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Based on an analysis of mode of action, styrene-induced mouse lung tumors are not a human cancer concern

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ABSTRACT

Based on 13 chronic studies, styrene exposure causes lung tumors in mice, but no tumor increases in other organs in mice or rats. Extensive research into the mode of action demonstrates the key events and human relevance. Key events are: metabolism of styrene by CYP2F2 in mouse lung club cells to ring-oxidized metabolites; changes in gene expression for metabolism of lipids and lipoproteins, cell cycle and mitotic M-M/G1 phases; cytotoxicity and mitogenesis in club cells; and progression to preneoplastic/neoplastic lesions in lung. Although styrene-7,8-oxide (SO) is a common genotoxic styrene metabolite in in vitro studies, the data clearly demonstrate that SO is not the proximate toxicant and that styrene does not induce a genotoxic mode of action. Based on complete attenuation of styrene short-term and chronic toxicity in CYP2F2 knockout mice and similar attenuation in CYP2F1 (humanized) transgenic mice, limited metabolism of styrene in human lung by CYP2F1, 2 + orders of magnitude lower SO levels in human lung compared to mouse lung, and lack of styrene-related increase in lung cancer in humans, styrene does not present a risk of cancer to humans.

1. Introduction

The relationship of styrene toxicity and cancer has been reviewed previously based on data that did not include more recent updates of the styrene epidemiology cohorts and animal mode of action studies (IARC, 2002; ATSDR, 2010; NAS, 2014). These recent data can now inform a current assessment of the mouse lung tumor MOA and its quantitative and/or qualitative relevance to human cancer risks.

2. Background: styrene toxicity and metabolism

1. The only tumorigenic response in rats and mice to styrene exposure is mouse lung tumors.

There are 8 chronic studies of styrene in rats (Table 1, reviewed in Cruzan et al., 2002). There are no increases in lung tumors or consistent increases in tumors at other sites. Malignant mammary tumors were increased in one inhalation study in Sprague-Dawley rats (Conti et al., 1988), but not in a gavage study conducted at similar doses at the same laboratory at the same time in the same strain. No increase in mammary tumors was reported in 3 other gavage studies (F344 or BDIV rats) or 1 drinking water study in S-D rats. Malignant mammary tumors were found at 600 ppm styrene by inhalation (within historical control range), but not at 1000 ppm (Jersey et al., 1978) in S-D rats. No increase in mammary tumors occurred from exposure to 50 or 200 ppm styrene by inhalation in S-D rats (Cruzan et al., 1998) and a dose-related decrease in malignant mammary tumors was found at 500 and 1000 ppm. Mammary tumors are extremely common in rats, especially the S-D strain. Thus the weight of the evidence indicates that styrene does not induce mammary gland tumors.

In contrast to rats, multiple studies in mice indicate that lung is the only cancer target organ following either oral or inhalation exposures. There are 4 long-term gavage studies of styrene in mice and 1 by inhalation (Table 2). Oral gavage of 300 mg/kg/day styrene in corn oil 5 days/week for 78 weeks, with additional observation to week 91, resulted in increased lung tumors in male, but not female, B6C3F1 mice

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Table 1
Long-term studies of styrene in rats.

Study	Strain	Route	Doses	Tumor Result
Ponomarkov and Tomatis, 1978	BDIV	Gavage	500 m/k/wk	No increase
Jersey et al., 1978	SD	Inhalation	600, 1000 ppm	Increased mammary at 600, not 1000, within historical control range
NCI, 1979a	F344	Gavage	500, 1000, 2000 m/k/d	No increase
NCI, 1979b	F344	Gavage (mix with nitroS)	175, 350 m/k/d 3x/wk	No increase
Beliles et al., 1985	SD	Water	125, 250 ppm	Increased fibroadenoma, not malignant mammary tumors
Conti et al., 1988	SD	Gavage	50, 250 mkd 12 mo, obs, to death	No increase
Conti et al., 1988	SD	Inhalation	25, 50, 100, 200, 300 ppm 12 mo, obs to death	Increased malignant mammary tumors all groups
Cruzan et al., 1998	SD	Inhalation	50, 200, 500, 1000 ppm	Decreased malignant mammary tumors 500 and 1000 ppm

nitroS = 30% b-nitrostyrene,70% styrene; doses are styrene administered as part of mixture. Taken from Cruzan et al. (2002.

Table 2
Long-term studies of styrene in mice.

Study	Strain	Route	Dose	Tumor Result
NCI, 1979a	B6C3F1	Gavage	150, 300 mkd, 5 d/wk	Increased benign lung tumors high dose males, within historical control
NCI, 1979b	B6C3F1	Gavage	204, 408 mkd 3x/wk w/nitroStyrene	No increase in tumors
Ponomarkov and Tomatis, 1978	C57	Gavage	300 m/k 1x/week	No increase in tumors
Ponomarkov and Tomatis, 1978	O20	Gavage	1350 m/k 1x/week for 16 wk, obs to 104	50% mortality by week 16; increased lung tumors in survivors
Cruzan et al., 2001	CD-1	Inhalation	0, 20, 40, 80, 160 ppm for 104 wk	Increased lung tumors at 40–160 ppm males, 20, 40, 160 ppm females

Taken from Cruzan et al. (2002).

