SB LATEX COUNCIL

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July 7, 2008

Dr. Ruth M. Lunn
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Via e-mail: lunn@niehs.nih.gov

Re: Comments on NTP Draft Background Document for Styrene

Dear Dr. Lunn:

The SB Latex Council (SBLC) appreciates the opportunity to provide the attached comments on the National Toxicology Program's (NTP) "Report on Carcinogens Draft Background Document for Styrene," as announced in the May 20, 2008 *Federal Register* (73 Fed. Reg. 29139) and scheduled for peer review on July 21-22, 2008. SBLC is comprised of the major North American producers of water-based emulsions produced from styrene and butadiene, commonly referred to as SB latex. Members of the SBLC include: BASF Corporation; Mallard Creek Polymers, Inc.; OMNOVA Solutions, Inc.; and, The Dow Chemical Company.

Given the importance of this issue, we appreciate your attention to this matter.

Respectfully Submitted,

Robert J. Fensterheim
Executive Director

SB LATEX COUNCIL
COMMENTS ON NTP REPORT ON CARCINOGENS
DRAFT BACKGROUND DOCUMENT FOR STYRENE

The Report on Carcinogens Draft Background Document for Styrene contains an evaluation of the cancer studies of workers exposed to styrene, cancer studies of experimental animals exposed to styrene and mode of action analyses.

The draft document appears to have an assumption that styrene is carcinogenic and data to support that assumption are cited, but data that do not support the assumption are not cited. This approach is found in the human cancer studies, animal cancer studies, and mode of action analysis.

In the human studies, slightly higher SMRs or RRs based on few cases and not statistically significant are cited as evidence of carcinogenicity, while statistically significant decreases in cancer risk are ignored; increases attributed by study authors to other chemicals are attributed to styrene in the draft report. In rats, IARC, EU, Harvard Panel, ATSDR have all concluded styrene does not increase cancer in rats, while the NTP draft document asserts increased mammary tumors and leukemia/lymphoma.

IARC's 2002 review of styrene concluded that "The lung tumors in mice probably develop as a result of in-situ formation of styrene 7,8-oxide which causes cytotoxicity and increased cell proliferation, but the roles of circulating styrene 7,8-oxide and of DNA adducts cannot be discounted. Based on metabolic considerations, it is likely that the proposed mechanism involving metabolism of styrene to styrene 7,8-oxide in mouse Clara cells is not operative in human lungs to a biologically significant extent."

However, since 2002, a number of studies have indicated that styrene 7,8-oxide is not the causative agent.

An integration of the whole rat database does not support the conclusion of increased cancer in rats. The analysis of mode of action also picks certain data to emphasize and ignores contrary data. These comments focus on a mode of action assumption.

The 2005 US EPA Cancer Risk Assessment Guidelines assert that one should develop a hypothesis for the mode of action of tumor formation by a chemical, and test that hypothesis against the facts. The NTP Styrene draft document proposes a hypothesis for carcinogenic action of styrene: "The potential mechanisms for the carcinogenicity of styrene are related primarily to the metabolic conversion of styrene to styrene oxide."

These comments evaluate whether the review the data on styrene and styrene oxide support this hypothesis.

Hypothesis: Styrene-7,8-oxide resulting from the metabolism of styrene causes genotoxic events, leading to cancer.

Facts that support this hypothesis:

a. There is ample evidence that styrene is metabolized to styrene-7,8-oxide in liver and lung (IARC, 1994, 2002).
b. Styrene inhalation exposure in animals and humans results in circulating levels of styrene-7,8-oxide. (Cruzan et al., 1998, 2001; reviewed in IARC, 2002)
c. Low levels of SO-DNA adducts have been reported in animals and humans exposed to styrene (reviewed in IARC, NTP draft document).
d. *In vitro* genotoxicity studies of styrene-7,8-oxide are positive, including bacterial mutagenicity, chromosomal aberrations (IARC, 1994; NTP draft document).
e. Increased cytogenetic endpoints in some human studies (IARC, NTP draft document).

