SIRC Styrene Information and Research Center (SIRC)
1300 Wilson Boulevard, Suite 1200, Arlington, Virginia 22209 (703) 741-5010 Fax (703) 741-6010 Website www.styrene.org

June 3, 1999

RECEIVED
JUN 4 1999

Dr. C.W. Jameson
National Toxicology Program
79 Alexander Drive
Building 4401/Room 3127
MD: EC-14
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Re: Information Supporting the Removal of Styrene Oxide From NTP's Candidate List

Dear Dr. Jameson:

The National Toxicology Program (NTP) recently solicited comments on the nomination of styrene oxide (SO) for listing in the Report on Carcinogens, Tenth Edition. 64 Fed. Reg. 15984 (April 2, 1999); 64 Fed. Reg. 19188 (April 19, 1999). The Styrene Information and Research Center, Inc. (SIRC) is taking this opportunity to submit these comments to the NTP related to the nomination of styrene oxide. SIRC respectfully recommends, for the reasons discussed below, that styrene oxide should not be considered for listing in the Report on Carcinogens because it does not meet the NTP's criteria for listing either as "Known to be a Human Carcinogen" or "Reasonably Anticipated to be a Human Carcinogen."

SIRC is a non-profit organization formed in 1987 to explore the health effects of styrene and to act as a liaison between the styrene industry and U.S. and international regulatory agencies in disseminating the results of state-of-the-art research. SIRC's membership includes styrene manufacturers and users representing more than 95% of the industry. SIRC member companies are either directly involved in the manufacturing or processing of styrene monomer or the fabrication of consumer products from styrene derivatives.

Styrene oxide has been reported to increase the incidence of forestomach tumors in both rat and mouse chronic studies when administered by gavage. No other increases in tumors have been reported. Cell damage and repair, and increased cell proliferation with marginally detectable DNA adducts have been reported to occur in the forestomachs of rats after being given oral doses of SO. It is likely that SO causes tumors in the forestomach of rodents by a mode-of-action that is mainly the result of increased cell turnover as a result of the cellular damage caused by high concentrations of SO. For this reason, as described in more detail below, it appears that SO is not an appropriate candidate for listing.
For a substance to be listed as "Known to be a Human Carcinogen," NTP requires "sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between the substance and human cancer." For a substance to be listed as "Reasonably Anticipated to be a Human Carcinogen," three alternative criteria may apply: (1) if there is limited evidence of carcinogenicity

from studies in humans, which indicates that causal interpretation is credible, but alternative explanations such as chance, bias or confounding factors cannot adequately be excluded; (2) if there is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (a) in multiple species, or at multiple tissue sites, or (b) by multiple routes of exposure, or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or (3) there is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however, the substance belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

In its criteria for listing, NTP has stated that "conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information." SIRC believes that the forestomach tumors observed in rodents given high oral doses of styrene oxide are the result of tissue damage at the site of contact, and are not relevant to humans given the high concentrations of styrene oxide needed to induce tumors.

If NTP concurs with SIRC's own conclusions regarding these results, then styrene oxide would not meet criterion (2) for listing as "Reasonably Anticipated to be a Human Carcinogen." The NTP review committee may wish to consider the following data when evaluating SO for listing in the Report on Carcinogens. F344 rats receiving 250 and 500 mg/kg of SO in corn oil three times per week for 104 weeks *via* oral gavage developed squamous cell carcinomas and papillomas of the forestomach (Lijinsky, 1986). Less than 10% of the carcinomas metastisized to the liver and other organs. Conti et al. (1988) gave Sprague-Dawley rats either 50 or 250 mg/kg of SO in olive oil 4 to 5 days per week for 52 weeks *via* oral gavage and then maintained the animals until death. The rats developed simple hyperplasia, squamous cell dysplasia, acanthomas, papillomas, and invasive squamous cell carcinomas of the forestomach. Ponomarkov et al. (1984) orally dosed female BDIV pregnant rats with 200 mg/kg SO in olive oil on Gestational Day 17. Their offspring received 100-150 mg/kg of SO once a week for 96 weeks by gavage and developed hyperkeratosis, hyperplasia, dysplasia, carcinomas, and papillomas of the forestomach. Although SO causes forestomach tumors when given orally, dermal administration in C3H or Swiss-Mullerton mice has not caused tumors (Weil, 1963; Van Duuren et al., 1963) in the forestomach or other sites.

Criterion (3) for listing as "Reasonably Anticipated" would appear to rely on the genotoxic activity of SO. Whereas the genotoxicity of SO *in vitro* has been well documented, *in vivo* tests have been largely negative. When BALB/c male mice were dosed intraperitoneally (250 mg/kg), results were negative on the induction of chromosomal aberrations in bone marrow cells, on the production of micronuclei in polychromatic erythrocytes, on the spermatocyte test (examination for the presence of reciprocal translocations), and on the dominant lethal test (Fabrey et al., 1978). Chinese hamsters receiving a single intraperitoneal dose of 250 mg/kg SO did not have increased chromosomal aberrations or number of micronuclei in the bone marrow (Pentilla et al., 1980). Inhalation exposure to SO at 25, 50, 75, and 100 ppm had no effect on chromosomal aberrations or SCE frequencies in Chinese hamsters

(Norppa et al., 1979). SO injected into male NMRI mice (ip, 216-841 mg/kg) did not induce single-strand breaks in the DNA of liver, lung, kidney, testis, or brain (Walles and Orsen, 1983). Thus, SO has weak (if any) genotoxic activity *in vivo* and criterion (3) for listing as "Reasonably Anticipated to be a Human Carcinogen" would not appear to apply to SO.

