2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO STYRENE IN THE UNITED STATES

Styrene is a high production chemical; the production capacity for styrene in the United States was over 12 billion pounds in 2008. Small amounts of styrene are naturally present in foods such as legumes, beef, clams, eggs, nectarines, and spices. It can also be present in packaged foods by migration from polystyrene food containers and packaging materials. Styrene is a combustion product of cigarette smoke and automobile exhaust. Manufactured styrene is primarily used in the production of polystyrene plastics and resins used principally for insulation or in the fabrication of fiberglass boats; production of copolymers such as styrene-acrylonitrile and acrylonitrile-butadiene-styrene, which are used to manufacture piping, automotive components, and plastic drinking glasses; production of styrene-butadiene rubber used to manufacture car tires, hoses for industrial purposes, and shoes; or formulated with unsaturated polyester resins used as fiberglass reinforcement materials. Styrene copolymers are also frequently used in liquid toner for photocopiers and printers.

Median styrene concentrations in urban and rural/suburban air samples are 0.07–4.6 ppb and 0.06–0.1 ppb. The median styrene concentration in indoor air samples ranged from 0.07 to 11.5 ppb; the primary sources of styrene in indoor air are cigarette smoke and photocopiers. Styrene is rarely detected in drinking water samples and is rarely detected in soil samples.

General population exposure to styrene in air and food has been estimated to be 18–54 and 0.2–1.2 μg/person/day, respectively, with a total daily exposure of 18.2–55.2 μg/day or 0.0003–0.0008 mg/kg/day (assuming a 70-kg reference body weight).

2.2 SUMMARY OF HEALTH EFFECTS

Styrene-induced neurotoxicity has been reported in workers since the 1970s. Studies over the last 15 years have firmly established the central nervous system as the critical target of toxicity. Both short- and long-term exposures to styrene can result in neurological effects. Acute exposure data are limited to the finding of impaired performance on tests of vestibular function in test subjects exposed to 87–376 ppm for 1–3 hours and studies finding no alterations in performance of neurobehavioral tests (reaction time, color discrimination, and tests of memory or attention) in subjects exposed to 20 or 49 ppm. A variety of neurological effects have been observed in chronically exposed styrene workers;

these effects include decreased color discrimination, vestibular effects, hearing impairment, symptoms of neurotoxicity, particularly "feeling drunk" and tiredness, delays in reaction time, impaired performance on tests measuring attention and memory, increased vibration perception thresholds, impaired nerve conduction velocity, and EEG alterations. The LOAELs for these effects range from about 10 ppm to 93 ppm. In most of the occupational exposure studies, neurological function tests were conducted in the morning before work, suggesting that the deficits were not acute effects. Results of a meta-analysis suggest that the severity of the some of the neurological symptoms increases with exposure duration. For example, 8, 15, 25, and 35% increases in reaction time were observed in workers exposed to 100 ppm for 2, 4, 6, and 8 work-years, respectively. However, this may also be reflective of higher exposure levels in the past rather than a duration-related increase in severity. The existing data are inadequate to determine whether chronic styrene exposure results in permanent damage. Mixed results have been found in studies examining workers before and after an extended period without styrene exposure. Neurotoxicity studies in animals have primarily focused on effects on hearing and damage to the organ of Corti.

Other effects that have been observed in animal studies include damage to the nasal olfactory epithelium and liver necrosis; testicular damage and developmental effects have also been reported, but the weight of evidence does not support concluding that these are sensitive targets. Damage to the nasal olfactory epithelium was observed in mice after 3 days of exposure. The severity of the lesion progressed from single cell necrosis to atrophy and respiratory metaplasia with increasing exposure duration. The lowest-observed-adverse-effect levels (LOAELs) for these lesions are 80, 50, and 20 ppm for acute, intermediate, and chronic exposure, respectively. Rats do not appear to be as sensitive as mice to the nasal olfactory epithelial damage; an intermediate-duration study identified a no-observed-adverse-effect level (NOAEL) and LOAEL of 500 and 1,000 ppm for focal hyperplasia and a chronic study identified a LOAEL of 50 ppm for atrophy and degeneration. The observed species differences may be due to differences in styrene metabolism in the nasal cavity. In particular, rats have a higher capacity to detoxify styrene oxide with epoxide hydrolases and glutathione S-transferase. Humans are not likely sensitive to the nasal toxicity of styrene because styrene oxide has not been detected and high levels of epoxide hydrolases have been found in *in vitro* assays of human nasal tissue.