Facts that contradict this hypothesis

a. Genotoxic carcinogens normally cause tumors at multiple sites in multiple species. **This is not true for styrene.** The only tumor type increased in 8 rat studies and 5 mouse studies is mouse lung tumors (IARC, 2002; EU, 2007, Cohen et al., 2002).

b. **Administration of SO (up to 550 mg/kg) to mice did not result in increased lung tumors.** Lijinski et al., 1985 administered SO to rats and mice at 275 and 550 mg/kg/day. Severe necrosis of the forestomach and forestomach tumors were found. In low-dose males, there was an increase in liver tumors. There was no increase in lung tumors. Dose-response for cell proliferation parallels dose response for tumors; cell-proliferation plateaued at 200 mg/kg (Dalbey et al., 1996). Lutz and coworkers () found very low level of DNA adducts, and proposed that genotoxicity did not explain the forestomach tumors..

c. **Increased mouse lung tumors not related to level of SO in lungs.** Inhalation of styrene at 40 ppm resulted in increased mouse lung tumors (estimated SO level 4.38 nmoles/mL); gavage of SO at 550 mg/kg/day did not cause increased lung tumors (estimated SO level 5.56 nmoles/mL). Increased mouse lung tumors not related to level of SO in lungs. Sarangapani et al., 2002.

d. **Lung level of SO does not explain rat/mouse difference (Cohen, 2002).** Hofmann et al., 2005 demonstrated 8 fold higher SO in rat lung ex vivo exposed to 1000 ppm (2.05 nmole/ml) than in mouse lung exposed to 40 ppm (0.25 nmole/ml). They concluded that mouse lung tumors were not related to the presence of SO.

e. **There is no increase in DNA adducts in target tissues.** Levels of SO-DNA adducts are very low – <1 in 10^7 nucleotides and are not higher in mouse than rat or in mouse lung than mouse liver.

f. **Genotoxicity studies in mouse lung are negative**. There was no increase in chromosomal aberrations in the lungs of mice exposed to 125, 250 or 500 ppm styrene for 2 weeks (Kligerman et al, 1992). There was no increase in lung tumors after the 20 weeks observation period when styrene was administered ip for 6 weeks in a lung tumor initiation in A/J mice (Brunnemann et al., 1994).

Specific Editorial Comments

p. 219, line 13: Hofmann et al. demonstrated that rat lungs exposed to 1000 ppm styrene produced 8 times as much SO as mouse lungs exposed to 40 ppm. The conclusion from the Hofmann paper was that SO is not responsible for producing mouse lung tumors.

p. 222, line 31: The conclusion of Cohen was based on their PBPK model. Other models give different results. It is important to understand the construction of the 3 models because they largely explain the different outputs. The Cohen model assumes that all styrene metabolism takes place in the liver and that lung SO level is determined by the blood SO level. The Filser model assumes that styrene is metabolized in both the liver and the lung, but averages styrene lung metabolism over the whole lung, although only the Clara cell are capable of metabolizing styrene; this model assumes a uniform distribution of styrene in all lung cells. The Sarangapani model is based on metabolism of styrene in both the liver and specifically in the terminal bronchioles of the lung and predicts styrene oxide levels in the terminal bronchioles.

p. 226, line 4: The report states that there is some evidence that O^6 adducts build up over time. Very low levels were found after 95 weeks of exposure to 1000 ppm in rats and were not detected in mice exposed to 160 ppm for 2 weeks (Oteneder et al., year).

p. 325, line 5: Statement by Huff is inappropriate for SO: SO is not highly reactive, it has a half-life in blood in vitro of ~30 minutes. There is no indication of increases in “liver, harderian gland, and circulatory system neoplasms in mice”, “Zymbal’s gland and brain tumors in rats” or “mammary gland tumors in both rats and mice” from exposure to SO. There were only forestomach tumors in rats and mice, and liver tumors in the low dose of male mice, as stated in the next sentence. There is no reason to put this sentence in and the data indicate that SO is NOT like other epoxides described by Huff.

