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The Styrene Information & Research Center

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Dr. Michael D. Shelby
Director
Center for the Evaluation of Risks to Human Reproduction
National Institute of Environmental Health Sciences
P.O. Box 12233
MD EC-32
Research Triangle Park, NC 27709
[E-mail: shelby@niehs.nih.gov]

RE: Comments on the Draft Expert Panel Report on Styrene (Sections 1-4), Per 70 *Federal Register* 11,680 (March 9, 2005)

Dear Dr. Shelby:

The Styrene Information and Research Center (SIRC)[1] is pleased to submit comments to the National Toxicology Program's (NTP's) Center for the Evaluation of Risks to Human Reproduction (CERHR) on the above-referenced Draft *NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Styrene* ("Draft Report"), sections one through four.

In a *Federal Register* notice dated December 8, 2004, CERHR announced the creation of an expert panel to evaluate the available scientific evidence regarding the potential reproductive and developmental toxicity associated with exposure to styrene.[2] Styrene was proposed for evaluation based on "public concern about styrene exposure" and "recently available exposure studies."

SIRC commends CERHR's commitment to "provide a strictly scientifically based, uniform assessment of the evidence for reproductive and developmental toxicity of man-made or

[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 helping ensure that regulatory decisions are based on sound science. For more information, visit http://www.styrene.org.

[2] 69 Fed. Reg. 71,067 (December 8, 2004).

naturally occurring chemicals or chemical mixtures."[3] The CERHR panel reports serve to inform the public and regulatory agencies concerning reproductive and developmental health effects associated with exposure to specific chemicals and to identify knowledge gaps to help establish research and testing priorities.

In response to the December 8, 2004 *Federal Register* notice, SIRC provided the Panel with a summary of the key studies that address the reproductive and developmental toxicity of styrene, including a recently completed definitive two-generation reproductive and developmental neurotoxicity study on rats.[4] We noted then that the most recent reviews of styrene's effects on reproduction and development had concluded that there is little evidence that styrene causes any specific developmental or reproductive effects.

SIRC remains eager to assist the CERHR Panel to ensure the accuracy and proper context of the information reviewed and summarized in the Panel's draft assessment. Accordingly, SIRC respectfully submits the following comments on the Draft Report for styrene. Our comments follow the sequence of the Draft Report and, where relevant, we identify specific discrepancies in the document by page number, and also suggest additional sources of information for the consideration of the Expert Panel.

Section 2: Comments on the General Toxicology and Biological Effects Section

Page 21, 2.1.1.3. *The Draft Report characterizes styrene oxide as "the species suspected of carcinogenicity."*

Characterizing styrene oxide as "suspected" at this early stage in the Report, absent the subsequent discussions of more recent mode of action data, appears premature. Perhaps line seven could be reworded as "Styrene oxide, an IARC 2A carcinogen, is metabolized to…"

Page 25, 2.1.2. *Typographic error*

It would appear the first sentence was intended to read "The most thorough reviews on animal toxicokinetic…"

Page 27. *The Draft Report cites to a physiologically based pharmacokinetic (PBPK) model developed by Ramsey and Andersen.*

While the PBPK model by Ramsey and Anderson may be of historical importance because it was one of the first models published, it is extremely outdated. At least three newer models have been published. Of these, the most complete model is by Sarangapani et al., 2002, which describes not only metabolism by the liver, but also metabolism in the terminal bronchioles and nasal epithelium, where toxicity occurs.

[3] 63 Fed. Reg. 68,782 (December 14, 1998).

[4] Letter from John O. Snyder, SIRC Executive Director, References for CERHR Review of Styrene Reproductive and Developmental Toxicity Data, January 21, 2005

Page 27, last paragraph. *The Draft Report states "In a study by Cruzan et al. (37), 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 and 1000 ppm styrene."*

We suggest that this sentence be reworded as follows: "In a study by Cruzan et al. (37), blood levels of styrene in a chronic inhalation study in rats were proportional to dose; blood levels of styrene oxide were generally proportional to dose, but showed some degree of saturation between 200 and 1000 ppm."

Page 30, paragraph 4, lines 5-9: *Characterization of "color vision loss."*

Using the term "color vision loss" suggests that the workers become "color blind." This is not the case, as this represents a very slight decrease in color discrimination, which as described by Harvard is subclinical. Further, there is no evidence that this results from impairment of the optic nerve.