Unlike the nasal lesions, the severity of hepatic lesions decreases with increased exposure durations. Severe hepatocellular necrosis was observed in mice exposed to 250 ppm for 3 days; however, continued exposure at this concentration resulted in focal necrosis and an increase in pigmented macrophages. Centrilobular aggregates of siderophages were observed in mice exposed to 200 ppm for 13 weeks; no liver effects were observed at 160 ppm after 2 years of exposure. Rats are less sensitive than mice to liver toxicity; no liver effects were observed in an intermediate-duration study in which rats were exposed to a styrene concentration 10-fold higher than the concentration eliciting hepatic effects in mice. No alterations in serum markers of liver damage were observed in styrene workers exposed to 40 ppm for approximately 5 years. Liver effects have not been observed in rats orally exposed to 35 mg/kg/day for 105 weeks. Some hepatic alterations (increases in liver weight and small areas of focal necrosis) have been reported in rats exposed to 400 mg/kg for an intermediate duration; however, the studies are poorly reported and lack statistical comparisons with controls. No studies examined systemic end points following acute exposure.

Occupational exposure studies have not found significant increases in the occurrence of stillbirth, infant death, malformations, or low birth weight. An increase in fetal deaths were observed in hamsters exposed to very high concentrations (1,000 ppm on gestation days 6–18) and in rats exposed to 300 ppm on gestation days 6–20. However, most single and multigeneration inhalation and oral exposure animal studies did not find significant alterations in fetus/pup survival, growth, or incidence of abnormalities in rats, mice, rabbits, and hamsters exposed to styrene. Two studies have examined neurodevelopmental effects in rats; one study found some minor effects (slight delays in some developmental landmarks). The other, higher-quality study did not find any significant alterations in a number of neurodevelopmental end points. The National Toxicology Program (NTP) Expert Panel examining the developmental potential of styrene concluded that the human data are not sufficient to evaluate the potential developmental toxicity of styrene in humans and that there was no convincing evidence of developmental toxicity in animals.

Although several epidemiology studies have examined potential reproductive effects in male and female styrene workers, adequate analysis of the data is limited by the lack of exposure information and concomitant exposure to other compounds. Mixed results have been found for increased occurrence of spontaneous abortions and oligomenorrhea. In male workers, sperm abnormalities have been reported (Kolstad et al. 1999a), but not alterations in time-to-pregnancy or fertility rates. No adverse reproductive effects were observed in inhalation and oral multigeneration studies in rats. A series of studies found decreases in spermatozoa counts in rats exposed as adults, as neonates, and through lactation. However, as noted by the NTP Expert Panel, this finding is not consistent with the lack of reproductive effects found in the inhalation two-generation study. The NOAEL identified in the two-generation inhalation study was 500 ppm (6 hours/day), which is roughly equivalent to 230 mg/day using a reference inhalation rate of 0.42 m^3/day. The LOAEL for spermatozoa effects in adult rats was 400 mg/kg (6 days/week), which is roughly equivalent to 158 mg/day using a reference body weight of 0.462 kg.

There are several epidemiologic studies of workers at styrene manufacturing and polymerization facilities and reinforced plastics facilities that suggest an association between occupational exposure and an increased incidence of cancer of the lymphatic and hematopoietic tissues in styrene. However, the reported studies are inconclusive due to exposure to multiple chemicals (including benzene) and the small size of the cohorts. Other studies have reported negative results. More consistent results for increases in the risk of lymphatic and hematopoietic cancers have been observed among workers at styrene-butadiene manufacturing facilities. There is suggestive evidence that these increased risks may be due to exposure to 1,3-butadiene rather styrene exposure; however, it is difficult to separate the risks for styrene and 1,3-butadiene because the exposure is highly correlated. There are no reports of cancer resulting from styrene exposure by the oral or dermal routes in humans. Species differences in styrene carcinogenicity have been detected in animal studies. Inhalation and oral exposure studies in rats have not found significant increases in neoplastic lesions. However, increases in lung tumors have been found in mice following inhalation and oral exposure. The increased production of styrene 7,8-oxide in lung Clara cells and the higher ratio of styrene oxide R- to S-enantiomers likely resulted in the increased sensitivity of mice. Overall, human and animal studies suggest that styrene may be a weak human carcinogen. The International Agency for Research on Cancer (IARC) has assigned styrene to Group 2B, possibly carcinogenic to humans. EPA and DHHS have not evaluated the carcinogenic potential of styrene. One study lists a cancer classification of A4, not classifiable as a human carcinogen based on a 1996 evaluation of the available data.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for styrene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