Cell proliferation was reported in the rat forestomach following oral administration of SO three times per week for four weeks at doses of 137, 275, and 550 mg/kg (Cantoreggi et al., 1993). Subsequently, another study demonstrated increased cell proliferation from oral gavage administration of 50 mg/kg or higher SO, with a plateau above 250 mg/kg and a NOEL of 20 mg/kg (Dalbey et al., 1996). No adducts were reported in forestomach DNA in F344 rats exposed to 240 mg/kg SO by gavage, with a limit of detection of approximately 1 in 10^7 nucleotides (Cantoreggi and Lutz, 1992). By pooling DNA from six rats which improved the limit of detection, they were just able to detect DNA adducts at a level of 0.4 in 10^7 nucleotides in forestomach but not in liver, indicating a very low level of binding (Lutz et al., 1993).

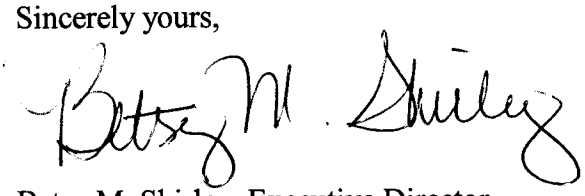
Finally, SIRC is not aware of human studies involving styrene oxide that are relevant to NTP's criterion for listing as "Known to be a Human Carcinogen" or for criterion (1) for listing as "Reasonably Anticipated to be a Human Carcinogen."

Based on the available evidence in animals summarized above, SIRC believes that it would not be appropriate for styrene oxide to be listed in the NTP Report on Carcinogens. SIRC respectfully requests that NTP consider this information and make a formal recommendation not to go forward with the nomination and listing of styrene oxide.

References for the studies discussed in SIRC's comments are provided below. Should you have any questions or require further information, please do not hesitate to contact us.

Thank you for your consideration.

Sincerely yours,



Betsy M. Shirley, Executive Director
Styrene Information and Research Center, Inc.

References

Cantoreggi, S., and Lutz, W.K. (1992) Investigation of the covalent binding of styrene-7,8-oxide to DNA in rat and mouse. Carcinogenesis 13: 193-197.

Cantoreggi, S., Dietrich, D.R., and Lutz, W.K. (1993) Induction of cell proliferation in the forestomach of F344 rats following subchronic administration of styrene-7,8-oxide and butylated hydroxyanisole. Cancer Res. 53: 3505-3508.

Conti, B., Maltoni, C., Perino, G., and Gilberti, A. (1988) Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats and para-methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice. Ann. N.Y. Acad. Sci. 534: 203-234.

Dalbey, W.E., Rodriguez, S.C., Cope, C., and Cruzan, G. (1996) Cell proliferation in rat forestomach following oral administration of styrene oxide. Fundam. Appl. Toxicol. 30: 67-74.

Fabrey, L., Leonard, A., and Roberfroid, M. (1978) Mutagenicity tests with styrene oxide in mammals. Mutat. Res. 51: 377-381.

Lijinsky, W. (1986) Rat and mouse forestomach tumors induced by chronic oral administration of styrene oxide. J. Natl. Cancer Inst. 77: 471-476.

Lutz, W.K., Cantoreggi, S., and Velic, I. (1993) DNA binding and stimulation of cell division in the carcinogenicity of styrene 7,8-oxide. In: Butadiene and Styrene: Assessment of Health Hazards, Sorsa, M., Peltonen, K., Vaino, H., and Hemminki, K., IARC Scientific Publication No. 127, pp. 245-252, International Agency for Research on Cancer, Lyon.

Norppa, H., Elovaara, E., Husgafvel-Pursianen, K., Sorsa, M., and Vaino, H. (1979) Effects of styrene oxide on chromosomal aberrations, sister chromatid exchange and hepatic drug biotransformation in Chinese hamsters *in vivo*. Chem.-Biol. Interact. 26: 305-315.

Pentilla, M., Sorsa, N., Vaino, H. (1980) Inability of styrene to induce nondisjunction in Drosophila or a positive micronucleus test in the Chinese hamster. Toxicol. Lett. 6: 119-123.

Ponomarkov, V., Cabral, J.R.P., Wahrendorf, J., and Galendo, D. (1984) A carcinogenicity study of styrene-7,8-oxide in rats. Cancer Lett. 24: 95-101.

Van Duuren, B.L., Nelson, N., Orris, L., Palmes, E.D., and Schmitt, F.L. (1963) Carcinogenicity of epoxides, lactones, and peroxy compounds. J. Natl. Cancer Inst. 31: 41-55.

Walles, S.A.S., and Orsen, Il (1983) Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide. Cancer Lett. 21: 9-15.

Weil, C.S., Condra, N., Haun, C., and Striegel, J.A. (1963) Experimental carcinogenicity and acute toxicity of representative epoxides. Am. Ind. Hyg. Assoc. J. 24: 305-325.