Page 30, last paragraph. *The Draft Report states a review sponsored by the Styrene Information and Research Center disputed claims that neurotoxicity occurs at concentrations below 50 ppm styrene.*

The conclusions from this review should be attributed to the authors, Rebert and Hall (Ref. 28), and not to SIRC. SIRC sponsored the review, but the article is the authors' interpretation of the data, which was peer reviewed and published. The same characterization of Rebert and Hall as being a SIRC study is also made at the top of page 32.

Page 33, first paragraph, last sentence: *The Draft Report states "Harvard (6) 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]."*

We suggest that a more accurate statement of the Harvard conclusion would be: "Harvard (6) evaluated numerous *in vitro* studies demonstrating that styrene oxide is more consistently genotoxic than styrene and effects are generally observed with lower concentrations of styrene oxide than styrene, but the results of mutagenicity tests of styrene oxide in animals or humans is less clear [styrene oxide data not shown in CERHR report]."

Page 44. *The Draft Report cites the Harvard Panel and IARC reports as the most recent and complete evaluations of cancer hazard in humans. With respect to reinforced plastic workers, CERHR states "[t]hough not consistently observed among different studies, lung/respiratory and lymphatic/hematopoietic cancers were most often reported in reinforced plastics workers."*

The Draft Report's statement on the conclusions of the IARC and Harvard human cancer studies of reinforced plastics workers is misleading, because it modifies an IARC statement regarding all studies of workers in various industries where there is styrene exposure, to apply it specifically to the reinforced plastics industry, where it does not fit. In actuality, there are only three independent cancer studies of reinforced plastics workers:

1. Wong (1990, 1993);
2. Kogevinas, 1993,1994, Coggon 1987, Kolstad 1993, 1994, 1995. and
3. Okun, 1985, Ruder 2004.

Only the Kogevinas-associated studies report borderline increased lymphatic/hematopoietic cancers; this relationship was not present in the others. The Wong study was the only one of the three studies to report increased lung cancer, and this was attributed to smoking, not styrene.

Page 45, Table 20. *In Table 20, CERHR cites to Kolstad as if it were multiple studies.*

Table 20 of the Draft Report should be revised to reflect that Kolstad, 1993 is a preliminary report, and should not be reported or implied to be separate from Kolstad, 1994, which is the complete assessment of the same data.

In addition, SIRC has concerns about the value of the Kolstad study. The study contains no information that can be used to conclude that "2/3 of exposed employees worked at companies where about ½ of employees were involved in reinforced plastic manufacturing." According to the paper, 23,688 workers were employed in companies where the *resin suppliers*, as opposed to the reinforced plastics companies themselves, estimated that less than 50% of workers were involved in reinforced plastics; and 12,837 were in companies where it was estimated that more than 50% were involved in reinforced plastics. The authors estimated that 43% of the workers in these companies may have actually been involved in reinforced plastics manufacture. No attempts were made to determine which workers were in highly exposed jobs (laminators) and which were in lower exposure jobs. It should be added that *all* increases in cancer incidence were among those employed for less than one year and that no attempt was made to determine whether any of the cancer deaths occurred in workers who were ever exposed to styrene.

Page 47. *In Table 20, the Draft Report cites to Kogevinas as if it were multiple studies.*

Kogevinas 1993 and 1994 should not be listed as separate studies. The 1993 article was a preliminary report made to a conference. The 1994 report contains the same data as the 1993 report. The relative risk (RR) increases were only relative to average or intensity of exposure, not to duration or cumulative exposure. We also note that the Kogevinas study used only employees of companies estimated by the Kolstad study to have over 50% of their workers involved in reinforced plastics production. These workers were categorized during the study as "other exposed workers," not laminators. Also the Coggon, 1987 cohort was included in the Kogevinas study. Thus, there was no increased cancer risk relative to duration of exposure or cumulative exposure, but there was to intensity of exposure (calculated as cumulative exposure/duration of exposure). Thus, this study does not indicate increased cancer risk from styrene exposure.

Page 48. *In Table 20, the Draft Report presents the Wong 1990 and 1994 studies as a single study.*

The two Wong studies were conducted over 12 years apart and, unlike the Kolstad and Kogevinas studies, could be listed separately on Table 20, although the 1994 paper was an update of the information contained in the 1990 paper.