Acute-Duration Inhalation MRL

An MRL of 5 ppm has been derived for acute-duration inhalation exposure (14 days or less) to styrene.

The acute-duration inhalation toxicity database for styrene consists of several human experimental studies primarily examining neurotoxicity (Ödkvist et al. 1982; Seeber et al. 2004; Ska et al. 2003; Stewart et al. 1968), systemic toxicity studies in mice (Cruzan et al. 1997, 2001; Morgan et al. 1993a, 1993b, 1993c), neurotoxicity studies in rats (Campo et al. 2001; Crofton et al. 1994; Lataye et al. 2003), mice (Cruzan et al. 1997; DeCeaurriz et al. 1983), and guinea pigs (Lataye et al. 2003), a reproductive toxicity study in mice (Salomaa et al. 1985), and developmental toxicity studies in rats (Murray et al. 1978), mice (Kankaanpää et al. 1980), hamsters (Kankaanpää et al. 1980), and rabbits (Murray et al. 1978). Eye irritation was reported in humans exposed to 99 ppm for 7 hours or 376 ppm for 1 hour (Stewart et al. 1968); nasal irritation was also reported at 376 ppm. A significant inhibition of the vestibular-oculomotor system was observed in subjects exposed to 87 ppm for 1 hour (Ödkvist et al. 1982). Studies by (Stewart et al. 1968) found alterations in tests of balance or coordination in subjects exposed to 376 ppm for 1 hour, but not after exposure to 99 ppm for 7 hours or 216 ppm for 1 hour; the test used in the Stewart et al. (1968) studies is probably less sensitive than those used by Ödkvist et al. (1982). No significant alterations in performance on tests of reaction time were observed in subjects exposed to 20 ppm for 3 hours (Seeber et al. 2004) or 49 ppm for 6 hours with or without four 15-minute peak exposures to 98 ppm) (Ska et al. 2003). Additionally, no significant alterations in color discrimination, olfactory threshold, or performance on neurobehavioral tests of memory or attention were observed in subjects exposed to 49 ppm for 6 hours with or without four 15-minute peak exposures to 98 ppm (Ska et al. 2003).

In mice, the most sensitive target of styrene toxicity appears to be the nasal olfactory epithelium; single cell necrosis was observed following exposure to 80 ppm 6 hours/day for 3 days (Cruzan et al. 2001). At 250 ppm, hepatocellular necrosis and degeneration have been observed (Cruzan et al. 1997; Morgan et al. 1993a, 1993b, 1993c). The severity of this lesion appears to be inversely related to the duration of exposure, with more severe damage observed in mice killed within 3 days of exposure (Morgan et al.

1993a, 1993b, 1993c) compared to animals killed after 2 weeks of exposure (Cruzan et al. 1997; Morgan et al. 1993a). Exposure to 250 ppm 6 hours/day, 5 days/week for 2 weeks also resulted in lethargy and unsteady gait in mice (Cruzan et al. 1997). Impaired performance on a swimming test was observed in mice exposed to 610 ppm for 4 hours, but not in animals exposed to 413 ppm (DeCeaurriz et al. 1983). Exposure of rats to high concentrations (1,000 or 1,600 ppm) 6–8 hours/day for 5–14 days resulted in auditory threshold shifts (indicative of hearing loss) and loss of outer hair cells (OHC) in the organ of Corti (Campo et al. 2001; Crofton et al. 1994; Lataye et al. 2003). No alterations in sperm morphology were observed in mice exposed to 300 ppm styrene 5 hours/day for 5 days (Salomaa et al. 1985) and no developmental effects were observed in rats or rabbits exposed to 600 ppm 7 hours/day on gestational days 6–15 or 6–18, respectively, (Murray et al. 1978) or mice exposed to 250 ppm 6 hours/day on gestational days 6–16 (Kankaanpää et al. 1980). An increase in fetal deaths or resorptions was observed in hamsters exposed to 1,000 ppm 6 hours/day on gestational days 6–18 (Kankaanpää et al. 1980).