(NCI, 1979a). The evidence of lung cancer was considered “Suggestive” by the NCI review panel. Oral gavage of 0, 204, or 408 mg/kg/dose of 30% β-nitrostyrene/70% styrene 3 days/week for 78 weeks with observation to week 91 (styrene dose adjusted to 5 days/week = 0, 122 or 244 mg/kg/day) did not result in increased lung tumors (NCI, 1979b). When styrene was administered by gavage at 1400 mg/kg/dose on day 17 of gestation and once weekly to offspring of O20 mice, there was severe lung congestion and 50% mortality by week 16, when dosing was stopped. Survivors were observed until week 120. Increased lung tumors compared to solvent controls, but not untreated controls were found (Ponomarkov and Tomatis, 1978). A similar study of styrene in C56BL/6 mice dosed at 300 mg/kg/dose did not find lung toxicity or tumors (Ponomarkov and Tomatis, 1978). None of these studies evaluated the development of proliferative lesions during the course of the exposure.

In the inhalation study, there were increased lung tumors in male CD-1 mice at 40, 80 and 160 ppm and at 20, 40, and 160 ppm (not at 80 ppm) in females (6 h/day, 5 days/week, 104 weeks). These were mostly benign and the increase was not seen until after 18 months of exposure (Cruzan et al., 2001). Tumors in these studies were found in the periphery of the lung, which encompasses areas of terminal bronchioles and alveoli. Because the origin of the tumors cannot be determined by cellular anatomy or location, these are referred to as bronchioloalveolar adenomas or adenocarcinomas.

2. There are species and organ differences in styrene metabolism.

Many metabolites of styrene have been identified in various organs or urine. These can be collated into 4 general pathways: 1. Styrene-7,8-oxide (SO) followed by epoxide hydrolase to mandelic acid; 2. Styrene-7,8-oxide followed by GSH transferases resulting in conjugation and progression to mercapturic acids. 3. Oxidation of vinyl side chain to phenylacetaldehyde (or rearrangement from SO) and further oxidation; and 4. Oxidation of the aromatic ring, with or without vinyl side chain oxidation. Differences in the relative contribution of each pathway exist among rat, mouse and human. The relative contribution of each pathway to urinary metabolites of styrene is shown in Table 3. (Cruzan et al., 2002).

Styrene metabolism to SO is the primary pathway for removal of styrene from animals, including humans. SO formation is catalyzed primarily by CYP2E1 in all species; however, other CYPs may also be involved, but such alternative CYP-oxidations to SO may be species- and organ-dependent. For example, in CYP2E1-knockout mice (KO), SO is still produced in liver and lung. Shen et al. (2010) reported a 44% decrease in styrene glycol in liver microsomes of CYP2E1-KO mice compared to wild-type mice and no reduction in lung microsomes. In contrast, they reported a 25% decrease in styrene glycol in microsomes from the livers of CYP2F2-KO mice and a 64% decrease in lung microsomes of the CYP2F2-KO mice. The SO-EH pathway accounts for > 95% of styrene metabolism in humans (Cruzan et al., 2002). The relative roles of CYP2E1 and CYP2F2 in the mouse-specific lung tumor MOA will be discussed later.

Table 3
Styrene metabolic pathways based on urinary metabolites.

Species	SO-EH (%)	SO-GSH (%)	PAA (%)	Ring (%)
B6C3F1 mouse	49–52	33–35	12–17	4–8
CD-1 mouse	51–59	20–27	21–22	4–8
F344 rat	68–72	23–26	3–5	< 1
Human (2–4 h)	95	ND	5	ND
Human (4–9 h)	100	ND	ND	ND

ND = not detected.
SO-EH = epoxide hydrolase reaction with SO, leading to mandelic acid, etc. SO-GSH = GSH conjugation products of SO. PAA = phenylacetaldehyde and phenylacetic acid (may or may not involve SO). Ring = hydroxylation of benzene ring (may also include side chain oxidation). Taken from Cruzan et al. (2002).

Ring-oxidized metabolites of styrene include 2-hydroxystyrene, 3-hydroxystyrene, 4-hydroxystyrene (2-, 3-, or 4-vinylphenol), 3,4-dihydroxystyrene (4-vinylphenol catechol) and 4-hydroxystyrene-7,8-oxide (4-(2-oxiranyl)-1, 2-benzenediol) (Shen et al., 2010; Zhang et al., 2011). There was an > 80% reduction in formation of these metabolites by inhibition of CYP2F2 by 5-phenyl-1-pentyne (5P1P) (Zhang et al., 2011) and complete attenuation of 2-, 3- and 4-vinylphenol formation in lung microsomes from CYP2F2-KO mice (Shen et al., 2014). The role of ring-oxidized metabolites in the mouse lung tumor MOA will be discussed later.

A framework for analyzing modes of action/human relevance has been proposed and updated (Meek et al., 2013, 2014). The induction of