Page 48. *Okun et al. listed in Table 20 does not reflect most recent information on that cohort.*

The Draft Report should indicate that the Okun study was recently updated by Ruder, and that this study now has the longest follow-up period and indicates no styrene–related increases in cancer. Ruder, A.M., Ward, E.M., Dong, M., Okun, A.H., Davis-King, K., 2004. Mortality patterns among workers exposed to styrene in the reinforced plastic boatbuilding industry: an update. *Am. J. Ind. Med.* 45: 165-176.

Section 2.4. 2. Paragraphs 3 & 4. *Addition of Harvard conclusions where only IARC conclusions are currently cited.*

We suggest that paragraph three should also reference the Harvard Report's conclusion, since Harvard's evaluation of the data is discussed in the preceding section. The Harvard Report said "Styrene via inhalation increases the incidence of mouse lung tumors."

For the same reason, we suggest that the Harvard Report's conclusion also be added to paragraph four: "Mice and rats develop forestomach tumors following administration of styrene oxide by gavage."

Page 54, Section 2.4.3. *The cancer mode of action discussion relies on outdated information.*

The mode of action discussion in Section 2.4.3 is based on data available before 2002. The Draft Report should include in this discussion the more recent data summarized below:

> Cruzan et al., 2002 summarized the mode of action data and concluded that it is metabolites produced by the CYP2F family that produces cytotoxicity from styrene. It was found that the inhibition of CYP2F2 in mice inhibits the nasal and lung cytotoxicity from styrene inhalation.
>
> Further experiments have indicated that ring-oxidized metabolites of styrene are much more cytotoxic than either styrene or styrene-7,8-oxide. These metabolites are produced by members of the CYP2F family (based upon experiments with inhibitors of various CYPs). These findings are summarized in Cruzan et al., 2005.
>
> These findings are important in understanding the relevance of mouse lung tumors from styrene because CYP2F1 (the member of the family found in humans) has very little, if any, ability to metabolize styrene. The relevant citations are as follows:
>
> > Cruzan, G., Carlson, G.P., Johnson, K.A., Andrews, L.S., Banton, M.I., Bevan, C., Cushman, J.R. (2002). Styrene Respiratory Tract Toxicity and Mouse Lung Tumors Are Mediated by CYP2F-Generated Metabolites. *Reg. Toxicol . Pharmacol.* 35: 308-319.
> >
> > G. Cruzan, G.P. Carlson, M. Turner, W. Mellert. (2005). Ring-Oxidized Metabolites of Styrene Contribute to Styrene-Induced Clara Cell Toxicity in Mice. *J. Toxicol. Environ. Health, Part A* 68: 229-237.

Sarangapani, R., Teeguarden, J.G., Cruzan, G., Clewell, H.J., and Andersen, M.E. (2002) Physiologically based Pharmacokinetic Modeling of Styrene and Styrene Oxide Respiratory Tract Dosimetry in Rodents and Humans. *Inhal. Tox.*,14: 781-834.

Section 3: Comments on Developmental Toxicity Data

In this section, we address the relevancy of developmental toxicity findings, including data consistency, in light of questions posed to the Expert Panel on page 98 of the Draft Report. We also respond to the Panel's comments on a 2005 developmental neurotoxicity study sponsored by SIRC.[5] Our overall interpretation of the data is that styrene has consistently been shown to lack the potential to cause prenatal mortality or malformations, but with sufficiently high exposure styrene may delay overall growth of offspring.

Page 62, Section 3.1. *Studies by Holmberg*

Serious limitations in many of these studies include mixed solvent exposures, lack of quantification of the exposures by plasma concentrations with estimation of exposure by industry and job description. When exposure was quantified (*e.g*. Ref. 63), there was a lack of an exposure response relationship. The outcome of these studies is that no increase in congenital malformations was evident. The early increase in exposed women decreased as more pregnancies were included. The bias of the initial cases eventually was diluted out.

Although Holmberg stresses the objectivity of his interviewing technique, similar interviews have been subject to reporting bias (Hertz-Picciotto, I. et al. Reporting bias and mode of interview in a study of adverse pregnancy outcomes and water consumption. *Epidemiology* 1992;3:104-112).

Page 63, Section 3.1. *Ref. 64, 65*

The study showed a lower rate of spontaneous abortions for pregnancies that occurred during styrene exposure than before or after styrene exposure, and that the risk of malformations for infants born to mothers who were members of the Union during the first 2 months of pregnancy did not have an increase in congenital malformations.

