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Dear Dr. Shelby:

The CERHR has assembled an Expert Panel to review the risk to human reproduction from exposure to styrene. That Panel has issued a draft report, held a public forum to review and take comments on that report and has issued their final report. Part of that report includes a summary of data on the general toxicity and metabolism of styrene. Although these sections did not directly impact the assessment of risks to reproduction from styrene, it is important that these section be accurate because some people may use these sections as their evaluations of styrene toxicity. The metabolism and toxicity sections relied almost exclusively on three reviews (ATSDR, 1992; Cohen et al., 2002; IARC, 2002). Those reviews were 3 to 13 years out of date at the time of the CERHR Report and no accounting of the more recent literature was included. Since 2002, the understanding of styrene metabolism and mode of action has increased dramatically. A Federal Register Notice (vol. 70, No. 139, July 21, 2005, pp. 42064-42065) invited comments on the Expert Panel Report. Therefore, I request that the CERHR monograph be modified to reflect the current understanding of styrene metabolism and toxicity.

Page 23, paragraph 5, line 2

Contrary to the statement in the CERHR review, there is no evidence that CYP2F1 (the human relative of mouse CYP2F2) is active in metabolizing styrene. To date, no metabolism of styrene has been found by cells rich in CYP2F1, and metabolism of styrene by human whole lung preparations is below the limit of detection in nearly all samples tested.

Page 28, Section 2.1.2.3 Metabolism, after 3rd full paragraph on p. 28, please add:

In mice about 6% of urinary metabolites from styrene are ring-oxidized (Sumner et al., 1995), while in rats they account for about 1% (Sumner et al., 1995) and were not detected (limit of

detection 0.1%) in humans exposed to 50 ppm for 2 hours (Johanson et al., 2000; CERHR #33). Ring oxidation of styrene would be expected to lead primarily to the formation of 4-vinylphenol (assuming no reaction on the vinyl side chain). However, 4-vinylphenol (4-hydroxystyrene) is a very minor urinary excretion product in animals or humans exposed to styrene (Bakke and Scheline, 1970), suggesting further metabolism. Incubation of hepatic microsomes from CD-1 mice with styrene resulted in the formation of SO, but the formation of 4-vinylphenol was not detected (Carlson et al., 2001). The authors reported that 4-vinylphenol readily disappeared when incubated with microsomes from rat and mouse liver and lung in the presence of NADPH, indicating oxidative metabolism. Mouse liver was about 3 times as active as rat liver, and mouse lung was about 8 times as active as rat lung. Further studies indicated that CYP2E1 and CYP2F2 were important in the metabolism of 4-vinylphenol in mouse liver, but that CYP2F2 was more important in the metabolism of 4-vinylphenol in mouse lung than was CYP2E1, similar to the metabolism of styrene to SO. They were not able to identify the metabolite(s) formed, but lack of UV absorbance suggests the metabolites are not aromatic; i.e., they may be ring-opened. Further evidence of ring-opening metabolism of styrene is provided by excretion of $^{14}CO_2$ from a 6 hour nose-only inhalation exposure to 160 ppm [ring-U-^{14}C]styrene, which was 3-4 fold greater in mice (6-8%) than rats (2%) (Boogaard et al., 2000). Using selected CYP inhibitors, Carlson (2004) found that several CYPs contributed to the metabolism of 4-vinylphenol in CYP2E1-knockout mice, but CYP2F2 was most important in the lung.

Page 46. *The 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 "[tThough 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, 1994);
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 47, Table 20. *In Table 20, CERHR cites 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, I have 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."as stated in the CERHR report. 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 within reinforced plastics manufacture. 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 48 leukemia deaths occurred in workers who were ever exposed to styrene.

Page 49. *In Table 20, the Draft Report cites 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 increased cancer risk based on intensity of exposure (calculated as cumulative exposure/duration of exposure), but there was no increase in cancer risk based on duration of exposure or cumulative exposure. Thus, this study does not indicate increased cancer risk from styrene exposure.

Page 50. *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 cohort described in the 1990 paper.