These data suggest that the nervous system is the most sensitive target of styrene toxicity in humans following acute-duration inhalation exposure. The lowest LOAEL for a relevant end point in humans is 87 ppm for vestibular impairment in subjects exposed to styrene for 1 hour (Ödkvist et al. 1982). A similar LOAEL (80 ppm) was identified for nasal effects in mice exposed to styrene for 3 days (Cruzan et al. 2001). Although nasal irritation has been observed in humans exposed to 376 ppm for 1 hour (Stewart et al. 1968) and focal hyperplasia in the nasal olfactory epithelium was observed in rats exposed to 1,000 ppm (NOAEL of 500 ppm) for 13 weeks (Cruzan et al. 1997), mice appears to be unusually susceptible to this effect. As discussed in Section 2.2, mice appear to have a greater capacity than humans to generate the reactive metabolite, styrene oxide, in the nasal cavity and a lower capacity to detoxify styrene oxide (Green et al. 2001a). Thus, nasal lesions in mice were not considered suitable as the basis of an MRL. The identification of the nervous system as the critical target of toxicity for styrene is supported by a large number of occupational exposure studies. Delays in reaction time have been observed in workers exposed to 21.9–92 ppm (Cherry et al. 1980; Fallas et al. 1992; Gamberale et al. 1976; Jegaden et al. 1993; Mutti et al. 1984a; Tsai and Chen 1996) and vestibular effects have been observed at 18–36 ppm (Calabrese et al. 1996; Möller et al. 1990; Toppila et al. 2006).

The Ödkvist et al. (1982) study did not identify a NOAEL for vestibular effects; however, a NOAEL of 49 ppm for performance on several tests of reaction time, memory, attention, color discrimination, and olfactory threshold was identified by Ska et al. (2003) in subjects exposed to styrene for 6 hours. Although there is some uncertainty whether deriving an MRL based on a 6-hour exposure study would be protective of continuous exposure to styrene for 2 weeks, the Ska et al. (2003) study was selected as the basis of an acute duration inhalation MRL for styrene.

In this study (Ska et al. 2003), groups of 24 healthy men (aged 20–50 years) were exposed to 1 ppm (control exposure), 24 ppm, and 24 ppm with four 15-minute exposures to peak concentrations of 49 ppm, 49 ppm, or 49 ppm with four 15-minute exposures to peak concentrations of 98 ppm for 6 hours. The subjects were exposed to each concentration with a 2-week period between each session. The subjects did not have a history of styrene exposure. At the end of the exposure session the subjects were tested for color discrimination (using the Lanthony D-15 desaturated panel), vision contrast, olfactory threshold, simple reaction time, color word stress (response time), symbol digit matching, digit span memory, and continuous tracking. The subjects were also given a questionnaire to assess mood and symptoms. No significant styrene-related alterations in performance on color discrimination, olfactory threshold, neurobehavioral tests, mood, or subjective symptoms were found.

The NOAEL of 49 ppm was selected as the point of departure for the MRL; it was not adjusted for intermittent exposure because the study involved a single exposure for 6 hours. The NOAEL of 49 ppm from the Ska et al. (2003) study was divided by an uncertainty factor of 10 to account for human variability resulting in an acute-duration inhalation MRL of 5 ppm.

Intermediate-Duration Inhalation MRL

No human intermediate-duration studies were identified. Animal studies examining systemic, neurological, reproductive, and developmental toxicity have identified the respiratory tract as the most sensitive target of toxicity. Atrophy of the olfactory epithelium, hypertrophy/hyperplasia of Bowman's gland has been observed in mice exposed to 50 ppm 6 hours/day, 5 days/week for 13 weeks (Cruzan et al. 1997), decreased nasal cilia activity has been observed in rats exposed to 150 ppm 4 hours/day, 5 days/week for 21 days (Ohashi et al. 1986), and focal hyperplasia has been observed in rats exposed to 1,000 ppm 6 hours/day, 5 days/week for 13 weeks (Cruzan et al. 1997). As discussed previously, the mouse does not appear to be a good model for nasal effects in humans due to metabolic differences. Other systemic effects that have been observed include eye irritation in rats exposed to 200 ppm 6 hours/day, 5 days/week for 13 weeks (Cruzan et al. 1997) and centrilobular aggregates of siderophages in the livers of mice exposed to 200 ppm 6 hours/day, 5 days/week for 13 weeks (Cruzan et al. 1997).

