is absorbed through the respiratory tract. A number of studies conducted in volunteers or workers reported that pulmonary retention of inhaled styrene is 60–70% of the dose (ATSDR, 1992; IARC, 2002). Blood levels in humans inhaling styrene vapors are summarized in Table 16. A small number of studies suggested limited absorption of styrene through skin. In studies reviewed by ATSDR, dermal absorption rates were reported at ~1 μg/cm^2/min in volunteers who dipped their hands in styrene and 9–15 mg/cm^2/hr when styrene was placed on the forearms of volunteers. ATSDR noted that the higher rate likely included disappearance of styrene from the skin surface. At styrene air concentrations of 300 or 600 ppm, it was estimated that dermal penetration was 0.1–2% the amount absorbed through the respiratory tract (ATSDR, 1992).

2.1.1.2 Distribution: Styrene distribution in humans is widespread, with the highest concentrations found in adipose tissue (ATSDR, 1992). IARC (2002) noted that older studies reported accumulation of styrene in adipose tissues; however, no accumulation of styrene, as determined by measurement of urinary metabolites of workers, was suggested by more recent studies. **[No reasons were stated for discrepancies between older and more recent studies.]** Volume of distribution in humans was reported as 99 L or 1.4 L/kg (Wigaeus et al., 1983).

2.1.1.3 Metabolism: Styrene is highly metabolized in humans, with an estimated 97% eliminated through metabolic pathways (ATSDR, 1992). Styrene metabolic pathways adapted from the IARC review (IARC, 2002) are outlined in Figure 2. A metabolic scheme presented in the ATSDR profile was not as complete and differed slightly from the scheme presented by IARC. Because the IARC review was more recent, it was assumed to have more up-to-date information. In the main metabolic pathway in humans, styrene is converted to styrene 7,8-oxide (further referred to as styrene oxide) by cytochrome P450 (CYP) enzymes. Styrene oxide is metabolized to phenylethylene glycol (styrene glycol) by epoxide hydrolase. Phenylethylene glycol is metabolized to mandelic acid, which is then converted to phenylglyoxylic acid. Smaller amounts of mandelic acid are converted to benzoic acid. Mandelic acid and phenylglyoxylic acid are the predominant urinary metabolites in humans and are commonly used as biomarkers of exposure. Mandelic acid was reported to represent 57–80% of a styrene dose and phenylglyoxylic acid, 10–33% (ATSDR, 1992; Rebert and Hall, 1994). In four male volunteers who inhaled 50 ppm styrene vapors for 2 hr, mandelic acid represented 6–29% of the retained dose, and phenylglyoxylic acid represented 4–6% of the dose (Johanson et al., 2000). **[The Expert Panel noted that lower metabolite recoveries in the Johanson et al. (2000) study likely reflected an unknown amount reaching blood.]** IARC reported that in humans, metabolites generated through ring epoxidation of styrene (i.e., vinylphenol), conversion of styrene to 1-phenylethanol (i.e., ultimately leading to formation of hippuric acid), glutathione conjugation of styrene oxide, and glucuronidation of phenylethylene glycol represent <1% of the retained styrene dose.

ATSDR (1992) noted some differences in metabolism of styrene in humans compared to experimental animals; metabolism in experimental animals is discussed in greater detail below. Mercapturic acids generated through glutathione conjugation are major metabolites in rodents. Mice and rats have greater capacity to produce styrene oxide than humans. Humans seem to have a greater ability to convert 7,8-styrene oxide to styrene glycol than rats or mice.

Löf and Johanson (1993) examined dose-related kinetics in one man and one woman (age 41–43 years) inhaling 26, 77, 201, or 386 ppm styrene for 2 hr while lightly exercising on four different occasions. Blood samples were collected during exposure and for up to 5 hr after exposure to measure styrene levels by gas chromatography (GC). Urine samples were collected for up to 24 hr after exposure for measurement of mandelic acid level by high-performance liquid chromatography (HPLC). After 2 hr of exposure, the study authors noted

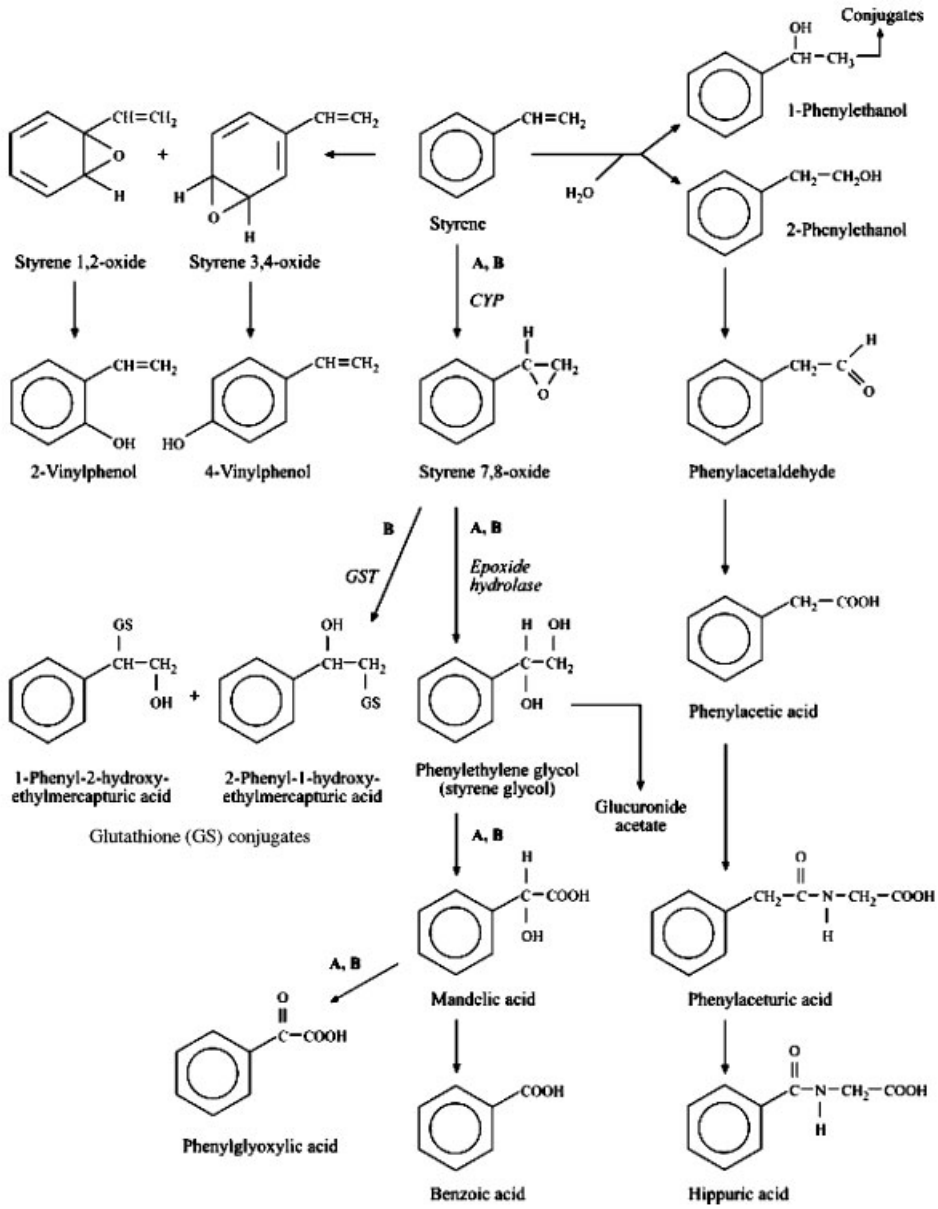

Fig. 2. Metabolism of Styrene in Humans and Rodents. Adapted from IARC (2002). **A**, major pathway in humans; **B**, major pathway in rodents.

nonlinear increases in blood styrene levels (see Table 16) and blood AUC values **[~8, 20–85, 120–180, and 325–415 µmol-hr/L at each respective dose, as estimated by CERHR from a graph]**. According to study authors, concentrations at which responses deviated from those predicted by a linear model were 201 ppm in one volunteer and 386 ppm in both volunteers. Cumulative urinary mandelic acid excretion plateaued at 0–5 hours but was proportional to styrene concentration at 0–24 hr; the study authors interpreted the finding as indicating a delay in excretion most likely resulting from saturated metabolism. A V_{max} of 2.9 mmol/hr was estimated using a PBPK model. The study authors concluded that saturation of styrene metabolism occurs between concentrations of 100 and 200 ppm and is dependent on activity level.

According to a PBPK model developed by Ramsey and Andersen (1984), saturation of styrene metabolism in humans occurs at blood levels exceeding 1.7 mg/L styrene or 200 ppm styrene in air. Below those concentrations, the rate of styrene metabolism is limited by the rate of blood perfusion in liver or other organs involved in styrene elimination. The model was verified using actual blood concentrations of styrene in humans and rats. A more recent model by Sarangapani et al. (2002) compared dosimetry of styrene and styrene oxide in the lung of humans, rats, and mice, which has been stated to be more relevant to toxicity than blood concentrations (Cruzan

Table 16
Styrene and Styrene Oxide Blood Levels in Humans and Experimental Animals

Subjects	Exposure		Blood level		Reference
	Duration (hr)	Concentration	Styrene	Styrene oxide	
Children in School Health Initiative: Environment, Learning, Disease (SHIELD) study	NA	NA	Mean = 0.17 ng/mL **[0.00017 mg/L]** 95th percentile = 0.50 ng/mL **[0.0005 mg/L]**	NR	Sexton et al., 2005
Adults in National Health and Nutrition Examination Survey (NHANES) III survey	NA	NA	Mean = 0.07 ng/mL **[0.00007 mg/L]** 95th percentile = 0.18 ng/mL **[0.00018 mg/mL]**		Sexton et al., 2005
General population	NA	NA	0.4 μg/L **[0.0004 mg/L]**		ATSDR, 1992
Workers	NS	20 ppm		0.001 mg/L	IARC, 2002,
One man and one woman exposed while lightly exercising	2	26 ppm 77 ppm 201 ppm 386 ppm	~7–7.5 μmol/L[a] **[0.73–0.78 mg/L]** ~18.5–29 μmol/L[a] **[1.9–3.0 mg/L]** ~60–80 μmol/L[a] **[6.2–8.3 mg/L]** ~150–200 μmol/L[a] **[16–21 mg/L]**		Löf et al., 1986
Four male volunteers exposed while lightly exercising	2	50 ppm	7.0–8.6 μmol/L **[0.73–0.89 mg/L]**	2.5–12.2 nmol/L **[0.00025–0.0012 mg/L]**	Johanson et al., 2000
Eight men working in reinforced plastics industry; exposed while lightly exercising	0	0	0.3 μmol/L **[0.031 mg/L]**		Löf et al., 1986
	2	296 mg/m^3 **[68 ppm]**	14.7 μmol/L **[1.53 mg/L]**	≤0.03 μmol/L **[0.003 mg/L]**	
Seven men with no occupational exposure; exposed to styrene while lightly exercising	2	296 mg/m^3 **[69 ppm]**	21.2 μmol/L **[2.2 mg/L]**	0.05 μmol/L **[0.005 mg/L]**[b]	Löf et al., 1996; Wigaeus et al., 1983
Workers in a styrene polymerization plant				0.03 μmol/L **[0.003 mg/L]**	Wigaeus et al., 1983
Four men	6	80 ppm	~0.9 mg/L[a]		Ramsey and Andersen, 1984
Rats	6	80 ppm 200 ppm 600 ppm 1200 ppm	~0.9 mg/L[a] ~2 mg/L[a] ~40 mg/L[a] ~80 mg/L[a]		Ramsey and Andersen, 1984
Rats	6 (over period of 5 days/week for 95 weeks	0 50 ppm 200 ppm	<100 ng/mL **[<0.1 mg/L]**[c] 290–430 ng/mL **[0.29–0.43 mg/L]**[c] 1950–2780 ng/mL **[1.95–2.78 mg/L]**[c]	<10 ng/mL **[<0.01 mg/L]**[c] <10 ng/mL **[<0.01 mg/L]**[c] 28–66 ng/mL **[0.028–0.066 mg/L]**	Cruzan et al., 1998

		500 ppm	9460–12,490 ng/mL **[9.46–12.49 mg/L]**[c]	92–116 ng/mL **[0.092–0.116 mg/L]**[c]		
		1000 ppm	29,680–33,210 ng/mL **[29.680–33.210 mg/L]**[c]	153–185 ng/mL **[0.153–0.185 mg/L]**[c]		
Mice	6 (over period of 5 days/week for 74 weeks	0	<10 ng/mL [<**0.010 mg/L]**[c]	<1 ng/mL **[0.001 mg/L]**[c]	Cruzan et al., 2001	
		20 ppm	30–69 ng/mL **[0.030–0.069 mg/L]**[c]	<1 ng/mL **[0.001 mg/L]**[c]		
		40 ppm	106–177 ng/mL **[0.106–0.177 mg/L]**[c]	1.4–2.5 ng/mL **[0.0014–0.0025 mg/L]**[c]		
		80 ppm	527–654 ng/mL **[0.527–0.654 mg/L]**[c]	6.2–11.8 ng/mL **[0.0062–0.0118 mg/L]**[c]		
		160 ppm	1461–1743 ng/mL **[1.461–1.743 mg/L]**[c]	20.1–33.5 ng/mL **[0.0201–0.0335 mg/L]**[c]		

[a]Estimated from a graph by CERHR.
[b]Collected in a complementary study of four individuals.
[c]Means in males and females.
NA, non-applicable; NR, not reported; NS, not specified.

et al., 2002). The model is discussed in greater detail in Section 2.4.3.

Studies reviewed by IARC (2002) demonstrated that CYP2B6 and CYP2E1, from liver and lung, and CYP2F1, from lung, were the most active isoforms in metabolizing styrene. One study conducted with human liver microsomes identified CYP2E1 and CYP2C8 as the most prominent isoforms at low styrene concentrations (0.085 mM) and CYP2B6 and CYP2C8 as the most significant enzymes at high styrene concentrations (1.8 mM).

IARC (2002) reviewed studies examining activity of styrene metabolism by human pulmonary microsomes. Pulmonary microsomal activity was found to be very low ($\leq$0.088 nmol/mg protein/min) and far below values reported for hepatic microsomal activity (1.07–1.91 nmol/mg protein/min). IARC noted that the whole organ homogenates do not indicate differences in activity among different cell types. Metabolism of styrene was also examined in nine human nasal tissues, and no activity was detected; as discussed in more detail below, styrene metabolism was observed in nasal tissues of rats and mice.

2.1.1.4 Elimination: As noted above, styrene is highly metabolized, and the majority is eliminated through urine, primarily as mandelic acid and phenylglyoxylic acid. ATSDR (1992) and IARC (2002) report that a limited amount of styrene (0.7–4.4%) is present unchanged in exhaled air.

Styrene clearance from blood is biphasic, indicating a two-compartment toxicokinetics model. Half-lives for inhaled styrene were reported at 0.6 hr for the first elimination phase and 13 hr for the second elimination phase (ATSDR, 1992). The half-life for styrene oxide **[assumed to be for the first elimination phase]** was reported at 1.8 hr (Johanson et al., 2000). Half-lives for urinary elimination of mandelic acid were reported at 3–4 hr and 25–40 hr for each respective phase (ATSDR, 1992; Rebert and Hall, 1994). Half-lives for the first urinary elimination phase of phenylglyoxylic acid were reported at 3.5–13.9 hours (ATSDR, 1992; Johanson et al., 2000).

Clearance rate for styrene was reported at 1.7 L/min (Wigaeus et al., 1983); the study authors noted that the rate was similar to that of total blood flow through liver, thus suggesting that styrene is metabolized in a high-affinity pathway limited by perfusion.

2.1.2 Experimental animal. The most thorough reviews on experimental animal toxicokinetics and metabolism were conducted by IARC (2002) and ATSDR (1992), and they were the basis for the summary presented below. Additional sources were used as needed and are referenced below.

2.1.2.1 Absorption: Studies in rats demonstrated rapid absorption of styrene through the gastrointestinal and respiratory tracts (ATSDR, 1992). Blood styrene levels in rodents inhaling styrene vapors are summarized in Table 16. One study of rats exposed to styrene vapors reported that 9.4% of total uptake was through dermal absorption (IARC, 2002); a second study in rats reported a peak blood styrene level of 5.3 μg/mL occurring 1 hr after the application of 2 mL neat styrene to skin. A study in which rats' tails were dipped in styrene for 1 hr reported styrene levels in liver and brain that were estimated to be 50–70% of levels measured after

inhalation exposure to 11,800 mg/m^3 **[2700 ppm]** styrene for 4 hr (ATSDR, 1992).

2.1.2.2 Distribution: As in humans, styrene is widely distributed in rats and mice with highest concentrations found in fat (IARC, 2002). Two studies examined distribution of styrene to fetuses.

Brown (1991) reviewed a Russian-language study of placental styrene transfer. Styrene was measured in maternal and fetal blood and amniotic fluid in rats after inhalation exposure of dams to 3.6 or 10 ppm styrene on gestation day (GD) 18–21. Styrene levels measured in the 3.6 ppm group were 1–3 μg/mL in maternal blood, 1–2 μg/mL in fetal blood, and 1–2 μg/mL in amniotic fluid. In the 10 ppm group, styrene was measured at 11–12 μg/mL in maternal blood, 8–9 μg/mL in fetal blood, and 2–3 μg/mL in amniotic fluid.

Withey and Karpinski (1985) exposed six pregnant Sprague–Dawley rats/group to 1000 or 2000 ppm styrene vapors for 5 hr on GD 17 **[plug day not specified]**. Rats were killed after exposure for measurement of styrene levels in maternal blood and in each homogenized fetus. **[Methods for measuring styrene were not discussed.]** Styrene exposure did not affect fetal weight. Geometric means for fetal levels of styrene per litter ranged from 12.63–21.82 μg/g ($n = 6$ litters) in the 1000 ppm group and 35.14–65.49 μg/g ($n = 4$–5 litters) in the 2000 ppm group. Levels in the 2000 ppm group were more than twice the levels in the 1000 ppm group (ratio = 2.73). Maternal blood styrene in the 2000 ppm group was more than twice the level in the 1000 ppm group (ratio = 2.44) **[values of 89.05 and 36.44 were reported for maternal blood levels, but units were not provided]**. It seems that data from the 2000 ppm group were presented briefly in a study by Grice et al. (1981).

Kishi et al. (1989) studied placental transfer of styrene in mice. CD-1 mice were given 8-^{14}C-styrene **[purity not specified]** in corn oil by intravenous (i.v.) injection on GD 16 **[plug day not specified]**. Mice were killed at 1 and 30 min and at 1, 2, and 6 hr after dosing for examination by autoradiography. To differentiate between volatile styrene and its non-volatile metabolites, some sections were exposed at −70°C to prevent volatilization; other sections were heated before autoradiography to drive off the volatile fractions. Separate groups of mice were killed at 5 and 30 min and at 1, 2, 6, and 24 hr after exposure for measurement of total radioactivity associated with styrene plus its metabolites in unsectioned maternal and fetal tissues. At time periods ≤1 hr after exposure, the highest concentrations of unmetabolized styrene were found in maternal lung, kidney, liver, and adipose tissue. At ≥2 hr after exposure, the highest styrene concentrations were observed in maternal lung, liver, kidney, and intestine. Styrene metabolites were detected at the greatest concentrations in lung, liver, kidney, and intestine throughout the exposure period. Styrene and metabolites were detected in fetuses from 5 min to 24 hr after styrene exposure. Within the first hour after exposure, styrene equivalents in whole fetuses, as determined by total radioactivity, were about 3–5 times lower than maternal blood levels, 15–25 times lower than maternal liver levels, and 200–400 times lower than maternal lung levels. Styrene equivalents in fetuses were about equal to levels in amnion and amniotic fluid, but were about half the levels of placenta and uterus within 1 hr after exposure. At ≥2 hr after exposure, styrene equivalents were nearly equal in fetuses, placenta, amniotic fluid, and amnion. Although levels of radioactivity began rapidly declining in maternal tissues within 2 hr after exposure, there were no reductions in fetal radioactivity levels for up to 6 hr after exposure. The study authors concluded that the placenta seems to act as a barrier against styrene, but fetuses seem to metabolize styrene more slowly than the dam.

The review by Brown (1991) discussed studies examining distribution of styrene or its metabolites to reproductive organs. One study reported that gavage dosing of rats with 20 mg/kg bw styrene resulted in peak tissue concentrations in 2 hr for most organs. Peak styrene level in testes (3.5 μg/g) and ovaries (2 μg/g) were similar to levels measured in brain and muscle, but lower than levels measured in liver, pancreas, and kidney. In a second study where mice were intraperitoneally (i.p.) injected with 3.3 mmol/kg bw **[344 mg/kg bw]** 7-^{14}C-styrene, styrene and metabolite levels (measured by HPLC) peaked in testes within 30 min after exposure (Löf et al., 1983). Testicular styrene levels were less than half those measured in subcutaneous (s.c.) adipose tissue, pancreas, liver, and kidney. Testicular concentrations of styrene glycol, a metabolite, were similar to those measured in liver and pancreas but half the levels found in lung and kidney. Conjugated styrene glycol levels were low in testes. The same study reported a linear increase in testicular styrene glycol levels that was not proportional to dose after exposure to 1.1–4.9 mmol/kg bw styrene **[115–510 mg/kg bw]**. A third study in mice did not discuss reproductive organs but reported that the levels of the metabolite styrene oxide were <10% of the styrene concentration in all organs examined. Some correlations were noted between cytochrome P450 activity of organs and styrene oxide levels (Brown, 1991).

2.1.2.3 Metabolism: As in humans, the metabolism of styrene in rodents begins with conversion of styrene to styrene 7,8-oxide (styrene oxide) by CYP enzymes and conversion of styrene oxide to styrene glycol by epoxide hydrolase. In rodents, styrene glycol is partially metabolized to mandelic acid and then phenylglyoxylic acid. In contrast to humans, a significant portion of styrene oxide is conjugated to glutathione. The glutathione conjugates are converted to mercapturic acid compounds that are excreted in urine. It is estimated that ≥10% of the styrene dose is converted to mercapturic acid in rats inhaling ≤300 ppm styrene or i.p. injected with ≤250 mg/kg bw styrene and in mice i.p. injected with 400 mg/kg bw styrene (IARC, 2002). A study by Truchon et al. (IARC, 2002) reported dose-dependent excretion of mercapturic acid compounds in rats inhaling 25–250 ppm styrene for 6 hr/day, 5 days/week, for 4 weeks. The predominant urinary metabolites in rats are mandelic acid, phenylglyoxylic acid, hippuric acid, and glucuronide (ATSDR, 1992) **[there was no discussion of glucuronide formation by ATSDR, and it is possible they meant glutathione conjugation to form mercapturic acid compounds]**.

Although not described as a major pathway by IARC (2002), the pathway involving conversion of styrene to 1- and 2-phenylethanol and ultimately to hippuric acid was described as more important in experimental animals than humans. Ring epoxidation to form vinylphenol seems to be a minor pathway in rodents. One study

reviewed by IARC reported that exhaled $^{14}CO_2$ represented 6.4–8.0% of the retained ring-U-^{14}C-styrene dose in mice and 2% of the retained dose in rats; the observation led study authors to postulate a possible pathway involving ring hydroxylation followed by ring opening.

According to ATSDR (1992), studies examining in vitro hepatic metabolism reported that mice have greater ability to produce styrene oxide than rats, but both rats and mice have a greater capacity to produce styrene oxide than humans. Both rodent species were found to be less effective than humans at converting styrene oxide to styrene glycol. IARC (2002) reported that V_{max} ratios for epoxide hydrolase versus styrene monooxygenase are 3.9 in rat and 1.4 in mouse. **[The Expert Panel notes that this indirect evidence suggests that the risk of styrene oxide-related toxicity may be less for humans that for experimental animals.]**

IARC (2002) reviewed several studies indicating that the primary CYP isoforms involved in styrene metabolism in mice are CYP2E1 in liver and lung and CYP2F2 in lung. In contrast to humans, CYP2B was found to have a minor role in styrene metabolism.

Examination of metabolism in different rodent pulmonary cells found high styrene metabolism activity by Clara cells but little to no activity in Type II pneumocytes (IARC, 2002). Styrene metabolism by mouse Clara cells was found to be several fold higher than metabolism by rat Clara cells. Microsomes from nasal epithelium of rats and mice were also observed to metabolize styrene, and activity was similar in microsomes from rats and mice. As described above, microsomes from human nasal epithelium were not found to have styrene-metabolizing activity. IARC reports that metabolism of styrene oxide by epoxide hydrolase and glutathione-*S*-transferase is higher in nasal tissues of rats than mice.

Brown (1991) reviewed studies evaluating activity of styrene metabolizing enzymes in reproductive organs. CYP activity was reported to be low in testes and ovaries of rats. In contrast, rat and mouse testes were found to be abundant in epoxide hydrolase and in rat testes, epoxide hydrolase activity was second only to that in liver. Although epoxide hydrolase activity is not as high in rat ovaries, it was still considered to be significant and similar to that of lung and kidney. Glutathione-*S*-epoxide transferase activity in rat ovary and testis is about 60–70% that in adult rat liver. Activities were reported to be increased in testes of immature animals and in ovaries of pregnant or lactating rats.

According to a PBPK model developed by Ramsey and Andersen (1984), saturation of styrene metabolism in mice and rats occurs at concentrations $\geq$200 ppm styrene in air. Below those concentrations, the rate of styrene metabolism is limited by the rate of blood perfusion in metabolizing tissues. The model was verified using actual blood concentrations of styrene in humans and rats. Several studies reviewed dose-related toxicokinetics in rodents. A nonlinear increase in blood styrene levels was noted in rats inhaling 80–1200 ppm styrene, and evidence of saturated metabolism was observed after 6 hr (Ramsey and Young, 1978). A 112-fold increase in styrene area under the time versus concentration curve (AUC) was observed from 80–1200 ppm styrene. In a styrene inhalation study comparing male mice and rats, metabolic capacity was limited at concentrations $\geq$300 ppm and was saturated at concentrations of $\sim$700 ppm in rats and 800 ppm in mice. In a study by Cruzan et al. (1998), one section stated that blood levels of styrene and styrene oxide were proportional to dose in rats inhaling 50–1000 ppm styrene; another section of the report stated that some degree of saturation was noted between 200–1000 ppm styrene. A study in mice reported that blood styrene and styrene oxide levels were proportional to dose at styrene vapor concentrations of 20–160 ppm (Cruzan et al., 2001). Blood levels are summarized in Table 16.

A more recent mode of action based PBPK model by Sarangapani et al. (2002) compared dosimetry of styrene and styrene oxide in the lung of humans, rats, and mice, which has been stated to be more relevant to toxicity than blood levels (Cruzan et al., 2002). The model is discussed in detail in Section 2.4.3.

Some studies examining stereo-selective metabolism were reviewed by IARC (2002). In studies examining metabolism by liver or lung microsomes, larger amounts of styrene were converted to *S*-styrene oxide by rats and to *R*-styrene oxide by mice.

2.1.2.4 Elimination: As in humans, styrene elimination in rats and mice occurs mainly through biphasic urinary excretion of metabolites (ATSDR, 1992).

2.2 General Toxicity

2.2.1 Human data. The summary of systemic toxicity in humans is primarily based on reviews conducted by ATSDR (1992), an IARC Working Group (IARC, 2002), and the Harvard Panel (Cohen et al., 2002).

ATSDR (1992) noted that exposure of the general public to high levels of styrene in air is unlikely, and illness or injury due to styrene exposure is most commonly reported in workers. Exposure to significant amounts of styrene through the oral or dermal routes is not commonly reported in workers or the general public. Information on human general toxicity is based on occupational and controlled laboratory studies; the studies indicate that the respiratory system and CNS are primarily affected by styrene exposure.

Irritation of the eyes and upper respiratory tract and CNS depression are the acute effects of styrene exposure reported most commonly. In the workplace, irritation was reported at styrene levels exceeding 105 mg/m^3 **[24 ppm]** (IARC, 2002). Harvard noted that styrene-induced symptoms of acute CNS depression are similar to those observed with many solvents and include drowsiness, dizziness, headache, and impaired balance; CNS depression is typically reported in workers exposed to $>$100 ppm styrene (Cohen et al., 2002).

Other neurological signs reported in workers or clinical study volunteers were alterations in nerve conduction, neurobehavioral test performance, electroencephalogram results, and psychiatric function. Harvard noted that studies examining neurobehavioral effects were inconsistent, poorly controlled, and lacking in dose-response information, but overall they suggested slower reaction time, impaired short term memory, and reduced manual dexterity (Cohen et al., 2002). Impaired color vision discrimination was reported in styrene-exposed workers. Impaired color vision discrimination was noted at air styrene levels at and above 20–50 ppm (Cohen et al., 2002; Benignus et al., 2005). One study

reported that vision effects were correlated with urinary levels of the styrene metabolite mandelic acid (Cohen et al., 2002; IARC, 2002). A study reviewed by IARC found decreased color discrimination to be reversible in 4 weeks, whereas a study reviewed by Harvard did not find the effect to be reversible. Hearing loss has also been reported in styrene-exposed workers. However, study descriptions presented by Harvard and IARC suggest that the effect is observed primarily in inadequately controlled studies and not in studies that control for confounders such as background noise. According to IARC, neurological effects usually occur at styrene concentrations exceeding 100 ppm but have been reported with exposures as low as 10–30 ppm.

A review by Rebert and Hall (1994), sponsored by the Styrene Information and Research Center, disputed claims that neurotoxicity occurs at concentrations below 50 ppm styrene. The review concluded that positive findings in many neuroepidemiological studies were due to Type I statistical error, confounding by factors other than styrene, and misinterpretation of the data. Authors concluded "Despite the study of workers exposed for many years, no indications of persisting damage to the nervous system were evident from this review." A meta-analysis of human neurobehavioral effects of long term exposure to styrene, performed at EPA, pooled exposure and outcome data from studies on reaction time and color discrimination (Benignus et al., 2005). The authors estimated that 8 work-years of exposure to styrene at 20 ppm would produce a 6.5% decrease in choice reaction time, which they considered significant in terms of potential effects on traffic accident rates. They estimated that the same exposure would decrease color discrimination to an extent equivalent to an additional 1.7 years of age in men.

IARC (2002) noted styrene-induced changes in lymphocyte numbers and alterations in T-lymphocyte response in workers. Based on limited evidence of lymphocyte effects in workers and effects in experimental animal studies, Harvard concluded that styrene should be investigated further as a potential immunotoxicant (Cohen et al., 2002).

2.2.2 Experimental animal data. IARC (2002) reported that acute inhalation exposure to a single high concentration of styrene resulted in weakness, unsteadiness, loss of consciousness, and death in rats. LD50s reported in the styrene Registry of Toxic Effects of Chemical Substances (RTECS) file (NIOSH, 2004a) are listed in Table 17.

Table 17
LD_{50}s Reported for Styrene Exposures[a]

Species	Route	LD_{50}
Mouse	Inhalation	21,000 mg/m^3/2 hr **[4830 ppm/2 hr]**
Mouse	Inhalation	9,500 mg/m^3/4 hr **[2185 ppm/4 hr]**
Mouse	i.p.	660 mg/kg bw
Mouse	i.v.	90 mg/kg bw
Mouse	Oral **[gavage assumed]**	316 mg/kg bw
Rat	Inhalation	11,800 mg/m^3/4 hr **[2710 ppm/4 hr]**
Rat	i.p.	898 mg/kg bw
Rat	Oral **[gavage assumed]**	2650–5000 mg/kg bw

[a]RTECS (NIOSH, 2004b).

The most recent and thorough reviews of styrene-induced systemic toxicity in experimental animals were conducted by an IARC working group (IARC, 2002) and the Harvard Panel (Cohen et al., 2002). The reviews identified liver and the nervous, respiratory, and immune systems as targets of styrene-induced toxicity in rodents.

Evidence of neurological effects was observed in repeat dose studies reviewed by IARC (2002) and, to a limited extent, Harvard (Cohen et al., 2002). Repeated inhalation exposures to styrene have resulted in decreased brain dopamine levels and increased homovanillic acid levels in rabbits ($\geq$750 ppm), depletion of retinal dopamine levels in rats (300 ppm), increased brain glial fibrillary acidic protein in rats (320 ppm), and mild neurobehavioral disturbances in rats (1400 ppm) and mice (425 ppm). Neurobehavioral tests conducted in rodents exposed to styrene during prenatal or postnatal development are addressed in Section 3.2. Neurological effects observed with repeated oral exposures included decreased brain monoamine oxidase levels (1200 mg/kg bw) and decreased brain dopamine and metabolite levels and loss of motor function in rats (0.5 mg/kg bw). Hearing impairment was observed in rats after repeated inhalation exposure to 850 ppm styrene but not to 200 ppm styrene in a second study. Styrene (2 mM) and styrene oxide (0.2 mM) were cytotoxic to neuronal and non-neuronal cells in cultures of dissociated murine spinal cord-dorsal root ganglia-skeletal muscle. **[It is not clear if many of the studies examining neurotoxicity used multiple dose levels, and in some cases effects were observed at the lowest dose level. Therefore it seems that a LOAEL and NOAEL cannot be identified for neurotoxicity in experimental animals.]** In a brief review of experimental animal neurotoxicity studies sponsored by the Styrene Information and Research Center, it was concluded that few studies exposed the animals to $<$300 ppm styrene (Rebert and Hall, 1994).

Styrene produces respiratory toxicity in mice and rats (Cruzan et al., 1997), reviewed by IARC (2002) and Harvard (Cohen et al., 2002). Nasal lesions were observed after repeated inhalation exposure of both rats and mice. Lesions were characterized by disorganization and atrophy of olfactory mucosa in mice and rats and basal cell proliferation in rats. Mice had more extensive lesions characterized by hyperplasia of submucosal glands, complete loss of olfactory cells, scarring, and replacement of olfactory cells with ciliated columnar epithelial cells. Nasal lesions were observed after chronic exposure of rats to 50 ppm and mice to 20 ppm; the lesions were thought to result from conversion of styrene to styrene oxide by nasal epithelium, a process that does not seem to occur in humans. Repeated inhalation exposure to styrene caused pulmonary lesions in mice but not rats. Pulmonary toxicity in mice began with decreased eosinophilia followed by hyperplasia of terminal bronchiolar epithelium that later extended into alveolar ducts. Pulmonary lesions in mice were observed after subchronic exposure to 50 ppm styrene and chronic exposure to 20 ppm styrene.

Hepatic toxicity characterized by glutathione depletion, changes in liver enzyme activities, or necrosis were noted in studies reviewed by IARC (2002). Mice were

more sensitive than rats, and the lowest repeated inhalation dose reported to cause hepatic toxicity was 50 ppm. Cruzan et al. (1997) reported hepatic toxicity characterized by inflammation and hepatocyte loss in mice exposed to 200 ppm styrene vapors for 13 weeks. Signs of pancreatic damage were observed in mice and rats repeatedly dosed with 25 and 50 mg/kg bw styrene orally in one study (IARC, 2002).

Evidence of possible immunotoxicity was noted in studies reviewed by IARC (2002) and Harvard (Cohen et al., 2002). Styrene suppressed mouse splenic T-lymphocyte killer cell activity in vitro. Repeated oral exposure of mice to styrene doses of 25–50 mg/kg bw/day impaired humoral and cell-mediated immunity and reduced resistance to viral and malarial infections. Resistance to hookworm infections was reduced in rats treated with 295 mg/kg bw/day.

2.3 Genetic Toxicity

The most complete reviews of genetic toxicity were conducted by the Harvard Panel (Cohen et al., 2002) and IARC Working Group (2002). The Harvard review is based largely on a review by Scott and Preston (1994). The most recent review reported only in vivo genetic toxicity findings in animals and was sponsored by the European Chemical Industry Council (Speit and Henderson, 2005).

Harvard reported that styrene does not interact with DNA, but its metabolite, styrene oxide, forms stable adducts with DNA in humans and laboratory animals (Cohen et al., 2002). Styrene oxide binds to guanine at N^7, N^2, and O^6 positions. The O^6 adduct, which represents 3.7% of adduct formation, is reported to be persistent and tends to accumulate after chronic exposures, but not after a single high-level exposure. Persistent O^6 adducts were detected in lymphocytes of styrene workers. The adducts indicate styrene exposure, but implications regarding possible health effects are not known. Increased frequencies of DNA strand breaks were observed in white blood cells of workers exposed to styrene (Table 18). It seems that the breaks are quickly repaired with findings at the end of a work shift being absent at the beginning of the next day's work shift (Walles et al., 1993; as cited by Cohen et al., 2002).

The majority of in vitro studies examining the effects of styrene on chromosomal aberrations and sister chromatid exchanges (SCE) resulted in positive findings in human and experimental animal cells (Table 19). However, it seems that results are affected by the metabolic system utilized. Effects seem to be mitigated if conversion to styrene oxide is slowed or blocked or if styrene oxide detoxification proceeds rapidly. Harvard (Cohen et al., 2002) evaluated numerous studies demonstrating that styrene oxide is more consistently genotoxic than styrene, and effects are generally observed with lower concentrations of styrene oxide than styrene **[styrene oxide data not shown in CERHR report]**.

According to Harvard (Cohen et al., 2002), the majority of styrene in vivo animal studies result in negative chromosomal aberration and micronuclei findings but positive SCE findings (Table 20). Genotoxicity occurs only at high dose levels and usually with more than 1 day of exposure. Harvard noted that exposure levels in experimental animal studies were higher than expected human exposures, but duration of exposure was days or weeks, a time period much shorter than many potential human exposures.

Variable results were obtained in human studies that examined the effects of occupational styrene exposure on chromosomal aberrations, SCEs, and micronuclei frequencies (Table 18). In evaluating the cytogenetic endpoints, Scott and Preston (1994) concluded that there were no relationships between exposure levels and positive or negative responses, there was no convincing evidence of positive dose–response relationships, the human studies produced opposite findings (i.e., greater sensitivity to aberrations and reduced sensitivity to SCEs) to those of in vitro studies, and the effects of confounders could not be ruled out. Possible confounders noted by Harvard (Cohen et al., 2002) included smoking and occupational exposures to other chemicals (e.g., organic peroxides, dichloromethane, hydroquinone, dimethylaniline, and maleic anhydride). Harvard noted that variability among occupational studies has resulted from artifacts arising from chance and design differences. Harvard examined the studies further by conducting additional statistical evaluations, such as regression analyses. They noted that four studies demonstrated evidence of a positive association between frequency of chromosomal aberrations and styrene exposure (Fleig and Theis, 1978; Cammuri et al., 1983; Artuso et al., 1995) or urinary mandelic acid level (Pohlova et al., 1985), but an association was not observed in most studies. The Harvard Panel concluded that evidence of an association between occupational styrene exposure and chromosomal aberrations is compelling, whereas the association with SCEs is less certain. None of studies provided evidence of an association between styrene exposure and micronuclei frequency.

Harvard reported that mutagenicity studies in several strains of *Salmonella* usually resulted in negative findings in the absence of metabolic activation and occasionally positive results with metabolic activation (Cohen et al., 2002). IARC (2002) reported that increased mutations occurred only in strains TA1530 and TA1535, with metabolic activation. According to Harvard, styrene oxide was usually found to be mutagenic in *Salmonella*. Results of mutagenicity assays in experimental animals are variable, possibly due to conditions affecting generation or detoxification of styrene oxide.

Harvard (Cohen et al., 2002) concluded, ''Studies of genotoxicity of styrene have found it to be a negative or weakly positive mutagen in vitro, although it does show some clastogenic activity. Styrene does not seem to bind covalently to cellular macromolecules, although styrene oxide does. Styrene oxide is mutagenic in vitro.''

