SIRC

The Styrene Information & Research Center

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January 21, 2005

Dr. Michael Shelby, Director
CERHR
P.O. Box 12233
National Institute of Environmental Health Sciences
MD EC-32
Research Triangle Park, NC 27709

RE: References for CERHR Review of Styrene Reproductive and Developmental Toxicity Data

Dear Dr. Shelby:

The *Federal Register* of December 8, 2004, contained a notice that the National Toxicology Program's (NTP's) Center for the Evaluation of Risks to Human Reproduction (CERHR) will be convening an expert panel to evaluate the scientific evidence regarding the potential reproductive and developmental toxicity associated with exposure to styrene. 69 Fed. Reg. 71067 (December 8, 2004). Styrene was proposed for evaluation based on "public concern about styrene exposure" and "recently available exposure studies."

The *Federal Register* notice also solicited nominations of scientists qualified to serve on an expert panel.

In this cover letter, the Styrene Information and Research Center (SIRC)[1] is pleased to offer a summary of the key studies that address the reproductive and developmental toxicity of styrene. In particular, enclosed are copies of two manuscripts that have been accepted for publication by *Developmental and Reproductive Toxicology* (Cruzan et al., submitted 2004 a & b) that report on recently completed, definitive two-generation reproductive and developmental neurotoxicity inhalation studies in rats. Also included in this submittal is an electronic version of the full reports on these studies, which were conducted by WIL Research Laboratories. We feel that these data should contribute significantly to the panel's assessment.

Under separate cover, SIRC also has submitted a list of nominees to the expert panel for CERHR's consideration.

[1] The Styrene Information and Research Center's (SIRC's) mission is to evaluate existing data on potential health effects of styrene, and develop additional data where it is needed. SIRC has gained recognition as a reliable source of information on styrene and for helping to ensure that regulatory decisions are based on sound science. For more information, visit http://www.styrene.org.

Data Available

Several reviews of styrene effects on reproduction and development have been published recently (Brown et al., 2000; Cohen et al., 2002; IARC, 2002). These reviews have concluded that there is little evidence that styrene causes any specific developmental or reproductive effects. The chief limitation was the lack of a two-generation reproduction study at relatively high exposure concentrations. Beliles et al. (1985) reported no reproductive effects from exposure to styrene in drinking water for three generations at 125 and 250 ppm (maximum 21 mg/kg/day). The styrene concentration (and dose) was limited by styrene's low solubility in water.

SIRC recently completed a two-generation reproduction study by inhalation (exposures at 50, 150, and 500 ppm). During lactation days 1-4 (when inhalation exposures would result in absence of the dam and resulting excessive stress on offspring), styrene was administered by gavage using a physiologically based pharmacokinetic (PBPK) model to estimate oral doses equivalent to the inhalation exposure. A selected portion of the pups from the F2 generation were evaluated for development of the neurological system. Manuscripts of these studies have been accepted for publication (Cruzan et al. submitted 2004a,b). The full report is also included with this submittal (CD WIL Laboratories Study 419002).

Exposures to styrene have been summarized recently (Cohen et al., 2002; IARC, 2002).

Data Summary

The reproductive and developmental effects of styrene have been extensively reviewed by Brown et al. (2000).

Reproduction

Reports of styrene-related effects on human reproduction are limited and conflicting. A large study of US women concluded that styrene exposure did not affect menstrual cycle (Lemasters et al., 1985); however, Cho et al. (2001) concluded that exposure to styrene increased the risk of menstrual cycles longer than 35 days. One study (Jelnes, 1998) suggested increased sperm abnormalities in workers exposed to high levels of styrene in the reinforced plastics industry. A later study of 23 workers (Kolstad et al., 1999) found no effect on sperm abnormalities, but reported a decrease in sperm density during the first 6 months of exposure to styrene in the reinforced plastics industry. In a study of 220 male reinforced plastics workers exposed to high levels of styrene, there was no relationship between exposure and time to pregnancy of their partners (Kolstad et al., 2000). A study of female reinforced plastics workers reported a possible decrease (4%) in birth weight of offspring of mothers exposed to styrene above 80 ppm and other solvents (Lemasters, 1989). Birth weights were taken from mothers' memory, not birth records, and the difference was not statistically significant.

No effects on ovarian or testicular pathology have been reported in several of the subchronic or chronic toxicity studies in rats [500 to 2000 mg/kg/day gavage, 50-1500 ppm inhalation] and mice [150-300 mg/kg/day gavage, 20 to 200 ppm inhalation] (NCI, 1979; Cruzan et al., 1997, 1998, 2001; Roycroft et al., 1995). On the other hand, testicular pathology and decreases in sperm count were reported in rats treated with 400 mg/kg/day styrene by gavage for 60 days (Srivastava et al., 1989). Decreased free testosterone in plasma was reported in pre-pubertal male C57BL/6 mice exposed to 50 mg/l styrene in drinking water for 4 weeks (12 mg/kg/day).

