Styrene Expert Panel Report

Part B — Recommendation for listing status for "styrene" in the Report on Carcinogens and scientific justification for the recommendation

The Report on Carcinogens (RoC) expert panel for styrene met at the Radisson Hotel, Durham, North Carolina on July 21-22, 2008, to peer review the draft background document on styrene and make a recommendation for the listing status in the 12th Edition of the RoC. Members of the expert panel are as follows:

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The recommendation for listing status and scientific justification for the recommendation follow this page.

Overall Evaluation

Following a discussion of the draft document, together with additional recommended inclusions and some re-analysis of published data, the expert panel reviewed the RoC listing criteria and made its recommendation for the listing status of styrene in the RoC. The expert panel recommended by a vote of 8 yes/2 no that styrene should be listed in the RoC as *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity in humans and sufficient evidence in animals. The two members of the panel who voted against the motion did so because, in their opinion, styrene should be listed as *known to be a human carcinogen*.

The major considerations discussed by the panel that led to its majority recommendation included: (1) evidence of past and present human exposure to styrene in the United States; (2) evidence of cancer in styrene-exposed workers; (3) induction of lung tumors in mice by styrene by two routes of exposure; (4) the established carcinogenicity in animals and genotoxicity of a styrene metabolite, the 7,8-oxide; (5) evidence for styrene-related DNA adducts and cytogenetic effects in styrene-exposed workers.

This evidence is described in further detail below.

Human Exposure

There is more than sufficient data to show that humans are exposed to styrene by inhalation, skin exposure, and oral absorption in such situations as the workplace (inhalation and skin exposure predominantly), food and drinking water (oral ingestion), and in the ambient environment (levels found in ambient air, surface and groundwater, and soils) in the United States.

Human Cancer Studies

The expert panel concluded that there was limited evidence for the carcinogenicity of styrene in humans, although some members were of the opinion that the epidemiological data provided sufficient evidence. The strongest evidence for cancer in humans is the association between styrene exposure and non-Hodgkin lymphoma (NHL). This evidence comes from the Delzell *et al.* (2006) analysis in the styrene-butadiene industry and the Kogevinas (1994a) study in the reinforced plastics industry. In the Delzell study there was an exposure-response relationship for NHL and NHL plus chronic lymphocytic leukemia (CLL) that was not attenuated by control for butadiene and only mildly attenuated by control for dimethyldithiocarbamate (DMDTC) (which may not have been appropriate to control for). It is very unlikely that such a strong exposure-response trend could be due to chance, bias, or confounding. These findings are supported by increases in RR for all lymphomas with time since first exposure and estimated average exposure in the multiplant-cohort studied by Kogevinas. Many of the smaller studies found excesses in lymphohematopietic cancers, although these were not statistically significant. They provide further evidence of consistency in the risk. In addition, there is some evidence for increased risk of pancreatic and esophageal cancer. It should be noted that increased risk of solid tumors was not evaluated in many studies. Studies in workers have provided evidence for DNA adducts and chromosomal aberrations in lymphocytes of styrene-exposed workers which supports a genotoxic mechanism for styrene.

Studies on Experimental Animals

Evidence on the carcinogenicity of styrene comes from a number of studies in rats and mice. There are robust studies in male and female mice and rats by oral gavage (NCI 1979a) and inhalation (Cruzan *et al.* 2001, Cruzan *et al.* 1998) routes, along with several other studies more limited in their ability to detect carcinogenic effects because of study design (low doses, short treatment, short study duration, small group size), high early mortality, or limited reporting (tumor diagnosis) (Beliles *et al.* 1985, Brunnemann *et al.* 1992, Conti *et al.* 1988, Jersey *et al.* 1978, Ponomarkov and Tomatis 1978).

Sufficient evidence of carcinogenic activity comes from multiple studies in mice exposed to styrene by multiple routes. Styrene induced benign and malignant lung tumors in male and female mice by inhalation (Cruzan *et al.* 2001) and in male mice by oral intubation (NCI 1979a). This is supported by findings of lung tumors in both sexes of mice in studies of more limited design (Ponomarkov and Tomatis 1978). There is also the finding in rats of malignant mammary tumors by inhalation (Conti *et al.* 1988) and a small increase in mammary fibroadenoma in a relatively low dose drinking water study (Beliles *et al.* 1985), but mammary tumors were not increased in an adequate inhalation study in the same rat strain exposed for two years (Cruzan *et al.* 1998), limiting the weight given to the mammary tumor findings. In earlier reviews (IARC 1994b, NTP 2002), sufficient evidence in animals was found for the carcinogenicity of styrene-7,8-oxide.

Mechanistic Concerns

There are at least two reasonable, literature-supported, and scientifically valid mechanisms for styrene-induced cancer. These mechanisms are not mutually exclusive. These mechanisms supported the panel's decision to recommend listing styrene in the Report on Carcinogens.

- Styrene is genotoxic to human lymphocytes through sister chromatid exchange processes and chromosomal aberrations (e.g., micronuclei and aneuploidy). Styrene is mutagenic through formation of styrene-7,8-oxide. Cyp2f2 in mouse lung cells efficiently oxidizes styrene to styrene-7,8-oxide. In addition, CYP2F1, CYP2A13, and CYP2S1 are expressed in many extrahepatic organs, and their known catalytic activity with styrene supports a plausible mechanism by which styrene is bioactivated to styrene oxide in many human tissues. Styrene-7,8-oxide forms multiple DNA adducts, primarily O^6 and N7 of guanine, in cultured human lymphocytes and embryonal lung fibroblasts. In addition, lymphocytes from styrene-exposed workers possess the same DNA adducts.

- Styrene bioactivation to the 7,8-epoxide or 3,4-epoxide is cytotoxic to mouse lung cells. Specifically, the 3,4-epoxide could form 4-vinylphenol which could be oxidized to dihydrodiols and quinones by P450 enzymes and produce cytotoxicity through reactive oxygen mechanisms. Subsequent cellular proliferation and clonal expansion would then constitute an epigenetic mechanism. Recent evidence suggests that mice may be especially sensitive to lung carcinogenesis through this mechanism, and it may be operative in humans as well.

Organ Selectivity

- Styrene is potentially carcinogenic to lung tissues because sufficient evidence exists in animals for bioactivation to the mutagenic epoxide, and tumors are produced within the same sites where P450s are expressed, and DNA adducts are also identified.

- Support for a mechanism of mammary tissue as a site includes limited human evidence for elevated prolactin levels from styrene-exposed workers.

- Styrene produces lymphohematopoetic cancer in humans, and this effect has the strongest epidemiological support. However, bioactivation of styrene to styrene-7,8-oxide by cytochrome P450 enzymes in blood cells has not been reported. Thus, carcinogenic mechanisms for lymphomas would likely depend on direct interactions of preformed styrene-7,8-oxide in blood cells.

Report Approved Redacted

David Phillips, Ph.D. (Chair)

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Date

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