2.4 Carcinogenicity

2.4.1 Human. The most recent and complete evaluations of cancer hazard in humans exposed to styrene were conducted by a Harvard Panel (Cohen et al., 2002) and IARC (2002). Both groups evaluated occupational studies. IARC also reviewed a limited study in individuals who attended a high school next to a styrene butadiene manufacturing plant. The occupational studies reviewed by Harvard and IARC were conducted in workers employed in industries manufacturing reinforced plastics, styrene or polystyrene, and styrene

Table 18
In Vivo Genetic Toxicity Studies of Styrene in Occupationally Exposed Humans[a]

Styrene dose (urinary mandelic acid)[b]	Years exposure duration	Tissue or cell	Endpoint	Result	Study
NS	NS	Mononuclear leukocytes	DNA strand breaks	↑ correlated with air styrene levels and years of exposure	Somorovska et al., 1999
~70 ppm (estimated from blood metabolite levels)	NS	NS	DNA strand breaks	↑	Maki-Paakkanen et al., 1991
NS	NS	Leukocytes	DNA strand breaks	↑ at end of shift but not the following morning	Walles et al., 1993
NS	NS	Lymphocytes	DNA strand breaks	↑ correlated with level of O^6 adducts	Vodicka et al., 1995, 1999
		Lymphocytes	HPRT mutations	↔ to weak ↑, not correlated with level of O^6 adducts	
Mean TWA of 37 ppm	NS	Erythrocytes	Mutations in glycophorin A	↑ 20 ppm	Bigbee et al., 1996
NS (mean = 328)[c]	10–22	Lymphocytes	Chromosomal aberrations	↑	Anwar and Shamy, 1995
0.5–28 ppm (NS)	NS	Lymphocytes	Chromosomal aberrations	↔	Artuso et al., 1995
20–326 ppm (NS)	NS	Lymphocytes	Chromosomal aberrations	↑	Artuso et al., 1995
27–199 mg/m^3 **[6–46 ppm]** (NS)	14	Lymphocytes	Chromosomal aberrations	↑	Somorovska et al., 1999
≤300 ppm (23–3257)[c]	0.6–8.5	Lymphocytes	Chromosomal aberrations	↑	Meretoja et al., 1977
≤300 ppm (23–3257)[c]	1–15	Lymphocytes	Chromosomal aberrations	↑	Meretoja et al., 1978
≤10 ppm (19–40)	14–25	Lymphocytes	Chromosomal aberrations	↔	Meretoja et al., 1978
0–47 ppm (<5–100)	3–39	Lymphocytes	Chromosomal aberrations	↔	Fleig and Thiess, 1978
50–300 ppm (42–1500)[c]	2–24	Lymphocytes	Chromosomal aberrations	↑	Fleig and Thiess, 1978
14–192 ppm (225–2100)[c]	0.5–10	Lymphocytes	Chromosomal aberrations	↑	Hogstedt et al., 1979
0.7–178 ppm (0–320)	4–27	Lymphocytes	Chromosomal aberrations	↔	Theiss et al., 1980
0–232 ppm (NS)	0.3–12	Lymphocytes	Chromosomal aberrations	↑	Anderson et al., 1980
1–211 ppm (9–4300)	0.6–9.3	Lymphocytes	Chromosomal aberrations	↔	Watanabe et al., 1981
40–50 ppm (0–1041)	0.2–30	Lymphocytes	Chromosomal aberrations	↑ (borderline significance)	Watanabe et al., 1983
Mean = <23 ppm (measurement not accurate) (NS)	1–30	Lymphocytes	Chromosomal aberrations	↑	Dolmierski et al., 1983
7–96 ppm (45–1440)	1–22	Lymphocytes	Chromosomal aberrations	↑	Cammurri et al., 1983, 1984
2–44 ppm (NS)		Lymphocytes	Chromosomal aberrations	↑	Hansteen et al., 1984
Mean = 24 ppm (<152–304)	1–26	Lymphocytes	Chromosomal aberrations	↔	Nordenson and Beckman, 1984
<0.1–1.4 ppm (NS)	NS	Lymphocytes	Chromosomal aberrations	↔	Van Sittert and de Jong, 1985
1–236 ppm (35–972)	1–11	Lymphocytes	Chromosomal aberrations	↑ at 1 or 2 sample times	Pohlova and Stram, 1985
9–132 ppm (40–3000)	1–11	Lymphocytes	Chromosomal aberrations	↑ at 1 or 2 sample times	Pohlova and Stram, 1985
8–63 (possibly as high as 145 ppm based on earlier measurements) (0–1064)	1–25	Lymphocytes	Chromosomal aberrations	↔	Maki-Paakkanen, 1987
NS (NS)	4.5±3.4 (SD)	Lymphocytes	Chromosomal aberrations	↑ in chromatid breaks and exchanges but not total aberrations	Forni et al., 1988
28–140 ppm (NS)	Mean = 10	Lymphocytes	Chromosomal aberrations	↔	Jablonicka et al., 1988
1–38 ppm (NS)	0.1–25.4	Lymphocytes	Chromosomal aberrations	↔	Hagmar et al., 1989
NS (≤3268)	Mean = 6.4	Lymphocytes	Chromosomal aberrations	↔	Hagmar et al., 1989
NS (≤2523)	Mean = 7.2	Lymphocytes	Chromosomal aberrations	↑	Maki-Paakanene, 1991

Mean = 70 ppm estimated from urinary metabolite levels (≤3268)	Mean = 6.7	Lymphocytes	Chromosomal aberrations	↔	Maki-Paakanene, 1991
5–182 ppm (NS)	NS	Lymphocytes	Chromosomal aberrations	↔	Sorsa et al., 1991
1–133 ppm (NS)	NS	Lymphocytes	Chromosomal aberrations	↔	Sorsa et al., 1991
5–24 ppm (46–345)[c]	1–18	Lymphocytes	Chromosomal aberration	↔	Tomanin et al., 1992
27–104 ppm (423–1325)[c]	1.5–15	Lymphocytes	Chromosomal aberrations	↑	Tomanin et al., 1992
0–140 ppm (NS)	NS	Lymphocytes	Chromosomal aberrations	↑	Tates et al., 1994
125–180 mg/m^3 **[29–41 ppm]** (mean = 652)	1–26	Lymphocytes	SCE	↑	Hallier et al., 1994
2.2–111 mg/m^3 **[0.51–26 mg/m^3]** (11–649)[c]	Mean = 2.9	Lymphocytes	SCE	↔	Van Hummelen et al., 1994
0.5–28 ppm (NS)	NS	Lymphocytes	SCE	Weak ↑	Artuso et al., 1995
20–326 ppm (NS)	NS	Lymphocytes	SCE	↑	Artuso et al., 1995
85–1280 mg/m^3 **[20–300 ppm]** (14–1482)[d]	Mean = 9.9	Lymphocytes	SCE	↑	Karakaya et al., 1997
≤300 ppm (23–3257)[c]	1–15	Lymphocytes	SCE	↔	Meretoja et al., 1978
0–232 ppm (NS)	0.3–12	Lymphocytes	SCE	↑	Anderson et al., 1980
1–211 ppm (90–4300)	0.6–9.3	Lymphocytes	SCE	↔	Watanabe et al., 1981
40–50 ppm (0–1041)	0.2–30	Lymphocytes	SCE	↔	Watanabe et al., 1983
7–96 ppm (45–1440)	1–22	Lymphocytes	SCE	↑	Cammurri et al., 1983, 1984
2–44 ppm (NS)	NS	Lymphocytes	SCE	↔	Hansteen et al., 1984
8–63 (possibly as high as 145 ppm based on earlier measurements) (0–1057)	1–25	Lymphocytes	SCE	↔	Maki-Paakkanen, 1987
1.7–131 ppm (mean = 275)[c]	Mean = 8.6	Lymphocytes	SCE	↔	Kelsey et al., 1990
5.8–130 ppm (mean = 323)[c]	Mean = 7.2	Lymphocytes	SCE	↔	Kelsey et al., 1990
NS (mean 3268)[c]	Mean = 6.4	Lymphocytes	SCE	↔	Kelsey et al., 1990
NS (≤2523)	Mean = 7.2	Lymphocytes	SCE	↔	Maki-Paakkanen, 1991
Mean = 70 ppm, estimated from urinary metabolite levels (≤3268)	Mean = 6.7	Lymphocytes	SCE	↔	Maki-Paakkanen, 1991
1–44 ppm (96–2495)[c]	Mean = 2.7	Lymphocytes	SCE	↔	Brenner et al., 1991
5–182 ppm (NS)	NS	Lymphocytes	SCE	↔	Sorsa et al., 1991
1–133 ppm (NS)	NS	Lymphocytes	SCE	↔	Sorsa et al., 1991
0.2–56 ppm (NS)	0.5–27	Lymphocytes	SCE	↑	Yager et al., 1993
0.2–56 ppm (NS)	0.5–27	Lymphocytes	SCE	↑	Rappaport et al., 1996
2.2–111 mg/m^3 **[0.51–26 mg/m^3]** (11–649)[c]	Mean = 2.9	Lymphocytes	Micronuclei	↔	Van Hummelen et al., 1994
NS (mean = 328)	10–22	Lymphocytes	Micronuclei	↔	Anwar and Shamy, 1995
85–1280 mg/m^3 **[20–294 ppm]** (14–1482)[d]	Mean = 9.9	Lymphocytes	Micronuclei	↔	Karakaya et al., 1997
≤300 ppm (23–3257)[c]	0.6–8.5	Lymphocytes	Micronuclei	↑	Meretoja et al., 1977
1–40 ppm (9–316)[c]	1–23	Lymphocytes	Micronuclei	↑	Hogstedt et al., 1983
Mean = 24 ppm (<152–304)	NS	Lymphocytes	Micronuclei	↑	Nordenson and Beckman, 1984
8–63 ppm (possibly as high as 145 ppm based on earlier measurements) (0–1064)	1–25	Lymphocytes	Micronuclei	↔	Maki-Paakkanen, 1987
1–38 ppm (NS)	0.1–25.4	Lymphocytes	Micronuclei	↔	Hagmar et al., 1989
1–44 ppm (96–2495)[c]	Mean = 2.7	Lymphocytes	Micronuclei	↑	Brenner et al., 1991

Table 18
(Continued)

Styrene dose (urinary mandelic acid)[b]	Years exposure duration	Tissue or cell	Endpoint	Result	Study
NS (< 3268)	Mean = 6.4	Lymphocytes	Micronuclei	↔	Brenner et al., 1991
NS (≤ 2523)	Mean = 7.2	Lymphocytes	Micronuclei	↔	Maki-Paakanene, 1991
Mean = 70 ppm, estimated from urinary metabolite levels (≤ 3268)	Mean = 6.7	Lymphocytes	Micronuclei	↔	Maki-Paakanene, 1991
5–182 ppm (NS)	NS	Lymphocytes	Micronuclei	↔	Sorsa et al., 1991
1–133 ppm (NS)	NS	Lymphocytes	Micronuclei	↔	Sorsa et al., 1991
5–24 ppm (46–345)[c]	1–18	Lymphocytes	Micronuclei	↔	Tomanin et al., 1992
27–104 ppm (423–1325)[c]	1.5–15	Lymphocytes	Micronuclei	↔	Tomanin et al., 1992
0.2–56 ppm (NS)	0.5–27	Lymphocytes	Micronuclei	↔	Yager et al., 1991

[a]Harvard (Cohen et al., 2002), Scott and Preston (1994), and IARC (2002) reviews.
[b]Expressed as mg/L unless otherwise specified.
[c]Expressed as mg/g creatinine.
[d]Expressed as mandelic acid + phenylglyoxylic acid in mg/g creatinine.
↑, significant increase; ↔, no effect of treatment; HPRT, human hypoxanthine-guanine phosphoribosyltransferase gene; NS, not specified; TWA, time weighted average.

butadiene rubber. Studies in reinforced plastics workers were judged to be most informative because styrene exposures were generally the highest, and there was less confounding by other chemical exposures. Harvard noted that exposure to workers in reinforced-plastics industries are generally highest because open processes are used to manufacture products, in contrast to enclosed processes usually employed in styrene/polystyrene and styrene butadiene rubber production. In addition, there is less probability of confounding by other potential chemical exposures, such as benzene, ethylbenzene, and acrylonitrile in styrene production, and butadiene in the manufacture of styrene butadiene rubber. However, IARC identified shorter job tenure as a limitation of studies in reinforced-plastics workers. Because the studies in reinforced-plastics workers are most informative, they are summarized in Table 21.

Although not consistently observed among different studies, lung/respiratory and lymphatic/hematopoietic cancers were most often reported in reinforced plastics workers. However, Harvard noted that increases in lung/respiratory cancers were often not related to estimated exposure level or duration and may have been confounded by smoking (Cohen et al., 2002). Interpretation of lymphatic/hematopoietic cancers was difficult due to low numbers of observed and expected deaths, possible confounding, lack of consistent dose-response patterns, and small size of subgroups with high level and duration of exposure. IARC (2002) concluded that findings were not robust and may have been due to chance or bias, in addition to confounding. Harvard noted that generally the epidemiological studies were subject to bias from inadequate information on exposure and cancer diagnoses. For example, some of the studies in reinforced plastics workers lacked exposure information. In other studies, exposures could only be roughly estimated due to limited numbers of available measurements, especially for earlier time periods. Some of the studies ascertained tumor types from death certificates, which may have resulted in less accurate diagnosis compared to studies obtaining information from cancer registries. It was stated that biases could have obscured associations (i.e., favored the null hypothesis). Large confidence intervals in all studies suggested a high probability of chance findings.

The Harvard Panel (Cohen et al., 2002) concluded "…the balance of epidemiological evidence that is currently available does not suggest a hazard of cancer in humans from exposure to styrene. However, the possibility of a small elevation of risk for one or more cancers cannot be ruled out…the relevance of the mouse bioassay data to humans cannot be ruled out." Experimental animal studies are discussed below.

IARC (2002) concluded, "There is limited evidence in humans for the carcinogenicity of styrene."

2.4.2 Experimental animal. The most recent and complete evaluations of styrene animal cancer bioassays were conducted by a Harvard Panel (Cohen et al., 2002) and IARC (2002). Studies included oral and inhalation exposures. Rat studies are summarized in Table 22 and mouse studies in Table 23.

There was no evidence of increased tumor incidence after oral exposure of rats. Two studies in rats demonstrated increases in mammary tumors, but Harvard (Cohen et al., 2002) considered the results to be equivocal

Table 19
In Vitro Genetic Toxicity Studies of Styrene[a]

Species or cell type/strain	Concentration mM (duration in hr)	Metabolic activation	Endpoint	Result	Original study
Human/whole blood	2.6 (72)	No	Chromosomal aberrations	↑	Linnainmaa et al., 1978
	0.0005–0.5 (24)	No	Chromosomal aberrations	↑ at 0.5 mM	Pohlova et al., 1985
	0.5–6.0 (24)	No	Chromosomal aberrations	↑ ≥2.0 mM	Jantunen et al., 1986
	1.0–4.0 (24)	No	Chromosomal aberrations	↑ ≥1.0 mM	Jantunen et al., 1986
	0.3–4.0 (24)	No	SCEs	↑ ≥0.7 mM	Norpa et al., 1980
	0.5–4.0 (48)	No	SCEs	↑ ≥0.5 mM	Norpa et al., 1983
	2.6 (72)	No	Micronuclei	↑	Linnainmaa et al., 1978
	2.6 (72)	No	Hypoploidy	↑	Linnainmaa et al., 1978
Human/ lymphocytes	0.5–4.0 (48)	No	SCEs	↑ ≥2.0 mM	Norpa et al., 1983
	NS	No	SCEs	↑ at 1 μg/mL **[0.01 mM]**	Chakrabarti et al., 1993[b]
	NS	No	SCEs	↑ at 52 μg/mL **[0.5 mM]**	Lee and Norpa, 1995[b]
Rat/whole blood	0.5–1.0 (48)	No	SCEs	↑ at ≥0.5 mM	Norpa et al., 1983
Chinese hamster/ lymphocyte	2.4 (3)	No	Chromosomal aberration	↔	Matsuoka et al., 1979
	2.4 (3)	Yes, MR	Chromosomal aberration	↑	Matsuoka et al., 1979
Chinese hamster/ ovary	≤8.7 (1)	No	SCEs	↔	DeRaat et al., 1978
	≤8.7 (1)	Yes, PR	SCEs	↔	DeRaat et al., 1978
	4.4–8.7 (1)	Yes, PR+ CO	SCEs	↑ at ≥4.4 mM	DeRaat et al., 1978
	1.0–15.0 (4)	No	SCEs	↔	Norppa and Tursi, 1984
	2.0–12.0 (24)	No	SCEs	↔	Norppa and Tursi, 1984
	12.0–15.0 (34)	No	SCEs	↑ at ≥12.0 mM	Norppa and Tursi, 1984
	1.0–20.0 (4)	Yes, CR	SCEs	↔	Norppa and Tursi, 1984
	2.0–12.0 (24)	Yes, E	SCEs	↑ at ≥8 mM	Norppa and Tursi, 1984
	12.0–15.0 (34)	Yes, E	SCEs	↑ at ≥12 mM	Norppa and Tursi, 1984
Allium cepa	0.87–4.35 (2–12)	No	Chromosomal aberrations	↑ at ≥0.87 mM	Linnainmaa et al., 1978
	0.87–1.74 (2–12)	No	Micronuclei	↑ at 1.74 mM	Linnainmaa et al., 1978
	0.87–1.74 (2–12)	No	Anaphase aberrations	↑ at ≥0.87 mM	Linnainmaa et al., 1978
Salmonella typhimurium	52 μg/mL	Yes	Reverse mutation	TA100 weakly+	Vainio et al., 1976[b]
	0.5 μg/mL	Yes	Reverse mutation	TA1535+	Vainio et al., 1976[b]
	52 μg/mL	Yes	Reverse mutation	TA1537–	Vainio et al., 1976[b]
	52 μg/mL	Yes	Reverse mutation	TA1538–	Vainio et al., 1976[b]
	52 μg/mL	Yes	Reverse mutation	TA98–	Vainio et al., 1976[b]
	0 μg/mL [sic]	Yes	Reverse mutation	TA100–	de Meester et al., 1977[b]
	52 μg/mL	Yes	Reverse mutation	TA1535+	de Meester et al., 1977[b]
	0 μg/mL	Yes	Reverse mutation	TA1537–	de Meester et al., 1977[b]
	0 μg/mL	Yes	Reverse mutation	TA1538–	de Meester et al., 1977[b]
	0 μg/mL	Yes	Reverse mutation	TA98–	de Meester et al., 1977[b]
	500 μg/mL	Yes	Reverse mutation	TA100–	Stolz and Withey, 1977[b]
	500 μg/mL	Yes	Reverse mutation	TA1535–	Stolz and Withey, 1977[b]
	500 μg/mL	Yes	Reverse mutation	TA1537–	Stolz and Withey, 1977[b]
	500 μg/mL	Yes	Reverse mutation	TA1538–	Stolz and Withey, 1977[b]
	500 μg/mL	Yes	Reverse mutation	TA98–	Stolz and Withey, 1977[b]
	250 μg/mL	Yes	Reverse mutation	TA100–	Watabe et al., 1978[b]
	250 μg/mL	Yes	Reverse mutation	TA1535–	Watabe et al., 1978[b]
	250 μg/mL	Yes	Reverse mutation	TA1537–	Watabe et al., 1978[b]
	250 μg/mL	Yes	Reverse mutation	TA1538–	Watabe et al., 1978[b]
	250 μg/mL	Yes	Reverse mutation	TA98–	Watabe et al., 1978[b]
	104 μg/mL	Yes	Reverse mutation	TA100–	Busk, 1979[b]
	104 μg/mL	Yes	Reverse mutation	TA1535–	Busk, 1979[b]
	104 μg/mL	Yes	Reverse mutation	TA1537–	Busk, 1979[b]
	104 μg/mL	Yes	Reverse mutation	TA1538–	Busk, 1979[b]
	104 μg/mL	Yes	Reverse mutation	TA98–	Busk, 1979[b]
	250 μg/mL	Yes	Reverse mutation	TA100–	De Flora, 1979[b]
	250 μg/mL	Yes	Reverse mutation	TA1535–	De Flora, 1979[b]
	250 μg/mL	Yes	Reverse mutation	TA1538–	De Flora, 1979[b]
	250 μg/mL	Yes	Reverse mutation	TA98–	De Flora, 1979[b]
	312 μg/mL	Yes	Reverse mutation	TA100–	Florin et al., 1980[b]
	312 μg/mL	Yes	Reverse mutation	TA1535–	Florin et al., 1980[b]
	312 μg/mL	Yes	Reverse mutation	TA98–	Florin et al., 1980[b]
	521 μg/mL	Yes	Reverse mutation	TA1535+	Poncelet et al., 1980[b]
	1000 ppm in atmosphere	Yes	Reverse mutation	TA100+	de Meester et al., 1981[b]

Table 19
(Continued)

Species or cell type/strain	Concentration mM (duration in hr)	Metabolic activation	Endpoint	Result	Original study
	0.02 ppm in atmosphere	Yes	Reverse mutation	TA1530+	de Meester et al., 1981[b]
	1000 ppm in atmosphere	Yes	Reverse mutation	TA1535+	de Meester et al., 1981[b]
	1000 ppm in atmosphere	Yes	Reverse mutation	TA1537–	de Meester et al., 1981[b]
	1000 ppm in atmosphere	Yes	Reverse mutation	TA1538–	de Meester et al., 1981[b]
	1000 ppm in atmosphere	Yes	Reverse mutation	TA98–	de Meester et al., 1981[b]
	500 μg/mL	Yes	Reverse mutation	TA100–	Brams et al., 1987[b]
	500 μg/mL	Yes	Reverse mutation	TA98–	Brams et al., 1987[b]

[a]Harvard Review (Cohen et al., 2002) and Scott and Preston (1994).
[b]IARC (2002).
↑, significant increase; ↔, no effect of treatment; E, human erythrocytes; CO, cyclohexane oxide (inhibitor of epoxide hydratase); CR S9, from clophen 50-induced rats; MR S9, from 3-methylcholanthrene-induced rats; NS, not specified; PR S9, from phenobarbital-induced rats; SCE, sister chromatid exchange.

due to lack of dose-response relationships. In addition, other studies conducted with higher doses and more animals did not confirm the findings of increased mammary tumors. Similar conclusions regarding carcinogenicity status in rats were reached by Harvard and IARC. Harvard (Cohen et al., 2002) concluded that the rat studies "…do not offer compelling evidence that rats develop tumors in response to styrene exposure." IARC (2002) concluded "Overall, there was no reliable evidence for an increase in tumor incidence in rats."

Both IARC (2002) and Harvard (Cohen et al., 2002) noted that a study by Cruzan et al. (2001) provided evidence of increased pulmonary adenomas in male and female mice and an increase in pulmonary carcinomas in female mice after inhalation exposure to styrene. Two gavage studies demonstrating an increase in pulmonary tumors in mice given styrene were considered inadequate by IARC. IARC (2002) concluded "There is *limited evidence* in experimental animals for the carcinogenicity of styrene."

Harvard (Cohen et al., 2002) also reviewed studies of styrene oxide, a major styrene metabolite, in rats and mice. Three gavage studies in rats and one in mice consistently demonstrated an increase in forestomach tumors after exposure to ≥50 mg/kg bw styrene oxide. Two dermal studies in mice did not demonstrate an increase in tumors. In a previous evaluation, IARC (2002) concluded "…there was *sufficient evidence* in experimental animals for the carcinogenicity of styrene 7,8-oxide."

2.4.3 Mechanisms of carcinogenicity. Possible mechanisms of styrene-induced carcinogenicity were discussed in reviews by a Harvard Panel (Cohen et al., 2002) and an IARC Working Group (2002). According to IARC, two plausible mechanisms of toxicity for tumor formation involve styrene oxide-induced DNA damage and pulmonary cytotoxicity. Genetic toxicity is discussed in detail in Section 2.3. IARC and Harvard noted some evidence that hyperplasia precedes tumor formation in mice. In the most informative cancer study, conducted by Cruzan et al. (2001), an increase in pulmonary hyperplasia was observed in mice after 12 months of styrene inhalation exposure and incidence of lung tumors increased by the end of the study. In a rat study by Cruzan et al. (1998), pulmonary hyperplasia was not increased, and the rats did not develop increased numbers of tumors. Harvard (Cohen et al., 2002) and IARC (2002) reviewed a study demonstrating an increase in pulmonary markers of toxicity (e.g., γ-glutamyltranspeptidase and lactate dehydrogenase) after i.p. injection of mice with styrene oxide; the study suggested that styrene oxide is the chemical species inducing pulmonary cytotoxicity that can lead to cell replication and proliferation.

IARC (2002) and Harvard (Cohen et al., 2002) evaluated interspecies differences in metabolism or toxicokinetics as possible explanations for variable cancer responses between mice and rats. In mice, rats, and humans, styrene is converted to styrene oxide by CYP2E1 and CYP2F2. Articles by Cruzan et al. (2002, 2005a) noted that in the mouse, CYP2F2 is also involved in the production of 4-vinylphenol, a styrene metabolite reported to be more potent than styrene oxide in inducing pulmonary toxicity. Inhibition of CYP2F2 by 5-phenyl-1-pentyne blocked styrene-induced pulmonary toxicity in mice. Compared to rats, pulmonary Clara cells of mice are reported to have higher levels of CYP2E1 and CYP2F2 activity and to produce more *R*-styrene oxide, 4-vinylphenol, and 4-vinylphenol-derived metabolites. Although pulmonary production of styrene oxide is higher in mice, styrene oxide levels are reported to be two orders of magnitude higher in blood of rats compared to mice after exposure to equal concentrations of styrene. IARC noted that cancer risk depends on whether locally produced or extrapulmonary styrene is critical in inducing toxic effects. According to IARC, evidence suggests that locally-produced styrene oxide is the most likely cause of lung tumors in mice, though other mechanisms cannot be ruled out. Harvard reported

Table 20
In Vivo Genetic Toxicity Studies of Styrene in Experimental Animals[a]

Species, sex if specified	Styrene dose (route)	Tissue or Cell	Endpoint	Result	Study
Mouse	177–1050 mg/kg bw (i.p.)	Kidney	DNA strand breaks	↑ as linear function of dose	Walles et al., 1983
C57BL/6 mouse	NS, 1 time dose (i.p.)	Liver, kidney, bone marrow, lymphocytes	DNA strand breaks	↑ at 250 mg/kg bw (350 mg/kg bw in bone marrow)	Vaghef and Hellman, 1998
CD-1 mouse, male, female	500 or 1000 mg/kg bw (oral)	Bone marrow	Chromosomal aberration	↔	Loprieno et al., 1978
CD-1 mouse, male	500 mg/kg bw for 4 days (oral)	Bone marrow	Chromosomal aberration	↔	Sbrana et al., 1983
CD-1 mouse, male	200 mg/kg bw for 70 days (oral)	Bone marrow	Chromosomal aberration	↔	Sbrana et al., 1983
$B6C3F_1$ mouse, female	124–491 ppm (inhalation) 6 hr/day for 14 days	Blood, spleen, lymphocytes	Chromosomal aberration	↔	Kligerman et al., 1992
C57Bl/6 mouse, male	50–1000 mg/kg bw (i.p.)	Bone marrow	Chromosomal aberration	↔	Sharief et al., 1986
BDF_1 mouse, male	565 ppm (inhalation) 6 hr/day for 4 days	Liver	SCE	↑	Connor et al., 1979
BDF_1 mouse, male	565 ppm (inhalation) 6 hr/day for 4 days	Bone marrow	SCE	↑	Connor et al., 1979
BDF_1 mouse, male	104–922 ppm (inhalation) 6 hr/day for 4 days	Liver	SCE	↑ ≥387 ppm	Connor et al., 1980
BDF_1 mouse, male	922 ppm (inhalation) 6 hr/day for 1 or 2 days	Liver	SCE	↑	Connor et al., 1980
BDF_1 mouse, male	104–922 ppm (inhalation) 6 hr/day for 4 days	Bone marrow	SCE	↑ ≥387 ppm	Connor et al., 1980
BDF_1 mouse, male	922 ppm (inhalation) 6 hr/day for 1 day	Bone marrow	SCE	↔	Connor et al., 1980
BDF_1 mouse, male	922 ppm (inhalation) 6 hr/day for 2 days	Bone marrow	SCE	↑	Connor et al., 1980
BDF_1 mouse, male	104–922 ppm (inhalation) 6 hr/day for 4 days	Alveolar macrophage	SCE	↑ ≥387 ppm	Connor et al., 1980
BDF_1 mouse, male	922 ppm (inhalation) 6 hr/day for 1 day	Alveolar macrophage	SCE	↔	Connor et al., 1980
BDF_1 mouse, male	922 ppm (inhalation) 6 hr/day for 2 days	Alveolar macrophage	SCE	↑	Connor et al., 1980
C57Bl/6 mouse, male	50–1000 mg/kg bw (i.p.)	Bone marrow	SCE	↑ ≥500 mg/kg bw	Sharief et al., 1986
$B6C3F_1$ mouse, female	124–491 ppm (inhalation) 6 hr/day for 14 days	Blood, spleen, lung, lymphocytes	SCE	↑ ≥250 ppm	Kligerman et al., 1992
$B6C3F_1$ mouse, female	124–491 ppm (inhalation) 6 hr/day for 14 days	Blood, spleen, lung, erythrocytes	Micronuclei	↔	Kligerman et al., 1992
$B6C3F_1$ mouse, male	50 ppm (inhalation) for 8 hr	Bone marrow polychromatic erythrocytes	Micronuclei	↔	Leavens et al., 1997
NMRI mouse, male	750 and 1500 mg/m^3 **[173 and 345 ppm]** (inhalation) 1, 3, 7, or 21 days	Bone marrow cells	Micronuclei	↑ at 345 ppm for 7 days, ↔ all other time points	Vodicka et al., 2001

Table 20
(Continued)

Species, sex if specified	Styrene dose (route)	Tissue or Cell	Endpoint	Result	Study
NMRI mouse	750 and 1500 mg/m^3 **[173 and 345 ppm]** (inhalation) 6 hr/day for 1, 3, 7, 14, or 21 days	Bone marrow polychromatic erythrocytes	Micronuclei	↔	Engelhardt et al., 2003
C57Bl/6 mouse, male	240–1500 mg/kg bw (i.p.)	Bone marrow polychromatic erythrocytes	Micronuclei	↑ ≥250 mg/kg bw	Norppa, 1981
LACA Swiss mouse	150–600 mg/kg bw (i.p.)	Bone marrow polychromatic erythrocytes	Micronuclei	↑ 600 mg/kg bw	Simula and Priestly, 1992
Wistar rat, male, female	300 ppm (inhalation) 6 hr/day, 5 days/week for 2–11 weeks	Bone marrow	Chromosomal aberration	↑ at 9 weeks	Meretoja et al., 1978
Sprague–Dawley rat	600, 1000 ppm (inhalation) 6 hr/day, 5 days/week for 12 months	Bone marrow	Chromosomal aberration	↔	Sinha et al., 1983
Fisher 344 rat	150–1000 ppm (inhalation) 6 hr/day, 5 days/week, for 4 weeks	Lymphocytes	Chromosomal aberration	↔	Preston and Abernethy, 1993
Wistar rat, male	300 ppm (inhalation) 6 hr/day, 5 days/week for 2–11 weeks	Bone marrow	Aneuploidy	↔	Meretoja et al., 1978
Wistar rat, male	300 ppm (inhalation) 6 hr/day, 5 days/week for 2–11 weeks	Bone marrow	Polyploidy	↑	Meretoja et al., 1978
Fisher 344 rat	150–1000 ppm (inhalation) 6 hr/day, 5 days/week, for 4 weeks	Lymphocytes	SCE	↔	Preston and Abernethy, 1993
Chinese hamster, male	300 ppm (inhalation) 6 hr/day, 5 days/week for 4 days or 3 weeks	Bone marrow	Chromosomal aberration	↔	Norppa et al., 1980
F344 rat, female	125–500 ppm (inhalation) for 14 days	Bone marrow normochromatic erythrocytes	Micronuclei	↔	Kigerman et al., 1993
Porton rat	300–3000 mg/kg bw (i.p.)	Bone marrow polychromatic erythrocytes	Micronuclei	↔	Simula and Priestly, 1992
Chinese hamster, male	1 g/kg (i.p.)	Bone marrow polychromatic erythrocytes or non-polychromatic erythrocytes	Micronuclei	↔	Penttila et al., 1980

[a]Harvard (Cohen et al., 2002), Scott and Preston (1994), IARC (2002), and Speit and Henderson (2005) reviews.
↑, significant increase; ↔, no effect of treatment; F, female; M, male; NS, not specified; SCE, sister chromatid exchange.

Table 21
Summary of Epidemiologic Cancer Studies Conducted in Reinforced-Plastics Industry Workers[a]

Study details	Cancer findings	Notes	Original study
Study of 64,181 workers **[~83% male and 17% female based on original enrollment numbers]** in 552 Danish companies with ≥1% of employees involved in reinforced plastics production since the 1960s. Subjects worked at least 10 hr/week at some point between 1964 and 1988. The follow-up period was from 1970–1989.	Significant positive findings presented as cancer type: SIR, 95% CI: –Respiratory cancer in males: 1.19, 1.09–1.30; –Lung cancer in males: 1.15, 1.04–1.27. Non-significant or negative findings: –Increases for respiratory and lung cancers in females and lymphatic/hematopoietic cancers in males; –Fewer than expected lymphatic/hematopoietic cancers in females.	–Study authors note that a high percentage of cohort may have been unexposed, with ~25% of cohort involved in reinforced plastic production on any given day. –IARC reported that duration of exposures was misclassified for many workers. –IARC noted that majority of women were not involved in reinforced plastic production. –Study authors cautioned about interpretation of results due to possible confounding by other occupational exposures or smoking and no exposure in many cohort members.	Kolstad et al., 1993
Continuation of Kolstad et al., 1993 study with focus on males and more exposure information; 36,525 male workers "exposed" during reinforced plastic production, 14,254 not exposed, 2941 with unknown exposures; further stratified by employment duration (<1 year, ≥1 year) and time since first employment (<10 year, ≥10 years). Women were excluded from analyses since the majority were not involved in reinforced plastic manufacture.	Significant positive findings presented as cancer type: SIR, 95% CI: –Lymphatic or hematopoietic cancers in group employed <1 year and hired ≥10 years earlier: 1.65, 1.18–2.26; –Leukemia in group employed <1 year and hired ≥10 years earlier: 2.34, 1.43–3.61; –Leukemia in group hired ≥ 10 years earlier (1964–1970): 1.57, 1.07–2.22; –Non-Hodgkins lymphoma in group hired <10 years earlier: 2.35, 1.42–3.67. Non-significant or negative findings: –No increase in lymphatic/hematopoietic cancers or leukemia in workers employed >1 year; –In a limited number of companies classified as low (<50 ppm) or high (>50 ppm) exposure, Hodgkins disease was non-significantly associated with exposure, but other lymphatic/hematopoietic cancers were not associated with exposure.	–60% of cohort employed in industry for <1 year, 2/3 of exposed employees worked at companies where about 1/2 of employees were involved in plastic manufacture. –Study authors postulated that increased cancer in short term workers may have due to chance, carcinogen exposures in other jobs, or from cancer-associated behaviors. –Excess of non-Hodgkins lymphoma was limited to subgroup employed at companies where <1/2 workers were involved in reinforced plastic production.	Kolstad et al., 1994
Continuation of Kolstad et al., 1994 study including 36,310 workers from reinforced plastics companies and 14,293 workers from similar industries not manufacturing reinforced plastics; workers divided into categories of those directly involved with reinforced plastics work: 0%, 0–50%, and ≥50%; exposures in 1960s measured at 180 ppm and in further decades at 43–54 ppm.	Significant positive findings: –Poisson regression analysis revealed significant increase in pancreatic cancer (RR = 2.2; 95% CI = 1.1–4.5). Non-significant or negative findings: –No increased SIRs for any type of cancer, but SIR for lung cancer (1.1) was slightly increased.		Kolstad et al., 1995
Cohort of 7949 subjects (6638 males and 1311 females) employed between 1947 and 1984 at 8 plants in the UK; exposures rated as high, moderate, low, or background based on job	Significant positive findings: None. Non-significant or negative findings: –Fewer than expected cancer deaths SMR: 80, 95% CI = 69–93);	–Data from one facility were analyzed separately due to particularly poor follow-up and were not reported in the Harvard Panel report. –Lung cancer was not associated with exposure	Coggon et al., 1987

Table 21
(Continued)

Study details	Cancer findings	Notes	Original study
description; potential exposures to styrene were <1 year in 32%, 1–9 years in 26%, ≥10 years in 5%, and non-classifiable in 5% of cohort.	–Increases in deaths from cancer of larynx, lung, skin, cervix, and ovary; –SMR for lymphatic/hematopoietic cancer deaths: <100.	level, duration, or period or time since first hire.	
Includes cohorts from Kolstad and Coggon studies as well as workers from Finland, Italy, Norway, Sweden; total cohort size = 40,683 (34,556 males and 6127 females); workers classified according to job type (laminators 26%, unspecified 48%, other 13%, unexposed 10%, and unknown 3%) and exposure duration (<1 year 41%, 1–4 years (35%), 5–9 years (14%), and ≥10 years 9%). Exposures in Denmark in 1965 were 200 ppm for laminators; exposures in late 1980 s were 20–40 ppm. Study period was 1945–1991.	Significant positive findings: None. Non-significant or negative findings: –When analyzed by job category, total cancer SMR was <100; non-significant increase in lymphatic/hematopoietic cancer deaths for workers in unspecified task group.	–SMR for all cancers and lymphatic/hematopoietic cancers, higher in those working >1 year. –SMR for all lymphatic/hematopoietic cancers, non-Hodgkins lymphoma, Hodgkin's disease, and leukemia increased with time since first hire. –IARC noted very low SMI in exposure group.	Kogevinas et al., 1993
Continuation of Kogevinas et al., 1993 study. Exposures estimated for each country using two approaches. The first considered exposures prior to 1965 to be 200 ppm for laminators and exposures in the 1970 s and after to be 80–100 ppm. The second modeling approach assumed that earliest styrene level measured in each country applied to earlier time periods.	Significant positive findings in Poisson regression models presented as cancer type (styrene exposure level): RR, 95% CI: –All lymphatic/hematopoietic cancers (100–119 ppm): 3.1, 1.1–9.1; (120–199 ppm): 3.1, 1.0–9.1; (≥200 ppm): 3.6, 1.0–13.1; –Malignant lymphoma (120–199 ppm): 7.2, 1.2–42.1 (no dose-response noted and not significant at higher dose. Non-significant or negative findings: –Total cancer SMRs <100 for all job categories; –Increases in deaths from lung cancer (laminators), lymphatic/hematopoietic cancers (unspecified task), non-Hodgkins lymphoma (laminators and unexposed), Hodgkin's lymphoma (laminators and unspecified), multiple myeloma (unspecified and unexposed) and leukemia (unspecified and total cohort); –Borderline significance in trend between pancreatic cancer and cumulative exposure but not for any other cancer type.	–In styrene-exposed workers, higher SMRs for all lymphatic/hematopoietic cancers, non-Hodgkin's lymphoma, and Hodgkin's disease were associated with time since first exposures for ≥2 year versus <2 years; leukemia SMRs in styrene workers were also associated with exposures ≥2 years. –No other associations were noted.	Kogevinas et al., 1994
Cohort of 15,826 workers (11,958 males and 3868 females) from 30 US plants; workers employed for at least 6 months between 1948 and 1977. Follow-up from 1948–1989. Office workers with no previous production jobs eliminated from cohort. Cumulative exposures based on TWA measurements were calculated for workers prior to 1977. Numbers of workers were	Significant positive findings presented as cancer type: SMR, 95% CI: –All cancers: 1.16, 1.05–1.27; lung: 1.41, 1.20–1.64; esophagus: 1.9, 1.1–3.2; respiratory system 1.4, 1.2–1.6; cervix: 2.8, 1.4–5.2; and other female genitalia: 2.0, 1.1–3.5.	Positive, increasing trends noted for SMRs of all cancer and lung cancer and time since first exposure. No associations between exposure duration or cumulative exposure and SMRs. In a nested case–control sub-study, the author noted that respiratory cancer was positively associated with smoking but not styrene exposure.	Wong et al., 1990, 1994

approximately evenly divided in categories of employment duration (0.5–0.9, 1.0–1.9, 2.0–4.9, and ≥5 years) and exposure levels (<10, 10.0–29.9, 30.0–99.9, ≥100 ppm-years).	Non-significant or negative findings: No excess of lymphatic/hematopoietic cancers.		
Cohort of 5201 workers (4519 males and 682 females) at two US plants; workers employed between 1959 and 1978; based on styrene measurement, exposure in high exposure group ($n = 2060$) estimated at 42.5–71.7 ppm and minimal in other group ($n = 3102$). Styrene exposure durations were <1 month in 25%, ≤1 year in 74%, and >5 years in 6% of workers.	Non-significant increase in SMRs for total and respiratory, reduced SMR for lymphatic/hematopoietic cancers.	Low power exploratory study of predominately short duration workers noted increased urinary tract cancer possibly associated with styrene exposure. Inconsistent associations with duration of exposure.	Ruder et al., 2004 updating Okun et al., 1985.

[a]Harvard (Cohen et al., 2002) and IARC (2002) reviews.
CI, confidence interval; RR, relative risk; SIR, standardized incidence ratio; SMR, standardized mortality ratio.

that production of the *R*-enantiomer of styrene oxide exceeds that of the *S*-enantiomer in mouse lung, and it is believed that the *R*-enantiomer is slightly more toxic. Pulmonary metabolism was not reported for rats, but it was stated that hepatic production of *S*-enantiomer exceeds that of *R*-enantiomer. Another difference between mice and rats is that mice are more susceptible to depletion of glutathione, which is involved in the detoxification of styrene oxide in mice. Although metabolic and toxicokinetics differences were noted between rats and mice, Harvard and IARC concluded that the differences are not of sufficient magnitude to explain increased tumor susceptibility in mice.

The Harvard Panel (Cohen et al., 2002) utilized two PBPK models to further explore whether varying tumorigenic responses in rats and mice are due to differences in toxicokinetics. One model was developed by Filser et al. in 1999, and the second was developed by the Harvard Panel at the time of their review. The Filser model was developed through empirical measurements, realistically characterizes the respiratory tract, and can be used to quantify glutathione depletion in lung. However, the model cannot be used to quantify *R*- and *S*-styrene oxide in various tissues and does not provide sufficient information to permit computations and characterization of different exposure scenarios. The Harvard Panel model allows for prediction of *R*- and *S*-enantiomers of styrene oxide in almost any tissue. However, compared to the Filser model, the experimental basis for the Harvard model is not as sound, and the description of the respiratory tract is less realistic. Therefore, predictions from the Harvard model are uncertain. Harvard concluded that PBPK models confirmed empirical observations that increased sensitivity of mice compared to rats cannot be explained by quantitative differences in lung or blood styrene oxide levels, concentrations of *R*- versus *S*-styrene oxide, or glutathione depletion.

A mode of action-based model by Sarangapani et al. (2002) predicted ~10-fold lower styrene oxide concentrations in the terminal bronchioles of rats versus mice. The study authors noted both strengths and uncertainties of the model. Strengths included the multicompartment description of the respiratory tract; validation of data with measurements of styrene and styrene oxide levels in blood, liver, and whole lung tissue; and use of species-specific information on respiratory tract physiology, cell composition, and metabolic capability. Uncertainties of the model included inherent errors in measurement or intra-individual variations in target tissue blood perfusion rates and target tissue thickness and area; use of in vitro measurements for in vivo predictions; development of target tissue constants using whole lung or Clara cell values; inconsistencies in reporting of Clara cell density; and inability to measure styrene oxide concentrations in specific target regions.