Page 63, Section 3.1. *Ref. 66*

The frequency of malformations in infants born of styrene-exposed women from 1965 to 1979 was lower than values expected from general population data. This lower frequency also existed in infants born to these women before they were exposed to styrene. The frequencies were comparable for the children of exposed males.

[5] *See* Ref. 88, Cruzan, G., Faber, W. D., Johnson, K. A., Roberts, L. S., Maurissen, J., Beck, M. J., Radovsky, A., Stump, D. G. and Buelke-Sam, J. Developmental neurotoxicity study of styrene by inhalation in Crl-CD rats. *Birth Defects Res.* (Part B) 2005; in press:

Page 64, Section 3.1. *Ref. 67*

The odds ratio for adverse outcomes for pregnancies exposed to styrene compared with those not exposed was 0.8, indicating no increased risk for adverse outcome. There were deficiencies in this study related to estimation of exposure and mixed exposures (additional solvents in addition to styrene), but there is no significant difference and a numerical decrease in events (i.e. mixed events of malformations, stillbirths, newborn deaths, low birth weight or prematurity) for the styrene-exposed cases.

Page 64-66, Section 3.1. *Ref. 68, 69*

Women were identified from plastic companies, and menstrual histories were taken for women who agreed to participate (Ref. 69). Exposure was estimated by job description. Multiple regression analyses were done to account for the numerous risk factors associated with menstrual abnormalities. Although none of the menstrual factors showed a significant difference for exposed versus not exposed patients, there was a strong trend for exposed women to have lower rates of secondary amenorrhea (p=0.08).

In a similar study, the birth weights were evaluated (Ref. 68). The birth weights were the weight provided by the mother, converted to grams. Assessment of birth weight is often inaccurate and can vary when the infant does or doesn't void at delivery or whether the infant is naked or diapered. Furthermore, when the weight is given as pounds and ounces and then converted to grams, as was done in this study, it is even less accurate. Given this inaccuracy, any small differences between the groups cannot be considered as accurate.

The styrene exposures were mixed, in that many of the patients were exposed with other solvents. There were three low birth weight infants in the highest exposure group associated with lower gestational age. The authors note that most of the women left their jobs during the second trimester (mean gestational exposure was between 5 and 6 months). The dose response effect on birth weight was not significant. Given the difference in early deliveries and the potentially large variation in birth weights, this study should be considered hypothesis generating.

Section 3.2

With regard to the studies reviewed by the Panel in this section, SIRC concurs with the Panel's assessment that the Murray et al. (Ref. 75, page 68) study "is of high utility to the CERHR evaluation process." The strongest data to support styrene's lack of lethality or teratogenicity are from these studies, which had negative outcomes when rats and rabbits were exposed to styrene by inhalation, the most relevant exposure route, and in rats by oral gavage, the most widely used route of exposure for hazard identification. These studies had adequate statistical power and, at least for the rat, failed to induce developmental effects even at maternally toxic exposure levels. In the rabbit, no developmental or maternal effects occurred.

Although several other studies reviewed by the Panel are of limited utility to the CERHR evaluation process because of their exposure regimen (e.g., Daston et al., Ref. 79, GD 11 dosing only), or by an inadequate number of litters (multiple studies), they nevertheless add to the body of evidence that there are no indications of styrene-induced malformations.

With regard to prenatal lethality, the only data suggestive of such a finding occurred in the mouse and hamster studies, but the studies used inappropriate statistical criteria ($p< 0.10$ instead of $p < 0.05$), very low numbers of animals, or extreme levels of styrene exposure (1000 ppm). Even under these conditions, no malformations were induced by styrene exposure. The two reproductive toxicity studies (Beliles et al., Ref. 50, and Cruzan et al., Ref. 89) provide indirect support for an interpretation that styrene does not cause prenatal mortality, in that no differences to litter sizes at birth were detected in either study.

The findings on delayed growth have been inconsistent. Srivastava et al. (ref. 77) and Ninomiya (Ref. 81) reported decreased fetal body weight. Fetal weight was not affected or not reported in other studies (ex: Refs. 75, 77, 79). Birth weight was not affected in the inhalation two-generation reproduction study (Cruzan et al., Ref. 88, 89), although postnatal growth reduction was reported in the F2 offspring, but not the F1 offspring. In addition to the usual factors influencing outcome, such as exposure level and species, the Draft Report identified additional considerations, such as small group sizes and inappropriate statistical methods as possible explanations for inconsistent findings. If review is restricted to those studies that met regulatory guidelines for group sizes and conducted statistical evaluations on the basis of litters (Ref. 75, 88 [same study as 89]), there appears to be a low potential for prenatal growth delay, even in the presence of mild maternal toxicity. Postnatal growth appeared to be affected with continuing exposure of the dams during lactation.