p. 325, line 1. This section makes an assumption that the tumorigenic activity of styrene is caused by styrene oxide. This is not supported by the scientific data. Cohen et al. conclude that differences in SO levels do not explain mouse lung tumors, Hofmann et al., conclude that mouse lung tumors are not caused by SO, Cruzan et al. (2002, 2005) indicate that CYP2F2 generated metabolites are responsible and that ring-oxidized metabolites are likely the cause.

p. 325, line 7: Lijinsky reported increased liver tumors in the low dose only in male mice exposed to styrene. There was no increase in female or in high-dose males.

p. 325, line 23: IARC concluded that lung metabolism of styrene was the likely MOA and that is not a likely MOA in humans.

p. 329, line 18: The studies of Chung et al. 2006 are basically irrelevant to the lung cytotoxicity. They were conducted in a liver transgenic cell-line that overexpresses CYP2E1. Studies by Carlson lab have shown that inhibition of CYP2E1 or genetic removal (CYP2E1 knockout mice) does not reduce the lung cytotoxicity of styrene. Green et al. (2001) demonstrated that inhibition of CYP2F2 by 5P1P prevented the cytotoxicity of styrene in mouse lung and nasal epithelium.

p. 330, line 7: The studies by Gadberry do not necessarily indicate that SO is responsible for the lung cytotoxicity of styrene. 4-vinylphenol was toxic at 5 times lower concentration than was SO. It is likely that a further metabolite of both of them is responsible for the cytotoxicity. Studies by Bartels et al., 2005 indicated that 3,4-dihydroxystyrene and 4-hydroxystyrene-7,8-oxide could be trapped with excess GSH from incubation of styrene or 4-VP using lung microsomes.

Bartels M, Rick D, Zhang F, Leibold E, Gelbke H, Cruzan G. (2005). In vitro metabolism of 4-vinylphenol and styrene in mouse, rat and human microsomes. The Toxicologist 84: abstract 1563.

p. 330, line 15: Although Cohen et al. (2002) identified CYP2E1 as important in the cytotoxicity of styrene, later studies in Carlson's lab have demonstrated that CYP2E1 does not play an important role lung cytotoxicity.

p. 330, line 27: Remember that the Cohen model assumes that all metabolism of styrene occurs in the liver and does not include lung metabolism. Thus it cannot explain mouse and rat differences because at similar doses, there is no difference in blood SO between rats and mice (Cruzan et al., 1998, 2001).

p. 331, first paragraph: the conclusions of Cohen et al. about styrene oxide lead logically to the conclusion that SO is not responsible for the cytotoxicity from styrene in mouse lung terminal bronchioles.

p. 331, end of paragraph 1: Cruzan et al., 2002 proposed a mode of action based on the available metabolic, pathologic, and tumor data. This proposed MOA was that CYP2F2 in the terminal bronchioles of mice generates metabolites that cause cytotoxicity, leading to regenerative cell proliferation, hyperplasia and eventually tumors. Additional studies since then in Carlson's lab, in Cruzan et al., 2005 and Bartels et al., 2005 have further supported this MOA. This NTP document needs to address this proposed MOA in the same manner that it addressed the discussions of the MOA by Cohen et al. The hypothesis of the MOA needs to be stated, along with a discussion of the supporting data, with a conclusion of the strengths and weaknesses.

p. 331, section 5.5.4: The genotoxicity and cytotoxicity data cited in this section do not support any conclusion on the MOA. The role of CYP2F2 metabolism needs to be addressed.

Conclusions of Others

Cohen et al., 2002: Difference in lung tumor response is not explained by differences of SO in rat and mouse lung.