The Harvard Panel (Cohen et al., 2002) also examined differences in metabolic and toxicokinetic parameters in humans versus rodents in an attempt to estimate sensitivity of humans to tumorigenicity. Several differences were noted between humans and mice. Less styrene oxide is produced by CYP-dependent monooxygenases in human compared to mouse lung. The ability of pulmonary epoxide hydrolase to metabolize styrene oxide is comparable or moderately greater in humans than in mice. Glutathione transferase activity is

Table 22
Summary of Styrene Cancer Studies in Rats[a]

Strain	Route, dosing, exposure duration	Significant findings	Notes	Reference
BD IV	Gavage, 0 ($n = 10$) or 1350 mg/kg bw ($n = 21$) to pregnant rats on GD 17. Offspring gavaged with 0 (36–39/sex from control dams) or 500 mg/kg bw (71–73/sex from treated dams) once/week from weaning through Week 120.	No treatment-related effects on survival, body weight, or tumor incidence in offspring.		Ponomarkov and Tomatis, 1978
F344/N	Gavage, 0 for 104–105 weeks (two separate control groups), 500 mg/kg bw, 5 days/week for 103 weeks; 1000 or 2000 mg/kg bw, 5 days/week for 78 weeks. Each group had 20–50 rats/sex/dose.	No increase in tumor incidence.		NCI, 1979
Sprague–Dawley	Gavage, 50 or 250 for 4–5 days/week for 52 weeks, $n = 40$ rats/sex/group; study ended at death of last animal.	–Reduced survival of females in high-dose group. –No treatment-related increases in tumors or effects on body weight.	A review by McConnell and Swenberg (1994) considered this study inadequate due to short exposure period.	Conti et al., 1988
Sprague–Dawley	Drinking water, 0, 125, or 250 ppm for 104 weeks ($n = 76$–104 rats/sex/group in control group and 50–70 rats/sex/group in treatment groups). Authors estimated doses at 7.7–12 mg/kg bw/day and 14–21 mg/kg bw/day	–Significantly decreased water intake in both treated groups. –Transient decreases in body weights (<10% compared to control rats) in both groups. –No effect on survival, toxicity, or carcinogenicity.	McConnell and Swenberg (1994) considered this study inadequate because exposure levels were well below the MTD.	Beliles et al., 1985
Sprague–Dawley	Inhalation, 0, 600, or 1000 ppm for 6 hr/day, 5 days/week, for 18.3 months (males, $n = 96$/group) or 20.7 months (females, $n = 96$–97/group). Animals killed at 2 years of age.	–Mammary adenocarcinomas increased at 600 ppm (1/85, 7/85)[b].	–Study authors concluded there was no causal association since no mammary tumors were observed at 1000 ppm (0/85). –Study authors noted that tumor incidence in concurrent controls (1%) was lower than in historical controls (5.8%).	Jersey et al., 1978 (CERHR did not obtain original reference)
Sprague–Dawley	Inhalation, 0, 25, 50, 100, 200, or 300 ppm for 4 hours/day, 5 days/week, for 52 weeks; $n = 30$ rats/sex/dose; study ended at death of last animal.	–Treatment associated with non-dose-related increased incidence of combined benign and malignant mammary tumors (34/60, 24/30, 21/30, 23/30, 24/30, 25/30)[b] and malignant mammary tumors alone (6/60, 6/30, 4/30, 9/30, 12/30, 9/30)[b].	–Harvard noted that study authors provided no information on background tumor rates but noted that a high rate of background mammary tumors was reported for the laboratory in 1978. –IARC noted the short treatment duration, incomplete reporting of data, and high rate of spontaneous mammary tumors in this strain. –Harvard concluded that this study does not necessarily reflect a true increase in cancer risk.	Conti et al., 1988

Sprague–Dawley	Inhalation, 0, 50, 200, 500, or 1000 ppm for 6 hr/day, 5 days/week, for 104 weeks; $n = 60$/sex/dose.	–Treatment-related increase in incidence of benign interstitial cell testicular tumors **[reported only by Harvard, not by IARC]**. –Decreased incidence of malignant mammary tumors. –Females in the 500 and 1000 ppm group weighed less.	–Study authors did not attribute testicular tumors to exposure because rates were within historical control ranges, there were no pair-wise statistical differences between control and any treatment group, and there was no pathology typically associated with chemically induced testicular tumors. –Harvard concluded that these study results do not reflect a true elevation in risk from styrene exposure.	Cruzan et al., 1998
Sprague–Dawley	i.p., 0 or 50 mg/animal given in four injections at 2-month intervals; study ended with death of last rat; $n = 40$ sex/dose.	No increase in tumor incidence.	–IARC noted incomplete data reporting and single low-dose treatment.	Conti et al., 1988

[a]Harvard (Cohen et al., 2002) and IARC (2002) reviews.
[b]Tumor data presented as number affected/number observed in control to high dose groups.

substantially lower in humans, resulting in decreased susceptibility to glutathione depletion. Limited evidence suggests that the human lung produces approximately equal quantities of *R*- and *S*- styrene oxide. Harvard noted that toxicokinetic characteristics of humans would suggest lower susceptibility to tumorigenicity compared to mice. Because differences in metabolism or toxicokinetics did not explain increased sensitivity of mice compared to rats, Harvard stated it is not possible to make definitive conclusions about human susceptibility.

In closing, IARC (2002) concluded that the most likely cause of lung tumors in mice is in situ formation of styrene oxide, which leads to cytotoxicity and increased cell proliferation. However, IARC noted that effects from circulating styrene oxide and DNA adduct formation cannot be excluded. IARC noted that the proposed mechanism involving conversion of styrene to styrene oxide by mouse pulmonary Clara cells is not relevant to humans, who have much smaller numbers of Clara cells than mice; however, a similar mechanism in other organs cannot be ruled out. In their overall evaluation of carcinogenicity, IARC concluded that *"Styrene is possibly carcinogenic to humans (Group 2B)."*

The Harvard Panel (Cohen et al., 2002) concluded that ''…styrene's carcinogenicity in humans is only suggestive, but…styrene's carcinogenic potential in humans cannot be ruled out.'' Because there is only suggestive evidence of carcinogenicity and the mode of action for tumorigenicity in mice seems to involve growth promotion, Harvard concluded that dose-response modeling to predict risks at very low doses is unwarranted.

2.5 Potentially Sensitive Subpopulations

The main styrene-metabolizing enzymes identified in humans are CYP2B6, CYP2E1, CYP2F1, and epoxide hydrolase. The CYP enzymes activate styrene by converting it to the epoxide compound styrene oxide. Epoxide hydrolase detoxifies styrene oxide by converting it to styrene glycol. Ontogeny and polymorphisms of those enzymes have been examined to a limited extent.

Human ontogeny of CYP2E1 and CYP2F1 was reviewed by Hines and McCarver (2002). CYP2E1 was not detected in liver by some investigators, but others reported its presence in livers of 16–24-week-old fetuses at 10–30% of adult levels. CYP2E1 was also detected in brain of 7–9-week-old fetuses at levels higher than in liver. Expression of the enzyme is rapidly activated at birth and reaches 30–40% of adult levels at 1 year of age and 100% of adult levels at 10 years of age. CYP2B6 was not detected in livers of 11–24-week fetuses. Expression was reported at 10% of adult levels within the first year of life. No information was presented for CYP2F1.

Pacifici and Rane (1982) examined human fetal tissues for epoxide hydrolase (microsomal fraction) activity toward 7-^{3}H-styrene 7,8-oxide (>99% radiochemical purity). Activity was measured in placenta and fetal liver, lung, kidney, adrenal, and gut obtained from seven fetuses legally aborted in weeks 14–25 of pregnancy. Epoxide hydrolase activity was highest in liver (~6 nmol/min/mg protein), followed by adrenals (~4 nmol/min/mg protein), and lower in the other tissues (~0.3–0.6 nmol/min/mg protein). The authors stated that values may not represent total organ capacity for metabolism because yield of microsomes is known to

Table 23
Summary of Styrene Cancer Studies in Mice[a]

Strain	Route, dosing, exposure duration	Significant findings	Notes	Reference
O20	Gavage, 0 ($n = 9$) or 1350 ($n = 29$) mg/kg bw to pregnant mice on GD 17. Offspring gavaged with 0 (20–22/sex from control dams) or 1350 mg/kg bw (39–45/sex/group from treated dams) once/week from weaning through Week 16; surviving animals were killed at 120 weeks.	–Significant increase in incidence of lung adenomas and carcinomas in male (8/19, 20/23)[b] and female (14/21, 32/32)[b] offspring.	–The study authors noted that the experiment was limited by use of the high dose, causing severe toxicity and mortality. –Harvard concluded that this study does not provide clear evidence or increased risk of lung cancer due to the high dose used. –IARC noted that this mouse strain is very susceptible to lung tumors.	Ponomarkov and Tomatis, 1978
C57BL	Gavage, 0 ($n = 5$) or 300 ($n = 15$) mg/kg bw to pregnant mice on GD 17. Offspring gavaged with 300 mg/kg bw (27/sex/group from treated dams) once/week from weaning through Week 120).	No treatment-related effects on survival, body weight, or tumor incidence in offspring.		Ponomarkov and Tomatis, 1978
$B6C3F_1$	Gavage, 0, 150, or 300 mg/kg bw, 5 days/week for 78 weeks; $n = 50$/sex/treatment group and 20/sex in control group; mice observed for a 13-week period following dosing.	–Increased incidence of combined lung adenomas and carcinomas in males (0/20, 6/44, and 9/43).[b]	–Harvard noted that incidence of combined tumors in concurrent controls (0%) was lower than historical control values (12%, range 0–20%) and effects were inconsistent between males and females. **[The Panel notes that gender differences are not unusual.]** –NCI concluded that the study provides no convincing evidence of styrene's carcinogenicity in mice of either sex. –IARC noted incidence of adenomas and carcinomas combined was within historical control ranges. **[Use of experimental controls demonstrated a dose-response effect.]**	NCI, 1979
CD-1	Inhalation, 0, 20, 40, 80, or 160 ppm, 6 hr/day, 5 days/week, for 98 weeks (female) or 104 weeks (males); $n = 50$/sex/dose.	–Increased incidence of bronchioalveolar adenomas in females exposed to ≥20 ppm (6/50, 16/50, 16/50, 11/50, 24/50)a and males exposed to ≥40 ppm (15/50, 21/50, 35/50, 30/50, 33/50)a **[IARC noted increase at 80 ppm was not significant].** –Increased lung carcinomas in females exposed to 160 ppm (0/50, 0/50, 2/50, 0/50, and 7/50).[b] –Decreased body weights in 80 and 160 ppm groups.	–High mortality incidence noted in control females by Week 98 (23/50). –Harvard concluded that results of this study indicated that inhalation of styrene results in increased probability of benign lung tumors in male and female mice and malignant lung tumors in female mice.	Cruzan et al., 2001
A/J	i.p., 0 or 200 μmol total (~100 mg/kg bw, according to IARC) given in 20 injections (3 times/week) to female mice ($n = 25$/group). Mice killed 20 weeks after the last dose.	No significant increases in lung adenomas or adenocarcinomas.		Brunnemann et al., 1992

[a]Harvard (Cohen et al., 2002) and IARC (2002) reviews.
[b]Tumor data presented as number affected/number observed in control to high dose groups.

be low for liver and is unknown in other organs. The authors noted that in contrast to rats, which have negligible epoxide hydrolase activity before birth, the values for fetal epoxide hydrolase activity in this study were similar to values reported previously for adults.

In their review of epoxide hydrolase activity in humans, McCarver and Hines (2002) indicated that microsomal and cytosolic fractions are involved in the metabolism of styrene oxide. They reviewed the Pacifici and Rane (1982) study, described above, and a second study by the same group that reported weak evidence of increased activity in liver between 10–25 weeks of gestation. Another study detected microsomal epoxide hydrolase in livers of 17–27-week-old fetuses. **[Activity was ~3–53% of adult levels according to CERHR calculations.]** In a more complete study, microsomal epoxide hydrolase activity was detected in fetal livers at 7.5 weeks of gestation. Activity increased linearly up to 22 weeks of gestation, to reach levels that were 50% of values reported for adults. Microsomal epoxide hydrolase was detected in fetal lung at 12 weeks gestation, and the level of activity was within ranges reported for adults. In studies of cytosolic epoxide hydrolase, activity was detected as early as 14 weeks in fetal livers, and activity was reported to be 4–5-fold less than adult levels.

Stoming and Bresnick (1974) determined ontogeny of epoxide hydrolase in Sprague–Dawley rats using 3-methylchoanthrene as a substrate. Assays were conducted using microsomes from fetal rats **[age not specified]** and rats ranging in age from 1–150 days old. Diol formation was measured by GC. Epoxide hydrolase activity was barely detectable in fetuses **[data not shown]** and in neonates at postnatal day (PND) 1 but rapidly rose to reach adult levels by 27 days of age.

A review by Brown et al. (2000) noted that, in general, xenobiotic-metabolizing enzymes are detected at very low levels in rodents before birth, and their functional relevance has been highly debated. It has been suggested that activities are higher in primates than in rodent embryos, leading to further debate in extrapolation of data from rodents to humans.

Information on possible polymorphisms of styrene-metabolizing enzymes and potential effects on sensitivity is very limited. A review by Anzenbacher and Anzenbacherova (2001) noted that 10 alleles were identified for CYP2E1, some of which form proteins with altered activity; however, no confirmation of differing CYP2E1 phenotypes was known at the time of the review. In a study reviewed by IARC (2002), V_{max} for styrene metabolism varied 8-fold in 20 human liver samples. CYP2E1 was determined to be the most important isoform in styrene metabolism, but there was no correlation between enzyme activity (V_{max} or K_m) and genetic polymorphisms. A second study reviewed by IARC examined differences in styrene metabolism in 20 male volunteers exposed to styrene at 104 mg/m^3 **[24 ppm]** or 360 mg/m^3 **[83 ppm]** for 1 hr while exercising. No correlations were found between blood clearance of styrene and metabolic capacity, as determined by CYP2E1, CYP1A2, and CYP2D6 activity. The study authors postulated that metabolism of styrene is limited by blood flow to liver. A third study examined effects of genetic polymorphisms in 30 workers exposed to a mean level of 18.2 ppm styrene and reported that the rare CYP2E1*1B allele was associated with increased urinary excretion of mandelic and phenylglyoxylic acids and mercapturic acid metabolites.

A study reviewed by IARC (2002) examined metabolism of *R*- and *S*-styrene oxide in 20 human livers and found 3–5-fold differences in V_{max}, K_m, and V_{max}/K_m values.

The interpretation of the role genetic polymorphisms may play in determining sensitive subpopulations depends on the interplay of several factors, including at least:

- the chemical species responsible for toxicity,
- the rates of oxidation and hydrolysis and how do they vary among tissues, and
- blood flows to different tissues.

The Expert Panel has not evaluated the role polymorphisms may play in sensitive subpopulations.

2.6 Summary of General Toxicology and Biologic Effects

2.6.1 Toxicokinetics and metabolism. Styrene is absorbed through the respiratory tract of humans and pulmonary retention of inhaled styrene is reported at 60–70% (ATSDR, 1992; IARC, 2002). Studies in rats demonstrated absorption of styrene through the respiratory and gastrointestinal tracts (ATSDR, 1992). Limited dermal absorption studies in humans and rats suggest that styrene is absorbed through skin (ATSDR, 1992).

In humans, rats, and mice, styrene is widely distributed throughout the body and the highest concentrations are initially found in fat (IARC, 2002). Styrene is distributed to fetuses of rats (Withey and Karpinski, 1985) and reproductive organs of rats and mice (Brown, 1991). Volume of distribution in humans was reported as 99 L or 1.4 L/kg (Wigaeus et al., 1983).

The main pathway of styrene metabolism in humans begins with conversion of styrene to styrene oxide by CYP enzymes. Styrene oxide is metabolized to styrene glycol by epoxide hydrolase. Styrene glycol is metabolized to mandelic acid, which is then converted to phenylglyoxylic acid. These metabolites are excreted in urine and are often used as biomarkers of exposure to styrene. In humans, mandelic acid represents 57–80% of a styrene dose, and phenylglyoxylic acid represents 10–33% of the dose (ATSDR, 1992; Rebert and Hall, 1994). Small amounts of styrene oxide are conjugated with glutathione. The glutathione conjugates are converted to mercapturic acid products. Metabolites generated through ring epoxidation of styrene (i.e., vinylphenol), conversion of styrene to 1-phenylethanol (i.e., ultimately leading to formation of hippuric acid), glutathione conjugation of styrene oxide, and glucuronidation of phenylethylene glycol represent $<1\%$ of the styrene dose (IARC, 2002).

Styrene metabolism in mice and rats is similar to that of humans, but some differences were noted. It seems that the capacity to generate styrene oxide is greater in rodents (IARC, 2002). In addition to converting styrene oxide to styrene glycol and ultimately mandelic acid, rodents metabolize a significant portion of styrene oxide by conjugating it to glutathione. The glutathione conjugates are converted to mercapturic acid products. It is estimated that $\geq 10\%$ of the styrene dose is converted to

mercapturic acid in rats inhaling $\leq$300 ppm styrene or i.p. injected with $\leq$250 mg/kg bw styrene and in mice i.p. injected with 400 mg/kg bw styrene. Although not described as a major pathway, the pathway involving conversion of styrene to 1- and 2-phenylethanol and ultimately to hippuric acid was described as more important in experimental animals than humans.

According to a PBPK model developed by Ramsey and Andersen (1984), saturation of styrene metabolism in humans occurs at styrene blood levels exceeding 1.7 mg/L. The saturation point based on styrene levels in air is 200 ppm in humans, rats, and mice. Below those concentrations, the rate of styrene metabolism is limited by perfusion of blood in liver or other organs involved in styrene elimination. The model was verified using actual blood concentrations of styrene in humans and rats. In experimental animal studies involving a range of exposures from 80–1200 ppm, the slope of the relationship between blood styrene concentration and air concentration increased above about 300 ppm in rats and mice.

A more recent mode of action-based PBPK model by Sarangapani et al. (2002) compared dosimetry of styrene and styrene oxide in the lung of rats and mice. The model predicted ~10-fold lower styrene oxide concentrations in the terminal bronchioles of rats versus mice.

In humans, the most active isoforms involved in styrene metabolism are CYP2E1 in liver and lung (IARC, 2002). CYP2B6 is also active in the metabolism of styrene in liver and lung of humans. In mice, the most active isoform in the lung is CYP2F2.

In metabolism of styrene by humans, pulmonary microsomal activity is far below that of hepatic microsomal activity, and nasal tissues were found to have no activity (IARC, 2002). In contrast, rodents (especially mice) have a high capacity to metabolize styrene in lung and nasal tissues.

In humans, styrene clearance rate is reported at 1.7 L/min, a rate similar to that of total blood flow through liver (Wigaeus et al., 1983). Styrene is highly metabolized and the majority of metabolites are eliminated through urine. A limited amount of styrene (0.7–4.4%) is present in exhaled air unchanged (ATSDR, 1992; IARC, 2002). Styrene clearance from blood is biphasic, indicating a two-compartment toxicokinetic model. Half-lives for inhaled styrene were reported at 0.6 hr for the first elimination phase and 13 hr for the second elimination phase (ATSDR, 1992). Half-lives for urinary elimination of mandelic acid in humans were reported at 3–4 hr and 25–40 hr for each respective phase (ATSDR, 1992; Rebert and Hall, 1994). The half-life for the first urinary elimination phase of phenylglyoxylic acid was reported at 11 hr (ATSDR, 1992). As in humans, styrene elimination in rats and mice occurs mainly through biphasic urinary excretion of metabolites (ATSDR, 1992).

2.6.2 General toxicity. Acute effects that have been observed in workers exposed to styrene are irritation of the eyes and upper respiratory tract and CNS depression characterized by drowsiness, dizziness, headache, and impaired balance (Cohen et al., 2002). Irritation was reported at styrene levels exceeding 24 ppm and CNS depression has been reported at concentrations $>$100 ppm (IARC, 2002). Impaired color vision discrimination is another potential effect in styrene-exposed workers and the effect was found to correlate with urinary levels of mandelic acid (Cohen et al., 2002; IARC, 2002). These effects were noted at air styrene levels of 20–50 ppm and higher (Cohen et al., 2002; Benignus et al., 2005). Hearing impairment was observed in styrene-exposed workers, but the effect was mainly reported in inadequately controlled studies. IARC (2002) concluded that neurological effects usually occur at styrene concentrations exceeding 100 ppm but have been reported with exposures as low as 10–30 ppm. Additional neurological effects were noted in occupational and clinical studies. Although the studies were noted to be inconsistent, poorly controlled, and lacking in dose-response information, they suggested that styrene exposure could lead to slower reaction time, impaired short term memory, and reduced manual dexterity (Cohen et al., 2002). A review by Rebert and Hall (1994), sponsored by the Styrene Information and Research Center, disputed claims that neurotoxicity occurs in humans at concentrations below 50 ppm styrene, citing inadequacy in study designs or interpretations. The authors also concluded that there are no indications of persisting damage to the nervous system as a result of styrene exposure.

Consistent with human findings, evidence of neurological effects have been observed in repeat dose experimental animal studies reviewed by IARC (2002) and to a limited extent, Harvard (Cohen et al., 2002). Styrene inhalation studies reported decreased brain dopamine levels and increased homovanillic acid levels in rabbits ($\geq$750 ppm), depletion of retinal dopamine levels in rats (300 ppm), increased brain glial fibrillary acidic protein in rats (320 ppm), and mild neurobehavioral disturbances in rats (1400 ppm) and mice (425 ppm). Similar effects were observed in rats orally exposed to $\geq$0.5 mg/kg bw styrene. Hearing impairment was observed in rats after repeated inhalation exposure to 850 ppm styrene but not to 200 ppm styrene in a second study. It is not known if studies examined multiple dose levels, and a LOAEL for neurotoxic effects is therefore not known. The Styrene Information and Research Center review noted that few studies exposed the animals to $<$300 ppm styrene (Rebert and Hall, 1994).

Additional effects that have not been observed in humans were reported in experimental animal studies. Inhalation of styrene vapors produced nasal lesions in rats and mice and pulmonary lesions in mice (Cohen et al., 2002; IARC, 2002). The effects occurred with styrene exposures $\geq$20 ppm in mice and $\geq$50 ppm in rats and were thought to result from local conversion of styrene to styrene oxide. Some studies reported liver effects (e.g., glutathione depletion, changes in liver enzyme activities, or necrosis) in rats and mice; effects were seen at $\geq$50 ppm in mice, the more sensitive species (IARC, 2002).

Some studies provided limited evidence of immunotoxicity in humans (e.g., alterations in lymphocyte numbers and T-lymphocyte response) and rodents (e.g., impaired immune response or reduced resistance to viral or parasitic infections) exposed to styrene (IARC, 2002). Based on limited evidence of lymphocyte effects in workers and effects in experimental animal studies, Harvard (Cohen et al., 2002) concluded that styrene should be investigated further as a potential immunotoxicant.

2.6.3 Genetic toxicity. Styrene does not interact with DNA, but its metabolite styrene oxide forms stable,

persistent adducts with DNA in humans and experimental animals (Cohen et al., 2002). Adducts indicate styrene exposure, but implications regarding possible health effects are not known. Increased frequency of DNA strand breaks were observed in white blood cells of workers exposed to styrene, but the breaks seemed to be repaired from the end of one work shift to the beginning of the next day's work shift.

Variable results were obtained in in vitro and in vivo studies examining styrene exposure on chromosomal aberrations, SCEs, and micronuclei frequencies. The majority of in vivo experimental animal studies resulted in negative chromosomal aberration and micronuclei findings, but positive SCE findings. Genotoxicity occurred only at high dose levels and usually after >1 day of exposure. In contrast to experimental animal studies, the majority of in vivo human studies suggested positive chromosomal aberration findings and negative SCE results. Human studies were complicated by confounders such as other occupational exposures. Harvard conducted additional statistical evaluations on human studies (Cohen et al., 2002) and concluded that evidence of a possible association between occupational styrene exposure and chromosomal aberrations was compelling, whereas the association with SCEs was less certain. None of the studies provided evidence of an association between styrene exposure and micronuclei frequency. Mutagenicity studies in several strains of *Salmonella* usually produced negative findings in the absence of metabolic activation and occasionally positive results with metabolic activation (Cohen et al., 2002).

2.6.4 Carcinogenicity. Studies in reinforced plastics workers were judged to be most informative for evaluating cancer effects in humans because styrene exposures were generally the highest, and confounding by other chemical exposures was less likely (Cohen et al., 2002; IARC, 2002). Although not consistently observed among different studies, lung/respiratory and lymphatic/hematopoietic cancers were most often reported in reinforced plastics workers. However, increases in lung/respiratory cancers were often not related to estimated exposure level or duration and may have been confounded by smoking. Interpretation of lymphatic/hematopoietic cancers was difficult due to low numbers of observed and expected deaths, possible confounding, lack of consistent dose-response patterns, and small size of subgroups with high levels and durations of exposure. It was noted that findings were not robust and may have been due to chance or bias in addition to confounding. IARC (2002) concluded, ''There is *limited evidence* in humans for the carcinogenicity of styrene.''

In studies examining carcinogenicity, rats and mice were exposed through oral and inhalation routes. IARC (2002) and Harvard (Cohen et al., 2002) concluded that there was no compelling evidence that styrene exposure increased tumor frequency in rats. Both groups noted evidence of increased pulmonary adenomas in male and female mice and an increase in pulmonary carcinomas in female mice after inhalation exposure to styrene. IARC concluded ''There is *limited evidence* in experimental animals for the carcinogenicity of styrene.'' A review of styrene oxide data indicated that three gavage studies in rats and one in mice consistently demonstrated an increase in forestomach tumors after exposure to $\geq$50 mg/kg bw.

Mechanism of styrene-induced lung tumors in mice is not known, but IARC (2002) stated that the most likely mechanism is cytotoxicity and increased cell proliferation resulting from in situ formation of styrene oxide. IARC noted, however, that effects from circulating styrene oxide and DNA adduct formation cannot be excluded. IARC noted that the proposed mechanism involving conversion of styrene to styrene oxide by mouse pulmonary Clara cells is probably not relevant to humans, who have far fewer Clara cells than mice; however, a similar mechanism in other organs cannot be ruled out. Metabolism of styrene oxide by epoxide hydrolase is comparable or moderately higher in humans than in mice. Toxicokinetics differences between rats and mice were noted, including greater production of styrene oxide with higher levels of the *R*-enantiomer in lung, and increased sensitivity to glutathione depletion by mice. It was thought that the differences could account for increased tumor sensitivity in mice. However, it was reported that differences were not of sufficient magnitude to explain the increased susceptibility of mice. In their overall evaluation of carcinogenicity, IARC concluded that ''*Styrene is possibly carcinogenic to humans (Group 2B).*'' The Harvard Panel (Cohen et al., 2002) concluded that ''…styrene's carcinogenicity in humans is only suggestive but…styrene's carcinogenic potential in humans cannot be ruled out.''

2.6.5 Potentially sensitive subpopulations. The main styrene-metabolizing enzymes identified in humans are CYP2B6, CYP2E1, and epoxide hydrolase. There is some information regarding ontogeny and polymorphisms of those enzymes, which may affect levels of styrene and its metabolites. However, the roles of genetic polymorphisms and ontogenetic differences in determining sensitive subpopulations is complex. This Expert Panel has not evaluated the role polymorphisms may play in forming sensitive subpopulations, nor has it seen information regarding other potentially sensitive subpopulations.

3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

Brown (1991), supported by the Styrene Research and Information Center, reviewed two papers from the Russian literature, which the author read in the original Russian. One paper (Vergieva et al., 1974), published in 1974, described pregnancy outcome in 47 styrene-exposed women who worked throughout pregnancy. It was reported that 18.3% of the women had ''early toxicosis,'' 42.5% of the women had ''late toxicosis,'' and 38.3% of the women had ''abnormal deliveries.'' These diagnostic terms were not described. Brown described this report as providing little useful information. Another report (Loseva et al., 1983), published in 1983, involved 287 women who worked in polystyrene and polyethylene processing. Spontaneous abortion was said to have occurred in 11.3% of the women. No control information was given. ''Toxicoses,'' premature rupture of the membranes, and preterm delivery were said to be more common among these women than expected, but no details were given. Besides styrene, women were exposed to ethylene oxide, carbon dioxide, heat, and noise. Brown described the significance of this report as

"obscure." **[The Expert Panel notes the summaries of the Russian reports for completeness, but did not read these reports and cannot evaluate their reliability. The summaries as presented in the Brown review are not useful for the evaluation process.]**

Holmberg (1977, 1979), Holmberg et al. (1982, 1986), Holmberg and Nurminen (1980), and Kurppa et al. (1983) published a series of papers describing a case-control study using data from the Finnish Register of Congenital Malformations. This register received notifications of all infants with congenital malformations born since January 1, 1963. Children with selected "marker defects" (central nervous system defects, orofacial clefts, cardiovascular defects, and musculoskeletal abnormalities) were identified, and mothers were interviewed a mean of 84 days after birth for information on occupational exposures. A reference group was constructed of mothers who delivered a normal child immediately before each case mother in the same district. Referent mothers were interviewed an average of 91 days after delivery. For this series of papers, the exposure of interest was to a heterogeneous group of chemicals called, "organic solvents." This diverse group of chemicals included styrene. **[Styrene was mentioned on lists of exposures in these papers, although it is not clear whether identification of styrene exposure was made by participant report, by inference from job title, or by a combination of these methods.]** The first articles in this series (Holmberg, 1977, 1979; Holmberg and Nurminen, 1980), which concerned CNS defects, mentioned a child with hydrocephaly and a child with anencephaly, each born to a woman employed in plastics manufacturing with exposure to styrene and acetone, among other chemicals. A subsequent article (Holmberg et al., 1982) focused on oral clefts and reported one case and one control mother with exposure to styrene through handicrafts at home. A summary paper on all of the marker defects (Holmberg et al., 1986) identified three cases and three referents with exposure to styrene and acetone in the reinforced plastics industry and four cases and two referents with exposure to styrene and acetone in boat laminating performed outside of their employment. The authors concluded, "…our explorative study has afforded limited evidence to suggest a possibility for organic solvents being causally related to human teratogenesis. Such a possibility needs to be further evaluated in other sets of mothers exposed to solvents during pregnancy."

Strengths/Weaknesses: The use of a population-based registry system is a strength, as is the short birth-to-interview interval. Weaknesses include the small sample size, potential for recall bias, and the lack of clarity regarding the methods of exposure assessment. It is not possible to identify an effect estimate for styrene alone in this group of individuals with heterogeneous chemical exposures.

Utility (Adequacy) for CERHR Evaluation Process: These reports are of limited utility in the evaluation process.

Hemminki et al. (1984), support not indicated, gave information in a book chapter on an unpublished study of congenital anomalies in women in the plastics industry. **[The results may have been obtained as part of a spontaneous abortion study (Hemminki et al., 1980), reviewed in Section 4.1, to which there are methodologic resemblances.]** Women who were members of the Finnish Union of Chemical Workers were identified from Union records and information on congenital malformations was obtained from the Finnish Register on Congenital Malformations for the years 1973–1979 (this registry was estimated to have "about 50% coverage"). Deliveries were identified from the Hospital Discharge Registry. Each Union member who was identified as having a malformed child was matched to two Union members according to date of delivery (within 1 year) and Union chapter. The cases and controls were evaluated to determine if they were working "at the time of early pregnancy." For women in the plastics industry, the relative risk (95% CI) based on nine exposed cases and 18 controls was 1.0 (0.2–5.5). There was no assessment of the specific chemicals to which these women were exposed.

Strengths/Weaknesses: The use of Union records to identify potentially exposed workers and the use of a national birth registry system are strengths of this study; however, exposure was only defined by Union status, which is a weakness. The sample size was small, producing a wide confidence interval around the risk estimate for plastics work, with low power for detection of an effect.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Härkönen et al. (1984), support not indicated, studied the incidence of congenital malformations among children born to styrene-exposed workers in Finland. Workers were identified from 160 workplaces in which styrene was used in the manufacture of reinforced plastics, with inclusion of 1698 men and 511 women. The time period during which styrene exposure occurred was determined **[by an unspecified method]** for each worker. Births between the years 1963–1979 were identified from the Population Register, and malformations were ascertained through the Register of Congenital Malformations. Children were assessed as having been born during or before the period of exposure of the parent. Rates of malformation obtained in children born within and outside periods of parental styrene exposure were compared to population rates calculated as total malformations in the Register divided by total live births recorded for 1963–1979. There were two malformed children born to 346 female workers before styrene exposure, and there was one malformed child (with hydrocephalus) born to 79 female workers during the period of styrene exposure. There were 4 malformed children born to 771 male workers before styrene exposure, and seven malformed children born to 630 male workers during styrene exposure. All of the rates for offspring of exposed male and female workers were lower than the expected population rates, and the 95% CI based on the Poisson distribution were overlapping. The authors concluded that the results of the study were inconclusive and that the numbers of malformations were so small as to make further analysis impossible.

Strengths/Weaknesses: The use of national registry data is a strength of this study, but the sample size is small with low power, especially for individual defects. The use of external rates for comparison and the lack of individual exposure data are additional weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Ahlborg et al. (1987), support not indicated, reported a case-control study of adverse pregnancy outcome among female employees of Norwegian and Swedish companies producing plastic polymers. Companies were asked to produce records of women who were employed any time during the period 1972–1980. Cooperation was obtained from 43 Swedish companies and 35 Norwegian companies **[the total number of companies approached was not indicated]**. National birth registers and congenital malformation registers were used to identify cases, defined as stillbirths, postnatal deaths **[not otherwise defined]**, any of a list of selected malformations **[not specified]**, or low birth weight (<2000 g in Sweden, <1500 g in Norway). Each case was matched to two controls on date of birth within 2 months, maternal age within 5 years, and parity (1, 2, >2). In ''a few'' cases, date of birth was matched within 1 year to permit selection of controls. One Norwegian woman contributed two case deliveries, and one Norwegian woman contributed two control deliveries. For each case and control woman, a questionnaire was sent to the employer requesting information about the type of plastic produced, production methods, and other exposures during the year before the delivery. Exposures were classified by study personnel as not involving plastics, involving unheated plastics, or involving heated plastics. Type of plastic was categorized as polyvinylchloride, polyurethane, or styrene (with or without other components). Odds ratios and 95% CI were calculated. **[The women were not contacted and there was no mention of identification or evaluation of possible confounders.]**

In the Swedish sample, there were 1397 deliveries of which there were 18 liveborn children with congenital malformations, 10 children with low birth weight, and 16 dead children or fetuses (10 stillbirths, 1 death in the first week of life, and 1 death after the first week of life). In the Norwegian sample, there were 282 pregnancies of which there were four liveborn children with malformations, two children with low birth weight, and four dead children or fetuses (1 stillbirth and 3 deaths in the first week of life). The odds ratio for adverse outcomes was not significantly different from unity for any generalized plastic work category (heated plastics, thermoplastics, thermosetting plastics). There were 18 cases in which styrene exposure was determined, giving an odds ratio (95% CI) of 0.8 (0.4–1.6). The authors indicated that they had not gotten information on lifestyle factors or social characteristics, and that the combining of diverse perinatal outcomes was a weakness of their approach. They also indicated that the size of their sample gave a $<50\%$ likelihood of identifying a 2-fold increase in risk of adverse pregnancy outcome associated with styrene exposure.

Strengths/Weaknesses: The use of national pregnancy/birth outcome registries and the use of company data to define potential exposure are strengths, as is the evaluation of multiple outcomes. Weaknesses include the potential for selection bias by company participation, the lack of individual exposure data, mixed exposures, and the small sample size. Potential confounding factors were not considered.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Lemasters et al. (1989), supported by the EPA, evaluated birth weight in children born to women working in reinforced plastics companies selected based on type of process and products and number of English-speaking employees. Of 120 companies contacted by the authors, 36 agreed to participate, and 2177 women employees of these companies were invited to be interviewed. Inclusion criteria included age <36 years at the time of hiring, employment for at least 6 months during the 7-year study period, and a current or previous marriage. There were 1535 subjects who were successfully contacted and agreed to be interviewed. The 642 non-participants, most of whom were not located or had no telephone, were less likely than the participants to be currently employed or white. The women who were interviewed included 1050 women who were considered exposed to styrene based on a site visit and inspection of processes and work records by one of the investigators (described in Lemasters et al., 1985a) and 485 unexposed individuals. Unexposed women were selected from employees of the plastics companies and other local companies based on job titles such as secretary or typist. Because of the smaller number of unexposed than exposed women, additional births were included from pre-employment pregnancies of women who contributed no exposed births. As a result of information obtained in the telephone interview, there were 229 exposed and 819 unexposed births. Maternal diabetes, infant congenital anomalies, and multiple births were excluded, and only one birth from each woman was selected.

In a study published previously on spontaneous abortion (Lemasters et al., 1985b), reviewed in Section 4.1, exposure was considered high if the individual worked in open molding with direct styrene contact (weighted mean exposure = 52 ppm) and low if involved in other open molding work or in press molding (weighted mean exposure = 13 ppm). The exposures were estimated from historically collected industrial hygiene data. In the current report, low exposure was considered <30 ppm and high exposure was ≥ 30 ppm in any month of pregnancy. A subset of 50 women who worked in the highest styrene-exposed jobs was considered in an additional analysis. Styrene exposures in these women averaged 82 ppm.

Telephone interviews were conducted by professional interviewers. Questions sought information on occupational and reproductive history as well as general health history and exposure to radiation, medication, cigarettes, and alcohol. Reproductive histories were obtained before information on exposures. Birth weight was obtained solely from the telephone questionnaire. The reliability of maternal reporting was checked by re-asking the birth weight 6 months later in 129 randomly selected participants. Reliability was described as ''good'' based on 86% agreement within $\frac{1}{2}$ pound.

Results were analyzed using multiple regression. Exposure was incorporated in the analysis as a categorical variable (none, low, high) and by multiplying mean exposure level and duration of exposure during pregnancy to produce three categories (cut-offs not given). Month- and trimester-specific versions of this cumulative exposure index were also considered in the regression. Gestational age and weight-for-gestational age were also considered. Results are shown in Table 24. There was no statistically significant effect of styrene exposure on birth

Table 24
Birth Weights in Pregnancies Exposed and Unexposed Occupationally to Styrene[a]

Parameter	Styrene exposure category		
	None ($n = 819$)	Low ($n = 154$)	High ($n = 75$)
Birth weight, g (mean ± SD)	3298 ± 595	3284 ± 565	3253 ± 594
Infants <2501 g, *n* (%)	67 (8.2)	13 (8.4)	3 (4.0)

[a]Lemasters et al. (1989).

weight or proportion of low birth-weight babies. When the 50 pregnancies with the highest exposures were considered separately, there was a 4% decrease in mean birth weight (95% CI = −7.7 to +0.6%; $p = 0.08$). The authors concluded that this effect was comparable to that of cigarette smoking. They cautioned, however, that the women who were the most highly exposed to styrene were also exposed to other occupational chemicals. In addition, they noted that exposure estimates were based on historically collected industrial hygiene data and were not specific to the individual pregnancy.

Strengths/Weaknesses: The use of work records to assess individual exposures and the adjustment for multiple confounding factors are strengths of this study. Weaknesses include potential selection bias by company and worker participation, the small sample in the highest exposure category, and potential confounding by other workplace exposures. In addition, the individual exposure assessments were incomplete.

Utility (Adequacy) for CERHR Evaluation Process: This study is of moderate utility in the evaluation process.

3.2 Experimental Animal Data

3.2.1 Chickens. Studies in which chicken eggs were treated are presented for completeness but are not used in the evaluation process.

McLaughlin et al. (1963, 1964), from FDA, injected the yolks of fertile White-Leghorn chicken eggs before incubation with styrene at 2.3, 4.6, or 18 mg/egg **[chemical purity and numbers of eggs not specified]**. In the low-dose group, 95% of eggs hatched (similar to uninjected eggs separately reported), in the middle-dose group, 40% of eggs hatched, and in the high-dose group, no eggs hatched. Malformations were not mentioned, but were noted after treatment with other chemicals in this report, suggesting that none were seen in the styrene-exposed chicks.

Vainio et al. (1977), of the Finland Institute of Occupational Health, treated fertile White-Leghorn eggs by injecting styrene or styrene oxide (''purum grade'') into the air space. The chemicals were dissolved in 99.5% ethanol and the solution further diluted in olive oil. Eggs were treated after 3 days of incubation, and embryos were evaluated 11 days later. Styrene treatments were 2, 5, 20, 50, and 100 μmol/egg **[0.11, 0.52, 2.08, 5.21, and 10.42 mg/egg]**. Styrene oxide treatments were 0.5, 1, 2, 2.5, or 5 μmol/egg **[0.06, 0.12, 0.24, 0.30, and 0.60 mg/egg]**. Uninjected and injected control eggs were also used. There were 10 or 20 eggs/treatment group. Among the control eggs, 80 or 90% contained live embryos at the time of evaluation. Live embryos were reduced by styrene 50 μmol/egg to 30%. There were no live embryos in eggs treated with styrene 100 μmol/egg. Styrene oxide treatment reduced live embryos to 30% at 2 μmol/egg. There were no live embryos after treatment with styrene oxide 5 μmol/egg. Malformations were identified in up to 20% of embryos exposed to styrene or styrene oxide. The average incidence of malformations in styrene-treated eggs was 15%, and the average incidence of malformations in styrene oxide-treated eggs was 7% **[the proportion of embryos malformed at each exposure level was not given]**.

Kankaanpää et al. (1979), of the same laboratory, reported experiments in which styrene treatments of fertile White-Leghorn eggs were given with trichloropropylene oxide, an inhibitor of epoxide hydrolase. It was hypothesized that the toxicity of styrene was due, at least in part, to styrene oxide, and that inhibition of styrene oxide detoxification by epoxide hydrolase would result in augmentation of toxicity. Styrene (''purum grade'') or styrene oxide (97% purity) were administered in polyoxyethylated vegetable oil at 10 μmol/egg **[1.04 mg/egg]** for styrene and 0.8 μmol/egg **[0.10 mg/egg]** for styrene oxide, with or without trichloropropylene oxide 0.1 μmol/egg **[16 μg/egg]**. Eggs were treated by injection into the air space after 3 days of incubation, and embryos were evaluated 11 days later. Uninjected and vehicle-injected controls were used. There were 15–74 eggs/treatment group. Endpoints included embryo mortality and the proportion of live embryos with malformations **[method of evaluation not given except as ''macroscopic inspection''; the malformations that were mentioned were all external]**. Results were presented without statistical analysis. Mortality rates were: untreated control 5.5%, vehicle-treated control group 18%, styrene 29%, styrene oxide 38%, trichloropropylene oxide 23%, styrene+trichloropropylene oxide 72%, and styrene oxide+trichloropropylene oxide 62%. Malformation rates as a percentage of live embryos were: untreated controls 0, vehicle-injected controls 4.9%, styrene 15%, styrene oxide 20%, trichloropropylene oxide 11%, styrene+trichloropropylene oxide 33%, and styrene oxide+trichloropropylene oxide 27%. The malformations included stunting, exteriorization of viscera or brain, an/microphthalmia, and limb defects. The authors concluded that the results support the relationship of styrene embryotoxicity and teratogenicity to the presence of the epoxide.

Shanker et al. (1984), funded by the Indian government and ''ICMR,'' treated fertile White-Leghorn eggs with styrene **[purity not specified]** in ethanol and olive oil at 0, 0.25, 1.25, 2.50, and 5.00 μmol/egg **[0.03, 0.13, 0.26, and 0.52 mg/egg]** injected into the yolk on the 3rd, 7th, or 14th day of development. An uninjected control group was also used. Hepatic heme levels and δ-aminolevulinic acid synthetase activity were assayed. **[The number of eggs per treatment group was not given, although results are given for 16 eggs/group treated on Day 14. The age at which livers were harvested was not given. Statistical methods were not provided, although *p*-values were given in the data table.]** Embryo mortality seemed to be increased in a dose-related manner with about 60–75% mortality after Day 3 treatment, 30–70% mortality after Day 7 treatment,

and 15–30% mortality after Day 14 treatment **[estimated from a graph]**. Heme levels were increased and δ-aminolevulinic acid synthetase levels were decreased in a dose-dependent manner after treatment on Day 14 **[only Day 14 data were given, and data for the 0.25 µmol/egg treatment group were not shown]**. The authors proposed that heme might be increased by inhibition of heme degradation by the styrene binding of CYP enzymes and that δ-aminolevulinic acid synthetase activity might be decreased by styrene-associated depletion of glycine. They suggested that a pathway not involving δ-aminolevulinic acid synthetase might be involved in heme synthesis.