Several studies evaluated postnatal function. Evaluations of both the nervous and reproductive systems noted no structural or functional deficits due to styrene exposure. Three studies (Ref. 82, 84, 86) suggest that styrene exposure may influence brain neurotransmitter levels. However, these studies utilized very small numbers of animals and/or inappropriate statistical methods, further complicating the interpretation of non-routine endpoints. The data with the strongest statistical power was the developmental neurotoxicity study conducted on the second generation of a two-generation reproduction study (Ref. 88). Although this study did not evaluate neurotransmitter levels, morphological evaluation of the nervous system indicated no changes to microscopic structure, and analysis of behavioral performance suggested that very slight differences from the controls were attributable to an overall delay in animal growth. The Draft Report correctly points out that the brain is usually an organ system refractory to body weight changes, but seemed to express concern about the increase in relative brain weight (brain/body weight ratio) observed in the offspring. This difference in relative weight was attributable to lower body weight, and was also observed in parental animals. Detailed brain morphometry, as required in a guideline developmental neurotoxicity study, indicated no differences between offspring exposed via dams at the high dose, 500 ppm, and their control counterparts. Microscopic evaluation of the F1 offspring gonads and spermatogenic endpoints when the animals reached adulthood found no differences attributable to maternal styrene exposure during gestation and lactation or direct exposure post weaning (Ref. 89).

Further, we have reservations about the following references cited in the Draft Report:

- **Hardin et al. (Ref. 76).** This study evaluated methyl styrene, not styrene. Methyl styrene is not a synonym for styrene and is also not a metabolite of styrene. This reference is inappropriate to the styrene Draft Report and should be deleted. Note also, the reference to styrene oxide is only in “weaknesses,” not in study description. No adverse effects of pregnancy from inhalation of styrene oxide were seen.

- **Srivastava et al. (Ref. 77).** In addition to the strengths/weaknesses already identified by the authors of the Draft Report, we were unable to determine from the methods section of the paper if the doses of styrene, provided on a body weight basis, were determined based upon initial (*i.e*., gestation day 0) body weight, or interim body weights, as this information was not found in the manuscript.

- **Kankaanpaa et al. (Ref. 80).** We noted that the statistical significance for the number of dead or resorbed fetuses was at the $p < 0.10$ level, rather than $p < 0.05$, the "cutoff" criteron most often used in scientific comparisons.

SIRC supports and emphasizes the following conclusions, which appear in or can be inferred from the Draft Report.

- **Malformation risk:** There was no increase in malformations with styrene exposure in either females (Ref. 58-66), after inspection of the entire range of studies rather than looking at interim data (Ref. 58-63), or in males (Ref. 107).

- **Spontaneous abortion risk:** There was no increase in spontaneous abortions with styrene exposure (Ref. 64-65, 102-105)

- **Low birth weight:** One study evaluated the effect of styrene exposure on birth weight. The study suggested a slight decrease in the birth weight of newborns to styrene-exposed women (Ref. 68). However, this small effect was not statistically significant, *i.e*. potentially spurious, is subject to measurement and recall errors, and had the potential to have been caused by other solvent exposure, not styrene.

Section 4: Comments on Reproductive Toxicity Data

A. General Comments

For the most part, the human studies cited in this section are not well conducted, owing in part to the complexities of the population and the lack of control over worker conditions and behaviors. Almost all the study authors expressed an expectation of finding an association of styrene exposure and abnormalities in the measured results. As a consequence most have concluded that they were unable to detect an exposure effect due to various methodological or sample size reasons. Indeed, the studies performed by Cruzan et al. (Ref. 88, 89) suggest that many of the reported effects of styrene on animal reproduction are due to either methodological or strain effects.

There is an apparent increase in prolactin related to acute styrene exposure (Ref. 99-101). Although this reported magnitude of prolactin increase might have consequences on animal reproduction, no adverse consequences in humans were demonstrated as a result of this increase (Ref. 99, 101). One study (Ref. 100) did note menstrual irregularities, but the study was highly confounded; other studies reported no menstrual irregularities.