A number of studies in rats have reported outer hair cell loss in the organ of Corti in rats exposed to 600–650 ppm for 4 weeks (Loquet et al. 2000; Makitie et al. 2002; Pouyatos et al. 2002) and hearing loss at

750–1,000 ppm for 3–4 weeks (Campo et al. 2001; Lataye et al. 2000, 2001; Loquet et al. 1999, 2000; Pouyatos et al. 2002). A NOAEL of hearing effects of 300 ppm was identified in rats exposed for 12 hours/day, 5 days/week for 4 weeks (Makitie et al. 2002). Other neurological effects include alterations in astroglial cells in rats continuously exposed to 320 ppm for 3 months (Rosengren and Haglid 1989) and decreased sensory nerve conduction velocity in rats exposed to 2,000 ppm 8 hours/day, 5 days/week for 32 weeks (Yamamoto et al. 1997). No reproductive, developmental, or neurodevelopmental effects were observed in a two-generation study (Cruzan et al. 2005a, 2005b); the NOAEL was 500 ppm. In contrast, an increase in neonatal deaths, developmental landmark delays, and alterations in neurochemical levels were observed in the offspring of rats exposed to 300 ppm 6 hours/day on gestational days 6–20 (Katakura et al. 1999, 2001).

Chronic-duration studies suggest that the most sensitive target of styrene toxicity is the nervous system. It is likely that this would also be the most sensitive effect following intermediate-duration exposure. In the absence of human neurotoxicity data, an intermediate-duration inhalation MRL is not recommended at this time.

Chronic-Duration Inhalation MRL

An MRL of 0.2 ppm has been derived for chronic-duration inhalation exposure (greater than 365 days) to styrene.

A large number of occupational exposure studies have examined the toxicity of styrene; however, most of these studies have focused on the potential neurotoxicity of styrene, which appears to be the most sensitive effect. Two common limitations of the occupational exposure studies are: (1) the range of current styrene levels for the workers is typically large and it is difficult to ascribe the observed effects to the mean or median exposure level and (2) historical exposure to higher styrene levels are not adequately taken into consideration. The use of urinary levels of mandelic acid, phenylglyoxylic acid, or mandelic acid plus phenylglyoxylic acid levels as biomarkers for styrene exposure eliminates another common limitation of styrene occupational exposure studies, which is poor characterization of styrene exposure levels due to the lack of personal air samples and the workers' use of respirators with or without canisters.

A variety of neurological effects have been reported in workers at reinforced plastic manufacturing facilities, including decreased color discrimination, slowed reaction time, impaired performance on other neurobehavioral tests, permanent hearing threshold shifts, vestibular effects, altered nerve conduction velocity, and increases in subjective symptoms. A summary of the results of studies for some of these neurological effects is presented in Table 2-1. An alteration in color discrimination is one of the more consistently found neurological effects; it may also be one of the more sensitive effects. Color discrimination was typically measured using the Lanthony desaturated panel D-15 test in which the subjects were asked to arrange 15 painted caps in a line with definite chromatic sequence; the total color distance score (TCDS) and color confusion index (CCI) are used to quantitatively analyze the results. LOAEL values of 6 to 93 ppm have been identified; however, these LOAELs often reflect the mean exposure level or the lower end of the range of exposure levels. Other neurological effects that have been frequently found include alterations in performance on neurobehavioral tests, particularly reaction time, in workers exposed to ≥21 ppm; vestibular alterations at ≥18 ppm; and increased frequency of clinical symptoms (e.g., tiredness and headaches in workers exposed to ≥6 ppm). Hearing loss and alterations in nerve conduction velocity have also been reported in some studies, but the finding is not consistent across studies.