3.2.2 Mammals treated during pregnancy. This section reviews studies in which rats, mice, rabbits, or hamsters were treated during pregnancy, with or without lactational treatment, and is divided based on whether neurotoxicity endpoints were the focus of the experiments.

3.2.2.1 Non-neurotoxicity endpoints: Brown (1991), supported by the Styrene Information and Research Center, reviewed a 1974 Russian study by Ragule, which he read in the original Russian. Two inhalation studies were described, with exposure levels of 0, 1.2, and 11.6 ppm and 0, 0.35, and 1.2 ppm. There was reportedly an increase in embryo or fetal death at 11.6 ppm in the first study and an increase in post-implantation death at both styrene exposure levels in the second study; however, Brown did not find evidence for these conclusions to be convincing. He concluded, ''The published report is lacking in experimental detail and is difficult to interpret.'' **[This review is presented for completeness. The Expert Panel did not read the study and cannot comment on its reliability.]**

Ponomarkov and Tomatis (1978), of IARC, presented a very limited amount of information on prenatal and preweaning mortality in a study that primarily focused on carcinogenicity in mice and rats treated with styrene during in utero development and after weaning. BD IV rats and O_{20} mice were gavaged with olive oil (n = 9–10/group) or 1350 mg/kg bw styrene (n = 21–29/group) on GD 17. C57BL mice were gavaged with olive oil (n = 5) or 300 mg/kg bw styrene (n = 15) on GD 17. Litter size was similar in control and treated rats and mice. In rats, preweaning mortality was 2.5% in the control group and 10% in the treated group. Preweaning mortality was higher in O_{20} mice treated with 1350 mg/kg bw styrene (43%) versus the control group (22%). Preweaning mortality was not affected by treatment with 300 mg/kg bw styrene in C57BL mice. **[Detailed data were not presented, and there seemed to be no statistical analysis.]** Treatment of offspring after weaning and a discussion of carcinogenicity are included in Section 2.

Strengths/Weaknesses: No data were presented on maternal toxicity of the single dose of styrene, except for tumors, although the histology of maternal organs is a strength. An adequate number of animals was used. The single dose on a single day of gestation is a weakness, and it is not clear that the gavage solution was analyzed. Administration in olive oil is a weakness. There was no statistical treatment of the data. Pup mortality seems to have been increased in both rats and mice, but no measure of variability was given. The mortality rate in the O_{20} mice was very high even in controls, suggesting either that this strain has a high background rate of early mortality or that there were husbandry issues in the lab. The lack of preweanling data is a weakness. The study did not use a regulatory compliant design for evaluating developmental toxicity.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study for the CERHR process is limited. It may be used only as corroboration for other studies.

Murray et al. (1978), supported by companies affiliated with the Manufacturing Chemists Association, treated pregnant Sprague–Dawley rats and New Zealand white rabbits with styrene (minimal purity 99.5%, 2–5 ppm *tert*-butylcatechol added to inhibit polymerization). Rats were exposed by inhalation 7 hr/day from GD 6–15 (plug = GD 0) to 0 or 300 ppm styrene in the first experiment and to 0 or 600 ppm styrene in a second experiment (n = 29 or 30/dose group). Additional rats were given styrene in peanut oil by gavage on GD 6–15 at 0 (n = 32), 90 (n = 24), or 150 (n = 24) mg/kg bw twice daily for total daily doses of 0, 180, or 300 mg/kg bw. Rabbits (20/dose group) were exposed by inhalation to styrene in two experiments using the same dose levels as in the rat experiments (300 and 600 ppm, each with its own 0 ppm control group). Rabbits were treated on GD 6–18 (day of breeding = GD 0). Rats were killed on GD 21 and rabbits were killed on GD 29 for evaluation of uterine contents. Apparently nonpregnant uteri were stained with 10% sodium sulfide for detection of implantation sites. All fetuses were weighed, measured, sexed, and examined for external abnormalities. One-third of fetuses were freshly dissected for visceral abnormalities, and all fetuses were cleared and stained with Alizarin red-S for skeletal evaluation. The incidence of fetal alterations and resorptions was evaluated using a modified Wilcoxon test, maternal and fetal weight were evaluated by ANOVA with post-hoc Dunnett test, and the incidences of maternal death and pregnancy were evaluated using the Fisher exact test.

There was one maternal death among styrene–exposed rats (at 300 ppm) but no other deaths or clinical signs. Rats given styrene at any dose and by either route showed a decrease in body weight gain on GD 6–9, which was attributed to a decrease in feed consumption. Inhalation exposure to styrene in rats at both exposure levels was associated with an increase in water consumption. There was no effect of styrene by either route of administration on litter size, resorptions/litter, or mean fetal body weight. A 2.2% mean decrease in crown-rump length in fetuses in the 300 ppm group was statistically significant **[a 1.8% mean decrease in fetal crown-rump length in the 600 ppm group was not statistically significant]**. There was no treatment effect by either route on malformations in rat fetuses. Skeletal variations (lumbar spurs and delayed ossification of sternebrae and vertebral centra) were increased in styrene-treated animals compared to controls but were within the historical control range **[data not shown]**.

There were no maternal clinical signs or effects on body weight gain in rabbits. There were no treatment effects on live litter size, resorptions/litter, fetal body weight or length, or incidence of malformations. Unossified 5th sternebrae were increased in fetal rabbits in the 300 ppm group but not the 600 ppm group.

The incidence of this variation was within the historical control range.

The authors concluded that styrene was not teratogenic at the exposure levels used in these experiments, including maternal toxic exposure levels in rats. The authors also concluded that embryotoxicity or fetotoxicity could not be attributed to styrene treatment because the decrease in crown-rump length seen at 300 ppm was not seen at 600 ppm, and because the skeletal variations that occurred in the offspring of treated animals had an incidence within the historical control range.

Strengths/Weaknesses: The protocol was a regulation-compliant (in its time) developmental toxicity study with the dosing period encompassing the major period of organogenesis in rats and rabbits. Although more recent protocols continue dosing through late gestation, the protocol used here was adequate to detect developmental toxicity. The number of animals per group provided good statistical power. The analysis of chamber styrene concentrations is a strength. An unusual design feature was that two legs of the inhalation study were run for each species, each with only one treatment group and with its own control group; the lack of additional dose levels is a weakness. Additional weaknesses are the lack of indication of the use of random procedures for assignment to groups at the start of the study and the lack of information on water consumption. The use of two routes of exposure in two species is a strength. It is a feature of the time period in which the study was carried out that one-third of fetuses were evaluated for visceral abnormalities; in modern protocols, the superior approach of evaluating half of fetuses for visceral abnormalities has been adopted.

Utility (Adequacy) for CERHR Evaluation Process: This study is of high utility to the CERHR evaluation process. The change in crown-rump length is not useful in the assessment because crown-rump length is hard to measure with precision. It is doubtful that a 2.2% decrease in crown-rump length would be reproducible, and given the lack of an effect on fetal body weight, the crown-rump length finding is suspect. It is not clear that the rationale for disregarding skeletal variations because of the historical control experience is defensible.

Hardin et al. (1981), of NIOSH, tested several workplace chemicals by i.p. administration in pregnant Sprague–Dawley rats. The animals were given methyl styrene **[purity not specified]** in corn oil i.p. at 0 or 250 mg/kg bw/day for 15 days beginning the day sperm were found in the vaginal smear. There were 10–15 rats/treatment group (the other treatment groups included treatments with other chemicals). Females were killed 6 days after the last treatment and uterine contents examined. Fetuses were weighed, measured for crown-rump length, sexed, and examined for external malformations. One-third to one-half of fetuses in each litter were evaluated for visceral abnormalities after Bouin fluid fixation, and the remainder were cleared, stained with Alizarin red S, and evaluated for skeletal abnormalities. Statistical methods were not discussed. There was no maternal toxicity, defined as alterations in maternal weight gain or the weights of two or more organs; there was no fetal toxicity, defined as reduced embryo fetal survival, body weight, or length; and there were no teratogenic effects. **[One experiment described in this study used styrene oxide and is described in Section 3.2.4.]**

Strengths/Weaknesses: Generally, this report contained insufficient information on methods. The protocol extended the dosing period to start on the first day of pregnancy instead of GD 6, but was otherwise comparable to a guideline-compliant developmental toxicity study. The i.p. route of exposure was not relevant for human exposures. There were no pharmacokinetic data and the lack of maternal toxicity raises the question of sufficiency of the exposure level. The use of only 10–15 litters per treatment group was not optimal.

Utility (Adequacy) for CERHR Evaluation Process: The irrelevant route of methyl styrene exposure limits the utility of this study for the CERHR process.

Srivastava et al. (1992a), of the Industrial Toxicology Research Centre, evaluated the activity of several hepatic enzymes in near-term fetal Wistar rats after maternal pregnancy exposure to styrene. The styrene **[purity not specified]** was dissolved in groundnut oil and given by mouth **[gavage assumed]** at 0, 200, or 400 mg/kg bw/day ($n = 12$/dose group) from GD 1 **[not defined]** until GD 20, when the pregnant animals were killed and fetal livers harvested. Livers from six fetuses in each of two litters were pooled, homogenized, and centrifuged at $9000 \times g$ to obtain the S-9 fraction. A portion of this fraction was assayed for glutathione and for the activity of aminopyrene N-demethylase, aniline hydroxylase, aryl hydrocarbon hydrolase, and glutathione-*S*-transferase **[methods were not specified except by citation to other articles]**. The remainder of the supernatant was centrifuged at $105{,}000 \times g$ to produce a microsomal fraction for the estimation of CYP protein **[methods not specified except by citation to another article]**. Assays were normalized to protein content, and comparisons were made using *t*-tests. Maternal mortality and behavior was described as unaffected by styrene administration **[feed consumption and maternal body weight were not given]**. Results are summarized in Table 25. Fetal weight decreased 17% and absolute fetal liver weight decreased 23% in the 400 mg/kg bw/day group; relative fetal liver weight was not affected. The activities of all enzymes and the glutathione and CYP content of the liver homogenates were decreased in the 400 mg/kg bw/day dose group compared to the controls. Aniline hydroxylase, aryl hydrocarbon hydrolase, glutathione-*S*-transferase, and glutathione were also decreased in the 200 mg/kg bw/day dose group. The authors concluded that exposure during pregnancy to styrene could interfere with the development of enzymes involved in the activation and inactivation of xenobiotics.

Strengths/Weaknesses: The dosing period covered most of developmental period and the route of exposure was relevant. The number of animals per group was lower than indicated by regulatory guidelines for developmental toxicity studies. The administration of styrene by mouth is a weakness with respect to modeling inhalation exposures, which are more typical for humans. Little information was provided on maternal parameters (feed consumption, water consumption, body weight), the fetuses were not sexed or counted, and it is not clear that the gavage solution was analyzed. It is also not clear how or why some of the fetal samples were pooled for biochemical analyses and how this pooling might have affected statistical analysis. The lack

Table 25
Effect of Oral Styrene During Pregnancy in Rats on Fetal Liver Endpoints[a]

	Control mean, %							
	Fetal weights		Enzymes[b]					
Exposure group	Body	Liver	1	2	3	4	GSH	CYP
Styrene 200 mg/kg bw/day	94	86	80	82[c]	70[c]	85[c]	88[c]	94
Styrene 400 mg/kg bw/day	83[c]	77[c]	65[c]	74[c]	51[c]	73[c]	82[c]	55[c]
BMD_{10}	279	175	113	149	81	149	218	234
$BMDL_{10}$	208	115	81	105	68	108	155	66
$BMD_{1\ SD}$	197	362	212	205	102	185	188	353
$BMDL_{1\ SD}$	110	221	135	131	74	122	123	182

[a]Srivastava et al. (1992a). **[Data converted to percent of control values and benchmark doses calculated by CERHR.]**
[b]1, aminopyrene-*N*-demethylase; 2, aniline hydroxylase; 3, aryl hydrocarbon hydroxylase; 4, glutathione-*S*-transferase.
[c]Different from control at $p < 0.05$ or less (Student *t*-test; $n = 12$ litters for fetal body and liver weights, $n = 6$ pooled samples for other measurements).
GSH, glutathione; CYP, cytochrome P450; BMD, benchmark dose. BMD_{10} is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. $BMDL_{10}$ represents the dose associated with the lower 95% CI around this estimate. A 10% alteration in a continuously distributed parameter is an arbitrary benchmark that may not be comparable to a similar alteration in any other endpoint. The $BMD_{1\ SD}$, which represents an alteration equivalent to 1 SD of the control distribution, may permit more appropriate comparisons of the responses of continuously-distributed parameters. Benchmark doses are used commonly in a regulatory setting; however, they are used in this report when the underlying data permit their calculation and are only supplied to provide one kind of description of the dose-response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making.

of assessment of reversibility of the enzyme changes and the lack of demonstrated relevance of these unconventional endpoints in human risk assessment are weaknesses of this study.

Utility (Adequacy) for CERHR Evaluation Process: The study is of limited utility for the CERHR process; however, there is no general consensus on whether changes in metabolizing enzyme levels in fetal liver constitute an adverse effect. It is possible, given the decreased fetal body weight, that the changes are really just developmental delays.

Chernoff et al. (1990), of the U.S. EPA, evaluated the relationship between maternal toxicity and developmental toxicity in Sprague–Dawley rats using a panel of chemicals that included styrene. The chemicals were given at single dose levels by daily gavage on GD 6–15 (vaginal sperm = GD 0). The dose levels were selected to produce significant maternal weight loss or mortality. The selected styrene dose level was 1147 mg/kg bw/day in corn oil. Groups of animals ($n = 3$–5/time point) were killed on GD 8, 12, and 16 for measurement of maternal thymus, spleen, and adrenal weight, and on GD 20 ($n = 13$) for measurement of these same organ weights and for assessment of litters. Half of each litter was fixed in formalin and dissected for soft tissue abnormalities, and the other half was cleared and stained with Alizarin red S for skeletal evaluation. Comparisons were made to a corn oil control group ($n = 6$ or 7 per time point except $n = 30$ on GD 20). The statistical methods involved correlation of developmental and maternal effects across the panel of chemicals; only the results for styrene will be considered here. All styrene-treated dams survived until the scheduled kills. Maternal body weight gain was reduced a maximum of 62.2 g on GD 12, with a lesser reduction (7.4 g) near term. Significant changes were also seen in maternal organ weights. **[Reductions in maternal body weight gain were taken from a table that reported weight deficits in animals killed on each of the reported days. A graph of maternal body weight gain for those animals killed on GD 20 showed a maximum weight gain deficit of about 28 g on GD 8.]** There were no adverse effects of styrene treatment on number of fetuses, fetal weight, or fetal abnormalities except for an increase in dilated renal pelvis (46.2% of fetuses/litter with left renal pelvis dilatation **[control percentage not given]**). The authors did not draw conclusions specific to styrene.

Strengths/Weaknesses: This study is not a traditional guideline study but does add to the weight of evidence that even high doses of styrene do not have adverse developmental effects. It is not clear if the increase in dilated renal pelvis was considered related to styrene or to maternal toxicity. Other weaknesses include the lack of a description of randomization of the animals, an insufficient number of animals per time point, use of a single dose level, fetal weight, fraction of fetuses dead, fraction of fetuses with supernumerary ribs, and lack of actual values for fraction of fetuses with IV and lateral ventricles and fetuses with kidney scores >1 (these parameters were described only in relation to control values, which were not stated).

Utility/Adequacy for CERHR Evaluation Process: This study is useful only in support of findings from studies for which hazard identification was the primary purpose.

Daston et al. (1991), supported by the U.S. EPA and the National Institutes of Health (NIH), evaluated alterations in metallothionein as a mediator of developmental toxicity in Sprague–Dawley rats, using urethane as an example of an agent that induces metallothionein and styrene as an example of an agent that does not. The hypothesis being tested was that alterations in zinc economy associated with metallothionein induction mediate the developmental toxicity of urethane at

maternally toxic doses. **[Styrene was used as a negative control because it does not induce metallothionein; only the styrene-related methods and results will be presented here.]** Styrene **[purity not specified]** 300 mg/kg bw was given by gavage to 18 pregnant animals on GD 11 (plug = GD 0). Controls ($n = 16$) received an equal volume of the corn oil vehicle. The styrene dose was selected as being maternally toxic and resulted in a decrease in maternal feed consumption and body weight over the day after treatment. A dietary control group ($n = 4$) was given 16 g feed for the 18-hr time period during which styrene-treated animals were recovering from treatment, to approximate the reduction in feed consumption that occurred in the styrene-treated group. This group was compared to a group of 4 ad lib fed rats. Animals were killed 18 hr after dosing for an assessment of hepatic metallothionein levels or on GD 20 for evaluation of uterine contents. Fetuses were examined for external abnormalities. Half of each litter was examined for visceral abnormalities and the other half cleared and stained for skeletal examination. An additional six dams/treatment group were killed 18 hr after GD 11 treatment for evaluation of radiolabeled zinc distribution. Styrene had no effect on the number of live embryos/litter or mean litter weight on GD 12 in the main experiment ($n = 9$ or 10 litters/group) or the zinc-distribution experiment ($n = 6$ litters/group). There were no adverse effects of styrene treatment on number of implantations, live fetuses, dead fetuses, or resorptions, on fetal weight, or on malformations evaluated on GD 20 (7 or 8 litters/group, malformations analyzed on a per fetus basis). Hepatic metallothionein was increased about 2.5-fold in the styrene-treated dams, with about the same magnitude of increase seen in the feed-restricted controls. Zinc distribution was not affected by styrene treatment. The authors concluded that styrene was not developmentally toxic under the conditions of the study.

Strengths/Weaknesses: This study was intended to evaluate potential mechanisms underlying maternally mediated developmental toxicity and was not designed to assess potential developmental effects of styrene. The single day of dosing is a weakness, but the use of a pair-fed control is a strength. The monitoring of feed and water consumption during gestation is also a strength. There were insufficient numbers of litters per group for evaluation of developmental toxicity, and there was no indication that random procedures were used for group assignment at the start of the study. The fetuses were weighed as litters; individual weights would have strengthened the study. Limited skeletal and visceral development data were presented, and analysis of fetal abnormalities did not include litter distribution.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is limited to support of findings from studies for which hazard identification was the primary purpose.

Two multigeneration studies in rats (Beliles et al., 1985; Cruzan et al., 2005b), discussed more fully in Section 4.2.3, provided information on developmental effects of styrene exposure. Rats in one study were exposed through drinking water and rats in the second study were exposed by inhalation; both studies were conducted according to regulatory guidelines in place at the time. These studies indicate growth effects at very high levels of exposure but no teratogenic or functional effects.

Kankaanpää et al. (1980), from the Finland Institute of Occupational Health, evaluated styrene effects on pregnancy in mice and hamsters. BMR/T6T6 mice were exposed by inhalation to styrene (>99% pure) 6 hr/day on GD 6–16 (plug = GD 0) at 0 ($n = 15$) or 250 ($n = 13$) ppm. Animals were killed on GD 16 after exposure and uterine contents were evaluated. Fetuses were weighed, measured, and evaluated for external abnormalities and cleft palates. Decapitated carcasses were cleared and stained with Alizarin red S for skeletal evaluation. Proportions were evaluated by Fisher exact test. There were no differences by treatment in the number of live fetuses/litter. The number of dead or resorbed fetuses was increased from 18.2% in the control group to 26.9% in the styrene-treated group. **[No litter analysis was performed; no data were presented on maternal condition.]** There was no treatment effect on the proportion of malformed fetuses.

Chinese hamsters were exposed 6 hr/day by inhalation on GD 6–18 (day of copulation = GD 0) to styrene (>99% purity) at 0 ($n = 15$), 300 ($n = 2$), 500 ($n = 3$), 750 ($n = 5$), or 1000 ($n = 7$) ppm. Hamsters were killed on GD 18 and uterine contents evaluated as for mice. There was no effect on the number of live fetuses/litter. There was a significant increase in the proportion of dead or resorbed fetuses at 1000 ppm. There were no malformed fetuses. **[No litter analysis was performed; no data were presented on maternal condition, although in the discussion the authors characterize maternal toxicity as "small."]** The authors concluded that styrene was embryotoxic in mice and hamsters.

Strengths/Weaknesses: The design of this study is appropriate for evaluating developmental effects, and the use of two species, a relevant exposure route, and a wide range of dose levels are strengths. The Expert Panel notes, however, that the mouse strain used is not conventional, and the use of hamsters in developmental toxicity studies is not routine. There was no indication that random procedures were used for group assignment at the start of the study. Other weaknesses include a lack of experimental detail, a suboptimal number of animals per group, and inadequate or incorrect statistical analysis. The high rate of embryo mortality in the mouse control is of concern. It is not clear that visceral evaluations were performed or why mouse fetuses were evaluated on GD 16 rather than the more conventional GD 17 or 18. Detailed data on skeletal development were not provided.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is limited because of apparent weaknesses in the study, although the interpretation of data from non-routine models can be helpful in evaluating the overall data set.

Ninomiya et al. (2000), support not indicated, treated pregnant ICR mice with styrene by inhalation and evaluated the number of implantations and live fetuses and fetal weights. **[The study was published in Japanese with an English abstract and English tables. Professional translation was obtained by CERHR.]** Styrene exposure levels were 0, 2, 20, and 100 ppm ($n = 18$ or 19/group) with exposures 24 hr/day on GD 0–15. Chamber concentrations measured by GC were 2.0 ± 0.5, 19.9 ± 2.7, and 111 ± 19 ppm. Animals were

killed on GD 15 and uterine contents evaluated for number of implantations, resorptions, live and dead fetuses, and malformed fetuses. **[Evaluation of fetal anatomy seems to have been limited to external examination.]** Statistical comparisons were made using χ^2, an unnamed median test, and a *t*-test. Dams were described as hyperkinetic after exposure to styrene 100 ppm. There was no treatment effect on proportion of animals pregnant, number of implantations, number of live fetuses, percent resorptions, or percent dead fetuses. In the 100 ppm group, maternal weight was decreased by 18%, and fetal weight was decreased by 25%. The authors considered 20 ppm to be a no-effect level and 100 ppm to be an effect level. **[It does seem seem that litter effects were considered. Using the benchmark dose approach, the BMD_{10} for fetal weight reduction was 36 ppm, and the $BMDL_{10}$ was 6 ppm. The $BMD_{1\ SD}$ was 49 ppm, and the $BMDL_{1\ SD}$ was 38 ppm. For maternal weight, the BMD_{10} was 63 ppm, and the $BMDL_{10}$ was 11 ppm. The $BMD_{1\ SD}$ was 47 ppm, and the $BMDL_{1\ SD}$ was 30 ppm.]**

Strengths/Weaknesses: The design of this study is unusual, especially the evaluation on GD 15, which limits the amount of fetal anatomical data that can be collected, and the 24-hr/day inhalation exposures. The use of external examination only and the low dose range are additional weaknesses, as is the lack of information on randomization of animals. Maternal toxicity was seen at 100 ppm, an exposure level that was not maternally toxic in other studies, although there was generally inadequate information on maternal toxicity (e.g., feed and water consumption). It is not clear whether visceral and skeletal examinations were performed, and the statistical treatment of data seems to be inadequate and or inappropriate. Strengths include the use of multiple exposure levels and measurement of chamber styrene concentrations.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility because of the apparent weaknesses but may still be useful for determining effects on embryo mortality and embryo growth.

3.2.2.2 Neurobehavioral endpoints:

Zaidi et al. (1985), from the Industrial Toxicology Research Centre, evaluated developmental effects of styrene on dopamine receptors and locomotor activity in rats. Albino rats **[strain not indicated]** were treated either during gestation, during lactation, or during both periods by gavage with styrene **[purity not specified]** in groundnut oil at 0 or 200 mg/kg bw/day. In the first experiment, treatment of 12 dams (6/dose group) extended from GD 1 **[not defined]** until parturition, after which three dams in each treatment group raised their own litters and the other three dams raised foster litters from the opposite treatment group. Litters were adjusted to eight randomized pups **[time of adjustment and sex not specified]**. In a second experiment, four pregnant dams/dose group were treated with styrene 0 or 200 mg/kg bw/day "throughout the gestation and lactation period up to 2 or 3 weeks." Litters were adjusted to eight randomized pups **[time of adjustment and sex not specified]**. In a third experiment, four dams/dose group were given styrene 0 or 200 mg/kg/day "from Day 1 after parturition up to 2 or 3 weeks." A fourth experiment was performed in which control pups were fostered to dams that had received styrene during gestation and continued to receive it during lactation. **[Styrene dose, vehicle, and route are specified only for some of these experiments and is assumed to have been uniform in all of the experiments.]** Pups were killed at "2 to 3" weeks of age, brains removed, and corpora striata dissected in the cold and frozen until assayed. Dopamine receptor binding was assessed using labeled spiroperidol binding. Six pups of "either sex" from each group were used. Amphetamine-stimulated locomotor activity and apomorphine-induced stereotypy were assessed in eight animals from litters exposed to vehicle during pregnancy and lactation and eight animals from litters exposed to styrene during pregnancy and lactation. Multiple treatment groups were compared using ANOVA followed by the Fisher least significant difference test. Comparison of means of two groups was performed using the Student *t*-test or, for stereotypy (which used a 6-point ranking scale), the Mann–Whitney *U*-test. **[Litter of birth or rearing seems not to have been considered in the analysis and is not mentioned with respect to selection of pups for testing.]**

Dam body weights at birth and during the lactation period and number of pups/litter were not affected by treatment **[data not shown]**. Pup body weight at 2 or 3 weeks of age and protein content in the striatum were not affected by treatment in any of the experiments. Dopamine binding in the offspring striatum was significantly increased by styrene treatment of the dam during the lactation period, whether or not the pups had been exposed during gestation. Scatchard analysis showed that the increase in binding was attributable to an increase in dopamine receptor number rather than an increase in affinity. Styrene treatment during the gestation and lactation period was associated with an increase in amphetamine-stimulated locomotor activity and an increase in apomorphine-induced stereotypy compared to vehicle treatment. The authors concluded that the increase in dopamine receptor number and the behavioral alterations were consistent with "denervation supersensitivity" and might have a correlate in the coordination and balance disturbances reported in styrene-exposed workers. The authors noted that the maturation of dopamine receptors in the rat occurs during PND 7–28, which is consistent with the effect of styrene during the lactation, but not the gestation, period.

Strengths/Weaknesses: The design of this study was not regulation compliant; the study was intended to evaluate selected neurochemical and neurobehavioral endpoints. Because the treatment was litter-based, the litter is the appropriate statistical unit, but there were far too few litters per group to make definitive conclusions from this study. Other weaknesses include not separating males and females for neurobehavioral testing, use of a single dose level, lack of information on maternal clinical signs, feed consumption, or weight, and assessment of only amphetamine-stimulated rather than spontaneous locomotor activity, which limits human relevance. The statistical treatment seemed to be inadequate, and there were a number of details missing from the study description. There was no indication of randomized assignment of animals to treatment groups, strain of animals, or analysis of the gavage solution. It is a strength that an effect was seen only during the relevant developmental period.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility because of missing

information and the small number of animals in the treatment groups.

Khanna et al. (1991), from the Industrial Toxicology Research Centre, evaluated the interaction of maternal styrene treatment and protein malnutrition on neurobehavioral parameters in rat offspring. Wistar rats were mated (plug = GD 1) and randomized to a 20% casein diet or an 8% casein diet. Animals were pair-fed **[the reference group is not indicated]**. On GD 6, the animals received styrene **[purity not specified]** in groundnut oil at 0 or 100 mg/kg bw/day orally **[gavage assumed]**. There were six animals in each of four treatment groups: normal protein–vehicle, normal protein–styrene, low protein–vehicle, and low protein–styrene. These interventions were continued through weaning. At parturition, litters were culled to eight **[using pups of both sexes, proportion of either sex not specified]**. Developmental landmarks and reflexes were evaluated during the lactation period. Ten pups from four litters in each group **[a figure legend says 15 pups representing four litters; sex not specified]** were killed on PND 22, and brains were used for determination of monoamine oxidase and succinate dehydrogenase activity in the mitochondrial fraction, sodium-potassium ATPase in the microsomal fraction, dopamine receptor in corpus striatum (using haloperidol as the ligand), and serotonin receptor in frontal cortex (using serotonin as the ligand). Six different pups from each dose group **[sex and litter origin not specified]** were tested for locomotor activity on PND 21 after injection of amphetamine 2.5 mg/kg bw i.p. Activity counts were recorded using a photocell method. Statistical analysis was performed using ANOVA. **[Post-hoc testing not mentioned; the comparisons were between pairs of interventions. Litter effects seem not to have been considered.]**

Maternal condition and weight gain were not discussed. Styrene treatment was associated with a decrease in pup body and brain weight and a delay in acquisition of developmental landmarks and reflexes in groups exposed to low protein, but not in groups exposed to a normal protein diet. Mean pup body weights on PND 22 **[estimated from a graph]** were: normal protein–vehicle 39.2 g, normal protein–styrene 36.7 g, low protein–vehicle 27.5 g, and low protein–styrene 17.5 g. Mean brain weights on PND 22 **[estimated from a graph]** were: normal protein–vehicle 1.25 g, normal protein–styrene 1.25 g, low protein–vehicle 1.01 g, and low protein–styrene 0.93 g. Enzyme activities were decreased, serotonin neuroreceptor binding increased, and amphetamine-stimulated locomotor activity was increased in the low protein-styrene group. There were no significant effects of styrene in the offspring of animals fed a normal protein diet. The authors concluded that the developmental neurotoxicity of styrene in protein-malnourished animals may be important for assessing the potential risk of styrene exposure in developing countries.

Strengths/Weaknesses: The design of this study was not regulation compliant; the study was intended to evaluate the effects of styrene/protein malnutrition interactions on postnatal development. The study was limited by the small number of litters, inadequate descriptions of results and methods, and inadequate statistical treatment, with lack of consideration of litter effects. There was no mention of randomization of animals to treatments. The use of a single styrene exposure group is a weakness. Maternal toxicity was not described, although feed consumption was measured, and the relevance of such a low-protein diet was not made clear. The very low PND 22 weights would be expected to influence neurobehavioral testing regardless of styrene exposure. An additional weakness is the assessment of only amphetamine-stimulated rather than spontaneous locomotor activity, which limits human relevance.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is limited because of the extreme under-nutrition of the animals.

Kishi et al. (1992, 1995), supported by the Japanese Ministry of Education, studied the effects of gestational exposure to styrene on neurochemical and behavioral endpoints in Wistar rats. These reports are presented together because they involve offspring of the same pregnant animals, which were exposed by inhalation to 0 ($n = 14$), 50 ($n = 3$), or 300 ($n = 7$) ppm styrene 6 hr/day on GD 7–21 (vaginal sperm = GD 0). Exposure chamber styrene concentrations were monitored hourly by GC and averaged 60.1 ± 18.9 and 292.7 ± 72.4 ppm **[errors defined as SD in the second article, but reduced by an order of magnitude to 1.9 and 7.2 ppm]**. Litters were standardized on PND 1 to six pups, except for one litter **[dose group not indicated]**, which was culled to five pups **[sex composition of litters not indicated]**. Ten culled pups per treatment group were decapitated, and cerebra and cerebella were homogenized. HPLC was used to estimate cerebrum and cerebellum levels of 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and norepinephrine. **[Statistical methods were not discussed for the comparison of neurotransmitter levels; litter effects do not seem to have been considered.]**

In the first article (Kishi et al., 1992), pup body weight on PND 1 was reduced in both styrene exposure groups (mean ± SD: 0 ppm, 6.2 ± 0.6 g; 50 ppm, 5.5 ± 0.7 g; 300 ppm, 5.7 ± 0.6 g; $p < 0.01$ **[BMD_{10} 413 ppm, $BMDL_{10}$ 205 ppm, $BMD_{1\ SD}$ 410 ppm, $BMDL_{1\ SD}$ 199 ppm]**). The second article (Kishi et al., 1995), using a subset of the same litters, reported no treatment effect on PND 1 pup body weight. Maternal weight gain during exposure was not shown to be affected by treatment (mean ± SD: 0 ppm, 48.1 ± 14.6 g; 50 ppm, 45.0 ± 18.0 g; 300 ppm, 39.1 ± 5.5 ppm; p = NS). Length of gestation, live litter size, brain weight, and brain protein were unaffected by treatment. Cerebrum 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, and homovanillic acid were decreased in offspring of the 300 ppm group at $p < 0.05$ (Table 26). Cerebrum dopamine and cerebellar 5-hydroxytryptamine were decreased at $p < 0.1$. Maternal and fetal brain, liver, lung, and kidney sections did not show histopathologic abnormalities by light microscopy.

For the neurobehavioral study, the number of litters evaluated was reduced to 12 (5 at 0 ppm, 2 at 50 ppm, 5 at 300 ppm) because of the limited number of exposure chambers available **[it seems that evaluation was restricted to litters exposed at the same time]**. Pups were evaluated daily for development of startle reflex, righting reflex, eye opening, incisor eruption, and vaginal patency. Behavioral tests included surface righting, pivoting locomotion, bar holding, negative geotaxis, and cliff avoidance. Pups were weaned on PND 22. Post-weaning tests included open field behavior, rotarod motor coordination, spontaneous activity monitoring,

Table 26
Significant Alterations in Cerebral Neurotransmitter Content in Newborn Rats After Gestational Exposure to Styrene[a]

	Control mean, %			
Exposure group	5-HT	5-HIAA	Dopamine	HVA
Styrene 50 ppm	91	78	102	110
Styrene 300 ppm	72[b]	59[b]	87[c]	84[b]
BMD_{10}	54	73	292	282
$BMDL_{10}$	28	48	117	99
$BMD_{1\ SD}$	276	311	309	302
$BMDL_{1\ SD}$	166	178	206	199

[a]From Kishi et al. (1992). **[Data converted to percent of control values and benchmark dose calculated by CERHR.]**
[b]Different from control value at $p < 0.05$.
[c]Different from control value at $p < 0.1$ ANOVA with post hoc Scheffe test; $n = 14$ at 0 ppm, $n = 3$ at 50 ppm, $n = 7$ at 300 ppm. 5-HT 5, hydroxytryptamine; 5-HIAA 5, hydroxyindolacetic acid; HVA, homovanillic acid; BMD, benchmark dose, (ppm). For an explanation of BMD, see footnote to Table 25.

operant conditioning, and sleep time in response to i.p. sodium pentobarbital. ANOVA was performed using the litter as the unit of analysis and excluding the 50 ppm group due to small numbers. If litter-based ANOVA showed a significant difference, ANOVA using individual offspring was performed with post-hoc Scheffé test.

Although pup body weight on PND 1 was not found to be affected by treatment in the second study, male offspring body weight on PND 21 and female offspring body weight on PND 77 were decreased at 300 ppm compared to the controls. Incisor eruption and eye opening were delayed an average of 0.7–0.8 days, and acquisition of auditory startle and righting reflexes were delayed an average of 1.3–1.8 days in the offspring of the 300 ppm group. The authors used the Scheffé test to evaluate per-offspring data and concluded that the 50 ppm group also had a delay in acquisition of auditory startle and righting reflex compared to the control group. Pivoting and bar-hold performance were impaired in the offspring of the 300 ppm group. Increased activity in the open field, a decrease in coordination in the rotarod test, and an increase in spontaneous activity during the dark period were seen in the offspring of the 300 ppm group during the first 2 months of life but not thereafter. Operant conditioning response was impaired in the 300 ppm group in Session 3 of 7. Pentobarbital-induced sleeping time was not altered by treatment. The authors concluded that styrene 300 ppm by inhalation during gestation caused neurobehavioral abnormalities in offspring without evidence of maternal toxicity. They proposed that the decreased 5-hydroxytryptamine brain levels identified on PND 1 may indicate a mechanism by which behavior would be altered. They indicated that the small number of litters at 50 ppm precluded evaluation of this exposure level on a litter basis, but that per fetus analysis suggested that 50 ppm was also an effect level. They recognized that the lack of a cross-fostering design precluded the elimination of dam factors as possibly mediating the effects of treatment on the offspring.

Strengths/Weaknesses: The study design was not regulation compliant. The number of litters was too small to support any definitive conclusions. Randomization of animals to treatment groups was not discussed. There were too few pups to assess a possible dose-response relationship. The contribution of the dam to the effects seen could not be evaluated. The assessment of developmental landmarks should have been correlated with pup weight. The lack of information on reversibility of the neurotransmitter alterations is a weakness. There was no information on cesarean sections, visceral development, or skeletal development. The culling to six per litter rather than eight or 10 was unusual. Strengths of the study are the 160-day follow-up of the animals, the mechanistic information on neurotransmitters, and the analysis of chamber styrene concentrations.

Utility (Adequacy) for CERHR Evaluation Process: The study is of limited utility because of apparent weaknesses in the study. Some information may be interpretable on developmental landmarks (pre-weaning and post-weaning behavior) after styrene exposure during gestation, 1-day litter index, length of gestation, and body weight gain.

Katakura et al. (1999, 2001), support not indicated, reported follow-up studies from the Kishi et al. (1995) laboratory using larger numbers of litters and pair-fed controls. The two studies by Katakura et al. are presented together because they involve offspring of the same pregnant animals. Treatment groups consisted of ad lib-fed controls exposed to styrene 0 ppm ($n = 14$), controls exposed to 0 ppm styrene and pair-fed to the high-dose group ($n = 12$), animals exposed to styrene 50 ppm and pair-fed to the high dose group ($n = 10$), and high-dose animals exposed to styrene 300 ppm ($n = 14$). Exposures to styrene or air occurred on GD 6–20 (vaginal sperm or plug = GD 0). Actual chamber concentrations of styrene were 50.1 ± 8.9 ppm and 295.5 ± 9.5 ppm **[error not defined but assumed to be SD based on Kishi et al. (1995)]**. Pups were counted, sexed, weighed, and examined under a stereomicroscope at birth. Three or four pups/litter **[sex not indicated]** were decapitated at birth and their brains dissected to isolate cerebrum and cerebellum for neurochemical analysis. Eight pups/litter **[sex not specified]** were reared by their dams until weaning on PND 21. During the pre-weaning period, offspring were observed for pinna unfolding, eye opening, incisor eruption, and acquisition of surface-righting and air-righting reflexes. Two males and two females per litter were killed at weaning and their brains dissected to isolate samples of prefrontal cortex, striatum, hippocampus, hypothalamus, and midbrain for neurochemical analysis. Neurochemical analyses were performed by HPLC for 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and norepinephrine on PND 1 and PND 21 brains. Statistical analyses were performed using ANOVA with post-hoc Scheffé test with χ^2 testing of proportions. **[The table legends indicate that 10 litters/dose group were used for the neurochemical analyses.]**

Feed consumption was decreased in dams in the 300 ppm styrene group to a mean $\pm$SD of 15.0 ± 2.4 g/day, compared to an ad lib-fed control value of 18.0 ± 2.3 g/day during GD 6–13 and 16.9 ± 1.0 g/day, compared to 21.4 ± 4.5 g/day during GD 14–20. Maternal weight gain during exposure was not significantly affected by treatment. There were no treatment-related differences in number of litters, liveborn litter size, pup

birth weight, or cerebellum weight on PND 1. Gestation length was decreased by an average of 0.5 days in the 300 ppm group compared to the ad lib-fed control, and the neonatal death rate (on a per fetus basis) was increased in the 300 ppm group compared to the ad lib-fed control. Cerebrum weight on PND 1 was reduced in the 300 ppm group compared to the ad lib-fed control group, but was not statistically different from the pair-fed control group. In the PND 1 cerebrum, the homovanillic acid levels were lower in the 300 ppm group than in either control. 5-Hydroxytryptamine was lower than in the ad lib-fed controls but was not different from the 5-hydroxytryptamine level in the pair-fed controls. There were no treatment effects on neurochemical levels in cerebellum on PND 1.

On PND 21, male body weight was decreased in offspring of the 300 ppm group compared to either control, and cerebrum weight (absolute and total) was decreased compared to the ad lib-fed control. 5-Hydroxytryptamine levels were statistically higher in the striatum of offspring in the 50 ppm but not the 300 ppm group. 5-Hydroxyindoleacetic acid was reduced in the hippocampus of offspring in the 300 ppm group compared to both controls and in neocortex in the 300 ppm group compared to ad lib-fed controls. The ratio of 5-hydroxyindoleacetic acid to 5-hydroxytryptamine was reduced in hippocampus in both styrene-exposed groups compared to either control. In measures of the dopaminergic system, there were no treatment effects except an increase in dopamine in striatum in the 300 ppm group compared to the ad lib-fed control, but not compared to the pair-fed control. Histopathologic evaluation of brain, lung, and kidney of dams and selected offspring did not show abnormalities at the light microscopic level. Statistically significant findings in comparison to the pair-fed control are shown in Table 27.

Lower incisor eruption, eye opening, and acquisition of air righting reflex were delayed in the 300 ppm group compared to either control. Upper incisor eruption was delayed in the 300 ppm group and air-righting was delayed in the 50 ppm group compared to the ad lib-fed control. The times of pinna unfolding and acquisition of surface righting were not affected by treatment.

The authors concluded that the effects noted in styrene-treated animals compared to either control may have been treatment-related because there was no significant difference in any endpoint between the two controls. They concluded that the PND 1 results suggested that styrene exposure during gestation causes effects in the serotonergic system of the fetal brain.

Strengths/Weaknesses: This study had improved statistical power compared to the Kishi et al. studies (1992, 1995), and the use of pair-fed controls was a useful addition to the study. Statistical treatment was better explained and was on a litter basis. Increased neonatal death and decreased weight at the high dose seemed to be the most severe effects. A dose-response relationship was not evident for 5-hydroxytryptamine. The use of a 50 ppm exposure level was a strength because in many countries, 50 ppm is the occupational threshold value. The information on methodology was generally complete; however, the number of animals per dose group and the number of dose levels were suboptimal. The lack of visceral development data is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the evaluation process.