B. Specific Comments

Page 102,103, Section 4.1. *Ref. 98*

Exposures were estimated from a prior study, but the specific exposures may have varied by the individual worker. The menstrual cycle duration was determined retrospectively by completion of a questionnaire at study entry.

This study used multiple comparisons for each solvent individually and in combination, as well as in repeated analyses using various models. For the minimally adjusted data, there were only 3 styrene-only (mutually exclusive)-exposed women and none had oligomenorrhea. For the mixed exposures including styrene, there was an increased percentage of oligomenorrhea (14.5% vs. 8.5% with no exposure). The percentages increased with longer duration of occupational exposure. There was a correlation of duration of work with age, but not using dichotomous and tertile age analyses. No mention was made of using age as a continuous variable. It is known that oligomenorrhea increases with age.

Page 104,105, Section 4.1. *Ref. 100*

This is an evaluation of 16 women who were exposed to styrene through their work. They evaluated 16 age-matched controls who were tested at the same stage of menstruation.

Because the subjects on average had elevated Hamilton Depression-Rating Scale scores (most had “high to very high” scores) and 50% of the women had menstrual abnormalities, the results are confounded. Although the paper states that the subjects were exposed to styrene through work, this was not fully documented and the possibility of additional solvent exposures is ignored. Additional issues are noted in the editor’s comments. Patients with depression might have impairments in monoamine systems. This may influence the results of the TRH stimulation test. This relationship of depression and result of the TRH stimulation test was not evaluated

One apparent finding is that the exposed subjects had prolactin concentrations that were significantly elevated over the controls. Additionally, one of the subjects had a decrease in basal prolactin concentration after discontinuing her exposure to styrene. Another had no change in basal prolactin concentration after discontinuing exposure but had return of menses after 3 years of amenorrhea during 3 years of exposure to styrene.

Page 105-107, Section 4.1. *Ref. 101*

The findings indicate that prolactin concentration is proportionate to the amount of acute exposure with styrene. Interestingly, the blood exposure to styrene was required to increase 10-fold to increase the prolactin concentration two-fold. It is not known what happens with greater increases in styrene since the effects may not be infinitely linear. This suggests that this relationship cannot be extrapolated beyond the range of results seen in this study.

Of particular importance, there is no mention of the known consequences of increased prolactin in the cohort of subjects. In particular, the discussion notes the potential for elevated prolactin to induce amenorrhea, but there is no corresponding statement regarding the subjects, rather

there is a theoretical description of the potential consequences of this elevation. This suggests that amenorrhea was not present to an unusual degree and probably not related to concentrations of prolactin. In the absence of reproductive problems, there is no clinical significance of these small elevations of prolactin.

Page 108-109, Section 4.1. *Ref. 102*

The study does not adequately assess the level of exposure or the potential for mixed exposures. Nevertheless, there is a non-significant but lower numerical rate of spontaneous abortions in the workers who were exposed to styrene during their pregnancy than those who were not exposed. So, even if the power of the study was low, one would *not* expect a larger study to produce a significantly greater risk for spontaneous abortion.

Page 108-109, Section 4.1. *Ref. 103*

The controls were age matched women who had never had a spontaneous miscarriage or malformed infant. Not all cases could be age-matched and there was imbalance with most cases having 3 controls, but a few having less and 2 older women (43, 44) having none. There may have been other imbalances and the cases and controls were not matched for socioeconomic class. Exposures may have been mixed and the exposures were estimated rather than directly measured. In addition, there was an imbalance due to inclusion of women with prior spontaneous abortions in the styrene-exposed group, but not the control group. This could have increased the rate of spontaneous abortion in the styrene-exposed group since couples with a prior spontaneous abortion have a higher rate of subsequent spontaneous abortion.

Nevertheless, the Odds ratio for styrene was significantly lower for styrene-exposed women than for control women.

Page 111, Section 4.1. *Ref. 105*

The raw data indicates that there was a numerically lower rate of spontaneous abortions for the polystyrene-only exposed pregnancies (19%) compared with the mixed exposures (26%). The total rate of observed spontaneous abortions with styrene was not statistically, but was numerically, greater than the expected rate.