There were no effects on body weight, testis weight, plasma cortisone, or plasma luteinizing hormone (Takao et al., 2000).

No effects on fertility or reproduction were found in three generations of male and female Sprague-Dawley rats exposed to 125 or 250 ppm styrene in their drinking water (Beliles et al., 1985). The concentration was limited by the solubility of styrene in water (approximately 300 ppm). Water consumption was significantly reduced in both groups compared to controls, indicating taste aversion. While this study demonstrated no effects on fertility, gestation, or reproduction, its value for risk assessment is limited due to the low doses achieved (<25 mg/kg/day).

Because data on the reproductive effects of styrene were limited, a two-generation reproduction study was conducted via whole-body inhalation according to current regulatory guidelines. Developmental neurotoxicity evaluation of selected offspring from the second generation are reported in an accompanying article (Cruzan et al., submitted 2004a). In most reproduction studies conducted by inhalation, exposure is stopped on day 20 of gestation and reinstated on lactation day 5 to minimize stress on the offspring from the more than six-hour separation that would occur during inhalation exposure of the dam. Because high concentrations of styrene may cause central nervous system (CNS) depression and significant development of the CNS occurs during the first few days after birth in rats, F0 and F1 dams were treated orally during lactation days 1-4 at doses estimated by PBPK modeling to mimic a six-hour inhalation exposure. No effects on reproduction were found at exposures up to 500 ppm for two generations (Cruzan et al., submitted 2004a).

Developmental Toxicity

A preliminary study suggested an association between styrene exposure in women and congenital central nervous system malformation; this has not been found in larger studies by the same and other authors (Brown et al., 2000). Likewise, an initial study suggested increased abortions in styrene exposed women, but subsequent studies have not confirmed this (Brown et al., 2000).

The potential developmental effects of styrene have been tested in multiple animal species including rabbit, rat, mouse, and hamsters. There is no evidence for malformations in these studies (Brown et al., 2000). Increases in fetal and neonatal death, as well as skeletal variations have been reported at inhalation concentrations that also caused maternal toxicity (Brown et al., 2000). The No-Observed-Adverse-Effect Level for these effects is around 250 ppm.

Developmental Neurotoxicity

Although few details are provided, Vergieva et al. (1979) reported no dose-related effects on body weights or offspring behavior when rat dams were exposed via inhalation to 163 ppm styrene 4 hours/day 5 days/week on gestational days 2-16 or to 47 ppm on gestational days 2-21. Zaidi et al. (1985) reported that gavage treatment of rat dams with 200 mg/kg/day styrene throughout gestation had no effect on the number of pups born per litter, pup body weight, protein content of the brain, or striatal dopamine receptors of pups. In contrast, Kishi and coworkers have conducted two studies on the effects of prenatal styrene exposure. In the first study (Kishi et al., 1992, 1995), pregnant Wistar rats were exposed via inhalation to 0 (14

litters), 60 (3 litters), or 293 ppm (7 litters) styrene 6 hours/day on gestation days 7 to 21. There was no effect on gestation length or average litter size, but pup weights in both styrene exposed groups were reduced compared to the controls. Neurobehavioral evaluation was conducted on 5 control litters, 2 litters exposed at 60 ppm and 5 litters exposed at 293 ppm. They reported differences in a number of developmental landmarks, as well as differences in open field activity, rota-rod activity and operant conditioning response for some but not all tested intervals. In the second study (Katakura et al., 1999, 2001), pregnant female rats were exposed to 0 (ad libitum feed, 14 litters), 0 (pair–fed to 300 ppm group, 12 litters), 50 (9 litters) or 300 (14 litters) ppm styrene by a static inhalation system. Compared to the pair-fed controls, exposure to 300 ppm styrene resulted in increased neonatal death, decreased pup weight on PND 21, increased time to lower, but not upper, incisor eruption, an increased time to development of air righting reflex, and decreased homovanillic acid in the cerebrum.