Cruzan et al. (2005c), supported by the Styrene Information and Research Center, reported a developmental neurotoxicity study in Crl:CD®(SD)IGS BR rats that was part of a two-generation reproductive study (Cruzan et al., 2005b; summarized in Section 4.2.3). F_0 and F_1 males and females ($n = 25$ sex/group) were exposed to styrene (≥99.9% pure) by inhalation at 0, 50, 150, or 500 ppm 6 hr/day, 7 days/week. Treatment began at least 70 days before mating and was continued through GD 20 (plug = GD 0). On PND 1–4, F_0 and F_1 dams received styrene in olive oil by gavage at 0, 66, 117, or 300 mg/kg bw/day, given in three divided doses, 2 hr apart. This treatment regimen was calculated based on a PBPK model to produce styrene blood levels at 2, 4, and 6 hr after onset of daily treatment similar to blood levels after inhalation exposure. Oral treatments were used during PND 1–4 to avoid the stress of separating dams from their litters for 6 hr/day. Inhalation exposure of dams resumed on PND 5. Offspring were weaned on

Table 27
Significant Alterations in Rat Pups After Gestational Exposure to Styrene, Compared to Pair-fed Control[a]

	Percent of pair-fed control mean			
Treatment group	Male body weight, PND-21	Neonatal death (per fetus)	Cerebral HVA, PND-1	Hippocampal 5-HIAA, PND 21[b]
Styrene 50 ppm	93	215	80	97
Styrene 300 ppm	92[c]	562[c]	69[c]	77[c]
BMD_{10}	498	408	113	139
$BMDL_{10}$	264	301	73	93
$BMD_{1\ SD}$	452	NA	276	247
$BMDL_{1\ SD}$	334	NA	166	146

[a]Katakura et al. (1999, 2001). **[Data converted to percent of control values and benchmark dose calculated by CERHR.]** Delay in developmental milestones and acquisition of reflexes not shown.
[b]Estimated from a graph.
[c]Different from control value at $p < 0.05$ (ANOVA with post-hoc Scheffe test; $n = 10$ litters/group except PND 21 body weight for which $n = 9$–14/group).
NA, non-applicable; HVA homovanillic acid, 5-HIAA 5-hydroxyindoleacetic acid. BMD, benchmark dose. For an explanation of BMD, see footnote to Table 25.

PND 21 and F_1 offspring were treated at their dams' exposure levels from PND 22. F_2 offspring were not directly exposed to styrene. Litters were standardized to 10 pups on PND 4, with equal sex distribution when possible. Pup developmental milestones were assessed, and two F_2 pups/sex/litter were selected for neurodevelopmental testing. A third F_2 pup/sex/litter was selected for neuropathologic evaluation on PND 21. Functional observation battery was performed on F_1 dams on GD 6 and 12 and PND 10 and 21 and on one subset of F_2 pups on PND 4, 11, 22, 45, and 60. The pups were also tested for locomotor activity on PND 13, 17, 21, and 61, for acoustic startle response on PND 20 and 60, and for swimming ability, learning, and memory in a water maze on PND 62. These pups were killed for central and peripheral nervous system pathology on PND 72. The second subset of F_2 pups was tested in the water maze only on PND 24. Statistical analysis was by ANCOVA with pup weights nested within litters and litter size as the covariate, by ANOVA with post-hoc Dunnett test, Kruskall–Wallis test, or Mann–Whitney *U*-test, or by Fisher exact test. Repeated measures ANOVA was used for results of tests conducted in sequential sessions.

Adverse effects on body weight gain were seen at 500 ppm in male and female F_0 rats and at 150 and 500 ppm during the premating interval in F_1 males and females. There were no treatment effects on feed consumption or maternal body weight gain during gestation in either generation, although water consumption was increased in F_0 and F_1 dams of the 500 ppm dose group. Reproductive parameters were not affected by treatment, as detailed more fully in Section 4.2.3. There was a decrease in F_2 offspring body weights from birth through PND 70 in the 500 ppm group; statistical significance was obtained from PND 1–21. In the 150 ppm group, pup body weight was decreased from PND 7 through adulthood; statistical significance was achieved in males on PND 13 and 21. There were no significant differences between treated and control animals in pinna detachment, surface righting response, hair growth, eye opening, and day or weight of onset of vaginal opening or balanopreputial separation. Incisor eruption was significantly delayed in the 500 ppm dose group. The authors identified "subtle indications of a delay in the acquisition of developmental landmarks," despite the lack of statistical significance, and stated that the later acquisition of landmarks in the presence of reduced body weight suggested "slight developmental delay."

There were no effects of treatment on functional observation battery in F_1 dams or in F_2 offspring. There was a decrease in fore- and hindlimb grip strength on PND 45 and 60 in the F_2 offspring, which the authors considered to be a further indication of developmental delay. There were no significant alterations in locomotor activity in the F_2 offspring, but the authors felt that a shift in the pattern of development of locomotor activity in the 500 ppm group was consistent with developmental delay. Auditory startle response was not affected by treatment. PND 24 swimming ability was described as "slightly decreased" in F_2 offspring of both sexes in the 500-ppm group, again suggesting slight developmental delay to the authors. Memory and learning in the water maze were not affected by treatment.

Brain weights in F_2 offspring on PND 21 or 72 were not altered by styrene exposure, but relative brain weight was increased in the 500 ppm group, attributed to reduced body weight. Brain length was decreased by 4% in the 150 and 500 ppm groups on PND 21, but brain length was described as increased (although not significantly) in all dose groups on PND 72, leading the authors to conclude a lack of treatment effect. No brain histopathologic alterations were attributed to styrene treatment. The authors concluded that their findings were attributable to slight developmental delay associated with impaired growth, with a NOAEL for growth of 50 ppm. They stated, "no specific effect on nervous system development was observed at exposures up to 500 ppm styrene."

Strengths/Weaknesses: This two-generation study with added developmental neurotoxicity endpoints was performed according to regulatory guidelines and with adequate statistical power. It is a strength that the study showed maternal/paternal toxicity at the highest exposure level. Other strengths include measurement of chamber concentrations of styrene, appropriate pathology examinations, careful observation of litters, and analysis of feed and water consumption. The consistent findings in the F_2 but not F_1 pups are unusual. The attribution of decreased brain weight to decreased body weight is a weakness in that brain weight is typically refractory to body weight changes.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful for the CERHR process.

3.2.3. Postnatal treatment. **Khanna et al. (1994)**, of the Industrial Toxicology Research Centre, evaluated the effects of protein malnutrition and styrene on neurobehavioral parameters in juvenile female Wistar rats. From weaning on PND 21, 50 animals/group were given a normal protein diet (20% casein) or a low-protein diet (8% casein). Animals were pair-fed **[the reference group was not indicated]**. From PND 36 through 51, half the animals in each diet group were given styrene 250 mg/kg bw/day orally **[purity not indicated; gavage assumed]**, and half were given the groundnut oil vehicle. An unspecified number of animals from each group were tested on PND 52 for foot shock-induced fighting behavior and amphetamine-stimulated locomotor activity. **[The methods indicate that 10 rats/group were screened for aggressivity resulting in the selection of a "pair of rats that exhibited at least one fighting episode in 1 min," suggesting that two rats per treatment group may have been evaluated. No indication was given of the number of animals tested for locomotor activity. Fighting behavior scores and locomotor activity counts were used to report the results but were not defined.]** Six animals per treatment group were decapitated at 7 weeks of age for evaluation of frontal cortex levels of norepinephrine, dopamine, and serotonin. Dopamine receptors in the corpus striatum and serotonin receptor levels in the frontal cortex were also assessed. Data were analyzed using Student *t*-test. Animals fed a low protein diet weighed 32% less than animals fed a normal protein diet, with an additional 30% weight decrement associated with styrene treatment. There was no significant effect of styrene on body weight in animals given a normal protein diet. Fighting behavior was increased by the low protein diet, with a further increase by styrene treatment. In the normal protein group, there was no

effect of styrene treatment. Locomotor activity was increased with the combination of styrene and low protein diet but was not affected by either treatment applied singly. Styrene treatment caused a significant 54% decrease in dopamine concentration in the frontal cortex of rats fed a normal protein diet, and a significant decrease in all three biogenic amines in rats fed a protein-deficient diet. Binding of spiperone (to estimate dopamine receptor activity) and serotonin binding were increased by styrene in rats fed the low protein diet but not in rats fed the normal protein diet. The authors concluded that ''protein malnutrition during the early developmental period is an important predisposing factor in the expression of the neurobehavioral toxicity of styrene.''

Strengths/Weaknesses: It is a weakness that dosing did not commence until after weaning. The interaction with protein deficiency was the focus of this study, but too low a protein level was used, causing excessive toxicity. There was insufficient detail about the behavioral assessments, which were unconventional, perhaps unvalidated, and difficult to interpret. The effects on dopamine were not consistent with other studies.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility. Although technically a developmental toxicity study, dosing with styrene did not begin until after weaning. It is not possible to conclude that the effects seen were organizational in nature.

Srivastava et al. (1992b), support not indicated, exposed lactating albino rats to oral styrene **[purity not specified; route not otherwise specified]** in groundnut oil at 0, 200, or 400 mg/kg bw/day from parturition (PND 0) until weaning on PND 21. **[The strain was not specified, but Wistar rats were used in other styrene studies from this laboratory.]** There were four dams in each dose group, with litters made up on the day of parturition using eight randomly assigned males per dam. After weaning, offspring were pooled by dose group, and six males/dose group/time point were decapitated on PND 31, 61, or 91. There were no unscheduled deaths or signs of toxicity among dams or offspring. Testes and epididymides were removed and weighed after decapitation. Offspring body and testis weights were described as unaffected by treatment **[data not shown; epididymal weights not discussed]**. Homogenized testes were assayed for the activity of six enzymes (acid phosphatase, β-glucuronidase, glucose-6-phosphate dehydrogenase, γ-glutamyl transpeptidase, lactate dehydrogenase, and sorbitol dehydrogenase; expressed with respect to tissue protein). Epididymal sperm were counted in a hemocytometer. Student *t*-test was used for statistical comparisons. Results are summarized in Table 28. Significant alterations were seen for most of the reported endpoints at 31 and 61 days of age in the offspring of the 400 mg/kg bw styrene group. Epididymal sperm count was not reported on PND 31 but was decreased in the 400 mg/kg bw group on PND 61 and 91. Histologic evaluation of paraffin-embedded, hematoxylin- and eosin-stained testis sections did not show abnormalities in any treatment group. The authors concluded that the changes in testicular ''marker'' enzymes without histologic alterations ''indicate that probably only enzymatic pathways involved in maturation of testis have been affected.''

Table 28
Testis Enzyme Activities and Epididymal Sperm After Lactation Period Treatment of Rats with Styrene[a]

Day of evaluation and treatment group	SDH	LDH	G6PD	â-GLU	ACID PHOS	GGT	Sperm[b]
PND 31 (Control mean, %)							
Styrene 200 mg/kg bw/day	97	106	94	92	92	106	Not reported
Styrene 400 mg/kg bw/day	94	111[c]	104	115[c]	81[c]	118[c]	
BMD_{10}	–	375	–	384	242	280	
$BMDL_{10}$	–	200	–	302	136	126	
$BMD_{1\ SD}$	–	487	–	379	300	324	
$BMDL_{1\ SD}$	–	222	–	284	164	169	
PND 61 (Control mean, %)							
Styrene 200 mg/kg bw/day	104	108	96	98	93	94	90
Styrene 400 mg/kg bw/day	80[c]	115[c]	91	113[c]	86[c]	122[c]	75[c]
BMD_{10}	383	266	–	392	288	380	152
$BMDL_{10}$	286	132	–	302	168	212	100
$BMD_{1\ SD}$	376	360	–	381	323	386	248
$BMDL_{1\ SD}$	254	189	–	242	178	259	141
PND 91 (Control mean, %)							
Styrene 200 mg/kg bw/day	97	105	96	96	96	103	93
Styrene 400 mg/kg bw/day	95	106	107	106	91	107	81[c]
BMD_{10}	–	–	–	–	–	–	258
$BMDL_{10}$	–	–	–	–	–	–	124
$BMD_{1\ SD}$	–	–	–	–	–	–	377
$BMDL_{1\ SD}$	–	–	–	–	–	–	194

[a]Srivastava et al. (1992b). **[Data converted to percent of control values and benchmark dose calculated by CERHR.]**
[b]Epididymal sperm ''count'' estimated from a graph, assumed based on Srivastava et al., (1992c) to be sperm concentration from minced epididymis.
[c]Different from control value at $p < 0.05$ (Student *t*-test; $n = 6$/group).
SDH sorbitol dehydrogenase; LDH lactate dehydrogenase; ACID PHOS, acid phosphatase; G6PD, glucose-6-phosphate dehydrogenase; β-GLU, β-glucuronidase; GGT, γ-glutamyl transpeptidase; BMD, benchmark dose. For an explanation of BMD, see footnote to Table 25.

Strengths/Weaknesses: This study used very few litters per group. It is a weakness that dosing solutions were not analyzed, epididymal weights were not reported, and the enzymes are not known to be biomarkers of spermatogenic, Sertoli, or Leydig cell function. There were only two dose levels, and the sample sizes were inadequate. Sperm motility data would have strengthened the study. The tissue fixative used for testis histopathology was not described. The enzyme alterations seem quite small. The conclusion that styrene causes adverse effects in testicular function is in conflict with the two-generation study that showed no effect on male reproductive development.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Srivastava et al. (1992c), support not indicated, treated male Wistar rat pups with oral styrene **[route not otherwise specified; purity given as "of the highest purity"]** in groundnut oil at 0, 100, or 200 mg/kg bw/day on PND 1–60 **[dose adjustment interval not described]**. Each treatment group consisted of three dams with seven male pups/dam. Males were weaned on PND 21. On PND 61, 24 hr after the last styrene dose, six males/treatment group were randomly selected and killed. Testes and epididymides were weighed, and homogenized testes were assayed for the activity of six enzymes (acid phosphatase, β-glucuronidase, glucose-6-phosphate dehydrogenase, gamma-glutamyl transpeptidase, lactate dehydrogenase, and sorbitol dehydrogenase; expressed with respect to tissue protein). Epididymal sperm were counted in a hemocytometer after mincing the epididymis in saline and filtering through a nylon mesh. Statistical comparisons were made to the control group using the Student *t*-test. Styrene-exposed rats did not show signs of toxicity or alterations in body weight **[data not shown]**. Results are summarized in Table 29. Alterations in all endpoints were identified in the 200 mg/kg bw/day group but not in the 100 mg/kg bw/day group. The authors concluded that the presence of these alterations at 200 mg/kg bw/day in the absence of histopathologic findings **[histopathology methods and findings were not mentioned]** "suggest that these enzymes may possibly be involved in the events [that] may ultimately be responsible for the damage of germ cells and decrease in number of spermatozoa."

Strengths/Weaknesses: The weaknesses of this study are similar to those of the previous study (Srivastava et al., 1992b). It is not clear why animals were treated 6 days/week instead of 7 days/week. In addition, no effect was seen at 200 mg/kg bw/day in this study in contrast to the preceding study in the same laboratory, raising the question of repeatability of the findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Takao et al. (2000), support not indicated, treated peripubertal male C57BL/6 mice with styrene **[purity not specified]** in drinking water for 4 weeks beginning at 5 weeks of age. Styrene was dissolved in ethanol (final ethanol concentration 0.005%) and provided at 0, 5, and 50 mg/L. **[Based on the average daily water intake and the mean body weights during the treatment period, styrene intake was 0, 1.2, and 12.3 mg/kg bw/day.]** Mice were decapitated after the treatment period, and testes and spleens were weighed. Trunk blood was taken for measurement of plasma free testosterone, corticosterone, and luteinizing hormone using commercial kits. One testis/mouse was prepared for light microscopy with hematoxylin and eosin staining **[fixation and embedding not specified]**. Data were analyzed with ANOVA followed by the Fisher protected least squares difference test. There were no effects of treatment on water intake, body weight, testis weight (absolute or relative), or spleen weight. Relative spleen weight was increased by both styrene exposure levels. In the 50 mg/L exposure group, plasma free testosterone was decreased **[to about 3% of the control level, estimated from a graph]**. There was no effect of the styrene lower exposure level on plasma free testosterone, and there was no significant effect of either exposure level on the other hormones or on testicular histopathology. The authors concluded that peripubertal exposure to styrene could disrupt male reproductive function. They believed that a direct testicular effect was at least partly responsible for the reduction in testosterone because pituitary function is not fully developed in the peripubertal mouse. However, effects of longer-term exposure on hypothalamic or pituitary function could not be excluded.

Table 29
Testis Enzyme Activities and Epididymal Sperm After 60-Day Treatment of Newborn Rats with Styrene[a]

	Endpoint, control mean %								
	Testis weight								
Treatment group	Absolute	Relative	SDH	LDH	G6PD	β-GLU	ACID PHOS	GGT	Sperm[b]
Styrene 100 mg/kg bw/day	92	102	96	106	109	110	104	101	89
Styrene 200 mg/kg bw/day	89[c]	84[c]	85[c]	119[c]	122[c]	160[c]	87[c]	140[c]	60[c]
BMD_{10}	177	194	158	133	111	100	196	159	97
BMDL	119	144	91	81	59	25	141	78	43
$BMD_{1\ SD}$	109	191	147	99	115	195	145	167	126
$BMDL_{1\ SD}$	68	121	74	51	64	135	76	100	63

[a]Srivastava et al. (1992c). **[Data converted to percent of control values and benchmark dose calculated by CERHR.]**
[b]Epididymal sperm concentration estimated from a graph.
[c]Different from control value at $p < 0.05$ (Student *t*-test; $n = 6$/group).
SDH sorbitol dehydrogenase; LDH lactate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; β-GLU, β-glucuronidase; ACID PHOS, acid phosphatase; GGT, γ-glutamyl transpeptidase; BMD, benchmark dose. For an explanation of BMD, see footnote to Table 25.

Strengths/Weaknesses: Weaknesses include administration in drinking water for only 4 weeks, lack of mention of random assignment of animals, insufficient numbers of animals per group, use of only two dose levels, lack of stability data on styrene in drinking water, lack of analysis of water for styrene concentration, and failure to specify the method of preserving testes. It is an important weakness that the extreme decreases in free testosterone levels were not accompanied by corresponding changes in testis weight and histopathology. The use of animals at 9 weeks of age was not optimal for understanding testis effects. The levels of bound or total testosterone should have been reported. The study would have also been strengthened by data on reversibility.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

3.2.4. Styrene oxide.

Sikov et al. (1986), preliminarily published as Hardin et al. (1983), funded by NIOSH, reported inhalation studies using styrene oxide (≥99% purity) exposure in pregnant Wistar rats and New Zealand white rabbits. For 3 weeks before mating, rats were exposed 7 hr/day, 5 days/week to filtered air ($n = 159$), styrene oxide 100 ppm ($n = 106$), or styrene oxide 300 ppm ($n = 106$). The 300 ppm rat dose group was discontinued because there was excess mortality after the initial 7-hr exposure; 42% of dams died within the first 3 days after this single exposure period. After mating, rats were exposed to 0 or 100 ppm styrene oxide. Gestational exposures were 7 hr/day, 7 days/week on GD 1–19 (vaginal sperm = GD 0). The rat study consisted of four exposure groups, identified by the styrene exposure in ppm before gestation and during gestation as 0–0 ($n = 41$), 100–0 ($n = 32$), 0–100 ($n = 48$), and 100–100 ($n = 31$). Rats were killed on GD 20 for evaluation of uterine contents. Fetuses were weighed, measured, and inspected for external malformations, after which slightly more than half the fetuses were dissected for visceral malformations after decapitation for serial section of the heads. The remainder of the fetuses were eviscerated without removal of the heads. All carcasses were cleared and stained with Alizarin red S for skeletal evaluation.

Rabbits were exposed to 0, 15, or 50 ppm ($n = 24$/dose group) 7 hr/day, 7 days/week on GD 1–24 (the day after insemination = GD 1). Rabbits were killed on GD 30 for evaluation of uterine contents in a manner similar to that used in the rats, except that all fetuses were dissected for visceral examination. Statistical analysis was performed using ANOVA followed by Dunnett test. The Fisher exact test was used for proportions. Multiple comparisons were adjusted using the Bonferroni method.

In the 100 ppm rat group, dam mortality was 16% during the pre-gestation exposure. An additional 16% of dams died during the gestational exposure period at this exposure level. Dam body weight was decreased (31–36%) by pre-gestation and gestation exposure to styrene oxide, attributable to a decrease in feed consumption. There was a decrease in the proportion of styrene oxide-exposed rats found to be pregnant at necropsy, without a significant decrease in corpus luteum number, which was interpreted by the study authors as consistent with an increase in preimplantation loss. There was no significant treatment effect on resorptions/litter. Fetal weight and length were reduced (32% for weight, 10% for length) in the fetuses of rats exposed to styrene oxide only during gestation, but were said not to be affected in the offspring of rats exposed both before and during gestation. **[Analysis of these data by CERHR using ANOVA with post-hoc Dunnett test shows a significant reduction in fetal body weight and length in both groups exposed to styrene oxide during pregnancy.]** There were increased incidences of ossification delays or defects in fetuses of both groups exposed during gestation to styrene oxide but no increase in malformations.

Maternal mortality in rabbits was 16.7% at 15 ppm and 79.2% at 50 ppm. There was a significant decrease in feed consumption in both styrene oxide-exposed groups, with dams in the 50 ppm dose group consuming just under half the amount of feed as dams in the control group. There was a significant 17% decrease in maternal weight gain in the 50 ppm group. The only significant alteration in litter or fetal parameters in rabbits was an increase in the percent of litters with resorptions from 18.8% in the control group to 66.7% in the 15 ppm group and 75.0% in the 50 ppm group. The authors concluded, ''styrene oxide may be regarded as producing reproductive and developmental toxicity at the levels studied, although it has not been established whether these are direct effects or are the result of maternal toxicity.''

Strengths/Weaknesses: There were a sufficient numbers of rats and rabbits per group, litters of rats and rabbits were evaluated for development, including soft-tissue and skeletal development, animals were assigned randomly by weight, chemical analysis was given, analysis of chamber concentrations was performed, and technique of cesarean section and fetal examination was described adequately. The large number of animals and the agreement with rat styrene studies on the lack of malformations even at maternally toxic doses are additional strengths of this study. Although the study design was appropriate for evaluating developmental toxicity, excessive maternal toxicity (>10% mortality in both species) clouded study interpretation. Regulatory guidance suggests that this level of maternal mortality is too high to permit definitive study interpretation. This problem is particularly important in rabbits, in which resorptions and abortions are clearly related to decrements in feed consumption. The exposure levels seem to have been too high. Other weaknesses include the cessation of exposure on GD 18 in rats and GD 24 in rabbits, the use of only one or two styrene exposure levels, the small number of rats examined for skeletal defects in one of the treatment groups, and the exposure 5 days/week instead of 7.

Utility (Adequacy) for CERHR Evaluation Process: The utility of this study is limited because of the excessive maternal toxicity, and because styrene oxide is not comparable to styrene. Styrene oxide is more reactive than styrene, and although styrene oxide is produced in specific tissues after styrene exposure, direct exposure of the whole lung to styrene oxide is qualitatively different.

3.2.5. In vitro studies.

Brown-Woodman et al. (1994), supported by Worksafe Australia and Cumberland College, evaluated the effects of styrene and styrene oxide on Sprague–Dawley rat embryos in culture. Embryos randomly selected from a pool of at least 3 litters were explanted in the afternoon of

GD 10 at about 10 somites and were cultured for 40 hr. The culture medium was heat-inactivated rat serum with added streptomycin and penicillin. Styrene and styrene oxide (both analytic grade) were dissolved in dimethylsulfoxide. The final concentration of dimethylsulfoxide in the cultures was 0.1 volume %, which had been found previously not to be toxic to cultured embryos. Styrene and styrene oxide were added to the cultures at a range of concentrations determined by GC to be 0.63–5.56 mM **[66–579 mg/L]** for styrene and 0.0240.175 mM **[3–21 mg/L]** for styrene oxide. **[The concentrations used here and in the results were measured after 16 hr of culture. Concentrations of styrene and styrene oxide in the culture medium decreased over time, presumably as a result of the chemicals coming out of solution into the headspace of the culture bottles. After 40 hr of culture, styrene concentrations in the medium were 51–83% of the 16-hr concentrations, and styrene oxide concentrations were about one-third of the 16-hr concentrations.]** At the end of the culture period, embryos were evaluated for yolk sac diameter, development of yolk sac circulation, dorsal convexity, heart beat, crown-rump length, somite number, and protein content of at least two embryos/culture bottle. The number of embryos evaluated per concentration ranged from 5–15, with 36 control embryos for the styrene group and 28 control embryos for the styrene oxide group. Statistical analysis was performed using ANOVA with post-hoc Dunnett test.

Results are summarized in Figure 3. There was a concentration-dependent decrease in most measures of embryo integrity. At the highest concentration of each chemical, there was no embryo development, although a yolk sac diameter was measurable in the styrene oxide experiment. Using statistical testing for difference from the control values, crown-rump length was the most sensitive parameter in the styrene experiment, with a significant decrease at or above a concentration of 1.00 mM **[104 mg/L]**. In the styrene oxide experiment, yolk sac diameter, crown-rump length, somite number, and embryonic protein content were significantly different from control at 0.038 mM **[4.6 mg/L]** and higher concentrations. The no-effect concentrations were 0.63 mM **[66 mg/L]** for styrene and 0.038 mM **[4.6 mg/L]** for styrene oxide. The authors compared these concentrations to human blood concentrations of styrene and styrene oxide under occupational exposure conditions: styrene 21.2 μM **[2.2 mg/L]** and styrene oxide 0.05 μM **[0.006 mg/L]** (Wigaeus et al., 1983). In another study cited by the authors, styrene concentrations in occupationally exposed workers were 15.0–21 μM **[1.6–2.2 mg/L]** (Löf et al., 1986). The authors concluded, ''…it would seem highly unlikely that the blood levels achieved after industrial exposure would reach the embryotoxic level demonstrated in the present study.''

Strengths/Weaknesses: Whole embryo culture is an investigative in vitro method that may be of some use in developmental hazard identification. However, the concentrations used in vitro need to have some relationship to maximally attainable levels in vivo to be of use. No information was given about how the concentrations were chosen, and the levels seem to have been excessive.

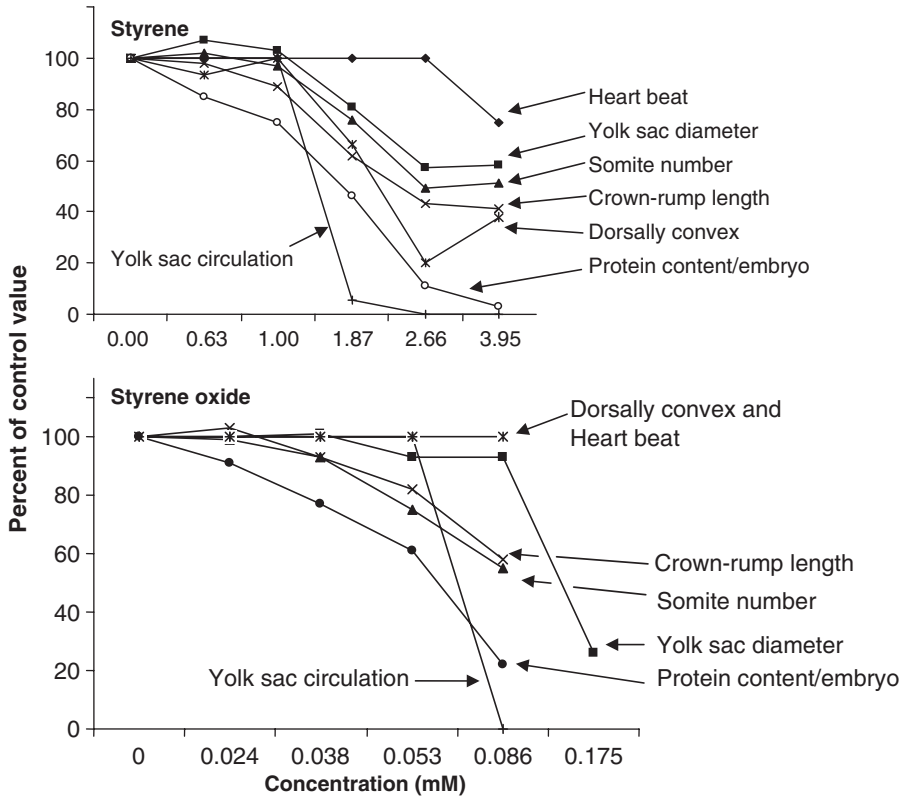

Fig. 3. Concentration–response relationship of styrene and styrene oxide in rat whole embryo culture. Figure drawn from the data of Brown-Woodman et al. (1994).

It is not clear if the concurrent control was run with the vehicle. It is a strength that culture concentrations were measured and put into perspective; that is, the exposure levels producing in vitro effects are unlikely to be seen after human exposures.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the CERHR process except in perhaps providing ancillary information to in vivo studies.

Gregotti et al. (1994), supported by NIH, NATO, and the University of Washington, evaluated styrene and styrene oxide in Sprague–Dawley rat brain and limb micromass cultures and, for styrene oxide, in rat whole embryo culture. Micromass culture refers to the cell culture of disaggregated cells from embryonic (12 days post coitum) brains or forelimbs, with a comparison of test chemical concentrations that interfere with differentiation, growth, or viability. Cells were disaggregated by trypsinization and trituration and placed in culture at an initial density of 5×10^6 brain cells/mL and 2×10^7 limb cells/mL. After a 2-hr interval to permit attachment, cultures were treated with styrene or styrene oxide, with or without a monooxygenase system (S9). Vehicle and vehicle+S9 control cultures were also run. **[The purities of styrene and styrene oxide were specified as "the highest purity commercially available." The styrene contained 10–15 ppm 4-*tert*-butylcatechol to inhibit spontaneous polymerization. The styrene concentrations tested were given as 100–300 μg/mL (0.967–2.900 mM); the styrene oxide concentrations were given in one figure legend as 0, 10, 20, and 40 μg/mL, but other figures also show data points for 1 and 60 μg/mL. Molar equivalents for these values are 0.008, 0.083, 0.166, 0.332, and 0.498 μM.]** Cultures were maintained for 5 days. Differentiation was assessed by microscopic evaluation of fixed and stained cultures and by uptake of radiolabeled sulfate (limb) and γ-aminobutyric acid (brain). Cell counts were determined by hemocytometer in single-cell suspensions prepared from cultures, and protein concentration was determined in cells harvested by scraping of cultures. Neutral red was used to assess viability of cultures. Statistical comparisons were made using calculated 50% effect levels with 95% CI. ANOVA and contingency trend tests were used for quantal data and lethality curves.

No adverse effects of styrene on limb bud or brain cell differentiation or viability were identified; however, the authors noted that styrene experiments yielded more variable results than previously obtained in the laboratory using these culture techniques; variability was attributed to the volatility of styrene. The addition of S9 did not apparently alter the toxicity of styrene in culture; the highest **[and perhaps the only]** styrene concentration tested with S9 was 100 μg/mL. Styrene oxide produced concentration-dependent decreases in limb and brain cell cultures in measures of differentiation, proliferation, and viability. The 50% effect levels are given in Table 30.

Table 30
Styrene Oxide 50% Effect Levels for Endpoints in Limb and Brain Micromass Culture[a]

	Concentration in μg/mL (mM)	
Endpoint	Limb	Brain
Formation of differentiated foci	6.7 (0.056)	9.2 (0.077)
Incorporation of sulfate (limb) or γ-aminobutyric acid (brain)	9.6 (0.080)	17.0 (0.142)
Cell number	13.5 (0.112)	15.5 (0.129)
Protein	26.5 (0.220)	15.5 (0.129)
Cytotoxicity	27.5 (0.229)	9.6 (0.080)

[a]Gregotti et al. (1994). Effect levels and conversion to molar concentration were calculated by the study authors and converted from μM to mM by CERHR for ease of comparison to the study of Brown-Woodman et al. (1994).

For the evaluation of styrene oxide in whole embryo culture, GD 9 Sprague–Dawley rats were used. **[The day of explantation and other details of culture, including the length of culture, were not given except by reference to a 1983 article and except for comments in the Discussion, indicated below.]** Tested concentrations of styrene oxide were 0, 4, 8, 20, 30, and 40 μg/mL **[the last of these concentrations was not mentioned in the study, but was estimated from a data figure]**. On pair-wise comparison with the control cultures, embryo lethality was increased at 40 μg/mL **[0.332 mM]**, and malformations were increased at 30 μg/mL **[0.249 mM]**. The estimated 50% effect level for lethality was 33.2 μg/mL **[275 μM]**, and the estimated 50% effect level for malformations was 20 μg/mL **[0.166 mM]**. Most of the malformed embryos had open neural tubes. Head length, somite number, and embryonic content of protein and DNA were significantly affected at and above styrene oxide concentrations of 8 μg/mL **[0.066 mM]**. The authors concluded that brain micromass culture results showed styrene oxide not to have adverse developmental effects except at concentrations that impaired viability, whereas limb bud cells were more sensitive to adverse developmental effects of styrene oxide at sub-cytotoxic exposures. The 50% effect level for styrene oxide for differentiation endpoints in limb bud cell cultures were estimated using data in an article by Ramsey and Anderson (1984) to be comparable to inhalation exposures of rats to 200–400 ppm styrene. The authors contrasted their results in whole embryo culture to the results of Brown-Woodman et al. (1994), who did not find neural tube closure defects in their cultures, and who found styrene oxide to be embryotoxic at a concentration of 4.6 μg/mL (38 μM), a concentration an order of magnitude lower than that associated with 100% lethality in the current study. The authors indicated that their exposure began 5 hr earlier than that of Brown-Woodman et al. (1994), and that their culture medium consisted of 50% Weymouth medium, 25% rat serum, and 25% human serum, in contrast to the use of 100% heat-inactivated rat serum by Brown-Woodman et al. (1994), perhaps accounting for some differences in outcomes.

Strengths/Weaknesses: The methods used are investigative in vitro tools that may be of some use in hazard identification, but only if the concentrations used in vitro have some relationship to maximally attainable levels in vivo. The ability of the system to distinguish styrene from styrene oxide effects may be a strength. The statistical determination of a 50% effect level is a strength. A weakness that illustrates the limitations of the in vitro system is the lack of consistency with in vivo effects at 200–400 ppm despite the stated equivalence of exposure levels. The lack of agreement with Brown-

Woodman et al. (1994) is a weakness, even though a plausible explanation concerning culture conditions was provided.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for CERHR process.

3.3 Utility of Data

There are three epidemiology studies in which styrene exposure was estimated or can be reasonably inferred for worker populations, including a study of reinforced plastics workers in Finland (Härkönen et al., 1984), a study of plastic polymer workers in Norway and Sweden (Ahlborg et al., 1987), and a study of reinforced plastic workers in the U.S. (Lemasters et al., 1989). The Scandinavian studies used information from registries to identify exposures and outcomes, and the U.S. study used a telephone questionnaire to identify outcomes and workplace visits to estimate exposure. The Finnish study focused on congenital malformations, the Norwegian–Swedish study evaluated a group of adverse perinatal outcomes (malformation, stillbirth, perinatal death, low birth weight), and the U.S. study considered only birth weight. Other reports on pregnancy outcome after styrene exposure include few if any women with documented styrene exposure, or provide inadequate detail for use in the evaluation process.

There is one regulation-compliant (for its time) developmental toxicity series of studies in rats using inhalation and gavage exposures and in rabbits using inhalation exposures (Murray et al., 1978). A rat study (Daston et al., 1991) using a single dose administered one time and a smaller number of litters than is typical for a developmental toxicity study was useful in showing a distinction between maternal toxicity and embryotoxicity. A rat study (Srivastava et al., 1992a) using oral doses of styrene during pregnancy evaluated xenobiotic-metabolizing enzymes in fetal liver, although the study lacked information on possible maternal effects of the treatment. Four reports from one laboratory evaluated brain neurochemicals and offspring behavior in rats after gestational exposure to styrene (Kishi et al., 1992, 1995; Katakura et al., 1999, 2001), and another study compared styrene exposure during the gestational and lactation periods with regard to dopamine receptor binding and neonatal behaviors (Zaidi et al., 1985). A two-generation rat study included neurobehavioral assessments in F_2 offspring (Cruzan et al., 2005c). A study using pregnant hamsters and mice (Kankaanpää et al., 1980) included a single high dose in the mouse study and small numbers of animals in the hamster study and lacked information on maternal toxicity. This report is considered to have utility for the evaluation process because it provides information, albeit limited, on a species other than the rat. Another mouse developmental study (Ninomiya et al., 2000) contained no information on malformations but could be used for an evaluation of effects of styrene exposure on maternal and fetal weight. A rat and rabbit inhalation study was carried out with styrene oxide (Sikov et al., 1986) but was limited by excessive maternal toxicity.

Three postnatal studies evaluated effects of styrene administration during the developmental period on testis parameters. One of these studies involved treatment of lactating dams and evaluation of male rat offspring (Srivastava et al., 1992b), one study involved direct treatment of male rats during the first 60 days of life (Srivastava et al., 1992c), and one study involved the treatment of peripubertal male mice (Takao et al., 2000).

3.4 Summary of Developmental Toxicity Data

3.4.1 Summary of human data. Two Scandinavian record linkage studies assessed malformation rates in styrene exposed workers. Neither study had high quality individual level exposure data, but the study by Ahlborg et al. (1987) assessed potential exposures using employer information. The Härkönen et al. (1984) study compared malformation rates during periods of potential styrene exposure with periods with no exposure within individuals, and the Ahlborg et al. (1987) study compared rates between workers in industries judged to have different styrene exposure potential. Both studies had limited power due to the small sample sizes of exposed individuals. Both studies found no increase in malformations in births to styrene-exposed workers. The Ahlborg et al. (1987) study looked at any adverse pregnancy outcomes (stillbirth, congenital malformation, low birth weight) and found no elevation in risk, although the power was limited as evidenced by the wide confidence intervals.

A U.S. study by Lemasters et al. (1989) assessed pregnancy outcomes in 229 possibly styrene-exposed and 819 unexposed births. Styrene exposure was based on historical industrial hygiene records and was not individually measured at time of pregnancy. There was no overall effect of putative styrene exposure on birth weight or the proportion of infants with low birth weight, but among a subgroup of 50 pregnancies with the highest styrene exposure potential, there was a modest and statistically non-significant decrease in mean birth weight. The study had limited power to detect adverse pregnancy outcomes as evidenced by the wide confidence intervals.

3.4.2 Summary of experimental animal data. The experimental animal studies on styrene treatment during pregnancy are presented in Table 31 and summarized here. Studies in which styrene oxide was administered during pregnancy or in which styrene was administered during postnatal developmental periods are summarized at the end of this section but do not appear in Table 31.

For sake of convenience, prenatal and postnatal studies will be discussed separately. There are two pivotal studies, one in rats and one in rabbits, that evaluate the potential prenatal developmental toxicity of styrene. Murray et al. (1978) treated pregnant Sprague–Dawley rats by inhalation 7 hr/day from GD 6–15 with 300 or 600 ppm styrene (each with its own 0 ppm control group). Additional rats were given styrene in peanut oil by gavage on GD 6–15 at 0, 180, or 300 mg/kg bw/day in two divided doses. Rabbits were exposed by inhalation to styrene in two experiments using the same dose levels as in the rat experiments (300 and 600 ppm, each with its own 0 ppm control group). Rabbits were treated on GD 8–18. Rats were killed on GD 21, and rabbits were killed on GD 29 for evaluation of uterine contents. Maternal toxicity consisted of a decrease in body weight gain on GD 6–9 in rats, attributed to a decrease in feed consumption. Inhalation exposure to styrene in rats at

both exposure levels was associated with an increase in water consumption. There was no maternal toxicity in rabbits. A small but statistically significant decrease in mean fetal length in rat fetuses in the 300 ppm group was not considered treatment-related because the comparable change in the 600 ppm group was not statistically significant. There were increases in the incidence of skeletal variations in styrene-treated fetuses in both species, which were not considered treatment-related because they were within the historical control range. There were no other effects of styrene on litter or fetal parameters in either species.

Table 31
Summary of Experimental Animal Studies on Pregnancy Effects of Styrene

Species and strain	Treatments	Effect levels/benchmark dose[a]	Reference
Rat, Sprague–Dawley	Gavage at 0, 180, or 300 mg/kg bw/day, GD 6–15	Maternal LOAEL 180 mg/kg bw/day (↓body weight gain) Developmental LOAEL > 300 mg/kg bw/day	Murray et al., 1978
Rat, strain not indicated	Gavage at 0 or 200 mg/kg bw/day during gestation or lactation period or both	No maternal toxicity. Developmental effects = altered dopamine binding in striatum with lactation exposure, altered amphetamine-stimulated locomotion, and apomorphine-induced stereotypy with gestation + lactation exposure (periods not evaluated singly for locomotion or stereotypy).	Zaidi et al., 1985
Rat, Sprague–Dawley	Single gavage administration on GD 11 at 300 mg/kg bw	Maternal toxicity with this treatment (↓body weight gain) without developmental effects.	Daston et al., 1991
Rat, Wistar	Oral administration of 0, 200, or 400 mg/kg bw/day GD 1–20	No maternal toxicity. Developmental LOAEL 200 mg/kg bw/day (↓hepatic enzymes, glutathione). **[Lowest BMD_{10} = 81 mg/kg bw/day; $BMDL_{10}$ 68 mg/kg bw/day; $BMD_{1\ SD}$ 102 mg/kg bw/day; $BMDL_{1\ SD}$ 74 mg/kg bw/day.]**	Srivastava et al., 1992a
Rat, Sprague–Dawley	Inhalation at 0, 300, or 600 ppm, GD 6–15	Maternal LOAEL 300 ppm (↓body weight gain). Developmental LOAEL > 600 ppm.	Murray et al., 1978
Rat, Wistar	Inhalation at 0, 50, or 300 ppm 6 hr/day, GD 7–21	Maternal LOAEL > 300 ppm. Developmental LOAEL 50 ppm (↓PND 1 body weight, delayed reflex acquisition) **[BMD_{10} 413 ppm, $BMDL_{10}$ 205 ppm, $BMD_{1\ SD}$ 410, $BMDL_{1\ SD}$ 199 based on PND 1 body weight]**. PND 1 cerebral neurotransmitters altered at 300 ppm **[lowest BMD_{10} 54 ppm, $BMDL_{10}$ 28 ppm, $BMD_{1\ SD}$ 276, $BMDL_{1\ SD}$ 166]**	Kishi et al., 1992, 1995
Rat, Wistar	Inhalation at 0, 50, or 300 ppm 6 hr/day, GD 6–20, one group of controls and 50 ppm group pair-fed to 300 ppm group.	Maternal LOAEL indeterminate. **[feed consumption reduced at 300 ppm; lower dose groups pair-fed]**. Developmental LOAEL 300 ppm (↓male weight on PND 21; ↓ in some neurochemical measures and delay in developmental landmarks, reflexes) **[lowest BMD_{10} 113 ppm, $BMDL_{10}$ 73 ppm, $BMD_{1\ SD}$ 276 ppm, $BMDL_{1\ SD}$ 166 ppm]**.	Katakura et al., 1999, 2001
Rat, Crl:CD®(SD)IGS BR	Inhalation at 0, 50, 150, or 500 ppm 6 hr/day as part of a two-generation study. F_2 offspring not directly exposed to styrene.	Parental LOAEL 150 ppm (reduced body weight during premating period); restricting observations to gestation, maternal NOAEL 500 ppm (highest dose level tested). Developmental LOAEL 150 ppm (↓growth, "slight developmental delay"), NOAEL 50 ppm.	Cruzan et al., 2005c
Mice, ICR	Inhalation at 0, 2, 20, and 100 ppm, 24 hr/day, GD 0–15	Maternal weight LOAEL 100 ppm **[BMD_{10} 36 ppm; $BMDL_{10}$ 6 ppm; $BMD_{1\ SD}$ 49 ppm; $BMDL_{1\ SD}$ 38 ppm]**. Fetal weight LOAEL 100 ppm **[BMD_{10} 63 ppm; $BMDL_{10}$ 11 ppm; $BMD_{1\ SD}$ 47 ppm; $BMDL_{1\ SD}$ 30 ppm]**.	Ninomiya et al., 2000
Mice, BMR/T6T6	Inhalation 6 hr/day, GD 6–16 at 0 or 250 ppm	Maternal effects not known. ↑Dead or resorbed fetuses at 250 ppm.	Kankaanpää et al., 1980
Rabbit, New Zealand white	Inhalation at 0, 300, or 600 ppm, GD 6–18	Maternal LOAEL > 600 ppm. Developmental LOAEL > 600 ppm.	Murray et al., 1978
Hamster, Chinese	Inhalation 6 hr/day from GD 6–18 at 0, 300, 500, 750, or 1000 ppm.	Maternal effects not known. ↑Dead or resorbed fetuses at 1000 ppm (small number of animals at lower exposure levels).	Kankaanpää et al., 1980

[a]BMD, benchmark dose. For an explanation of BMD, see footnote to Table 25.