Page 111, Section 4.1. *Ref. 106*

There were multiple comparisons of various sperm characteristics. The percent of live sperm was greater and the percent of immotile sperm was lower for the exposed patients and there were fewer immotile sperm. However, the percent of normal sperm and, thus, the percent of pyriform head and amorphous head sperm, was greater for the exposed patients. However, when the percent of live, motile, and normal sperm are calculated, the two groups hardly differ. The exposed patients had 26% and the reference group had 25%.

Page 112,113, Section 4.1. *Ref. 107*

Pregnancies of wives of men identified by the Finnish Institute of Occupational Health during 1965-1983 as being exposed to organic acids were assessed by the Hospital Discharge Register. The paternal exposures were classified as low/rare, intermediate, and high/frequent. There were no significant differences between the exposed cases and referents. The Odds Ratios were 1 or less for all the styrene-exposed levels and there was an inverse relationship with frequency and exposure. There were also no differences in congenital malformations.

Page 115-116, Section 4.1. *Ref. 110,111*

Although there was a decrease in sperm count and in normal morphology (possibly due to seasonal variation), the changes were not related to quantitative measures of styrene exposure. Changes in Chromatin structure were within the variability of the measures.

In this reference, the authors reported a comparison with 21 non-styrene-exposed farmers, matched for season, but not for age (farmers were an average of 11.5 years older) with the workers from study Ref. 110. The farmers showed no mean change in sperm count. The recruitment of the farmers was after the conclusion (and analysis) of the original study.

Page 116-118, Section 4.1. *Ref. 112,113*

The highest exposure group had numerically greater fecundity than the control group.

Page 118. *The Draft Report states that no other experimental animal studies on possible female reproductive toxicity of styrene have been identified.*

SIRC submits that the two 2-generation studies in rats (Ref. 88 and 89) conducted via inhalation would be useful for determining possible female toxicity of styrene.

Page 119. *The Draft Report states the use of four dose levels is a strength, and the lack of organ weights is a weakness.*

The Panel review of Cruzan et al. (Ref. 43) should be revised to reflect that ovarian and testicular weights were collected from the rats and mice, and uterine weights were collected from the mice only.

Page 119. *Summarizing Srivastava et al. (Ref. 114), the Draft Report states: 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.*

It is unlikely that this level of testicular lesions (including edema) could occur in these animals without change in testicular weights. Testicular weights are often a very sensitive indicator of edema in the testes. In addition, it is difficult to relate the level of edema (as described by the authors) with the supposed changes in intercellular enzymes. Intercellular enzymes usually decrease with edema as the protein is released to the extra cellular fluid and cleared by the blood circulation. Therefore, it would seem unlikely that there are increases in some of these

intercellular enzymes in the testicular fluid. Indeed, it is commonly accepted that these enzymes are not specific markers for any one cell type within the testes.

Page 120. *Salomaa et al (Ref. 115).*

This study is predicated on a hypothesis that has since been disproved and the study should therefore be removed from the analysis. At the time of this study, it was hypothesized generally that abnormal sperm morphology was an indication of DNA damage. Any altered shape supposedly then was the result of altered DNA. It is now understood that altered sperm shape does not necessarily reflect genetic damage and in fact there have been instances where grossly misshapen sperm have been directly injected into oocytes, resulting in normal mouse offspring. The reference for interpretation is a review entitled, "An evaluation and interpretation of reproduction endpoints for human health risk assessment," in the ILSI HESI review from November 1998, which can be found at: http://hesi.ilsi.org/publications/pubslist.cfm?publicationid=44.

Page 122. *The Draft Report review of Cruzan et al. (Ref. 89) states that 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 hours/day, 7 days/week for a minimum of 70 consecutive days before mating and during the mating period.*

The Draft Report should be revised to reflect that the males continued to receive styrene exposure following the mating period and up until euthanasia.

Page 123. *The Draft Report assessment of Strengths/Weaknesses of Cruzan et al. (Ref. 89) states that no mention was made of random assignment of animals.*

SIRC notes that the second line of the Panel's review of Cruzan et al. (page 122), previously had correctly noted that the animals were randomly assigned, therefore the notation that there was "no mention" of random assignment should be deleted.

While it is not stated explicitly in the published manuscript of Cruzan et al., the full report of the study, which was submitted to CERHR, clearly indicates that there was random assignment of animals to experimental or treatment groups.