Non-neurological effects observed in styrene workers include obstructive lung effects (Chmielewski and Renke 1975), mild hematological alterations (Checkoway and Williams 1982; Stengel et al. 1990; Thiess and Friedheim 1978), and impaired immune response to concanavalin (Somorowská et al. 1999; Tulinska et al. 2000). Although exposure levels were not reported in all of these studies, effects were typically observed at styrene concentrations of >20 ppm. Clinical chemistry studies did not find alterations indicative of impaired liver (Härkönen et al. 1984; Hotz et al. 1980; Lorimer et al. 1978; Thiess and Friedheim 1978) or kidney (Verplanke and Herber 1998; Viau et al. 1987; Vyskocil et al. 1989) function in workers exposed to ≥24 ppm.

Chronic-duration studies in laboratory animals identify the nasal olfactory epithelium as the most sensitive end point. Atrophic and/or degenerative changes were observed in rats exposed to 50 ppm styrene 6 hours/day, 5 days/week for 104 weeks (Cruzan et al. 1998) and respiratory metaplasia in the nasal olfactory epithelium were observed in mice exposed to 20 ppm 6 hours/day, 5 days/week for 98–104 weeks (Cruzan et al. 2001). As noted previously, mice do not appear to be a good model for potential respiratory effects in humans.

Alterations in color discrimination and reaction time are two neurological effects consistently found in styrene workers. Benignus et al. (2005) conducted a meta-analysis using color discrimination impairment data from the Campagna et al. (1996), Eguchi et al. (1995), Gobba et al. (1991), Gong et al. (2002), and Kishi et al. (2001) studies and choice reaction time data from the Jegaden et al. (1993), Mutti et al. (1984a), Triebig et al. (1989), and Tsai and Chen (1996) studies. Average styrene exposure

Table 2-1. Results of Selected Human Neurotoxicity Studies

Result	Reference	NOAEL ppm	LOAEL ppm
Decreased color discrimination	Chia et al. 1994		6
	Kishi et al. 2001	4	10
	Gong et al. 2002		10
	Gobba et al. 1991		16
	Triebig et al. 2001		20
	Iregren et al. 2005		22
	Fallas et al. 1992		24.3
	Campagna et al. 1996		26
	Eguchi et al. 1995	8	93
Neurological symptoms	Flodin et al. 1989	6	
	Edling et al. 1993	8.6	
	Checkoway et al. 1992	10.8	18.9
	Cherry et al. 1980		92
Vestibular effects	Möller et al. 1990		18
	Toppila et al. 2006		24.8
	Calabrese et al. 1996		36
Reaction time	Edling et al. 1993	8.6	
	Tsai and Chen 1996		21.9
	Jegaden et al. 1993		22.68
	Fallas et al. 1992		24.3
	Mutti et al. 1984a		25
	Gamberale et al. 1976		47
	Cherry et al. 1980		92
Hearing	Morata et al. 2002		3.68
	Śliwińska-Kowalska et al. 2003		15.6
	Morioka et al. 1999		16
	Möller et al. 1990	18	
	Calabrese et al. 1996	36	
	Triebig et al. 2009	40	50
Nerve conduction velocity	Seppäläinen and Härkönen 1976	30	
	Štětkářová et al. 1993		50
	Triebig et al. 1985	100	

concentrations were estimated from individual data reported in the papers; for studies reporting individual data as urinary mandelic acid levels, standardized methods for converting to styrene exposure levels were used. Cumulative styrene exposure was estimated by multiplying exposure level by length of employment. A common metric of effect magnitude (percentage of baseline) was calculated for the different neurological effects. The analysis found a significant linear relationship between choice reaction time and cumulative styrene exposure; cumulative exposure accounted for 91% of the variance in reaction time. Similarly, a significant relationship between CCI and cumulative styrene exposure was found, with cumulative exposure accounting for 35% of the variance in CCI. Using the regression equations for these two effects, Benignus et al. (2005) estimated that exposure to 150 ppm for 8 work-years would result in a 50% increase in choice reaction time and a 17% increase in CCI score; exposure to 20 ppm for 8 work-years would result in a 6.5% increase in choice reaction time and a 2.23% increase in CCI score. As discussed in Benignus et al. (2005), a 7% decrease in reaction time would prevent 58,000–70,000 injuries per year from automobile accidents. The investigators also noted that CCI increases with age, and the rate of increase is about 10% per 13 years of age; thus, a 2.23% decrease in color perception would be roughly equivalent to 2.9 additional years of age. Based on this analysis, 20 ppm is considered a LOAEL for neurological effects.