Other studies indicate that at high (maternally toxic) levels of styrene, there are growth effects on offspring. These kinds of non-specific developmental effects are often associated with maternal toxicity. Growth effects were observed in one generation of a multi-generation study in rats (Cruzan et al., 2005c) and mice (Ninomiya et al., 2000). These studies generally support the conclusions from the definitive studies that the developmental toxicity of styrene is minimal and only observed in the presence of maternal toxicity.

The pivotal studies for evaluating postnatal manifestations of developmental toxicity are two multi-generation studies (Beliles et al., 1985; Cruzan et al., 2005b), discussed more fully in Section 4.2.3. One of these studies was conducted by inhalation and the other via drinking water in rats according to regulatory guidelines in place at the time. The study by Cruzan et al. (2005b) also included a developmental neurotoxicity component (Cruzan et al., 2005c) that was consistent with regulatory guidelines. One of these studies indicates growth effects at very high levels of exposure, but neither study demonstrated teratogenic or functional effects.

Cruzan et al. (2005c) reported a developmental neurotoxicity study in Crl:CD®(SD)IGS BR rats that was part of a two-generation reproductive study (Cruzan et al., 2005b, summarized in Section 4.2.3). F_0 and F_1 males and females were exposed to styrene by inhalation at 0, 50, 150, or 500 ppm 6 hr/day, 7 days/week from at least 70 days before mating through GD 20. On PND 1–4, F_0 and F_1 dams received styrene in olive oil by gavage at 0, 66, 117, or 300 mg/kg bw/day to provide estimated styrene blood levels 2, 4, and 6 hr after onset of daily treatment similar to blood levels after inhalation exposure. Inhalation exposure of dams resumed on PND 5. Litters were standardized on PND 4 to 10 pups, with equal sex distribution when possible. Pup developmental milestones, neurobehavioral testing, and histopathologic assessments of the central and peripheral nervous system were performed in F_2 offspring. There was a decrease in F_2 offspring body weights from birth through PND 70 in the 500 ppm group. In the 150 ppm group, pup body weight was decreased from PND 7 through adulthood. The authors identified ''subtle indications of a delay in the acquisition of developmental landmarks,'' despite the lack of statistical significance, and stated that the later acquisition of landmarks in the presence of reduced body weight suggested ''slight developmental delay.'' Other indications of developmental delay included a decrease in fore- and hindlimb grip strength on PND 45 and 60, a shift in the pattern of development of locomotor activity in the 500 ppm group, and decreased PND 24 swimming ability in the 500 ppm group. The authors identified a NOAEL for growth of 50 ppm and stated, ''no specific effect on nervous system development was observed at exposures up to 500 ppm styrene.''

There are other studies (Zaidi et al, 1985; Kishi et al., 1992, 1995; Srivastava 1992b,c) that evaluated the effects of developmental styrene exposure on biochemical parameters in liver, testis, and brain. Although these studies do indicate some effects of styrene at high dose levels, it is unclear whether these effects represent adverse changes. Because they were measured at limited number of time points, it is possible that changes represent developmental delays or transitory changes and not permanent functional deficits.

There is an inhalation developmental toxicity study using styrene oxide. This study is of limited utility in this evaluation because it is unclear whether styrene oxide should be considered to be the relevant toxic metabolite to evaluate the developmental toxicity of styrene. Furthermore, the interpretation of this study is clouded by the excessive maternal toxicity. Even with this excessive toxicity, there were no terata observed. An inhalation study of styrene oxide in pregnant New Zealand white rabbits (Sikov et al., 1986) used exposure levels of 0, 15, or 50 ppm ($n = 24$/dose group) 7 hr/day, 7 days/week on GD 1–24 (the day after insemination = GD 1). Rabbits were killed on GD 30 for evaluation of uterine contents, including examinations for external, visceral, and skeletal abnormalities. Maternal mortality was 16.7% at 15 ppm and 79.2% at 50 ppm. There was a significant decrease in feed consumption in both styrene oxide-exposed groups, with dams in the 50 ppm dose group consuming just under half the amount of feed as dams in the control group. There was a significant (17%) decrease in maternal weight gain in the 50 ppm group. The only significant alteration in litter or fetal parameters in rabbits was an increase in the percent of litters with resorptions from 18.8% in the control group to 66.7% in the 15 ppm group and 75.0% in the 50 ppm group.

Expert Panel Conclusions

Data from human studies are not sufficient for an evaluation of the developmental toxicity of styrene after prenatal exposure. The studies were limited by low statistical power and lack of adequate individual-exposure information.

There are no human data on toxicity of styrene after exposure of children.

Data in experimental animals are sufficient to conclude that styrene does not cause developmental toxicity in rats as evaluated by structural endpoints at inhalation exposures of 600 ppm and oral exposures of 300 mg/kg bw/day and neurobehavioral endpoints at inhalation exposures of 500 ppm. Data in experimental animals are sufficient to conclude that styrene does not cause developmental toxicity in rabbits as evaluated by structural endpoints at inhalational exposures of 600 ppm. There were reduced growth and coincident developmental delays in some rat studies at levels of exposure associated with maternal toxicity. The growth effects occurred in the F_2 pups in a multi-generation study at 150 ppm. The experimental animal data are assumed relevant for the evaluation of human risk.

Note: The definitions of the term sufficient and the terms assumed relevant, relevant, and not relevant are in the CERHR guidelines at http://cerhr.niehs.nih.gov/news/guidelines.html.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

4.1.1 Female reproductive toxicity. Studies examining possible effects of styrene exposure on female reproduction have considered menstrual cycle

parameters and spontaneous abortion. Studies on serum prolactin in styrene-exposed women are included in this section because elevated prolactin could be a possible mediator of menstrual cycle disturbances or other reproductive effects.

4.1.1.1 Menstrual disorders and prolactin response.

Brown (1991), supported by the Styrene Research and Information Center, reviewed papers from the Russian literature, which Brown read in the original Russian. One author (Bondarevskaya) published reports in 1957 and 1961 suggesting that occupational exposure to styrene was associated with menstrual dysfunction in 21.1% of women during their first year of work, decreasing to 5.5% of women with 6 years on the job. Another group (Zlobina et al.) reported on 238 styrene-exposed women and an unspecified number of controls (deduced by Brown to be about 117). The exposed women were divided according to mean styrene exposure levels. Gynecologic problems did not vary by estimated styrene exposure. Abnormal cycles were assessed by vaginal cytology, but apparently control data were not reported. An additional paper from the same group reported that menstrual disturbances were more common in styrene-exposed women than in a control group (29.1% compared to 9.1%). Another group (Loseva et al.) reported gynecologic problems in 36.1% of women exposed to styrene and other chemicals compared to 12.3% of a comparison group; 33% of exposed women complained of menstrual disturbances compared to 8.6% of comparison women. Two other Russian authors (Bobrova, 1977; Gorobets, 1984) reported menstrual disorders in women exposed to styrene. Brown concluded in his review of the Russian reports that ''[t]hey all suffer from inadequate presentation, poor data, lack of appropriate controls and analysis, confounding exposures and conditions, etc.'' **[The Expert Panel notes this review for completeness, but does not find it suitable for the evaluation process. The original Russian reports were not evaluated by the Expert Panel, and no independent evaluation of the reliability of these reports is possible.]**

Lemasters et al. (1985b), supported by the EPA, evaluated menstrual cycle parameters in women working in reinforced plastics companies selected based on type of process and products and number of English-speaking employees. Of 120 companies contacted by the authors, 36 agreed to participate, and 2177 women employees of these companies were invited to be interviewed. Inclusion criteria included age <36 years at the time of hiring, employment for at least 6 months during the 7-year study period, and a current or previous marriage. There were 1535 subjects who were successfully contacted and agreed to be interviewed. Of these women, 912 were excluded from the menstrual cycle analysis because of conditions that might affect their menses (e.g., use of oral contraceptives, recent pregnancy, genital tract cancer). **[Some of these women were included in a study of birth weight, discussed in Section 3.1.]** The remaining 623 women included 174 women who were considered exposed to styrene based on a site visit and inspection of processes and work records by one of the investigators (Lemasters et al., 1985a) and 449 unexposed individuals. Information on exposure to other chemicals was limited, and only styrene exposure was considered. Exposure was considered high if the individual worked in open molding with direct styrene contact (weighted mean exposure = 52 ppm) and low if involved in other open molding work or in press molding (weighted mean exposure = 13 ppm). The exposures were estimated from historically collected industrial hygiene data. Of the unexposed individuals, some were employed by reinforced plastics companies in jobs that did not entail exposure to styrene or other chemicals used in processing, and some of the unexposed women had left styrene-exposed jobs at least 9 months before the interview. Interviews were conducted by telephone by professional interviewers who were initially unaware of the subject's exposure status. Questions related to menstrual cycle parameters were asked before exposure-related questions to reduce interviewer bias. Chi-square testing was performed, after which multiple logistic analysis was performed using as main effects age, age squared (to control for possible nonlinear trends), marital status, parity, chronic disease, and smoking status. Outcomes included severe dysmenorrhea (confined to bed), intermenstrual bleeding, secondary amenorrhea ($\geq$6 months duration), menstrual blood clots, and hypermenorrhea. Exposure was incorporated in the analysis as a categorical variable (none, low, high) based on the above criteria and by multiplying mean exposure level and duration of exposure to produce three categories (0, 1–14,000 ppm-days, and $>$14,000 ppm-days). There were no significant associations between exposed and unexposed women in any menstrual cycle parameter by χ^2 analysis and no significant association of styrene exposure with any menstrual cycle parameter in the regression analysis. The authors concluded that ''exposure to styrene and other solvents in the reinforced plastics industry is not associated with a significant increase in any menstrual disorder.''

Strengths/Weaknesses: The use of workplace exposure data and the adjustment for potential confounders are strengths of this study, as is the use of clear definitions of menstrual disorders. The sample size is relatively large, although the number of exposed women is limited, particularly in the most highly exposed group. Individual exposure data were not available and other occupational exposures were not accounted for. The low participation rates of companies and of contacted subjects are additional limitations.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited to moderate utility.

Cho et al. (2001), supported by the National Institute of Child Health and Human Development (NICHD), the National Institute of Environmental Health Sciences (NIEHS), NIOSH, and EPA, retrospectively evaluated the prevalence of oligomenorrhea, defined as average cycle length greater than 35 days, among female petrochemical workers in China. Women were recruited from employees in a governmental industrial park and included those women aged 20–40 who were applying for permission to marry or to become pregnant. Women were excluded if they had had a previous marriage, pregnancy, gynecologic disorder, or endocrinopathy. Data were obtained from 1408 women who met inclusion criteria. Exposure was assessed based on industrial hygienist evaluation of the work environments **[not individual subject exposure measurements, although average styrene concentration was said to be $<$1 ppm]**. Exposures to benzene, toluene, styrene, and xylenes were

specifically evaluated, and workers were asked by questionnaire about exposure to other chemicals. Information was obtained by questionnaire on menstrual pattern in the preceding year, including average cycle length, longest and shortest cycle, average duration of bleeding, perceived irregularity, intermenstrual spotting, and perimenopausal symptoms **[not specified]**. Information was also collected on age, body weight, height, date of marriage, contraception use, tobacco exposure, indoor coal or cooking oil fume exposure, alcohol consumption, diet, herbal medications, heavy lifting, body position during work, shift work, perceived work stress, and physical activities outside the workplace. Multiple logistic regression was used to evaluate associations between individual chemical exposures and menstrual cycle abnormalities. Only oligomenorrhea was prevalent enough for the authors to consider estimates reliable, and analyses for other cycle parameters were not performed.

There were 440 women with exposure to any aromatic solvent, of whom 276 were exposed to styrene (three were exposed only to styrene). Of women not exposed to any aromatic solvent, 8.5% reported oligomenorrhea. Among women exposed to any solvent, 12.7% reported oligomenorrhea, and among women whose exposure included styrene, 14.5% reported oligomenorrhea. After adjustment for age, body mass index, enrollment cohort (application for marriage versus pregnancy), passive smoking, and exposure to other chemicals, the OR (95% CI) was 1.24 (0.90–1.99) for any aromatic solvent and 1.65 (1.05–2.55) for styrene. The OR per year for any aromatic solvent was 1.07 (1.00–1.14). Per-year ORs were not calculated for individual aromatic solvents. The authors proposed that ''exposure-induced oligomenorrhea likely derives from a mechanism involving endocrine disturbance, and the relatively subtle outcome we describe may have been difficult to observe in the earlier studies.''

Strengths/Weaknesses: The use of industrial hygiene data, the large sample, and the adjustment for confounders are strengths of this study. Weaknesses include a lack of individual exposure data, exposure to multiple other chemicals, and the low average styrene concentration. The power to detect menstrual disorders, other than oligomenorrhea, was low.

Utility (Adequacy) for CERHR Evaluation Process: This report is of moderate utility and identifies oligomenorrhea as a possible risk.

Mutti et al. (1984b), supported by the Italian National Research Council and by a regional Department of Social Security, evaluated prolactin, thyroid stimulating hormone, growth hormone, follicle stimulating hormone, and luteinizing hormone in peripheral venous blood in 30 women occupationally exposed to styrene. Urine was collected just before venipuncture. The urine and blood were collected between 8–9:00 AM, before a work shift. **[The time of the month was specified as being during the proliferative phase of the menstrual cycle by questionnaire. The authors indicate further that the timing was between the 5th and 15th day of the cycle. The Expert Panel notes that this sampling frame is likely to have included large peri-ovulatory variations in gonadotropin levels.]** A comparison group of 30 factory workers living in the same area but not exposed to styrene was matched for age. All subjects were healthy with no history of endocrine or neurologic disease or psychotropic medication use. Urine samples were analyzed for the styrene metabolites mandelic acid, and phenylglyoxylic acid, and exposure intensity was represented by the sum of these measurements. The mean value ($\pm$SD) of the sum was 580 ± 290 mmol/mol creatinine, which the authors state corresponded to an 8-hr TWA styrene exposure concentration of 130 ppm (range = 65–300 ppm). Hormones were measured in serum using commercial radioimmunoassay kits. Statistical comparison of styrene-exposed and unexposed women was by Student *t*-test for prolactin and thyroid stimulating hormone results, which were normally distributed, and by Mann–Whitney *U*-test for the results of the other hormone tests. The Pearson correlation coefficient was used to assess the relationship between urine markers of exposure level and serum prolactin and thyroid stimulating hormone levels in styrene-exposed women **[these distributions were selected for correlation analysis because they were normal]**. Partial correlation methods were used to control for the effects of age and duration of exposure. Results of the hormone measurements are given in Table 32. Significant increases in prolactin and growth hormone serum concentrations were identified in styrene-exposed women. Prolactin and thyroid stimulating hormone concentrations were significantly positively correlated with urinary metabolite concentrations even when controlled for age and exposure time **[definition and ascertainment of exposure time not given]**. The authors concluded that their results supported the hypothesis that styrene exposure interferes with tuberoinfundibular dopaminergic activity, manifested as an increase in prolactin concentration in response to decreased dopaminergic inhibition of prolactin release.

Strengths/Weaknesses: The use of hormone and styrene assays is a strength. The plan to sample during

Table 32
Serum Pituitary Hormone Concentrations in Styrene-Exposed and Unexposed Women[a]

Hormone	Styrene-exposed	Unexposed
Prolactin (pM), mean $\pm$ SD	633 ± 372[b]	313 ± 175
Thyroid stimulating hormone (mIU/L), mean $\pm$ SD	2.3 ± 1.3	2.7 ± 1.8
Growth hormone (pM), median (range)	520 (65–1422)[c]	329 (67–1198)
Follicle stimulating hormone (mIU/L), median (range)	10.1 (3.6–118.8)	8.8 (1.6–18.7)
Luteinizing hormone (mIU/L), median (range)	13.4 (9.2–107.7)	11.7 (8.0–42.7)

[a]Mutti et al. (1984b).
[b]$p < 0.001$, Student *t*-test.
[c]$p < 0.05$ Mann–Whitney *U*-test, $n = 30$/group.

the follicular phase of the cycle is a strength, although the definition of this phase was imprecise. The sample size was too small to assess clinically meaningful abnormalities. Other occupational exposures were not considered.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in its assessment of endocrinologic effects but limited for clinical outcomes.

Arfini et al. (1987), supported by the Italian National Research Council and by the regional government, evaluated the response of prolactin to thyrotropin-releasing hormone in 16 women exposed to styrene and 16 unexposed, age-matched controls. Exposed women were described as euthyroid and aged 24.4 ± 5.6 years (mean $\pm$ SD). **[No other information was provided on the recruitment of exposed or unexposed subjects. This study is from the same authors as the study described previously (Mutti et al., 1984b); it is not stated whether any of the same women are represented in both articles.]** Women were evaluated on a Saturday morning at 8:00 AM after an overnight fast. Menstrual cycle phase was recorded, and each woman's control was evaluated in the same phase of the cycle. Urine was collected for GC evaluation of the styrene metabolites mandelic acid and phenylglyoxylic acid about 15 hr after the last styrene exposure **[urine was apparently not collected from control women]**. Blood was collected from an indwelling venous catheter 15 min before and 0, 10, 20, 30, 45, 60, and 90 min after infusion of 200 μg thyrotropin-releasing hormone. Sera were collected and frozen for later radioimmunoassay for prolactin using a commercial kit. Two subjects were excluded from statistical analysis, one of whom was using oral contraceptives and the other of whom had a prolactin-secreting adenoma. Two other subjects were re-evaluated an unspecified number of months after the first evaluation, when both had been removed from styrene exposure for at least 3 months. Comparisons were made between exposed and unexposed subjects using the Kruskall–Wallis analysis of variance followed by Mann–Whitney *U*-test, taking serum prolactin as a continuous variable. Chi-square testing with Yates correction was used to compare the proportion of subjects in each group with abnormal prolactin responses. Abnormal prolactin responses were defined based on any of three criteria: a prolactin increase of >60 ng/mL, a greater than a 5-fold increase in baseline prolactin, or area under the concentration-time curve >66 ng-h/mL **[it was not stated whether these criteria were determined before review of the results or determined post-hoc]**. The association between urinary excretion of styrene metabolites and prolactin response in styrene-exposed women was evaluated using the Spearman rank correlation coefficient.

Serum prolactin was higher in styrene-exposed women than in their matched controls at baseline and throughout the stimulation test. Median basal prolactin in unexposed women was 12–15 ng/mL, and median basal prolactin in styrene-exposed women was about 20 ng/mL **[estimated from a graph]**. Ten minutes after administration of 200 μg thyrotropin-releasing hormone, median peak serum prolactin levels were about 33 ng/mL in unexposed women and about 120 ng/mL in styrene-exposed women **[estimated from a graph]**. Using the three criteria for abnormal prolactin response, 15 of 16 styrene-exposed women satisfied at least one criterion, and 13 of 16 styrene-exposed women satisfied all three criteria. Of the 16 unexposed control women, three or four were abnormal by at least one of the criteria, and none were abnormal by all criteria. There was a significant difference between the exposed and unexposed groups in the proportion with any of the criteria for abnormal prolactin response ($p < 0.0001$). There was a significant correlation between urinary concentration of styrene metabolites in styrene-exposed women and the peak prolactin concentration in response to stimulation ($r = 0.52$, $p < 0.05$), with a stronger relationship when phenylglyoxylic acid was used alone as a surrogate for styrene exposure ($r = 0.62$, $p = 0.02$). **[The values for the styrene metabolite concentrations in urine were not given, but from a graph, it is estimated that the sum of mandelic acid and phenylglyoxylic acid concentrations in urine ranged from 100–800 mmol/mol creatinine.]** In the two subjects retested after removal from exposure, prolactin responses to stimulation seemed decreased, although no analysis was performed. One of these women was noted to have been amenorrheic for 1 year after being employed for 3 years, and to have re-established menstrual periods after being removed from exposure for 40 days. The authors concluded that the exaggerated prolactin response to thyrotropin-releasing hormone in styrene-exposed women was consistent with a reduction in dopaminergic modulation of pituitary activity. **[The authors added in the Discussion section of the article that most of the subjects had high scores in the Hamilton depression scale, and that half of the subjects had menstrual or sexual disturbances. No data were provided.]**

Strengths/Weaknesses: Matching for age and cycle phase is a strength of this study. The assessment of styrene metabolites in exposed subjects is a strength, but the lack of confirmation of exposure status in the ''unexposed'' comparison group is a limitation. Recruitment methods were uncertain, and occupational and other potential confounders were not considered. The use of stimulation testing with serial serum sampling is a strength of the design.

Utility (Adequacy) for CERHR Evaluation Process: The study is useful in evaluating endocrine effects but is limited with respect to clinical outcomes.

Bergamaschi et al. (1996), supported by the European Commission and the Italian National Institute of Occupational Safety and Prevention, compared styrene-exposed and unexposed workers with respect to serum prolactin and dopamine β-hydroxylase and platelet monoamine oxidase B. The styrene-exposed workers consisted of 33 men and 20 women employed for 1–22 years in the manufacture of glass-fiber reinforced plastics. Styrene exposure by ambient monitoring ranged from 5–100 ppm (8-hr TWA). Biological monitoring with urinary mandelic and phenylglyoxylic acids was carried out using pre-shift morning samples. The control group of presumably unexposed workers consisted of ''60 blue collar workers…recruited from local industries where occupational risk factors had been ruled out.'' **[Biological assessment of control subjects for styrene exposure was not mentioned.]** Pre-shift morning blood samples were drawn and used to prepare serum for prolactin determination by radioimmunoassay (RIA) and dopamine β-hydroxylase activity determination. Platelet rich plasma was prepared for determination of monoamine

oxidase B activity. Dopamine-β-hydroxylase activity was evaluated using HPLC with electrochemical detection of the norepinephrine product. Monoamine oxidase B was assessed in platelet-rich plasma using HPLC with colorimetric detection of the 2,4-dihydroxyphenylacetaldehyde product. Statistical analysis was performed using the Student *t*-test, ANOVA, Pearson correlation, and χ^2. Continuous variables were converted to dichotomous variables using 5th or 95th percentiles. Cox regression was used to evaluate the probability of abnormally high prolactin or abnormally low dopamine-β-hydroxylase activity as a function of urinary styrene metabolite concentration.

Mean prolactin was higher in men and women in the styrene exposed group than in the control group. The geometric mean ± SD prolactin concentrations were 8.90 ± 1.86 ng/mL in styrene-exposed men and 12.6 ± 2.39 ng/mL in styrene-exposed women, and 6.05 ± 1.58 ng/mL in control men and 9.33 ± 1.65 ng/mL in control women ($p < 0.01$ for group comparison of males, $p < 0.05$ for group comparisons of females). Mean serum dopamine-β-hydroxylase was lower in styrene-exposed workers than controls ($p < 0.01$). Monoamine oxidase B in platelet-rich plasma was lower in styrene exposed workers than control workers at a *p*-value of 0.07. Dopamine-β-hydroxylase activity in serum was negatively correlated with the sum of the styrene metabolite concentrations in urine ($p < 0.004$). When styrene-exposed workers were divided based on urinary metabolites into high-exposure and low-exposure subgroups, low dopamine-β-hydroxylase activity (less than the control 5th percentile) was found in 42% of the high-exposure group and 17% of the low-exposure group ($\chi^2 = p < 0.001$). Abnormally high prolactin was found in 4.8% of the unexposed controls, 20% of subjects in the styrene low-exposure group, and 38% of subjects in the styrene high-exposure group ($\chi^2 = p < 0.01$). The authors believed that styrene might deplete central nervous system dopamine, resulting in an increase in prolactin secretion, and that the most likely explanation for the decrease in dopamine-β-hydroxylase activity and the possible decrease in monoamine oxidase B activity in peripheral samples was a response to this dopamine depletion.

Strengths/Weaknesses: The exposure monitoring and urinary assay data are strengths of this study. Weaknesses include the relatively small number of exposed subjects, the uncertain exposure status of the controls, the use of only a single blood sample, and lack of consideration of potential confounders.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited to moderate utility.

Bergamaschi et al. (1997), supported by the Commission of the European Communities and the Québec Ministry of Research, Science, and Technology, compared styrene-exposed and unexposed workers with respect to plasma prolactin and dopamine β-hydroxylase and platelet monoamine oxidase B. Styrene-exposed workers consisted of 46 volunteers who worked in factories manufacturing reinforced plastics. Ambient monitoring of styrene exposure gave 8-hr TWA values of 5–120 ppm (median = 25 ppm). Individual subject exposure was assessed using pre-shift urinary mandelic and phenylglyoxylic acid concentrations at the end of the work week. Controls consisted of 30 ''blue-collar workers with no history of exposure to chemicals.'' **[No biologic assessment of possible styrene exposure was reported in the control subjects.]** Pre-shift blood samples were used for measurement of prolactin by RIA and dopamine-β-hydroxylase using HPLC with fluorometric detection of the norepinephrine product. Monoamine oxidase B was assessed in platelet-rich plasma using HPLC with electrochemical detection of the 2,4-dihydroxyphenylacetaldehyde product. Comparisons were made using the Mann–Whitney *U*-test, Fisher exact test, Spearman correlation, and logistic regression.

There were no significant differences between groups in demographic characteristics or in the number of positive answers to a 16-item symptom questionnaire. Plasma prolactin was higher among styrene-exposed workers compared to unexposed workers (median = 10.0, range = 2.8–32.0 μg/L compared to median = 5.7, range = 2.4–16.0 μg/L, $p = 0.0001$). Using the control group distribution to establish plasma prolactin percentiles, there were 14 (30%) of 46 styrene-exposed workers above the 95th percentile, compared to 2 (7%) of 30 unexposed controls. Plasma monoamine oxidase B was decreased in the styrene exposed group at a *p*-value of 0.06. Platelet dopamine-β-hydroxylase did not differ between exposed and unexposed subjects; however, styrene-exposed subjects in the highest exposure quartile based on individual urine monitoring had a significantly lower level of platelet dopamine-β-hydroxylase than did control subjects ($p = 0.01$). Overall, platelet dopamine-β-hydroxylase levels were negatively correlated with the sum of urinary mandelic and phenylglyoxylic acid concentrations ($p = 0.003$). The authors postulated that the styrene-associated increase in plasma prolactin was attributable to dopamine depletion in the tuberoinfundibular system. They further suggested that the decrease in peripheral levels of dopamine-metabolizing enzymes, whereas not necessarily reflecting central nervous system activity of these enzymes, could be evidence of a compensatory response to dopamine depletion. The authors concluded, however, that plasma prolactin concentration seemed to be a more sensitive indicator of ''catecholaminergic dysfunction'' than the peripheral enzyme activities.

Strengths/Weaknesses: The exposure monitoring and urinary assay data are strengths of this study. Weaknesses include the relatively small number of exposed subjects, the uncertain exposure status of the controls, and the use of only a single blood sample.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited to moderate utility.

Luderer et al. (2004), supported by NIH and the University of California at Irvine, evaluated the relationship between serum prolactin level and occupational exposure to styrene. Employees from 17 reinforced plastics workplaces were recruited as part of a larger study on neurological outcomes. Measurements were made for two or three ''sessions,'' approximately 1 year apart and included personal breathing zone styrene and acetone, blood styrene, and serum prolactin. Personal breathing zone measurements for each session were made over a full shift on 1–5 workdays (average = 3) in a month. On one of these days, a blood sample was collected at the end of the shift. On the workday when blood was sampled, an interview was conducted and the worker was observed in the performance of his or her job

tasks. An aliquot of blood was mixed with a deuterium-labeled styrene internal standard, and a pentane extract was frozen for subsequent analysis by isotope dilution GC-MS. Another aliquot of blood was centrifuged to obtain serum, which was frozen for subsequent estimation of prolactin using a commercial chemiluminescent immunoassay. Initial statistical comparisons were made using *t*-test and ANOVA. Pearson correlation was evaluated for continuous variables with respect to styrene and acetone exposure estimates. Variables that were considered plausibly related to styrene exposure were entered into a linear regression model. Models were developed for acute exposure (using only data from the first session), subchronic exposure (using all the sessions in subjects who were evaluated more than once), and chronic exposure (using the product of average air styrene concentration and number of years in the industry or number of years in the facility). For the subchronic and chronic models, the last serum prolactin measurement was used as the dependent variable.

The entire sample consisted of 259 men and 43 women. Mean time in the current job was 3.8 years (range = 0–30.7 years). Blood styrene and serum prolactin both were available for 173 men and 33 women evaluated in 386 sessions. Serum prolactin and air styrene concentrations were available for 193 men and 35 women evaluated in 417 sessions. Blood styrene ranged from <1–142 ppm (median = 9 ppm) **[<4.33–615 mg/m^3 (median = 39 mg/m^3)]**. Blood styrene concentrations ranged from <0.001–2.05 mg/L (median = 0.0089 mg/L). Blood and air styrene concentration were correlated ($r = 0.77$, $p < 0.001$). Age and income were negatively correlated with styrene exposure, reflecting the employment of younger individuals in highly exposed positions and older individuals in lower-exposure, managerial positions. In bivariate analysis, significant differences in blood styrene were observed by sex (men with levels twice as high as women), type of industry, glove use, prescription drug use, ethnicity (Hispanic higher than Caucasian), season (winter highest), and time of day (night shift highest). Serum prolactin was significantly associated with sex (women 22% higher). There was an inverse correlation of serum prolactin with income ($p = 0.013$) and age ($p = 0.085$) and a positive correlation of serum prolactin with blood styrene ($p = 0.016$) and air styrene ($p = 0.063$).

Coefficients for styrene exposure indices as independent factors in serum prolactin level were calculated in a multiple linear regression controlling for sex, age, and smoking (Table 33). The risk estimate calculated from the regression for the acute exposure model suggested a 2.06-fold increase in serum prolactin concentration for every 10-fold increase in blood styrene concentration. The authors sought to confirm the association of acute styrene exposure with serum prolactin by looking at the relation between blood styrene and serum prolactin collected individually in Session 1 and Session 2, controlled for sex, age, and cigarette smoking. A statistically significant association was shown for men but not for women for both sessions, possibly reflecting the smaller number of women in the sample. When the change in styrene concentration from Session 1 to Session 2 was evaluated against either Session 2 prolactin or against the change in prolactin from Session 1 to Session 2, a statistically significant association was shown for women but not men, perhaps reflecting the observation that prolactin concentration was more variable in women than in men between sessions. Using logistic regression to control for sex, age, and cigarette smoking, an average acute styrene exposure to more than 20 ppm styrene (the ACGIH TLV) was associated with a greater likelihood of a serum prolactin above the 95th percentile for the laboratory (15 ng/mL for men, 20 ng/mL for women), OR = 3.69 (95% CI = 1.39–9.79) **[OR not given by sex]**. The authors concluded that styrene exposure resulted in prolactin elevation and that this effect was related to acute rather than longer-term exposure.

Table 33
Effect of Styrene Exposure on Serum Prolactin, Controlling for Age, Sex, and Cigarette Smoking Using Multiple Linear Regression[a]

Model and exposure estimate	Coefficient ±SEM	*p*-value
Acute model[b]		
Blood styrene	0.314 ± 0.137	0.023
Air styrene	0.0548 ± 0.028	0.053
Subchronic model[c]		
Blood styrene	0.279 ± 0.171	0.105
Air styrene	0.0563 ± 0.031	0.074
Chronic model[d]		
Years in industry × average air styrene	0.0403 ± 0.021	0.060
Years in facility × average air styrene	0.0263 ± 0.023	0.255

[a]Luderer et al. (2004). $n = 175$ except in acute exposure where $n = 205$ for blood styrene and 227 for air styrene.
[b]Acute exposure evaluated using a single blood sample from the first evaluation session.
[c]Subchronic exposure evaluated using the mean of exposure measurements from all sessions for each subject and the last prolactin measurement.
[d]Chronic exposure evaluated using average air styrene measurements multiplied by the relevant exposure time. The last prolactin measurement was used as the dependent variable.

Strengths/Weaknesses: The individual exposure assessments, repetition of assays over time, comprehensive statistical analysis, and adjustment for possible confounders are strengths of the study. The sample size is adequate for men but small for women. Clinical outcomes were not evaluable in this study.

Utility (Adequacy) for CERHR Evaluation Process: The study is useful in evaluating endocrine effects but is limited with respect to clinical outcomes.

4.1.1.2 Spontaneous abortion:

Hemminki et al. (1980), funded by the Academy of Finland, evaluated the incidence of spontaneous abortion in female members of the Union of Chemical Workers. Information on spontaneous abortion, induced abortion, and births was obtained from a computerized registry of the Finnish National Board of Health, which recorded inpatient discharges from general hospitals. The authors estimated that 2.2% of obstetrics-gynecology hospital beds were private and not included in this registry, and that 15% of women with spontaneous abortions were not managed as hospital inpatients. Women with abortion ($n = 15{,}482$) and birth discharge diagnoses from 1973–1976 were linked by their personal identification numbers to members of the Union (the authors note that unionization is high in Finland). Spontaneous abortion

was assessed as a proportion of total pregnancies (spontaneous abortions + induced abortions + births) and as a proportion of births. Comparisons were made using χ^2 testing between abortion rates in Union members (including specific categories of Union membership) and nonmembers. Spontaneous abortion occurred in 5.52% of pregnancies in the general population during the study period and equaled 7.98% of births. Statistically significant increases were reported for spontaneous abortion among Union workers ($n = 52$ spontaneous abortions; 8.54% of pregnancies, 15.57% of births), plastic industry workers ($n = 21$ spontaneous abortions; 8.94% of pregnancies, 17.80% of births), and styrene workers **[presumably a subset of plastics workers]** ($n = 6$ spontaneous abortions; 15.00% of pregnancies, 31.59% of births). When worker age was considered, a particular increased risk was described for women aged 15–19 years. **[For comparison with other reports, unadjusted odds ratios and 95% CI were calculated by CERHR using the CDC Statistical Analysis Battery for Epidemiologic Research (SABER) program and the numbers given in the report. All women in Finland were used as the reference group. With respect to all pregnancies: Union members, 1.60 (1.19–2.14); plastic industry workers, 1.68 (1.04–2.68); styrene workers, 3.02 (1.14–7.52). With respect to births: Union members, 1.95 (1.44–2.64); plastic industry workers, 2.23 (1.36–3.62); styrene workers, 3.96 (1.42–10.47).]** The authors indicated that their study could not distinguish between effects due to occupational chemical exposures and other factors (nutritional, social) related to occupation, but they believed non-chemical factors unlikely to explain the increased abortion rate because abortion occurred more commonly in chemical workers than in other workers in industry and construction **[data presented as 5.8% of pregnancies and 9.2% of births for these latter groups, with citation to another article]**.

An extension of this study to 1979 was presented as a book chapter (Hemminki et al., 1984). Spontaneous abortions experienced by Union members during membership were compared to spontaneous abortions experienced before or after membership. There was no difference in abortions as a percent of pregnancies or of births for plastics industry workers or styrene workers (Table 34). The authors noted a greater incidence of spontaneous abortion among younger women and women whose pregnancies were during the early part of the study period. They also noted that the expected increase in spontaneous abortion incidence with older age did not occur in their sample of chemical workers, suggesting, ''the presence of some selection.''

Strengths/Weaknesses: The use of a computerized national registry was a strength. Weaknesses include the likelihood that spontaneous abortions were missed (the control rates are low, and rates did not increase with age). Exposure assessment was limited to industry codes, and potential confounders were not accounted for. The sample sizes were small and the times series was difficult to interpret.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited to moderate utility in the evaluation of spontaneous abortion in the plastics industry.

Lindbohm et al. (1985), supported by the Swedish Work Environment Fund, performed a case-control study of spontaneous abortion in women who belonged to the Finnish Union of Chemical Workers in 1979 or who had resigned from the Union in 1978 or 1979. Reproductive data for 1973–1980 were obtained from the hospital discharge register of the Finnish National Board of Health, supplemented with data **[source unspecified]** on outpatient management of spontaneous abortion. Cases included women who were recorded as having had a spontaneous abortion, and controls were women who had given birth and who had not had a registered spontaneous abortion. Controls were matched to cases based on age within 2 years. Three control women were matched to nearly all cases. Exposure information was obtained by questionnaire or telephone contact from occupational health physicians and used to assign women to one of four exposure categories: processing of unheated polymerized plastics, processing of heated plastics, processing of plastic monomers or epoxy resins, and not involved in processing plastics. Exposures were also divided into those involving vinyl chloride, polyurethane, or styrene (with or without butadiene or acrylonitrile). Odds ratios were estimated from a logistic regression model.

There were 44 cases and 123 controls. Details of employment were identified in 82% of cases and 72% of controls. No significant association was identified between spontaneous abortion and any category of plastics work or chemical exposure, except polyurethane; the OR (95% CI) was 3.0 (1.2–7.8) when all workers in a polyurethane plant were considered, but the OR (95% CI) was 1.9 (0.4–8.5, based on four exposed cases) when workers processing polyurethane were considered. Within exposure categories, the numbers of exposed women with spontaneous abortions were small (3–6 for most categories, 14 for polymerized plastics, and 12 for heated plastics). In univariate analysis, the spontaneous abortion OR for processing styrene was 0.4 (95% CI = 0.1–1.2, based on four exposed cases) and the OR for heating styrene plastics was 0.6 (95% CI = 0.2–2.3, based on four exposed cases). In the multivariate model, the OR was 0.4 for handling styrene plastics and 0.5 for heating styrene plastics (95% CI not given; association described as not

Table 34
Spontaneous Abortion Among Members of the Finnish Union of Chemical Workers 1973–1979[a]

	During membership			Before/after membership		
Exposure	*n*	Pregnancies, %	Births, %	*n*	Pregnancies, %	Births, %
Plastics work	59	8.6	13.3	65	10.1	17.3
Styrene work	9	6.8	10.2	19	12.2	22.1

[a]Hemminki et al. (1984).

statistically significant). The authors concluded that their results suggested no association between styrene plastics work and spontaneous abortion but cautioned that the small size of the study population resulted in low statistical power, with a <38% likelihood of being able to identify a two-fold increase in spontaneous abortion risk for specific types of plastics.

Strengths/Weaknesses: Strengths include the use of national data and the case-control design. Weaknesses include the small sample, the limited exposure assessment, and lack of consideration of non-hospitalized early abortions.

Utility (Adequacy) for CERHR Evaluation Process: This study has limited utility in the evaluation process.

Lindbohm et al. (1990), supported by NIOSH, carried out a case-control study of spontaneous abortion in women exposed to a diverse group of chemicals called, ''organic solvents.'' The study population was identified from records of women monitored for solvent exposure at the Finnish Institute of Occupational Health between 1965–1983, a Finnish national registry of pregnancies, and a Finnish national registry of congenital malformations. Pregnancies were eligible for inclusion if they occurred between 1973–1983. The registries that were used include 94% of all recorded births in Finland and were estimated to include 80–90% of recognized spontaneous abortions. Women with a spontaneous abortion were defined as cases, with one abortion/woman randomly selected if there were more than one. Three controls were matched to each case based on age, at conception within 2.5 years. Controls included women with at least one birth and without a pregnancy affected by abortion or congenital malformation. There were 115 cases identified. Three controls were available for 104 of the cases, two controls for eight of the cases, one control for eight cases, and zero controls for two cases. Each case and control woman was mailed a questionnaire soliciting information on workplace activities and exposures during the first trimester of the index pregnancy. Six specific chemicals, including styrene, were named with information requested on frequency of handling. In a separate appendix, biologic monitoring data for 37 women with styrene exposure during pregnancy were reported to have median urinary mandelic acid levels of 5.7 mM (mean $\pm$ SEM $= 8.0 \pm 1.6$ mM), with 73% of the women above the Finnish reference value for non-occupational exposure and 37.8% above the Finnish reference value for occupational exposure of 7.0 mM. **[The urine monitoring was not necessarily performed during the index pregnancy.]** Up to four mailings were used, with an overall response rate of 85.5%. Information was obtained for the index pregnancy for 78% of cases and 99% of controls. Odds ratios for exposure were estimated from a logistic regression model. There were three cases and 17 controls with a history of first trimester exposure to styrene. The OR adjusted for previous spontaneous abortion, parity, smoking, use of ethanol, and exposure to other solvents was 0.3 (95% CI = 0.1–1.0, $p = 0.05$). **[The Expert Panel finds this result extremely imprecise.]** The authors concluded that the decreased OR could be due to an increase in early subclinical abortion, producing a proportion of clinically recognized abortion lower than expected. They cited the increase in prolactin associated with styrene exposure by Mutti et al. (1984b) as suggesting a mechanism by which ''styrene might be able to interfere with reproductive events.''

Strengths/Weaknesses: Use of national registries is a strength. The responses rates were good except that the response rate was lower in cases than controls, which could have introduced bias. The small number of exposed cases limits the power of the study and the exposure assessment was limited.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Härkönen and Holmberg (1982), of the Finland Institute of Occupational Health, identified 67 female lamination workers occupationally exposed to styrene and compared their responses to an interviewer-administered questionnaire with the responses of a referent group of 67 textile and food production workers who were ''not occupationally exposed to any solvent.'' Referents were matched to styrene-exposed women by age. **[Whether the interviewer knew the exposure status of the subjects was not indicated. It is also not stated whether the subjects knew the purpose of the study and the hypotheses being evaluated.]** Menstrual cycle and pregnancy histories were assessed and considered in relation to the timing of employment in a styrene-exposed occupation. Comparisons were made within the styrene-exposed group between events occurring during and outside the estimated time period of occupational exposure. Comparisons were also made between each styrene-exposed woman and her referent by dividing time periods in the referent's life to match occupational periods in the styrene-exposed woman's life. **[The statistical methods were not given, except that a Poisson model was used for at least one analysis, suggesting that multiple events in the same woman were regarded as independent.]** Comparisons of births were made to age-specific fertility rates in the Finnish female population.