Because these studies used small numbers of litters and unusual methods of exposure (e.g., static inhalation exposure), a developmental neurotoxicity study was added to the two-generation reproduction study. Developmental landmarks were measured in both F_1 and F_2 offspring, selected F_2 offspring were tested for neurobehavioral effects and neuropathology. Styrene exposure of the F_0 and F_1 animals had no effect on survival, the clinical condition or necropsy findings of the F_2 animals. Functional observational battery evaluations conducted for all F_1 dams during the gestation and lactation periods and for the F_2 offspring were unaffected by styrene exposure. Swimming ability as determined by straight channel escape times measured on PND 24 were increased, and reduced grip strength values were evident for both sexes on PND 45 and 60 in the 500 ppm group compared to controls. There were no other parental exposure-related findings in the F_2 pre-weaning and post-weaning functional observational battery assessments, the PND 20 and PND 60 auditory startle habituation parameters, in endpoints of learning and memory performance (escape times and errors) in the Biel water maze task at either testing age, or in activity levels measured on PND 61 in the 500 ppm group. Taken together, the exposure-related developmental and neuromotor changes identified in F2 pups from dams exposed to 500 ppm occurred in endpoints known to be both age- and weight-sensitive parameters, and were observed in the absence of any other remarkable indicators of neurobehavioral toxicity. Based on the results of this study, an exposure level of 50 ppm was considered to be the NOAEL for growth of F_2 offspring; an exposure level of 500 ppm was considered to be the NOAEL for F_2 developmental neurotoxicity (Cruzan et al., submitted 2004 b).

Exposure

Styrene is a high production chemical that is used in the manufacture of a wide variety of plastics, primarily polystyrene, unsaturated polyester resins (fiberglass reinforced plastics), styrene-butadiene rubber, styrene-butadiene latex, acrylonitrile-butadiene-styrene, and styrene-acrylonitrile plastics. Manufacturing processes, especially fabrication of reinforced plastics, result in environmental releases mostly to air. Automobile exhaust also is a source of styrene in the air. Styrene is rapidly degraded in air, but there is an ambient environmental level of about 1 ppb styrene. For smokers, cigarettes can be the dominant source of styrene exposure.

In typical work environments, styrene exposures are well below 10 ppm; however, in the fabrication of reinforced plastics, styrene exposures can be 50 ppm or more.

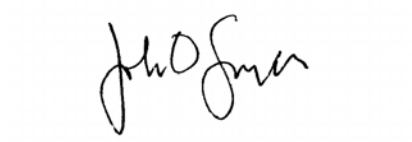
Styrene is naturally present in several foods including fruits and spices, and is naturally produced in the processing of foods such as beer, wine, and cheeses. Styrene is permitted as an indirect food additive as a residual in food packaging. Exposures are generally less than 10 µg/kg/day. Because of its low solubility in water and volatility, styrene is rarely found in drinking water.

Conclusion

SIRC believes that the collective available data, in particular the recently completed two-generation reproductive and developmental toxicity studies in rats, support the conclusion that exposure to styrene does not exert any significant effect on human reproduction, or on the development of offspring of persons exposed to styrene. This conclusion is supported by independent reviews of the styrene data.

SIRC appreciates the opportunity to provide this summary and the accompanying references in response to CERHR's request for input. We would be pleased to discuss these comments in detail, assist in providing any additional references required by the expert panel, or support CERHR in its styrene assessment in any way. Please contact me at the number below. A print copy of the documents cited below as attachments to this electronic submission will be sent under separate cover, including a CD of the full WIL Research Laboratories study data.

Very truly yours,



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Attachments

1. Manuscript, Developmental Neurotoxicity Study of Styrene by Inhalation in Crl-CD Rats (PDF file attachment; version as revised per journal comments)
    a. Letter of acceptance for publication from *Developmental & Reproductive Toxicology* (PDF file attachment)
2. Manuscript, 2-Generation Reproduction Study of Styrene by Inhalation in Crl-DC Rats (PDF file attachment; version as revised per journal comments)
    a. Letters of acceptance for publication from *Developmental & Reproductive Toxicology* (PDF file attachments)

3. Study Report, An Inhalation Two-Generation Reproductive Toxicity Study of Styrene in Rats, Including Developmental Neurotoxicity Assessment of the F2 Generation (electronic file sent via separate e-mail due to document size; CD to be provided under separate cover)
4. Article, A Review of the Developmental and Reproductive Toxicity of Styrene, Brown et al, 2000 (PDF file attachment)
5. Article, Reproductive and Developmental Toxicity of Styrene, Brown, 1991 (PDF file attachment)
6. Link to Harvard Center for Risk Analysis "Risk in Perspective": http://www.hcra.harvard.edu/pdf/May2002.pdf
7. *Journal of Toxicology & Environmental Health* / Part B: Critical Reviews, Vol. 5, No. 1-2, January – June 2002 (print copy being sent under separate cover)
8. Link to IARC Monograph Vol. 82 (2002), p. 437, styrene summary: http://www-cie.iarc.fr/htdocs/monographs/vol82/82-07.html
9. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 82, 2002, Some Traditional Herbal Medicines, Some Mycotoxins, Napthalene and Styrene (print copy being sent under separate cover)

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