In addition to the studies included in the Benignus et al. (2005) meta-analysis, a LOAEL of 20 ppm is supported by a color discrimination study conducted by Triebig et al. (2001). In this study, significant increases in CCI values were observed in styrene workers with urinary mandelic acid plus phenylglyoxylic acid levels of ≥472 mg/g creatinine (approximately 20 ppm air styrene concentration), when compared to >95th percentile age-dependent reference CCI values. An advantage of the Triebig et al. (2001) study is that individual exposure and CCI data were reported, which diminishes the problem of ascribing an observed effect to the mean or median concentration and the study addresses the issue of biological relevance because the CCI scores were compared to the 95th percentile of age-dependent reference values rather than values in the control group.

In comparisons between styrene workers and a control group employed at the same facility without styrene exposure, Triebig et al. (2001) found no significant differences in CCI scores between workers and controls when the tests were conducted on a Monday morning, but CCI scores were significantly different when measured on a Thursday afternoon. Within the styrene-exposed workers, CCI scores on Monday morning and Thursday afternoon were not significantly different. After a 4-week nonexposure period, the CCI scores were significantly reduced in the styrene workers. After styrene exposure levels were lowered, no difference between workers and controls was observed on Monday morning or

Thursday afternoon. However, among styrene workers, there were significant differences between Monday morning and Thursday afternoon measurements and between Monday morning and post-vacation levels. These findings provide suggestive evidence that the alterations in color discrimination were reversible.

The LOAEL of 20 ppm identified in the Benignus et al. (2005) meta-analysis was selected as the point of departure for the chronic-duration inhalation MRL. The LOAEL was adjusted for intermittent exposure (8 hours/day, 5 days/week) and divided by an uncertainty factor of 30 (3 for use of a minimal LOAEL and 10 for human variability), resulting in a chronic-duration inhalation MRL of 0.2 ppm. The LOAEL was classified as a minimal LOAEL based on the findings of Triebig et al. (2001) that alterations in color vision were reversible and the workers were not aware of any changes in color vision.

Oral MRLs

Acute-Duration Oral MRL

> An MRL of 0.1 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to styrene.

A limited number of studies have examined the acute toxicity of orally administered styrene; these studies have examined potential neurotoxicity and developmental toxicity. No developmental effects were observed in rats administered a single dose of 300 mg/kg on gestational day 11 (Daston et al. 1991) or administered 300 mg/kg/day (administered as two daily doses of 150 mg/kg) on gestational days 6–15 (Murray et al. 1978). Impaired learning was observed in rats administered via gavage 100 or 200 mg/kg/day for 14 days; increases in serotonin levels were observed in the hypothalamus, hippocampus, and midbrain (Husain et al. 1985). In another study, increases in dopamine receptor binding was observed in rats administered a single gavage dose of 200 mg/kg (Agrawal et al. 1982).

Although a limited number of toxicity end points have been examined following acute-duration oral exposure, longer-term oral studies examining systemic and reproductive end points have identified LOAELs that were higher than the 100 mg/kg/day LOAEL identified for neurotoxicity in the Husain et al. (1985) study. The lowest LOAEL identified for a systemic effect is 400 mg/kg/day for Heinz body formation in dogs administered styrene by gavage for 561 days (Quast et al. 1979); the NOAEL was 200 mg/kg/day. Decreased spermatozoa counts were observed in adult rats administered 400 mg/kg 6 days/week for 60 days (Srivastava et al. 1989), young rats exposed via lactation on postnatal days 1–

21 (maternal dose of 400 mg/kg/day) (Srivastava et al. 1992a), and young rats administered 200 mg/kg 6 days/week on postnatal days 1–61 (Srivastava et al. 1992b); the NOAELs identified in these three studies were 200, 200, and 100 mg/kg, respectively. Marked degeneration of the seminiferous tubules was also observed in the adult rats administered 400 mg/kg (Srivastava et al. 1989). Impaired learning observed in rats administered 500 mg/kg 5 days/week for 8 weeks (no NOAEL identified) (Bushnell 1994) also supports the identification of neurotoxicity as a sensitive end point following oral exposure. Additionally, the extensive inhalation toxicity database for styrene supports the selection of neurotoxicity as the most sensitive target of toxicity; both the acute- and chronic-duration inhalation MRLs are based on neurological effects in humans. Neurological effects observed in chronically exposed styrene workers include decreased color discrimination, slowed reaction time, increased prevalence of neurological symptoms, and ototoxicity (hearing and vestibular effects).