There were no significant differences between the styrene-exposed women and the referents in menstrual cycle parameters, contraception use, and use of recreational drugs, including tobacco and ethanol. There were no significant differences in number of women reporting pregnancies or in spontaneous abortion rates. Before styrene exposure, there were eight spontaneous abortions in each group ($n = 84$ pregnancies among the exposed women and 80 pregnancies among the referents), and during styrene exposure there were four spontaneous abortions in each group ($n = 16$ pregnancies among the exposed women and 22 pregnancies among the referents). The number of births was reduced in the styrene-exposed women during employment when compared to age-specific national fertility data and compared to the referent group. The authors postulated that a decrease in the number of births without an increase in spontaneous abortions suggested an increase in induced abortions in the styrene-exposed women, although a statistically significant difference in induced abortion was not demonstrated in this study.

Strengths/Weaknesses: The use of each woman as her own control is a strength, although the self-reported nature of the exposure assessment is a weakness. Possible control exposures were not ascertained. The small number of spontaneous abortions is a weakness, as is the lack of specification of the statistical methods.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

McDonald et al. (1988), supported by the Institut de Reherche en Santé et en Sécurité du Travail (a private non-profit in Québec), presented a report on outcome of 193 pregnancies in women employed in the plastics industry. The study was based on interviews with 56,012 women in Montreal between May, 1982 and May, 1984, representing 90% of births in the city. Women were contacted shortly after a delivery or a spontaneous abortion and were questioned in a standardized fashion using an 8-page questionnaire administered by a bilingual nurse. The current and past pregnancies were included in the questionnaire, giving information on 104,620 pregnancies. The current study consisted of pregnancies from this sample in which the woman worked at least 30 hr/week in plastics manufacture at the time of conception. There were 234 pregnancies identified (124 current and 110 previous), of which 10 were excluded due to lack of employment at conception and two due to elective termination of the pregnancy. Attempts were made to re-interview the remaining 222 women to obtain more detailed information about exposure. After exclusion of women whose workplaces could not be located or who did not work with plastics, 193 pregnancies remained for evaluation. Probabilities of spontaneous abortion were obtained from logistic regression models controlling for age, gravidity, history of previous abortion, ethnicity, educational level, smoking, and ethanol. The group of all working women was used to calculate the expected number of spontaneous abortions. Current and past pregnancies were analyzed separately and then combined. **[The results present only the combined data.]** Analysis was performed by calculation of 90% CI around the observed/expected ratios. Of the 184 pregnancies in which process work was performed by the mother, 19% ended in spontaneous abortion, compared to 14% of pregnancies in all working women. The observed/expected ratio for any process work was 1.27 (90% CI = 0.91–1.72). When the type of plastic with which the woman worked was distinguished as polyvinyl, polystyrene, polyurethane, or polyolefin, only for polystyrene did the 90% CI exclude unity: observed/expected ratio 1.58 (90% CI = 1.02–2.35). The authors concluded that their findings raised the suspicion that ''work in plastics production may carry a reproductive hazard.''

Strengths/Weaknesses: The large population-based cohort that served as the basis of the study is a strength; however, the exposed group was small. Adjustment for potential confounders is a strength. Exposure assessments were limited, however, and the comparison groups were heterogeneous.

Utility (Adequacy) for CERHR Evaluation Process: This report is of limited to moderate utility in the evaluation of spontaneous abortion.

4.1.2 Male reproductive toxicity.

Brown (1991), supported by the Styrene Research and Information Center, reviewed papers from the Russian literature, which Brown read in the original Russian. A study by Neshkov and Nosko from 1967 is cited as presenting information on sexual problems in men with 1–10 years in the reinforced plastics industry. Semen parameters were also reported. Brown (1991) indicates that the report does not allow conclusions because the men were exposed to a number of different chemicals, and because actual data were not presented. **[The Expert Panel notes this review for completeness, but does not find it suitable for the evaluation process. The original Russian report was not evaluated by the Expert Panel, and no independent evaluation of its reliability is possible.]**

An association of increasing serum prolactin with increasing styrene exposure has been identified in men (Bergamaschi et al., 1996; Luderer et al., 2004), discussed above; however, no reproductive endpoints were measured in these studies.

Jelnes (1988), Danish National Institute of Occupational Health, reported semen analysis results from 25 men employed in a reinforced plastics plant with exposure to styrene, acetone, and other unspecified chemicals. The men were studied because breathing zone measurements of styrene and acetone in the plant had shown values above the Danish TLV. Median (range) styrene concentrations were 294 (82–564) mg/m^3 **[68 (19–130) ppm]** 10 weeks before the semen collections, 362 (162–695) mg/m^3 **[83 (37–160) ppm]** 15 weeks before the semen collections, and 552 (261–961) mg/m^3 **[127 (60–221) ppm]** 28 weeks before the semen collections. The Danish TLV for styrene was $105\,mg/m^3$ **[24 ppm]**. Median acetone concentrations were less than the TLV of $600\,mg/m^3$; however, the high end of the acetone concentration range 15 weeks before the semen sampling was $1334\,mg/m^3$. Men provided a blood sample and a semen sample at a local fertility clinic. Comparisons were made with an age-matched reference group of 46 men attending the fertility clinic. Median age was 34.0 years in the styrene-exposed group and 32.5 in the reference group. **[No information was given on the reproductive experience of any of the men being studied or on the occupational exposures of the reference group. The men from the reinforced plastics plant will be referred to here as styrene-exposed for convenience; it is possible that some of the fertility-clinic men were also styrene-exposed. No information was given on use of tobacco, ethanol, medications, or other drugs for either group.]** Data were presented as medians without indications of variance and were analyzed with the Mann–Whitney *U*-test. There were no differences between the styrene-exposed men and the reference group in serum FSH or LH, semen volume, or sperm concentration. Sperm concentration in the styrene-exposed men was 56×10^6/mL. The percent live sperm was higher in the styrene-exposed men (80%) than in the reference group (68%), and the percent immotile sperm was lower in the styrene-exposed men (30%) than in the reference group (40%). The styrene exposed men had a lower percentage of normal forms (47%) than the reference group (60%), with pyriform and amorphous heads identified as particular abnormalities for which a significant difference was seen. The authors acknowledged that the use of a fertility clinic population as a reference group was potentially problematic, and that the styrene-exposed men may have experienced other exposures of importance, such as high temperature. The authors discounted tobacco, ethanol, and heat as likely explanations for their findings because these exposures are not known to interfere selectively with sperm morphology.

Strengths/Weaknesses: It is a strength that men in the exposed group may have been exposed to high levels of

styrene. Weaknesses include the small sample size, the lack of consideration of potential confounders, the lack of determination of individual exposures, and the use of fertility clinic controls.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Taskinen et al. (1989), funded by NIOSH, evaluated pregnancy outcome in the wives of men occupationally exposed to styrene and other industrial chemicals **[only the methods and results relevant to styrene are presented here]**. Men were recruited from workers monitored for organic solvent exposure by the Finnish Institute of Occupational Health between 1965–1983. Styrene exposure was assessed from the concentration of mandelic acid in urine. Wives were identified from a population registry using the personal identifying number of the men, and pregnancy outcomes were identified through pregnancy outcome registries, including a hospital discharger registry. Subjects were restricted to first marriages for the men and ages 18–40 at the end of the first trimester of pregnancy for wives. Pregnancies were not considered if they were estimated to have been conceived more than 9 months before marriage or at any time after divorce. Eligible pregnancies were used to set up a case-control study of spontaneous abortion and of congenital malformation. Women with the outcome of interest were identified as cases, and women with neither spontaneous abortion nor malformed children were eligible to be controls. Controls were selected by computer to match cases on maternal age within 30 months. Three controls were used for each spontaneous abortion case, and five controls were used for each congenital malformation case. Only one pregnancy per woman was used, randomly selected when there was more than one pregnancy that met eligibility criteria for case or control status.

Exposure information was obtained from postal questionnaires. Men were asked for information on employment, work tasks, and workplace during the year of conception and about exposure to chemicals, dusts, fumes, gases, and vapors. The questionnaires also requested information on frequency of exposure to chemicals and the presence of chronic diseases, tobacco use, and ethanol use. Exposure to styrene (and other chemicals of interest) was assessed based on occupation, job description, reported styrene use, and biological monitoring data when available. There were 81 of 120 cases for which biological monitoring data were available and 143 of 251 controls for which biological monitoring data were available **[monitoring data availability was given for chemicals as a group]**. The exposure period of interest was 80 days preceding the estimated onset of the study pregnancy **[80 days was used as the period of spermatogenesis]**. Men were categorized as unexposed, potentially exposed, or likely exposed, and exposure was further defined as high, intermediate, or low based on frequency of handling styrene or on biological measurements. Wives were assessed in a similar manner, although no biological measurements were available. In addition, wives were asked about lifting during the first trimester of pregnancy, and a score was constructed by assigning points to each lift based on weight categories and summing the scores of lifts at work and at home.

Response to the questionnaire in the overall sample (styrene plus other chemicals) was 79.1% for abortion cases and 73.3% for abortion controls. For congenital malformation cases and controls, the response rates were about 75%. Conditional logistic regression was used to calculate OR. **[Controlling was not mentioned for all comparisons, but for at least one comparison, controlling included likely paternal exposure to other organic solvents and dusts, maternal exposure to solvents, maternal heavy lifting, and previous spontaneous abortion.]** The OR (95% CI) for styrene exposure in the spontaneous abortion study was 1.3 (0.8–2.1), based on 37 cases and 66 controls. When exposure category was considered, the OR (95% CI) were: low exposure, 1.0 (0.3–3.1); intermediate exposure, 0.9 (0.4–2.1); high exposure, 0.7 (0.4–1.5). Results for congenital malformations were not reported by chemical (there were only 25 responding cases in the entire study). The OR for exposure to any organic solvent was 0.6 (95% CI = 0.2–2.0). The authors concluded that no increase in spontaneous abortion was found for styrene exposure, and that the number of congenital anomaly cases was too small for firm conclusions to be drawn.

Strengths/Weaknesses: The use of national registries is a strength, as are linking of male exposures to female pregnancy outcome and the offsetting of the male exposure period to account for spermatogenesis. The attempt to use biologic monitoring data is a strength, but this strength was offset by the incomplete availability of monitoring data. The reliance on questionnaire data for outcomes and the uncertainty about consistent adjustment for potential confounders are weaknesses. The sample size was not adequate to address congenital malformations.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility with respect to spontaneous abortions and is not useful with respect to congenital malformations.

Sallmén et al. (1998), supported by the Finnish Work Environmental Fund, evaluated time-to-pregnancy among the wives of men occupationally exposed to styrene and other occupational chemicals **[only the methods and results relevant to styrene are presented here]**. Subjects from a previous case-control study on spontaneous abortion (Taskinen et al., 1989) were recontacted 6 years later with a request for information on how many menstrual cycles it took to achieve the index pregnancy. Subjects had initially qualified for the spontaneous abortion study based on biological monitoring for exposure to styrene or one of several other workplace chemicals. Men in their first marriages and wives aged 18–40 at the end of their first trimester of pregnancy had been eligible for enrollment. Pregnancies and their outcomes were identified from national registries. Information on workplace and other exposures, including tobacco and ethanol, was collected by postal questionnaire as part of the original study, and the likelihood and amount of chemical exposure were assessed based on questionnaire response with or without biological monitoring data. On recontact for the time-to-pregnancy study, case and control wives from the spontaneous abortion study were asked to indicate if they were trying to get pregnant, whether they got pregnant during the first cycle of trying, and if not, whether they got pregnant in the 2nd, 3rd–4th, 5–6th, 7–12th, 13–24th, or after the 24th cycle. Additional information was collected on menstrual cycle

characteristics and frequency of intercourse. Results were expressed as fecundability density ratios, using discrete proportional hazards regression, adjusting for short menstrual cycle, long or irregular menstrual cycle, older age at menarche, frequency of intercourse, maternal age, maternal exposure to organic solvents, and a variable controlling for missing information. The wives of men listing their occupation as plastics worker **[presumably exposed to styrene]** had adjusted fecundability density ratios (95% CI) of 0.98 (0.50–1.92) for low/intermediate exposure ($n = 12$) and 0.94 (0.61–1.44) for high exposure. The wives of men in whom styrene exposure was considered likely had fecundability density ratios (95% CI) of 1.10 (0.73–1.66) for low/intermediate exposure ($n = 46$) and 0.98 (0.64–1.50) for high exposure ($n = 42$). An appendix was provided showing urinary mandelic acid concentrations (used to assess styrene exposure) among men in the study. There were 46 men considered to have a high level of exposure and 21 with a low level of exposure. **[No explanation was given for the difference in the number of men with high exposure in the study ($n = 42$) and in the appendix ($n = 46$).]** Median urinary mandelic acid in the men with high exposure was 2.49 mM (mean $\pm$ SD = 3.58 $\pm$ 3.75 mM). Median urinary mandelic acid in the men with low/intermediate exposure group was 0.23 mM (mean $\pm$ SD = 1.52 $\pm$ 2.73 mM). The authors noted that the information on time to pregnancy was collected 8–18 years after the event, and that the wives of men not exposed to solvents were more likely to have responded to the recontacting effort than the wives of men exposed to solvents (80% compared to 70% response rate). The authors concluded, ''Our work did not indicate any association between exposure to…styrene and fertility.''

Strengths/Weaknesses: Strengths include exposure biomonitoring and the linkage of male exposure to female time-to-pregnancy, with adjustment for multiple potential confounders. The 8–18 year lag between exposure and the events being recalled is a weakness. The response rates were moderate, and differed by exposure group.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Bonde et al. (1999) described the Asclepios project, a research initiative on occupational hazards to male reproduction sponsored by the EU and carried out in 14 European centers between 1993–1998. Some of the Asclepios studies involved men exposed occupationally to styrene (discussed below); this overview article noted that styrene concentrations in ambient air had been declining at Asclepios sites for 25 years. Concentrations during the time period of the Asclepios project were shown in a series of graphs to be about 3–30 ppm **[estimated from graphs]**. One of the aims of the Asclepios project was to evaluate semen parameters in men longitudinally, from before (or early in) employment to after some period of employment in a styrene-exposed workplace. This aim was frustrated in many of the participating centers by low worker turnover, and longitudinal studies of sperm quality were restricted to Danish workplaces.

Kolstad et al. (1999b,c) presented the results of the longitudinal semen quality studies from Asclepios **[the two reports seem to be duplicative, although Kolstad et al. (1999c)** is more complete]. Men hired by one of four Danish reinforced plastics companies were offered participation in the study on their first day of work. Of 131 invited workers, 37 (28% **[the article says 30%]**) agreed to participate. Three men were excluded (vasectomy, prior styrene exposure, previous welding exposure). Loss to follow-up occurred in 11 instances. The remaining 23 men provided semen samples during their first week of work and again after some months of work (6 months in 21 men, 2 and 3 months in 2 men). Each man served as his own control, but an external control group of farmers was included, matched by season of semen collection. **[The authors indicate that pesticide exposure among these farmers had been shown previously not to influence semen parameters.]** Semen samples were collected at home and evaluated within 1–2 hr by a technician who came to the home with a mobile laboratory. The semen samples were kept close to the body in the interim between collection and evaluation. Evaluations included semen volume, sperm concentration estimated in a hemocytometer, curvilinear velocity estimated by computer-assisted sperm analysis, and morphology evaluated from fixed, stained samples. A 0.1-mL aliquot of semen was diluted in saline buffer and frozen at -80°C. When all samples had been collected, they were shipped on dry ice to a single center for sperm chromatin structure assay. This analysis uses flow cytometry to estimate the extent of acid denaturation of sperm DNA, believed to be a marker of chemical-induced fertility impairment. Styrene exposure was assessed using post-shift urine concentrations of mandelic acid collected for 5 successive days just after hiring and for another 5 days after 6 months on the job. Semen parameters early in employment and months later were compared using a paired *t*-test. Changes in semen parameters in the plastics workers were compared to changes in the farmer controls using linear regression and were related to urinary mandelic acid concentrations by trend testing. Linear regression analysis was carried out after transformation of data to achieve normality. Information on medication use, smoking, ethanol consumption, and medical history was obtained but was not included in the analysis because these potential confounders were stable during the follow-up period.

The median urinary mandelic acid concentration in the 28 plastics workers was 45.0 mg/g creatinine (mean $\pm$ SD = 65.9 $\pm$ 73.8 mg/g creatinine). Values varied considerably depending on the frequency of involvement in lamination and the use of a respirator, ranging from undetectable (<25 mg/L) in individuals who did not laminate and who used a respirator to a median of 113.6 mg/g creatinine in workers who laminated daily and did not use a respirator. Median semen parameters in styrene-exposed workers are shown in Table 35. Styrene work was associated with a decrement in sperm concentration and percent normal forms and an increase in percent vital sperm (by eosin exclusion) and curvilinear velocity. The degree of change between the two samples in styrene workers was significantly greater than the degree of change in farmers for sperm concentration. Sperm chromatin structure assay results showed no significant changes in styrene exposed workers between the first and second samples. There was no significant trend in semen parameters with increasing urinary mandelic acid concentration, although the authors identified an association of the proportion of

Table 35
Longitudinal Study of Semen Parameters in Styrene-Exposed Workers and Unexposed Farmers[a]

	Styrene-exposed workers		Farmers	
Parameter	Sample 1	Sample 2	Sample 1	Sample 2
Sperm concentration, million/mL	63.5	46.0[b,c]	58.0	58.0
Normal sperm (%)	44.3	38.5[b]	38.5	45.0[b]
Vital sperm[d] (%)	85.5	94.5[b]	75.5	69.0
Sperm velocity (μm/sec)	66.1	77.8[b]	75.4	76.7

[a]Kolstad et al. (1999c). Values shown are medians.
[b]Significant difference ($p < 0.05$) from Sample 1 within occupational group by paired *t*-test on transformed means.
[c]Significant difference ($p = 0.04$) of the change in styrene-exposed workers compared to the change in farmers, from multiple regression model. $n = 21$–22 for styrene-exposed workers and 17–21 for farmers.
[d]Data in the study were reported as "nonvital sperm" and were converted by CERHR by subtracting from 100%.

nonvital sperm with increasing mandelic acid concentration ($p = 0.06$). This trend was due to improvements (decreases) in nonvital sperm in the second sample in the men with undetectable urinary mandelic acid and men in the lowest mandelic acid group rather than an increase in nonvital sperm in the highest two mandelic acid groups. Indices of sperm chromatin denaturability showed an increasing trend with increasing urinary mandelic acid, although the authors described the effect as weak and the change as being within the interassay variability of the method. The sperm chromatin structure assay was carried out on samples from only 14 men. The authors concluded that a declining sperm count and an increased susceptibility of sperm DNA to denaturation associated with styrene exposure were suggested by their data, but that interindividual variability was high and that the findings should be regarded as preliminary.

Strengths/Weaknesses: The use of each man as his own control is a strength as is the assay of styrene exposure and the use of multiple semen parameters. The low participation rate and small sample size are weaknesses, and it is not clear whether participation could have been selective. The use of farmer controls introduced possible selection bias and possible effects of farm-related exposures.

Utility (Adequacy) for CERHR Evaluation Process: This study is moderately useful in the evaluation of the change in semen parameters over time, correlated with urinary mandelic acid. Its utility is decreased by the possibility of selection bias and the inappropriate controls.

Kolstad et al. (1999a, 2000) evaluated time-to-pregnancy in male workers in the reinforced plastics industry in Denmark, Italy, and the Netherlands as part of the Asclepios project **[Kolstad et al. (1999a) was published as a preliminary study, and its methods and results are included in Kolstad et al. (2000)]**. Currently or recently employed workers from three Danish, nine Italian, and 14 Dutch reinforced plastics companies were invited to participate in the study. Workers were identified as using lamination techniques that were expected to result in styrene exposure. Of 1560 men who were invited, 213 were unmarried, 122 declined participation, and 296 did not respond, leaving 929 married men who participated. Information was obtained from the men by questionnaire ($n = 375$), telephone interview ($n = 393$), or face-to-face interview ($n = 161$). Pregnancy information was obtained for the youngest child in the 720 men who reported having fathered a pregnancy. There were 118 exclusions due to the pregnancy after contraceptive failure, due to inability to remember time-to-pregnancy, and due to pregnancy within 13 months of the start of data collection. Men with pregnancies within 13 months of the start of data collection were excluded to ensure comparability with more remote pregnancies for which time-to-pregnancy was censored at 13 months. There were 220 conceptions estimated to have occurred during employment in the plastics industry (considered exposed) and 382 conceptions estimated to have occurred before or after employment (considered unexposed). Time to pregnancy was estimated by asking the men how many months it took to conceive their youngest child. Styrene exposure was evaluated semi-quantitatively by incorporating biological measurements in a subset of workers, workroom air concentrations available from three Danish, one Italian, and five Dutch companies, and questionnaire data on the frequency of lamination and the use of a respirator. The questionnaire data on frequency of lamination and use of a respirator were used to weight the exposure estimates based on the data from Kolstad et al. (1999c), reviewed above. Subjects were categorized into four styrene exposure groups (none, low, medium, high). A Cox regression model was used to estimate fecundity odds ratios. The model included maternal age (<25, 25–29, 30–34, ≥ 35 years), smoking (husband/wife, yes/no), last contraceptive used (oral contraceptive/other), parity (1, >1), length of employment in plastics industry (<1, 1–4, ≥ 5 years), year of starting pregnancy attempts (1970–1979, 1980–1984, 1985–1989, 1990–1995), country, and an interaction term for oral contraceptive use and time to pregnancy. Data were censored after 13 months of attempting pregnancy to avoid including couples with fertility treatments.

The adjusted fertility OR (95% CI) for all exposed men was 0.79 (0.59–1.05). There was no significant impact on the fertility odds ratio of period of attempting pregnancy, length of time working in the plastics industry, or semi-quantitative exposure category (Table 36). A cumulative exposure index that included duration of exposure showed an increase in fecundity with increasing styrene exposure, and in a subset of 34 Italian men who began attempting pregnancy within 1 year of a urine sampling date when compared to 38 unexposed Italian men. **[Data were not shown for these two conclusions of increasing fecundity with increasing styrene exposure.]** The authors wrote that "the unexpected inverse exposure

Table 36
Fecundity Ratios for Men Working in Reinforced Plastics in Denmark, Italy, and the Netherlands[a]

Characteristic	*n*	Adjusted fecundity ratio (95% CI)[b]
Time-to-pregnancy starting year		
1970–1979	20	0.94 (0.49–1.80)
1980–1984	16	0.62 (0.20–1.27)
1985–1989	54	0.86 (0.52–1.41)
1990–1997	128	0.76 (0.52–1.11)
Length of exposure in the reinforced plastics industry (years)		
<1	23	0.81 (0.46–1.44)
1–4	76	0.98 (0.67–1.43)
≥5	96	1.08 (0.73–1.60)
All exposed	220	0.79 (0.59–1.05)
Styrene exposure		
None	283	Reference
Low	96	0.68 (0.48–0.97)
Medium	69	0.70 (0.47–1.04)
High	53	1.09 (0.69–1.72)

[a]Kolstad et al. (2000).
[b]Adjusted fecundity ratios were obtained from a proportional hazard model that included maternal age, use of oral contraceptives, maternal and paternal smoking, time to pregnancy starting year, length of employment in the reinforced plastics industry, and country. Test for trend $p = 0.19$.

response relation was probably due to regional differences in the reported time to pregnancy because no exposure response relations were seen within each participating center.'' The authors contrasted the findings of the current study to the decrease in sperm concentration identified in their previous study (Kolstad et al., 1999c). They concluded, ''…no consistent pattern of reduced male fecundity was observed as a result of styrene exposure. If the suggested styrene-dependent decline of sperm count is true, it is unlikely that the effect on fecundity is strong.''

Strengths/Weaknesses: The large sample size and adjustment for potential confounders (limited as it was) are strengths. Exposure data were, however, limited, and obtaining fecundity data retrospectively by interviewing men is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited to moderate utility in the evaluation process.

4.2 Experimental Animal Data

4.2.1 Endocrine assays.

Date et al. (2002), of Nissin Food Products Co., Japan, evaluated endocrine effects of styrene monomer (99% purity), dimer, and trimer in Sprague–Dawley and F344 rats. Estrogen- and androgen-receptor binding was assessed using receptor-rich cytosol obtained from uteri or ventral prostates of castrated 8-week-old Sprague–Dawley rats. Binding was assessed by competition with radiolabeled estradiol (for estrogen receptor) or methyltrienolone (for androgen receptor). A uterotropic assay was performed in 21-day-old (prepubertal) female rats and in 6-week-old castrated rats **[strain not specified]**. Animals were treated with three daily s.c. injections of test compound or corn oil vehicle. Styrene dose levels were 20 or 200 mg/kg bw/day in prepubertal animals. Styrene monomer was not tested in castrated adults. Styrene dimer and trimer were tested in animals at both ages at dose levels up to 200 mg/kg bw/day. Uterine weight was assessed 24 hr after the final treatment. A Hershberger assay was performed in 21-day-old castrated or sham-operated male rats **[strain not specified]**. Castrated rats were given s.c. testosterone propionate. Test chemicals were given by gavage and included styrene monomer, dimer, and trimer 20 or 200 mg/kg bw/day for 7 days. Weights of the seminal vesicles, ventral prostates, and levator ani/bulbocavernosus muscles were recorded 24 hr after the last treatment. A thyroid hormone-receptor binding assay was performed using a nuclear fraction isolated from 7-week-old Sprague–Dawley rat livers. Binding was assessed by competition with radiolabeled thyroxine. Prolactin was measured in 7-week-old castrated F344 rats after s.c. administration of styrene monomer, dimer, or trimer 20 mg/kg bw/day for 3 days. Rats were decapitated 24 hr after the final dose and trunk blood used for rat prolactin enzyme immunoassay.

Data were analyzed using one-way ANOVA followed by Dunnett, Aspin–Welch, or Student *t*-test. Positive and negative controls were run for all assays. Styrene monomer, dimer, and trimer failed to inhibit binding of estradiol, methyltrienolone, or thyroxine to their respective receptors. Tested concentrations ranged up to 10 μM **[1.04 mg/L for styrene monomer]**. Styrene monomer, dimer, and trimer did not increase reproductive organ weight in the uterotropic or Hershberger assays and did not increase serum prolactin. The authors concluded that styrene monomer, dimer, and trimer ''exhibit no apparent estrogenic, androgenic, anti-androgenic and thyroid activity.'' They recognized that their prolactin data were not consistent with studies in humans, citing Mutti et al. (1984b) and Arfini et al. (1987) but did not offer an explanation for the apparent discrepancy.

Strengths/Weaknesses: This study was carefully carried out according to standardized protocols for evaluating endocrine effects. The use of both in vitro and in vivo tests and the use of multiple dose levels are strengths, and the consistency of the negative findings across different assays is reassuring. Weaknesses include the lack of information on strain, numbers of animals, random allocation, and other design features.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in the assessment of reproductive effects of styrene. The results support the authors' conclusions.

Jarry et al. (2002, 2004), support not indicated, evaluated serum hormones and prolactin-related central neurotransmitters in styrene-exposed male Wistar rats. Animals at about 13 weeks of age were exposed to styrene by inhalation at nominal concentrations of 0, 150, 500, or 1500 ppm (0, 645, 2150, or 6450 mg/m^3). Mean concentrations determined by GC were 148 ± 5.2, 490 ± 13.2, and 1477 ± 53.5 ppm. Ten animals/dose group were exposed 6 hr/day for 5 consecutive days and were killed immediately after the last exposure period. An additional 10 animals/dose group were killed 24 hr after the last exposure to evaluate recovery from any identified exposure effects. Animals were decapitated under light ether anesthesia, and trunk blood was collected for determination of prolactin, dopamine, LH, and testosterone by RIA. Brains were immediately frozen

until analysis. Two micropunches of striatum were obtained from each brain and the mediobasal hypothalamus was dissected. Catecholamine and amino acid neurotransmitters were extracted from the brain samples and quantified using reversed-phase HPLC with electrochemical detection. Data were analyzed using ANOVA with post-hoc Dunnett test for multiple comparisons. There were no differences between groups killed at either time point in blood prolactin concentration. In the animals evaluated 24 hr after the last exposure, blood dopamine concentration was increased in the groups exposed to 150 and 1500 ppm styrene, but not in the group exposed to 500 ppm styrene. No alterations in blood dopamine were identified in any group among the animals evaluated immediately after exposure. Serum LH and testosterone were significantly increased in the 500 and 1500 ppm immediately evaluated groups, but not in the groups evaluated 24 hr after exposure. There were no treatment-related alterations in striatal levels of dopamine, dihydroxyphenylacetic acid, homovanillic acid, glutamate, or γ-aminobutyric acid, and there were no treatment-related alterations in mediobasal hypothalamic levels of dopamine, dihydroxyphenylacetic acid, norepinephrine, glutamate, or γ-aminobutyric acid. The authors concluded that there was no evidence of prolactin secretion induced by styrene exposure in rats, a finding supported by a lack of evidence of altered tuberoinfundibular dopamine or other neurotransmitters/metabolites in the striatum or mediobasal hypothalamus. They suggested that the increase in serum LH and testosterone in the two highest exposure groups may have reflected chance sacrifice of animals during an LH pulse, and they concluded that there would be no adverse effect of an increase in LH and testosterone on fertility.

Strengths/Weaknesses: The evaluation of multiple hormone-related endpoints, including brain catecholamines and not just serum levels, the use of three styrene exposure levels, the evaluation of animals immediately after exposure and 24 hr later, and the analysis of chamber styrene concentrations are strengths of this study. The two studies are consistent in their lack of observed effect on prolactin. The use of decapitation is a strength in studies evaluating prolactin and dopamine, both of which are sensitive to stress. The use of ether anesthesia may be stressful, however. The use of only 10 animals is a weakness given the variability in LH and testosterone values; use of a castrated rat model would have provided a more sensitive assessment of effects on LH. Frequent handling of animals to acclimate them to stress would also have been helpful. The 5 days of exposure may not have been sufficient to produce identifiable effects. Random assignment of animals to treatment groups was not specified.

Utility (Adequacy) for CERHR Evaluation Process: This study is minimally useful and is limited by the short duration of exposure, the number of animals, the suboptimal number of animals per treatment group, and physiological differences between rats and humans in the measured parameters.

[The Expert Panel notes that in separate experimental animal studies, the effects of styrene exposure on hypothalamic dopamine concentrations were evaluated. Decreased tuberoinfundibular dopamine levels were observed in rabbits exposed by inhalation to 1500 ppm for 12 hr/day for 3 or 7 days (Mutti et al., 1984a). Decreased hypothalamic dopamine levels were also observed in rats treated orally with styrene 500 mg/kg bw/day for 13 weeks (Chakrabarti, 2000). Because pituitary prolactin secretion is under tonic inhibitory control by tuberoinfundibular dopamine, one might expect a decrease in tuberoinfundibular dopamine to be associated with increased prolactin secretion; however, prolactin was not measured in these studies.]

4.2.1 Female reproductive toxicity.

Brown (1991), supported by the Styrene Research and Information Center, reviewed papers from the Russian literature, which the author read in the original Russian. One author (Bondarevskaya) published a report in 1957 in which disturbances in the reproductive cycle of rats were described at styrene exposures of about 3500 and 7000 ppm (subacute) and 465 ppm (chronic). Another study (Izyumova, 1972) described lengthening of the estrous cycle in rats exposed an unspecified number of hr/day to 1.2 and 11.6 ppm styrene. There were reductions in body weight at these exposure levels. The same author (Izyumova, 1972) reported an increase in estrous stage length in rats exposed by inhalation to styrene 0.2 and 1.2 ppm. Both exposure levels were associated with a decrease in body weight of the animals. In both studies, there seemed to be an increase in the cycle length alteration after a 1-month period without exposure. Brown (1991) found these reports to contain inadequate detail and to include some inconsistencies, but he was reluctant to discount the findings altogether. A study of ovarian response to hemicastration (Bakhtizina, 1981, 1982, 1983) found styrene 200 mg/kg bw by gavage to inhibit the increase in weight of the remaining ovary; however, it is not known whether the styrene treatment was given once or daily. Morphometric and histochemical changes in the ovaries were also described in these papers. **[The Expert Panel notes this review for completeness, but does not find it suitable for the evaluation process. The original Russian reports were not evaluated by the Expert Panel, and no independent evaluation of the reliability of these reports is possible.]**

No other experimental animal studies on possible female reproductive toxicity of styrene have been identified.

4.2.2 Male reproductive toxicity. Rat studies with testicular endpoints after lactational and juvenile styrene treatments (Srivastava et al., 1992b,c) and a mouse study with testicular and plasma testosterone endpoints after peripubertal styrene treatment (Takao et al., 2000) are discussed in Section 3.2.3 because exposures occurred during developmental periods.

Cruzan et al. (1997), supported by the Styrene Information and Research Center, conducted a limited examination of reproductive organs in rats and mice as part of a subchronic inhalation toxicity study. Whole-body styrene (>99.4% purity) inhalation exposures were conducted in 10 animals/sex/group for 6 hr/day, 5 days/week, for 13 weeks. Sprague–Dawley rats (>7 weeks old) were exposed to styrene concentrations of 0, 200, 500, 1000, or 1500 ppm. CD-1 mice (>4 weeks old) were exposed to styrene concentrations of 50, 100, 150, or 200 ppm. Among tissues preserved in neutral buffered formalin and examined histologically in control and high dose animals were uterus, cervix, vagina,

ovaries, prostate, seminal vesicles, epididymides, and testes. Neither male nor female reproductive organs of treated rats or mice were reported to have lesions **[data not shown]**. Systemic toxicity observed in this study is discussed briefly in Section 2.

Strengths/Weaknesses: Subchronic studies such as this are useful as screening tools to indicate potential for effect on reproductive organs; however, they cannot be used as definitive assessments of reproductive toxicity. The use of four dose levels is a strength and the lack of organ weights is a weakness. The use of formalin for fixation of male reproductive organs is a weakness; Bouin solution or glutaraldehyde with plastic embedding would have been preferable.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Srivastava et al. (1989), support not indicated, evaluated testicular effects of styrene (''of the highest purity'') in adult Wistar rats **[age not further specified, mean body weight 225 g]**. Styrene in groundnut oil was given orally **[route not otherwise specified]** at 0, 200, or 400 mg/kg bw/day 6 days/week for 60 days. Body weight was determined weekly. Animals were decapitated 24 hr after the last dose and testes and epididymides removed and weighed. Homogenized testes were assayed for the activity of six enzymes (acid phosphatase, β-glucuronidase, glucose-6-phosphate dehydrogenase, γ-glutamyl transpeptidase, lactate dehydrogenase, and sorbitol dehydrogenase; expressed with respect to tissue protein). Epididymal sperm were counted in a hemocytometer after mincing the epididymis in saline and filtering through a nylon mesh. Statistical comparisons were made to the control group using the Student *t*-test. Testis histopathology was evaluated in 5-μm formalin-fixed sections embedded in paraffin and stained with hematoxylin and eosin. Statistical comparisons were made using the Student *t*-test. No deaths were observed in any treatment group, and there were no alterations in body, testis, or epididymis weight **[data not shown]**. Results are summarized in Table 37. Alterations in testicular enzyme activities and epididymal sperm concentration occurred in males exposed to styrene 400 mg/kg bw/day. Rats in this group had abnormal testicular histopathology findings consisting of shrunken seminiferous tubules with some Sertoli-only tubule sections. The interstitium was edematous, and blood vessels were described as congested. There were no histopathologic alterations at 200 mg/kg bw/day. The authors concluded that short term exposure to styrene causes testicular degeneration.

Strengths/Weaknesses: The use of two styrene exposure levels and only six animals per dose group are weaknesses, as is the failure to report the purity of the styrene and the analysis of the gavage solution. It is not clear why dosing was 6 instead of 7 days/week. An insufficient number of endpoints was reported, and more detailed assessment of testicular histopathology utilizing Bouin's solution or glutaraldehyde fixative (with plastic embedding) would have been useful. The relevance of the testicular enzyme levels to testicular function is not clear. An adverse effect of treatment on male reproductive function would contradict the findings of the Cruzan et al. (2005b) study. Mesh-filtration of epididymal sperm is not an optimal method of specimen preparation. The testicular fixation method used was suboptimal and may have led to artifacts. The finding of shrunken tubules with some Sertoli-only tubules but without an effect on testis weight is inconsistent. It is not clear whether there were direct testicular effects or whether the effects were secondary to testicular edema. The dose-dependency of the findings is a strength of the study.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Salomaa et al. (1985), supported by the Swedish Work Environment Fund and the Academy of Finland, evaluated the effects of styrene on sperm morphology in mice. Styrene (''purum,'' stabilized with 40–50 ppm 4-*tert*-butylcatechol) was given by inhalation 6 hr/day for 5 days at exposure levels of 0, 150, or 300 ppm in one experiment and i.p. at 0, 175, 350, or 700 mg/kg bw/day in another group. The mouse strain was (C3H/He × C57Bl/6J)F_1, with 9–14 animals/dose group. Animals were killed 3 and 5 weeks after the last treatment. Cauda epididymal sperm were stained with eosin Y and morphology of 500 sperm heads/mouse were evaluated. Comparisons were made with the Mann–Whitney *U*-test (one-tailed). There was no significant effect of styrene treatment by either route on sperm head morphology. Testis weight was also not affected **[data not shown]**. Cyclophosphamide, used as a positive control, showed the expected increase in abnormal sperm heads. The

Table 37
Testis Enzyme Activities and Epididymal Sperm After 60-Day Treatment of Adult Rats With Styrene

	Control mean, %						
Treatment group	SDH	LDH	G6PD	β-GLU	ACID PHOS	GGT	Sperm[b]
Styrene 200 mg/kg bw/day	95	99	102	95	105	95	92
Styrene 400 mg/kg bw/day	67[c]	155[c]	117[c]	130[c]	72[c]	175[c]	67[c]
BMD_{10}	264	364	340	374	377	356	228
$BMDL_{10}$	148	118	153	183	236	69	101
$BMD_{1\ SD}$	274	381	349	397	379	386	257
$BMDL_{1\ SD}$	159	214	182	274	251	227	128

[a]Srivastava et al. (1989). [**Data converted to percent of control values by CERHR.**]
[b]Epididymal sperm concentration, estimated from a graph.
[c]Different from control value at $p < 0.05$ (Student *t*-test; $n = 6$/group).
SDH sorbitol dehydrogenase; LDH lactate dehydrogenase; G6PD, glucose-6-phosphate dehydrogenase; β-GLU, β-glucuronidase; ACID PHOS, acid phosphatase; GGT, γ-glutamyl transpeptidase; BMD, benchmark dose. For an explanation of BMD, see footnote to Table 25.

authors postulated that the lack of effect of styrene on sperm head morphology may have been due to impaired access of the chemical to the testis.

Strengths/Weaknesses: Styrene toxicity was evaluated in this study by two routes, one relevant, one not. The inhalation experiment included only two styrene exposure levels, and animals were treated only 6 hr/day for only 5 days. There was no analysis of the solution that was given i.p. Evaluations of testicular toxicity were limited. The mouse strain used in this study was not conventional, and there were too few animals per dose group. Sperm assessment 3 and 5 weeks after the dose did not assess all germ types that may have been affected by treatment in as much as spermatogenesis and epididymal transit take >5 weeks. The hypothesis that negative findings were due to impaired access of styrene to the testis is unproven and is considered unlikely. A strength was the use of a positive control, which performed as expected.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in the evaluation process.

Fabry et al. (1978), supported by the Fundamental Research Collective and by the Belgian Nuclear Energy Study Center, performed mutagenicity testing of styrene oxide in male BALB/c mice. The testing included a dominant lethal study **[presented because it reflects some aspects of male reproductive ability]**. Styrene oxide (97% purity) in paraffin oil was given i.p. to an unspecified number of animals **[$n = 5.67$ back-calculating from the number of mated females]** at 0 or 250 mg/kg. Males were immediately housed with three virgin females of the same strain, which were replaced after 7 and 14 days. Females were killed 17 days after the beginning of the mating period and pre- and post-implantation losses were calculated **[by a method referenced in a book chapter]**. Statistical comparisons made using χ^2 testing showed no differences between styrene- and vehicle-treated treated groups in pregnant females, corpora lutea, implantations, live embryos, dead embryos, or pre- or post-implantation loss. The authors noted mutagenicity in in vitro testing and postulated that styrene oxide access to the germ cells in vivo might have been reduced by uptake, distribution, excretion, and metabolism.

Strengths/Weaknesses: Dominant lethal studies can be of use in detecting effects on fertility, but because significant effects on testicular function must occur before there is a detectable decrease in fertility in rodents, the resolving power of these studies is limited. Furthermore, the test agent was styrene oxide, not styrene. The lack of specification of the number of treated animals is a weakness, as is the speculation that styrene oxide had limited access to the germ cells.

Utility (Adequacy) for CERHR Evaluation Process: This study is of limited utility in assessing styrene reproductive effects.

4.2.3 Multigeneration study.

Beliles et al. (1985), supported by the U.S. producers of Styrene Monomer (Chemical Manufacturers Association), performed a three-generation reproduction study of styrene in COBS (SD) BR rats. The reproductive study used a subset of animals from a 2-year chronic toxicity study. The animals were exposed to styrene (≥98.9% purity by GC) in drinking water at 0, 125, or 250 ppm (average concentration 88.5–89.8% of target). **[Water consumption was not reported for the rats participating in the reproductive substudy but was reported for a subset of animals in the 2-year study. Mating took place about 90 days into the study. Using the average of Week 11 and Week 17 water consumption data (Table 4 of the original article), which are the measurement times closest to 90 days, estimated styrene intake for the 125 ppm group was about 10 mg/kg bw/day for males and 12 mg/kg bw/day for females. Estimated styrene intake in the 250 ppm group was 18 mg/kg bw/day for males and 23 mg/kg bw/day for females. The authors' estimate of mean styrene intake over the 2-year study in rats not used as breeders was 7.7 mg/kg bw/day for males and 12 mg/kg bw/day for females in the 125 ppm group and 14 mg/kg bw/day for males and 21 mg/kg bw/day for females in the 250 ppm group. The authors indicated that styrene treatment did not alter body weight or feed consumption in the reproductive substudy, but no information was given on water consumption in any generation.]** The parental generations were cohabited after 90 days on study. Selected F_1 and F_2 animals were cohabited on about PND 110. For the F_0 matings, 15 males and 30 females were used for the control group; the styrene-exposed groups used 10 males and 20 females. For the F_1 matings, similar numbers were used, except only 19 females were mated in the 125 ppm group. For the F_2 matings, 14 control males were mated to 28 control females, 9 males were mated to 20 females in the 125 ppm group, and 10 males were mated to 20 females in the 250 ppm group. Endpoints were male and female fertility, pup survival and weight on PND 1, 7, 14, and 21, and sex ratio on PND 21. Gross physical abnormalities were assessed during the lactation period. Statistical analyses were by Dunnett *t*-test, contingency table analysis, or Wilcoxon rank-sum test. Formal litter analysis was not performed, although some litter-based observations were noted.