Page 57, Section 2.4.3. *The cancer mode of action discussion does not include the most recent information*

The mode of action discussion in Section 2.4.3 is based on data available before 2002. The Report briefly mentions two papers that have dealt extensively with the mode of action for styrene-induced mouse lung tumors, but present none of the findings. The following should be added:

Cruzan et al. (CERHR Ref # 37) proposed a mode of action for mouse lung tumors. In mice, but not in rats, repeated inhalation exposures resulted in toxicity to the Clara cells of the terminal bronchioles of the lung. Initially, there was increased cell proliferation and decreased staining of Clara cell cytoplasm. With continued exposure there was cell crowding in the terminal bronchioles (13 week study) with progression into hyperplasia of the terminal bronchiolar mucosa and extension into alveolar ducts over the course of the two-year study. Using Clara cell or Type II cell-enriched fractions, it was demonstrated that styrene metabolism takes place in Clara cells, the same cells where toxicity is seen, but not in alveolar cells (Hynes et al., 1999). These authors also reported that Clara cells from mice metabolize more styrene than Clara cells from rats and that mostly R-styrene oxide was produced (Hynes et al., 1999). Increased cell proliferation in the large and terminal bronchioles of mice exposed by inhalation of 40 or 160 ppm for three days, 6 hours/day, was decreased by inhibition of CYP2F2. This indicates that metabolites produced by the CYP2F pathway were important in the cytotoxicity from styrene (Green et al., 2001). It was

also demonstrated that Clara cells in mice have large amounts of both CYP2E1 and CYP2F2, while rats have much less (Green et al., 2001). The mode of action summary postulated that styrene respiratory tract toxicity and lung tumors were mediated by CYP2F-generated metabolites. The article proposed that these were either from styrene oxide (SO), particularly the R enamtiomer, or from ring-oxidized metabolites (Cruzan et al., 2002).

Toxic effects of styrene have generally been attributed to its metabolism to styrene-7,8-oxide (Bond, 1989; IARC, 2002; Gadberry et al., 1996; Cohen et al., 2002). However, styrene oxide is not much more potent as a pneumotoxicant than styrene. A single ip administration of 600 mg/kg styrene produced increased gamma-glutamyl transpeptidase and LDH activities in bronchiolar-alveolar lavage fluid; whereas, 300 mg/kg of styrene oxide was required (Gadberry et al., 1996). In comparison, pneumotoxicity has been demonstrated at 50 mg/kg for 4-vinylphenol (Carlson et al., 2002). 4-Vinylphenol is equally pneumotoxic in CYP2E1-knockout and wild-type mice (Vogie et al., 2004), providing further evidence of the role of CYP2F2. Using selected CYP inhibitors, Carlson (2004) found that several CYPs contributed to the metabolism of 4-vinylphenol in CYP2E1-knockout mice, but CYP2F2 was most important in the lung. Thus, the toxicity is not produced by 4-vinylphenol, but by a further metabolite. Taken together, these studies demonstrated that 4-vinylphenol is pneumotoxic at about 10-fold lower dose than styrene and 6-fold lower than styrene oxide following a single ip exposure, and that it produces a significantly more severe morphologic reaction in the terminal bronchioles than either styrene or styrene oxide. Intraperitoneal injections of 4-vinylphenol for two weeks produced lung, but not liver cytotoxicity in mice at 6, 20 or 60 mg/kg/day (CERHR ref #60).

The toxicity of styrene and a number of intermediate or final metabolites resulting from 3 days of ip dosing was measured by histopathology and cell proliferation in mouse lungs. Toxicity to terminal bronchioles was found only from exposure to styrene, styrene oxide, and 4-vinylphenol (BASF, personal communication, 2004); the increases in cell labeling over the control values were: 300 mg/kg/day styrene = ~75%, 300mg/kg/day styrene oxide = ~950%, 60 mg/kg/day 4-vinylphenol =~1870%. This study provides further evidence that 4-vinylphenol (or the ring-oxidized metabolite derived there from) is considerably more toxic to terminal bronchiolar cells than either styrene or styrene oxide. Effects were not seen in rats.

Sincerely,

George Cruzan, PhD, DABT

References (not included in CERHR):

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