The Husain et al. (1985) study was selected as the basis of the acute-duration oral MRL. In this study, groups of 15 male Wistar rats were administered by gavage 0, 100, or 200 mg/kg/day styrene in ground nut oil for 14 consecutive days. Spontaneous motor activity with or without amphetamine induction was observed 1 day after the last dose. Two days after exposure termination, the rats underwent acquisition training for 4 days. Learning was assessed by measuring the number of times the rat climbed the pole after the conditioned stimulus to avoid the foot-shock unconditioned stimulus. Noradrenaline, dopamine, and serotonin levels were measured in seven regions of the brain in six rats/group sacrificed after the acquisition training. No overt signs of toxicity were observed. No significant alterations in locomotor activity were observed with or without amphetamine induction. Significantly greater increases in percent avoidance response in the conditioned avoidance response test (indicative of impaired learning) were observed at 100 and 200 mg/kg/day; no difference was found between the two styrene groups. The effects were observed on test day 3 and 4. Significant increases in the level of serotonin in the hypothalamus (70%), hippocampus (51%), and midbrain (29%) were observed at 200 mg/kg/day. Styrene exposure did not affect brain noradrenaline and dopamine levels.

The LOAEL of 100 mg/kg/day was divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Intermediate-Duration Oral MRL

The systemic toxicity of styrene has not been investigated in intermediate-duration oral exposure studies. Neurotoxicity studies have identified a LOAEL of 200 mg/kg/day for increased dopamine receptor binding in rats (Agrawal et al. 1982), a LOAEL of 500 mg/kg (5 days/week) for impaired learning in rats (Bushnell 1994), and a LOAEL of 906 mg/kg/day for alterations in serotonin and noradrenaline levels in rats (Husain et al. 1980); none of these studies identified a NOAEL for neurological effects. An increase in dopamine receptor binding was also observed in the offspring of rats administered 200 mg/kg/day during gestation, lactation, or both (Zaidi et al. 1985). Reproductive and immunological effects were reported in the other intermediate-duration oral studies. Decreases in spermatozoa counts were observed in rats exposed as 400 mg/kg (6 days/week) as adults, 200 mg/kg (6 days/week) as neonates, or during lactation (maternal dose of 400 mg/kg/day) (Srivastava et al. 1989, 1992a, 1992b). Impaired immune function was observed in mice exposed to 30 mg/kg/day and in rats exposed to 294 mg/kg/day (Dogra et al. 1992); the NOAELs were 23 and 196 mg/kg/day.

Dogra et al. (1992) identified the lowest LOAEL following intermediate-duration oral exposure to styrene; however, there are limited data to support the identification of the immune system as a sensitive, relevant target for humans. Although, the sensitivity of the nervous system has been firmly established following inhalation and oral exposure, the LOAELs identified in the intermediate-duration studies are higher than the lowest LOAEL for neurotoxicity identified in an acute-duration study (Husain et al. 1985). An MRL based on the Dogra et al. (1992) study would be higher than the acute-duration oral MRL; thus, an intermediate-duration MRL is not recommended at this time.

Chronic-Duration Oral MRL

The available data on the chronic toxicity of styrene comes from three systemic toxicity studies. No adverse effects were observed in rats exposed to 35 mg/kg/day styrene in drinking water for 2 years (Beliles et al. 1985) and no liver or kidney alterations were observed in rats administered 500 mg/kg 1 day/week for 120 weeks (Ponomarkov and Tomatis 1978). Increase in Heinz body formation was observed in dogs administered 400 mg/kg/day for 561 days (Quast et al. 1979); the NOAEL for this effect is 200 mg/kg/day.

The chronic-duration inhalation database provides strong evidence that neurotoxicity is the most sensitive target of styrene toxicity. It is not known if this would also be true for chronic-duration oral exposure; the acute-toxicity oral database provides suggestive evidence that it would be a sensitive target. In the absence of a long-term oral study examining neurological end points, a chronic-duration oral MRL is not recommended.