In the chronic study, clinical signs were absent. Water consumption was decreased by styrene, an effect attributed to taste aversion. As indicated above, there were no effects of treatment on maternal body weight or feed consumption in any generation. There were no treatment-related effects on F_0 or F_1 fertility or F_1 pup measures except a decrease in PND 21 survival from a control rate of 97% to a rate of 89% in the high dose group ($p < 0.05$ on a pup basis). The authors discounted this effect as being due entirely to pup deaths between PND 15 and 21 in 2 of 20 litters in this dose group. Pup survival was decreased in the 250 ppm group in F_2 offspring (born to F_1 breeders) at birth and on PND 1, 7, and 14, to 94, 97, 91, and 88%. Control survival at these time points was 99, 99, 96, and 93%. On PND 21, the 88% survival rate among 250 ppm offspring was not statistically different from the 93% survival rate among control offspring. There were no treatment-related alterations in weight among F_2 pups. Fertility and pup survival was not affected by treatment for the F_2 matings. The F_3 offspring in the 250 ppm group were lighter than controls on PND 7 and 14, but not on PND 1 or 21. Testis and ovary weights were assessed in pups from all generations at weaning, and no treatment-related findings were reported. Bone marrow cells showed no chromosome aberrations. No congenital anomalies were reported. The authors concluded that the isolated

findings of impaired pup survival or weight were attributable to one or two litters, and that the absence of consistent effects across generations suggested a lack of relationship to the treatment. They concluded, "administration of styrene under these conditions produced no deleterious dose-related effects or decrements in reproductive function through three generations."

Strengths/Weaknesses: This multi-generation study was conducted according to regulatory guidelines in force at the time. Additional male reproductive endpoints have been added to reproductive studies since this study was performed; these additional endpoints add sensitivity. It is a strength of this study that the authors evaluated the total data set as a basis for their conclusions on reproductive function. Weaknesses include the lack of exposure estimation due to a lack of reporting of water consumption, the lack of litter analysis in the statistical methods, unusual pup mortality on PND 15–21 (recognized by the authors), and unusual decreases in F_3 pup body weight on PND 7 and 14 but not PND 0 or 21. The study would have been strengthened by reporting the solubility of styrene in water, more frequent preparation of styrene solutions, and inclusion of additional, higher exposure levels.

Utility (Adequacy) for CERHR Evaluation Process: This study is of high utility for the evaluation process.

Cruzan et al. (2005b), supported by the Styrene Information and Research Center, conducted a two-generation reproductive toxicity study of styrene in rats. Male and female Crl:CD®(SD)IGS BR rats were randomly assigned to groups (25/sex/group) that received whole-body inhalation exposure to styrene vapors (at least 99.9% purity) at 0, 50, 150, or 500 ppm for 6 hr/day, 7 days/week for a minimum of 70 consecutive days before mating and during the mating period. Styrene concentrations in inhalation chambers were verified. Estrous cycle length was assessed through vaginal smears obtained during the 21 days before mating or until necropsy in rats that did not become pregnant. Rats from the same exposure group were paired 1:1 until mating occurred or until 14 days elapsed. If mating did not occur, the female was paired for 7 days with a male that had previously mated. Reproductive parameters included mating, fertility, and gestation length. In females, inhalation exposures were continued through GD 20 (GD 0 = day of plug or sperm detection). On PND 1–4 (PND 0 = day parturition was completed), dams were gavage dosed with styrene in olive oil at 0, 66, 117, or 300 mg/kg bw/day, divided into three equal doses administered ~2 hr apart. The gavage dosing was designed to produce peak maternal blood levels that were observed with the inhalation exposure. Inhalation exposure of dams resumed on PND 5 and was continued until the day before kill. Pups were removed during inhalation exposure of dams. At birth, pups were sexed, weighed, and examined for viability and gross malformations. Litters were culled to five male and five female pups on PND 4. Pups were monitored for growth and developmental landmarks, and the results are reported in the developmental neurotoxicity study (Cruzan et al., 2005c) discussed in Section 3.2.2.2. On PND 21, two F_1 pups/sex/group were randomly selected from each litter. Exposure of F_1 pups began on PND 22 and was conducted as described for the F_0 generation. On PND 28, 1 or 2 rats/sex/litter were randomly selected to make up groups comprised of 25 rats/sex. F_0 and F_1 males were necropsied 3 weeks after the parturition period. **[It was not stated if males continued to receive styrene exposures after the mating period.]** F_0 and F_1 females that delivered were necropsied 6–10 days after weaning of their offspring. Parameters examined at necropsy were spermatogenic endpoints in F_0 and F_1 males and implantation sites, ovarian primordial follicle counts, and corpora lutea counts in F_1 females from the control group, high-dose group, and lower dose groups that did not produce litters. Statistical analyses included χ^2 test with Yates correction, parametric ANOVA, Dunnett test, Kruskal–Wallis nonparametric ANOVA, Mann–Whitney *U*-test, ANCOVA, or two-tailed Fisher exact test.

Evidence of systemic toxicity in parental rats included significantly reduced body weight gain in F_0 and F_1 males and females of the 500 ppm group and F_1 males of the 150 ppm group during the premating exposure period. Body weight gain during gestation was not affected by styrene treatment in F_0 dams, but was decreased in F_1 dams. Water intake was slightly increased in F_0 and F_1 dams during gestation. During the lactation period, water consumption was said to be increased in the 150 and 500 ppm F_0 dams but was unaffected in F_1 dams (data not shown). There were no major effects on feed consumption **[data not shown]**. An increase in liver weight relative to body weight in F_0 and F_1 males from the 150 and 500 ppm groups was the only organ weight effect reported **[data not shown]**. Lesions in olfactory epithelium were reported in males and females of the 500 ppm group.

Although estrous cycle length was significantly shorter in F_0 females of the 500 ppm group, the study authors reported that a value of 4.2 days in the 500 ppm group is within the historical range, whereas the value in the control group (5.8 days) exceeded historical control values. Styrene exposure had no effect on other F_0 reproductive parameters including mating, precoital interval, fertility, delivery of litters, gestation length, and implantation sites. Styrene treatment had no effect on sperm count in the F_0 500 ppm group or sperm motility or morphology in any treatment group. There were no effects on F_1 litter size, pup viability at birth, sex distribution, pup postnatal survival, or pup body weight gain on PND 1–4 or PND 21.

Styrene exposure had no effect on F_1 reproductive parameters including estrous cycle lengths, mating, precoital interval, fertility, delivery of litters, gestation length, or implantation sites. Styrene treatment did not affect the number of primordial follicles or corpora lutea in F_1 females from the 500 ppm group. There was no effect on sperm count in the F_1 500 ppm group or sperm motility or morphology in any treatment group. There were no effects on F_2 litter size, pup viability at birth, sex distribution, pup postnatal survival, or pup body weight gain on PND 1–4. On PND 21, body weights of F_2 male pups in the 150 ppm group and male and female pups in the 500 ppm group were significantly lower than controls. Compared to controls, mean body weights were 10% lower in 150 ppm males, 11% lower in 500 ppm males, and 13% lower in 500 ppm females. Significant organ weight effects in F_2 offspring were mainly observed in the 500 ppm group and included decreased

pituitary weight in males and decreased brain, uterus, thymus, and pituitary weight in females. Because relative organ weights were not reduced, the study authors attributed the effect to growth retardation and not to a direct chemical effect on the organs.

The study authors identified NOAELs of 50 ppm for parental toxicity and ≥500 ppm for reproductive toxicity. **[The Expert Panel concurs with the authors' conclusions.]**

Strengths/Weaknesses: This large, comprehensive two-generation study was conducted according to modern regulatory guidelines. The use of a dose level at which there was some systemic toxicity but not too much toxicity, is a strength. The findings in the F_2 but not F_1 offspring are a weakness, calling into question whether the effects were real.

Utility (Adequacy) for CERHR Evaluation Process: This study is of high utility for the evaluation process.

4.3 Utility of Data

There are three epidemiology studies (Härkönen and Holmberg, 1982; Lemasters et al., 1985b; Cho et al., 2001) on menstrual cycle parameters in women exposed occupationally to styrene or styrene plus other chemicals. Two other studies (Mutti et al., 1984b; Arfini et al., 1987) evaluated serum prolactin in women with occupational styrene exposures and correlated serum prolactin with urinary measurements of styrene metabolites and two studies (Bergamaschi et al., 1996; Luderer et al., 2004) evaluated the association between styrene exposure and serum prolactin in a mixed, but mostly male, population. There are five epidemiology studies (the first of which is presented in two citations) with information on spontaneous abortion in women with likely occupational exposures to styrene (Hemminki et al., 1980, 1984; Härkönen and Holmberg, 1982; Lindbohm et al., 1985, 1990; McDonald et al., 1988). None of these studies included measurements of styrene exposure in women but inferred exposure based on job descriptions.

A study of men with styrene exposure in a reinforced plastics plant (Jelnes, 1988) reported semen analysis results, but was not useful due to the presence of other exposures in the workplace and the lack of a suitable control group. There are four studies involving men exposed occupationally to styrene that can be used in the evaluation process. One study (Kolstad et al., 1999b,c) evaluated semen analysis parameters at early employment and after 2–6 months of employment. Another study (Taskinen et al., 1989) evaluated the incidence of spontaneous abortion in the wives of male workers. Two additional studies (Kolstad et al., 1999a, 2000; Sallmén et al., 1998) evaluated time-to-pregnancy in married male workers who had fathered pregnancies.

There are no studies specifically on female reproductive toxicity in experimental animals that can be used in the evaluation process. There is a two-dose (plus placebo) study using oral administration in male rats that evaluated specific endpoints (enzymes in testicular homogenates, epididymal sperm concentration). This study is of limited utility in the assessment of male reproductive toxicity. There is a rat multi-generation study using two drinking water exposure levels of styrene that can be used to assess treatment effects on reproductive success. A multidose level two-generation toxicity study was also conducted in rats exposed through inhalation.

4.4 Summary of Reproductive Toxicity Data

4.4.1 Summary of human reproductive toxicity data. Human studies on reproductive effects of styrene are summarized in Table 38 and Table 39.

4.4.1.1 Female: Two studies evaluated the endocrinologic effects of styrene exposure. Mutti et al. (1984b) assessed serum prolactin, thyroid stimulating hormone, growth hormone, FSH, and LH in 30 styrene exposed women compared to 30 age-matched styrene unexposed women. Styrene metabolites (mandelic acid and phenylglyoxylic acid) were determined in urine. Prolactin and growth hormone levels were significantly higher in the styrene-exposed subjects. Moreover, serum prolactin and thyroid stimulating hormone levels were significantly correlated with styrene metabolite excretion in urine, suggesting a dose-response effect. Arfini et al. (1987) assessed endocrine effects in 16 styrene-exposed women and 16 age-matched unexposed controls. Baseline serum prolactin was significantly higher in the styrene exposed women (20 ng/mL) compared to controls (12–15 ng/mL). After 200 μg thyrotropin-releasing hormone administration, prolactin levels were markedly increased in the styrene-exposed women (~120 ng/mL) compared to the controls (33 ng/mL). Moreover, after thyrotropin-releasing-hormone stimulation, prolactin responses exceeded a preset criterion in 94% of styrene-exposed compared to ~25% of unexposed women. There was also a significant correlation between urinary styrene metabolites and serum prolactin ($r = 0.52$, $p < 0.05$). In two women re-evaluated after terminating styrene exposure, prolactin response to thyrotropin-releasing hormone stimulation was reduced. One of these latter women experienced amenorrhea during styrene exposure and resumed menses 40 days after removal of exposure.

A European study (Bergamaschi et al., 1996) assessed 33 men and 20 women occupationally exposed to styrene with 8-hr TWAs ranging from 5–120 ppm (median = 25 ppm). A comparison group of 60 workers without evidence of styrene exposure was also assessed. Serum prolactin, dopamine β-hydroxylase, and monoamine oxidase were assessed pre-shift. Basal prolactin was significantly higher in styrene-exposed women than in unexposed controls. Monoamine oxidase and dopamine β-hydroxylase levels were significantly lower in the styrene exposed-subjects. Serum dopamine β-hydroxylase was negatively correlated with styrene urinary metabolites.

A U.S. study (Luderer et al., 2004), evaluated 173 men and 33 women employed in the plastics industry using personal breathing zone measurement as well as end of shift blood styrene and serum prolactin assessment. Air and blood styrene concentrations were correlated ($r = 0.77$), and there was a positive correlation between acute blood styrene and serum prolactin ($r = 0.31$) and acute air styrene and prolactin ($r = 0.06$), but no correlations were observed with subchronic or chronic exposures. Using a statistical model for acute exposure, it was estimated that each 10-fold increment of blood styrene was associated with a 2.1-fold increase in serum prolactin.

Table 38
Summary of Human Reproductive Studies on Styrene in Women

Sample	Styrene exposure	Endpoints	Results	Reference
Employees in reinforced plastics companies ($n = 174$ exposed to styrene, 449 not exposed to styrene).	None, low (mean = 13 ppm), and high (mean = 52 ppm) based on investigator assessment of work sites and records.	Severe dysmenorrhea, intermenstrual bleeding, secondary amenorrhea, blood clots, hypermenorrhea.	No significant association of endpoints with estimated styrene exposure	Lemasters et al., 1985b
Employees in petrochemical industry ($n = 1408$ total of whom 276 were exposed to styrene.	Not directly assessed, although average said to be <1 ppm.	Oligomenorrhea (average menstrual cycle length >35 days).	Adjusted OR = 1.65 (95% CI = 1.05–2.55).	Cho et al., 2001
Workers occupationally exposed to styrene and workers not exposed to styrene ($n = 30$/group).	Based on urinary styrene metabolite concentration, a workplace styrene concentration of 65–300 ppm (mean = 130 ppm) was estimated.	Serum prolactin, thyroid stimulating hormone, growth hormone, follicle stimulating hormone, luteinizing hormone.	Prolactin ↑2-fold Growth hormone ↑1.6-fold in styrene-exposed women. Prolactin and thyroid-stimulating hormone concentrations correlated with urinary concentrations of styrene metabolites.	Mutti et al., 1984b
Workers occupationally exposed to styrene and women not exposed to styrene ($n = 16$/group).	Not estimated, but mean urinary styrene metabolite concentration was in the same range as Mutti et al., (1984b).	Prolactin response to infusion of thyrotropin-releasing hormone.	Basal prolactin ↑1.3–1.7 fold, stimulated prolactin ↑3.6-fold **[estimated from a graph]**. Peak prolactin response correlated with urinary concentration of styrene metabolites.	Arfini et al., 1987
Spontaneous abortions in Finland 1973–1976 ($n = 15{,}482$). Case-control study.	Designated (without quantification) from records of the Union of Chemical Workers	Rates of spontaneous abortion compared between Union members and nonmembers using linkage with national registries.	**[OR (95% CI)** **Using all pregnancies:** **Union members 1.60 (1.19–2.14);** **plastics workers 1.68 (1.04–2.68);** **styrene workers 3.02 (1.14–7.52).** **Using only births:** **Union members: 1.95 (1.44–2.64);** **plastics workers 2.23 (1.36–3.62);** **styrene workers 3.96 (1.42–10.47).]**	Hemminki et al., 1980
Union of Chemical Workers in Finland 1973–1979 (extension of above study). Case-control study.	Pregnancy during period of union membership compared to pregnancy before or after membership as a surrogate for styrene exposure/non-exposure.	Rate of spontaneous abortion as a percentage of all pregnancies or of births only.	No significant difference between rates in pregnancies during union membership compared to pregnancies before or after membership.	Hemminki et al., 1984
Plastics workers in Union of Chemical Workers in Finland ($n = 167$). Case-control study.	Union records were used to identify the kind of plastics work. Styrene workers were identified without quantification.	Spontaneous abortion	No association between styrene plastics work and spontaneous abortion.	Lindbohm et al., 1985
Woman with biologic monitoring for organic solvents and women identified through national pregnancy registries.	Postal questionnaires were used to obtain information on exposures.	Spontaneous abortion	Adjusted OR (95% CI) for styrene exposure 0.3 (0.1–1.0) based on three exposed cases and 17 exposed controls.	Lindbohm et al., 1990

Table 38
(Continued)

Sample	Styrene exposure	Endpoints	Results	Reference
Women working in plastics manufacturing at the time of conception ($n = 193$).	Based on questioning women about their job exposures an unspecified time period after the pregnancy in question.	Spontaneous abortion	Observed: expected ratio for polystyrene work 1.58 (90% CI = 1.02–2.35).	McDonald et al., 1988
Female lamination workers compared to food and textile workers ($n = 67$/group).	Lamination workers were known to be exposed to styrene based on the process being used. Lack of exposure in the referent group was assumed.	Rates of spontaneous abortion before and during employment period.	No significant difference in rates of spontaneous abortion.	Härkönen and Holmberg, 1982

↑, statistically significant increase.

These four studies suggest a consistent and possibly causal effect of styrene or its metabolites on increased serum prolactin; however, most of the elevations in serum prolactin would not be considered indicative of clinically relevant hyperprolactinemia.

Three studies evaluated the effects of styrene exposure on the menstrual cycle. Lemasters et al. (1985b) assessed menstrual parameters in 174 exposed and 449 unexposed women employed in the U.S. reinforced-plastics industry. Styrene exposure was not associated with any menstrual cycle abnormalities (severe dysmenorrhea, intermenstrual bleeding, amenorrhea, and heavy menses), but the study had limited statistical power to detect effects. Cho et al. (2001) assessed oligomenorrhea (cycle length > 35 days) in 1408 Chinese petrochemical workers. Oligomenorrhea occurred in 8.5% of unexposed women compared to 14.5% of styrene-exposed women (adjusted OR = 1.65, 95% CI = 1.05–2.55). This study was limited by the concurrent exposure of these women to other petrochemicals. Härkönen and Holmberg (1982) observed no effect of styrene exposure on irregular menstruation or changes in menstrual patterns. Small sample size constrained statistical power.

A series of studies from Finland examined the risk of spontaneous abortion among female members of the Union of Chemical Workers. A national registry of inpatient hospital discharge data was used to ascertain spontaneous abortions. An initial analysis (Hemminki et al., 1980) suggested a three-fold elevated risk among a small number of women with a history of styrene-related work. Another analysis of the same data did not find increase in the proportion of spontaneous abortion stratifying employment before or after Union membership (Hemminki et al., 1984). A small case-control study of female Union of Chemical Workers found a decreased risk of spontaneous abortion associated with presumed styrene exposure (Lindbohm et al., 1985). A Finnish case-control study of women monitored for solvent exposure used national registry data to estimate the risk associated with styrene exposure (Lindbohm et al., 1990). The study reported an imprecise decreased risk of spontaneous abortion with presumed first trimester styrene exposure. Another Finnish study of a small number of female lamination workers reported no increase in spontaneous abortions related to the time period of employment with styrene exposure, compared to employment outside this period or in comparison to a referent group (Härkönen and Holmberg, 1982). A Montreal study of pregnancies among women employed in the plastics industry found weak to moderate elevations in risk for spontaneous abortion associated with plastics work (McDonald et al., 1988). The studies were generally limited by low statistical power, limited exposure data, and under-ascertainment of spontaneous abortions.

4.4.1.2. Male: An association of increasing serum prolactin with increasing styrene exposure has been identified in men (Bergamaschi et al., 1996; Luderer et al., 2004); however, no reproductive endpoints were measured in these studies.

A study of pregnancy outcome among wives of male workers monitored for organic solvent exposure was conducted by the Finnish Institute of Occupational Health (Taskinen et al., 1989). Styrene exposure was assessed from the concentration of mandelic acid in urine, the men's wives were identified from a population

Table 39
Summary of Human Reproductive Studies on Styrene in Men

Sample	Styrene exposure	Endpoints	Results	Reference
Wives of men occupationally exposed to styrene. Case-control study ($n = 37$ cases, 66 controls for spontaneous abortion endpoint).	Exposure assessment based on questionnaire and, in some men, biological measurements.	Spontaneous abortion (cases with spontaneous abortion were compared to controls without spontaneous abortion for husband exposure status during 80 days before pregnancy onset).	OR (95% CI). All styrene exposure 1.3 (0.8–2.1); low exposure 1.0 (0.2–3.1); intermediate exposure 0.9 (0.4–2.1); high exposure 0.7 (0.4–1.5).	Taskinen et al., 1989
Wives of men occupationally exposed to styrene recontacted 6 years after the above study ($n = 88$).	Same as the above. In a subset of men with biological monitoring of mandelic acid in urine, median mandelic acid concentration was 0.23 mM in men with low exposure to styrene and 2.49 mM in men with high exposure.	Time-to-pregnancy, assessed by questionnaire.	Fecundability density ratio (95% CI): Low/intermediate exposure 1.10 (0.73–1.66); High exposure 0.98 (0.64–1.50).	Sallmén et al., 1998
Men hired by reinforced plastics companies ($n = 23$); farmers served as external controls.	Assessed using urinary mandelic acid concentration collected daily for 5 consecutive days. Median value was 45.0 mg/g creatinine.	Sperm parameters early in employment compared to after 2–6 months of employment.	After employment compared to early in employment: Sperm concentration ↓28%; Normal sperm ↓13%; Vital sperm ↑11%; Sperm velocity ↑18%. No change in sperm chromatin structure assay.	Kolstad et al., 1999b,c
Male workers in reinforced plastics industry ($n = 602$).	Exposure estimate based on worksite data from some sites, and description of occupational practices of individual men.	Time-to-pregnancy, assessed by questionnaire.	Fertility OR (95% CI) 0.79 (0.59–1.05), adjusted for level of exposure and potential confounders.	Kolstad et al., 1999a, 2000

↑, statistically significant increase; ↓, statistically significant decrease.

registry using the personal identifying number of the men, and pregnancy outcomes were identified through pregnancy outcome registries, including a hospital discharge registry. A case-control analysis of spontaneous abortion found a weak and non-statistically significant elevation in risk. The risk of spontaneous abortion was decreased in the group with the presumed highest exposure. The study was limited by the small size and incomplete exposure and outcome determination.

Sallmén et al. (1998) evaluated time-to-pregnancy among the wives of men occupationally exposed to styrene and other occupational chemicals. Subjects were those from the previous case-control study on spontaneous abortion (Taskinen et al., 1989). There was no consistent pattern of decreased fecundability associated with styrene exposure level. The collection of time-to-pregnancy data many years later and the modest response rates limited interpretation of these findings.

Kolstad et al. (1999a, 2000) evaluated time-to-pregnancy in male workers in the reinforced plastics industry in Denmark, Italy, and the Netherlands as part of the Asclepios project, a multi-center study. Styrene exposure was evaluated semi-quantitatively by incorporating biological measurements in a subset of workers, workroom air concentrations, and questionnaire data on the frequency of lamination and the use of a respirator. There was an overall statistically non-significant decreased adjusted fertility odds ratio for all exposed men. The relatively large study size and adjustment for some potentially confounding factors are strengths, but error in the men's recall of time to pregnancy is a limitation.

Kolstad et al. (1999b,c) presented the results of the longitudinal semen quality studies from the Asclepios project. A total of 23 men hired by one of four Danish reinforced plastics companies participated in two separate semen collections. Each man served as his own control, and an external control group of farmers was included, matched by season of semen collection. Styrene exposure was assessed using post-shift urine concentrations of mandelic acid. Styrene work was associated with a decrement in sperm concentration and percent normal forms and an increase in percent vital sperm (by eosin exclusion) and curvilinear velocity. The degree of change between the two samples in styrene workers was significantly greater than the degree of change in farmers for sperm concentration. Sperm chromatin structure assay results from 14 men showed no significant changes in styrene-exposed workers between the first and second samples. There was no significant trend in semen parameters with increasing urinary mandelic acid concentration. Indices of sperm chromatin denaturability

Table 40
Summary of Experimental Animal Reproductive Studies of Styrene

Species and Strain	Treatments	Effect Levels	Reference
Rat, Wistar	Styrene 0, 200, or 400 mg/kg bw/day orally for 60 days in adult rats.	LOAEL = 400 mg/kg bw/day (alterations in testicular enzyme activities and epididymal sperm). **[Benchmark dose[a]: Lowest BMD_{10} = 228 mg/kg bw/day; $BMDL_{10}$ 101 mg/kg bw/day; $BMD_{1\ SD}$ 257 mg/kg/day; $BMDL_{1\ SD}$ 128 mg/kg bw/day.]**	Srivastava et al., 1989
Rats, COBS (SD) BR	Three-generation study of styrene in drinking water at 0, 125, or 250 ppm.	LOAEL > 250 ppm in drinking water **[estimated by CERHR at approximately 18 mg/kg bw/day for males and 23 mg/kg bw/day for females]**.	Beliles et al., 1985
Rats, Crl:CD®(SD)IGS BR	Two-generation study of styrene vapors at 0, 50, 150, or 500 ppm, 6 hr/day, 7 days/week.	LOAEL > 500 ppm in air (authors estimated that this concentration would result in peak blood levels observed following gavage exposure with ~300 mg/kg bw/day).	Cruzan et al., 2005b

[a]For an explanation of BMD, see footnote to Table 25.

showed an increasing trend with increasing urinary mandelic acid, although the results may be within the interassay variability of the method. The use of each man as his own control was a study strength, but the small study size and potential selection bias limit interpretation.

4.4.2 Summary of experimental animal reproductive toxicity data. Experimental animal reproductive studies are summarized in Table 40.

There were two thorough multigenerational studies conducted on styrene (Beliles et al., 1985; Cruzan et al., 2005b). One study used the inhalation route, and one study used drinking water exposures in rats. Even high doses had no effect on reproductive parameters. The Cruzan et al. (2005b) study evaluated not only fertility, but also parameters of sperm quality, estrous cyclicity, and ovarian follicle and corpora lutea numbers and found no effects at any dose level.

Beliles et al. (1985) performed a three-generation reproduction study of styrene in COBS (SD) BR rats, using a subset of animals from a 2-year chronic toxicity study. The animals were exposed to styrene in drinking water at 0, 125, or 250 ppm. **[Estimated styrene intake for the 125 ppm group was about 10 mg/kg bw/day for males and 12 mg/kg bw/day for females. Estimated styrene intake in the 250 ppm group was 18 mg/kg bw/day for males and 23 mg/kg bw/day for females.]** The parental generation was cohabited after 90 days on study. Selected F_1 and F_2 animals were cohabited on about PND 110. Endpoints were male and female fertility, pup survival and weight on PND 1, 7, 14, and 21, and sex ratio on PND 21. Gross physical abnormalities were assessed during the lactation period. There were no effects of treatment on maternal body weight or feed consumption in any generation. There were no treatment-related effects on F_0 or F_1 fertility or F_1 pup measures except a decrease in PND 21 survival from a control rate of 97% to 89% in the high dose group ($p < 0.05$ on a pup basis). The authors discounted this effect as being due entirely to pup deaths between PND 15 and 21 in 2 of 20 litters in this dose group. Pup survival was decreased in the 250 ppm group in F_2 offspring (born to F_1 breeders) at birth and on PND 1, 7, and 14 to 94, 97, 91, and 88%. Control survival at these time points was 99, 99, 96, and 93%. On PND 21, the 88% survival rate among 250 ppm offspring was not statistically different from the 93% survival rate among control offspring. There were no treatment related alterations in weight among F_2 pups. Fertility and pup survival was not affected by treatment for the F_2 matings. The F_3 offspring in the 250 ppm group were lighter than controls on PND 7 and 14 but not on PND 1 or 21. Testis and ovary weights were assessed in pups from all generations at weaning, and no treatment-related findings were reported. No congenital anomalies were reported. The authors concluded that the isolated findings of impaired pup survival or weight were attributable to one or two litters, and that the absence of consistent effects across generations suggested a lack of relationship to the treatment.

Cruzan et al. (2005b), conducted a two-generation reproductive toxicity study of styrene in rats. Male and female rats (25/sex/group) received whole-body inhalation exposure to styrene vapors (99.9% purity) at 0, 50, 150, or 500 ppm for 6 hr/day for a minimum of 70 consecutive days before mating and during the mating period. In females, inhalation exposures were continued through GD 20 (GD 0 = day of plug or sperm detection). On PND 1–4 (PND 0 = day parturition was completed), dams were gavage dosed with styrene in olive oil at 0, 66, 117, or 300 mg/kg bw/day, divided into three equal doses administered ~2 hours apart. Inhalation exposure of dams resumed on PND 5 and was continued until the day before kill. Pups were removed during inhalation exposure of dams. Litters were culled to five males and five female pups on PND 4. Exposure of F_1 pups/sex/group began on PND 22 and was conducted as described for the F_0 generation. On PND 28, 1 or 2 F_1 rats/sex/litter were randomly selected to make up groups comprised of 25 rats/sex. Evidence of systemic toxicity in parental rats included significantly reduced body weight gain in F_0 and F_1 males and females of the 500 ppm group and F_1 males of the 150 ppm group during the premating exposure period. In both the F_0 and F_1 generation, styrene treatment had no effect on reproductive parameters including estrous cycle length, mating, precoital interval, fertility, delivery of litters, gestation length, and implantation sites. Styrene treatment had no effect on

sperm count in the 500 ppm groups or sperm motility or morphology in any treatment groups. There was no effect on the number of primordial follicles or corpora lutea in F_1 females from the 500 ppm group. In F_1 and F_2 litters, there were no effects on litter size, pup viability at birth, sex distribution, pup postnatal survival, or pup body weight gain on PND 1–4. On PND 21, body weights of F_2 male pups in the 150 ppm group and male and female pups in the 500 ppm group were significantly lower than controls by 10–13%. The study authors identified NOAELs of 50 ppm for parental toxicity and $\geq$500 ppm for reproductive toxicity.

There are two studies (Srivastava et al., 1989; 1992c) that evaluated some aspects of testicular biochemistry after styrene exposure. These studies are limited in their scope. It is unclear what relevance the observations have to reproductive function. Given that two extensive multi-generation studies saw no effects on reproductive function, it is unlikely that the testicular biochemistry effects have functional significance.

Although the human data suggest effects of styrene on prolactin levels, two studies that evaluated prolactin levels in rats (Date et al., 2002; Jarry et al., 2002) failed to find any effect. The exposure levels used in these studies were 150–1500 ppm by inhalation for 5 days (Jarry et al., 2002) and 20 and 200 mg/kg bw/day s.c. for 7 days (Date et al., 2002). The Jarry et al. (2002) study also found no effect of styrene exposure on hypothalamic dopamine levels. This lack of effect, combined with a lack of any indication from the multi-generation or chronic studies of a perturbation in prolactin function, is inconsistent with the human data. In separate experimental animal studies, the effects of styrene exposure on hypothalamic dopamine concentrations were evaluated. Decreased tuberoinfundibular dopamine levels were observed in rabbits exposed by inhalation to 1500 ppm for 12 hr/day for 3 or 7 days (Mutti et al., 1984a). Decreased hypothalamic dopamine levels were also observed in rats treated orally with styrene 500 mg/kg bw/day for 13 weeks (Chakrabarti, 2000). Because pituitary prolactin secretion is under tonic inhibitory control by tuberoinfundibular dopamine, one might expect a decrease in tuberoinfundibular dopamine to be associated with increased prolactin secretion; however, prolactin was not measured in these studies

Expert Panel Conclusions

There is insufficient evidence in humans that styrene causes reproductive toxicity. There is suggestive evidence that exposure to styrene in occupational settings is associated with increased serum prolactin and depletion of peripheral blood dopamine metabolizing enzyme activities relative to unexposed individuals. The interpretation of the clinical relevance of these effects is uncertain because the average elevation was not outside the normal range and because menstrual function and other reproductive endpoints were not evaluated in these studies.

There is sufficient evidence in male and female rats that styrene does not cause reproductive toxicity evaluated by fertility as well as other testicular and ovarian parameters in multi-generation studies when exposure is by inhalation at 500 ppm or drinking water at 18–23 mg/kg bw/day. The data are assumed relevant to consideration of human risk.

Note: The definitions of the term sufficient and the terms assumed relevant, relevant, and not relevant are in the CERHR guidelines at http://cerhr.niehs.nih.gov/news/guidelines.html.

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.1.1 Developmental toxicity. Prenatal developmental toxicity has been assessed in rats, mice, rabbits, and hamsters by inhalation exposure or oral gavage. In these studies, there were few adverse developmental effects, which were observed only in the presence of maternal toxicity. These data are sufficient to assess the developmental toxicity potential of styrene and indicate little or no developmental toxicity. Maximum exposure levels for the most reliable studies were 600 ppm (inhalation) in rats and rabbits, and 300 mg/kg bw/day by oral gavage in rats. These doses represent developmental NOAELs. Postnatal development was assessed in two multi-generation studies in rats, one oral and one inhalation. There were effects on birth weight and growth and some delays in the achievement of developmental milestones at 500 ppm, which caused maternal toxicity. There were no other developmental effects. The developmental NOAEL from oral exposure via drinking water was 250 ppm (approximately 18–23 mg/kg bw/day). Based on a slight decrement in offspring body weight that was not considered adverse, the developmental NOAEL for inhalation was 150 ppm. Developmental neurotoxicity was assessed in the inhalation study. The NOAEL for developmental neurotoxicity was 500 ppm. These data are assumed relevant to the assessment of potential human hazard. There was insufficient information available on developmental outcomes in humans exposed to styrene to support conclusions.

5.1.2 Reproductive toxicity.

Male Effects: There were two multi-generation studies in rats, one via inhalation and one via drinking water. There were no effects on fertility, organ weights, or sperm parameters. The NOAELs from these studies were 500 ppm by inhalation and 250 ppm in drinking water (approximately 18 mg/kg bw/day). There are other studies in the literature that indicate an effect on testicular biochemistry and epididymal sperm count, but these papers are of inadequate quality to be considered for this assessment and are inconsistent with the higher quality studies.

Female Effects: There were two multi-generation studies in rats, one via inhalation and one via drinking water. There were no effects on fertility, organ weights, or estrous cyclicity. The NOAELs from these studies were 500 ppm by inhalation and 250 ppm in drinking water (approximately 23 mg/kg bw/day).

Collectively, these data demonstrate no reproductive toxicity of styrene in rats. These data are assumed relevant to the assessment of potential human hazard.

There was insufficient information available on reproductive outcomes in humans exposed to styrene to

support conclusions. There is suggestive evidence that exposure to styrene in occupational settings is associated with increased serum prolactin and depletion of peripheral blood dopamine metabolizing enzyme activities relative to unexposed individuals. The clinical relevance of these effects is uncertain because the average elevation was not outside the normal range, and because menstrual function and other reproductive endpoints were not evaluated in these studies.

5.2 Summary of Human Exposure

Humans may be exposed to styrene by ambient and indoor air, ingestion of food, cigarette smoke, consumer goods, and by occupational exposure to styrene vapors (Table 41).

Ambient and Indoor Exposures: Styrene is released to the atmosphere by industry and it is estimated that 47.7 million pounds of styrene were released to the environment in 2002. In addition to industrial releases, exhaust from gasoline-powered motor vehicles is a significant source of styrene in ambient air. Typical ambient exposures are between 0.1–2.35 μg/m^3 (median = 0.6 μg/m^3) with high-end ambient exposures up to 30 μg/m^3. Based on these estimates, styrene exposure in non-smokers was estimated at <0.25–<0.39 μg/kg bw/day (ages 12–19 years) and <0.20–<0.33 μg/kg bw/day (ages 20–70 years). In children, excluding adolescent smokers, the highest styrene exposures (<0.63–<0.79 μg/kg bw/day) were estimated for those 4 years or younger.

Dietary Intake: Styrene may be present in food as a result of natural processes or leaching from food packaging or contact materials. With the exception of raw cinnamon, naturally occurring styrene levels are low in foods such as meats, produce, and grains (≤6 ppb).

Table 41
Summary of Dose Estimates by Sources and Population Groups[a]

	Dose, μg/kg bw/day	
Source of exposure	Mean (except where indicated)	Upper boundary
Ambient and indoor exposure	0.2 (median)	9[b]
Dietary intake	<0.2	0.2
Drinking water	Negligible	NA
Cigarette Smoking[c]	3	42[d]
Occupational exposures[e]	1400–52,000	90,000[f]
General population		
Nonsmokers (ages 12–19 years)	<0.3	9[b]
Nonsmokers (ages 20–70 years)	<0.3	9
Smokers (ages 12–19 years)	3.51	42[d]
Smokers (ages 20–70 years)	2.86	42[d]
Children (< 4 years)	<0.7	9

[a]Exposure estimates are based on a 70-kg adult unless specified otherwise. Adapted from Health Canada (1993).
[b]Based on the maximum reported average ambient exposure (30 μg/m^3).
[c]Based on 20 cigarettes/day.
[d]Based on maximum concentrations reported in cigarettes (147 μg/cigarette).
[e]Range in reported mean 8-hr TWA levels of styrene in air of different worksites in polymer production and the production of glass-reinforced plastic products.
[f]Maximum individual 8-hr TWA level reported in recent reports.

Although the data do not provide information needed to calculate styrene residue estimates representative of typical diets in the U.S. or any other country, the data do provide an indication of the general range of residue values. Lickly et al. (1995) estimated the upper bound for total dietary styrene intake to be 0.2 μg/kg bw/day.

Drinking water intake: Styrene is not usually found in drinking water. When it is found in environmental water, the main source is usually industrial waste discharge from factories and coal gasification plants. Also styrene may leach into groundwater around hazardous waste sites. Soil may become contaminated with styrene by spills, landfilling with wastes, and industrial discharges. Because of its rapid biodegradation and volatility, styrene levels in surface water and groundwater are generally very low (<1 μg/L) or undetectable.

Cigarette Smoking Exposures: Styrene emissions from cigarette smoke have been estimated at 0.002–147 μg/cigarette **[assumed median/mean 10 μg/cigarette based on Health Canada estimate]**. If a 70-kg adult smokes 20 cigarettes/day, the average inhaled styrene dose would be 3 μg/kg bw/day. For smokers, Health Canada estimated styrene exposure at 3.51 μg/kg bw/day (ages 12–19 years) and 2.86 μg/kg bw/day (ages 20–70 years). Side-stream smoke has not been measured but probably also contains styrene, which will lead to styrene exposure of nonsmokers from indoor environmental tobacco smoke.

Consumer Products: Exposures from off-gassing and skin absorption from consumer products such as auto body filler, reinforced plastics patching products, and carpet glues are evident but no measurement data are available.

Occupational Exposures: The highest levels of styrene in air were measured in facilities using open processes to manufacture glass-reinforced polyester materials. In contrast, styrene monomers and polymers are manufactured using closed processes and generally result in lower styrene levels in air. The most recent data on these airborne exposure scenarios include manufacturing of glass-reinforced plastic products (arithmetic means = 13–355 mg/m^3) and in monomer and polymer production facilities (arithmetic mean = 9 mg/m^3). Inhalation doses from these reported arithmetic means will range from 1.4–52 mg/kg bw/day. The amount of exposure through the dermal route is unknown but generally believed to be minimal with the exception of exposure scenarios where there is prolonged and repeated skin contact with liquid styrene. In these situations the contribution of the dermal route could be similar to the dose achieved through inhalation exposure in the lower range of occupational inhalation exposures (1–2 ppm; 4–8 mg/m^3) **[estimated by Expert Panel]**.

5.3 Overall Conclusions

The human data are insufficient to conclude that styrene is a developmental toxicant. Available experimental data indicate little or no potential to produce developmental toxicity in laboratory animals. These data are assumed to be relevant for humans. Accordingly, dose levels were identified from experimental animal studies for use in this evaluation:

- A NOAEL of 600 ppm by inhalation and 300 mg/kg/day by oral gavage for prenatal developmental

toxicity were identified. These were the highest doses tested. A NOAEL of 150 ppm and LOAEL of 500 ppm (by inhalation) were identified for postnatal development. The 500 ppm level produced maternal toxicity.
- A NOAEL of 500 ppm by inhalation was identified for developmental neurotoxicity. This dose was the highest level tested.

The Expert Panel was not able to separate the developmental delays and growth effects of styrene from effects that may be due to maternal toxicity at the same exposure level.

The human data are insufficient to conclude that styrene is a reproductive toxicant. There is suggestive evidence that exposure to styrene in occupational settings is associated with increased serum prolactin and depletion of peripheral blood dopamine metabolizing enzyme activities relative to unexposed individuals. Although the clinical relevance of these findings is unclear, the findings warrant further investigation.

Available experimental data in rodents are sufficient to conclude that styrene is not a reproductive toxicant. These rodent data are assumed relevant for humans:

- A NOAEL of 500 ppm by inhalation for male reproductive toxicity was identified. This was the highest dose tested. A NOAEL of 250 ppm in drinking water (18 mg/kg bw/day) was identified. This dose was the highest level tested.
- A NOAEL of 500 by inhalation for female reproductive toxicity was identified. This was the highest dose tested. A NOAEL of 250 ppm in drinking water (23 mg/kg bw/day) was identified. This dose was the highest level tested.

Based on the styrene experimental animal data, the Expert Panel expressed negligible concern for reproductive or developmental toxicity in humans; however, there is insufficient epidemiologic evidence to support this conclusion. There is the outstanding question of the clinical relevance of the prolactin findings observed with occupational exposures.

5.4 Critical Data Needs

5.4.1 Experimental animal.

- There are no critical data needs for developmental toxicity. Styrene has been examined in multiple species by relevant routes, by acceptable study protocols, and no further studies are recommended.
- There are no critical data needs for reproductive toxicity. Styrene has been examined by relevant routes, by acceptable study protocols, and no further studies are recommended.

5.4.2 Human.

- Larger, appropriately powered epidemiologic studies of reproductive and developmental outcomes, targeting highly exposed groups should be conducted. These studies should include adequate measures of individual exposure levels, utilizing relevant biomarkers of exposure where possible.
- Epidemiologic studies of spontaneous abortion should include more complete ascertainment of early fetal losses.
- Studies are needed on time to pregnancy measures to assess fecundability in exposed women. Future studies of menstrual function should ideally be conducted prospectively and include a assessment of all menstrual parameters (e.g., cycle length, regularity, duration, and intensity of menses) and should incorporate endocrine measures.
- Further studies on the effects of styrene on prolactin and dopamine should include careful control of sample timing with respect to time of day, fasting, and recency of exposures. These studies should also assess other endocrine measures of ovarian function (e.g., urinary estrogen and progesterone metabolites, LH and FSH), as well as menstrual parameters, to assess whether observed perturbations of prolactin affect clinically relevant aspects of the menstrual cycle.

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