Page 124. *The Draft Report state that a two-dose (plus placebo) study using oral administration in male rats can be used to evaluate specific endpoints (enzymes in testicular homogenates, epididymal sperm concentration).*

CERHR has previously described this study, Srivastava et al. (Ref. 114), as being "of limited utility" for the evaluation process. SIRC questions whether this study provides any additional information given the problems with the underlying hypothesis (that certain enzymes are representative of specific cell types within the testes) described on page 120 of the Panel Report. We submit that the two multigenerational studies that were conducted on rats, one by drinking water and one by inhalation, would provide better data with which to evaluate the effect of styrene on the testes.

The multigenerational studies used the Sarangapani et al., 2002 PBPK model to derive inhalation concentrations comparable to the oral gavage doses used in the Srivastava studies. In Ref. 114, Srivastava et al. administered styrene 6 days/week to Wistar rats (average weight 225 grams) at 0, 200, or 400 mg/kg/day. The same area under the curve for blood styrene is achieved by inhalation 6 hours/day at 0, 413, or 764 ppm.

In Ref. 92, Srivastava administered styrene by gavage at 0,100, and 200 mg/kg/day from day 0 to day 60. Using the PBPK model and Charles River growth chart for Wistar rats, the equivalent inhalation concentrations were 0, 144, and 293 ppm. Thus the doses in the Srivastava studies were comparable to the inhalation concentrations used in the Cruzan et al., 2005 two-generation study of styrene. Accordingly, the Cruzan et al. study (Ref. 89) would be the most appropriate study for evaluation of styrene effects on male and female reproductive toxicity, specifically for effects on testicular development and spermatogenic endpoints.

We note that in the developmental toxicity section of the Draft Report, the Panel reviewed and evaluated two other Srivastava studies (Ref. 91 and 92),. We suggest that the Panel's comments concerning the appropriateness of the Srivastava methods be carried over into the reproductive toxicity section -- in particular those comments on the use of biochemical enzymatic markers as representative of a certain cell type or as indicators of any sort of damage. It is important to note that in Ref. 91 and 92, the enzymatic levels that rise and fall, or fall and rise, with the development of the testes during puberty, *do not change within the control population of animals used in those studies*. In other words, the authors themselves have demonstrated that these enzymes are not specific indicators either of pubertal development or of any representative cell type within the testes.

SIRC supports and emphasizes the following conclusions, which appear in or can be inferred from the Draft Report.

- **Female fertility:** There were mixed results related to oligomenorrhea or amenorrhea. Ref. 69 suggests that there might be a lower rate with styrene exposure. Ref. 98 showed an increased rate for mixed solvent exposure. Ref. 100, which was highly confounded, suggested anecdotally that there was an adverse effect on the menstrual cycle. The potential that any effects were due to mixed solvent exposure rather than styrene exposure was not ruled out (Ref. 98).

- **Male fertility:** There was no consistent or dose-related decrease in fecundity across the studies (Ref. 108,110-113).

- **Effects on spermatozoa:** There were variable effects on sperm across the studies (Ref. 106, 108, 110-113). Ref. 108 showed decrease in sperm count after work exposure began. Ref. 110 and 111 were of the same study that showed a decrease in sperm count in exposed workers. The comparison group was added retrospectively (Ref. 111). There was no apparent statistical significance related to any described effects.

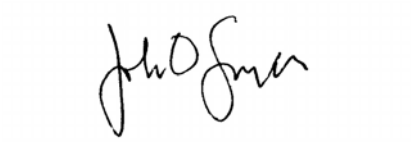
- **Stillbirth risk:** There was no difference in the rate of stillbirth for styrene-exposed pregnancies (Ref. 104).

Conclusion

SIRC appreciates the opportunity to comment on the CERHR Expert Panel's Draft Report. As our comments stress, it is essential to ensure the accuracy and proper context of the information reviewed and summarized by the Panel. Because no conclusions appear in the Draft Report, it is difficult to know whether we have properly interpreted all of the Panel's summaries of the existing literature. Nonetheless, we hope that our comments will be helpful, and we would welcome an opportunity to comment further once the Panel's conclusions become available.

SIRC and its member companies would be happy to provide additional information or discuss any questions that these comments might raise.

Sincerely,



John O. Snyder
Executive Director

Styrene Information and Research Center
1300 Wilson Boulevard – Suite 1200
Arlington, VA 22209
Phone: (703) 741-5010
Fax: (703) 741-6010
E-mail: Jack_Snyder@styrene.org