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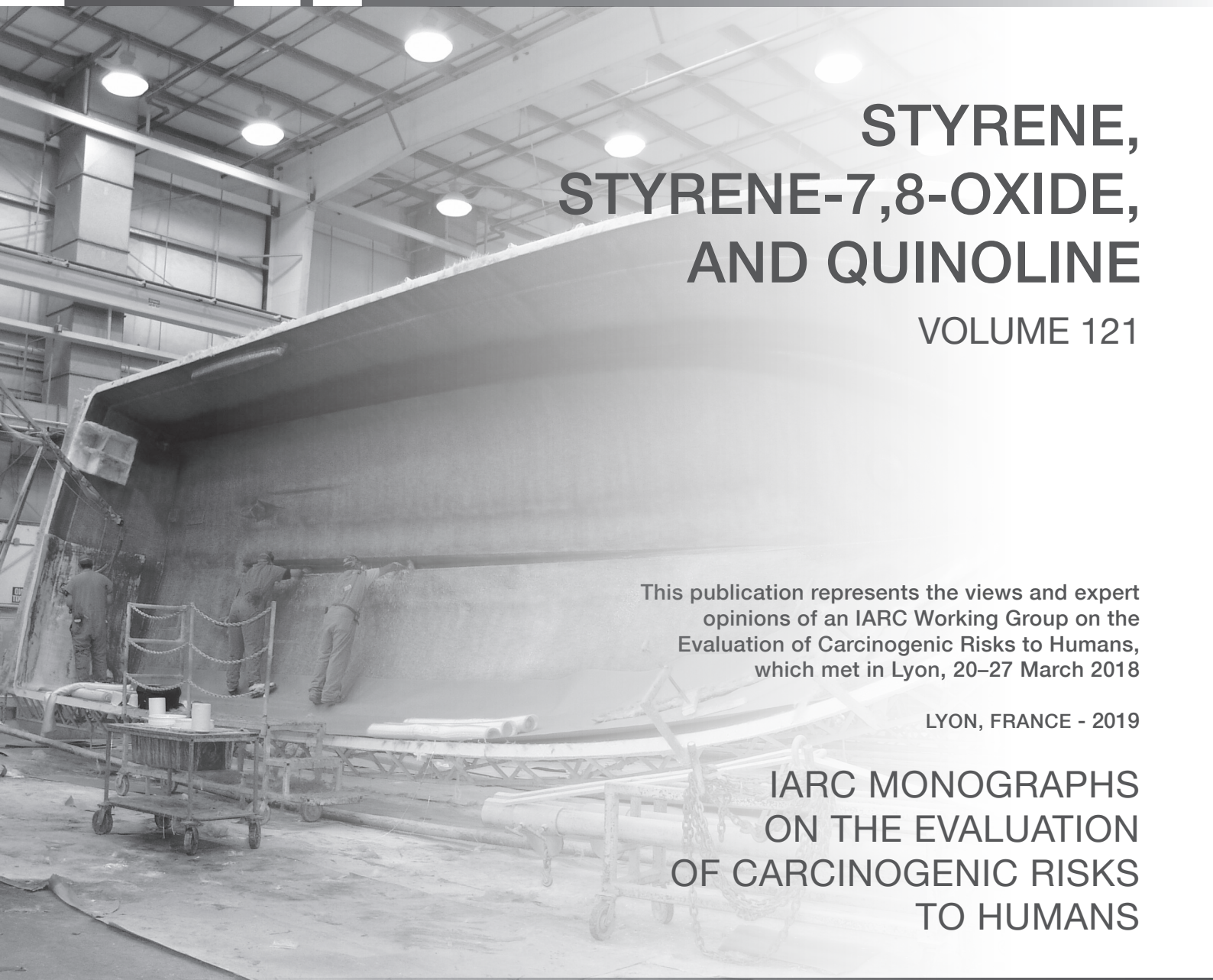

STYRENE, STYRENE-7,8-OXIDE, AND QUINOLINE

VOLUME 121

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 20–27 March 2018

LYON, FRANCE - 2019

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

2005). The Working Group concluded that there was no convincing evidence of an association.

2.4.8 Prostate

Two of the smaller studies – the case–control study reporting incidence of cancer of the prostate (Gérin et al., 1998) and the United States boatbuilding facility study assessing mortality from cancer of the prostate (Bertke et al., 2018) – showed positive associations, but the larger Danish and European studies of reinforced plastics workers found no positive association with incidence or mortality (Kogevinas et al., 1994; Christensen et al., 2017; Loomis et al., 2019).

2.4.9 Other cancers

Several other cancers were investigated in relation to styrene exposure, including cancers of the colon, rectum, stomach, pancreas, larynx, pharynx, brain, and central nervous system (including childhood cancers), and melanoma. However, the Working Group concluded that no reliable conclusions could be made either because of the small number of studies reporting results, inconsistencies in the findings, or the use of weak method(s) for assessing exposure to styrene.

3. Cancer in Experimental Animals

3.1 Styrene

3.1.1 Mouse

See Table 3.1.

(a) Transplacental exposure and oral administration (by gavage)

Female O20 or C57BL mice were exposed by gavage to a single dose of styrene (purity, 99%) at 0 (vehicle), 300 (C57BL), or 1350 (O20) mg/kg body weight (bw) in olive oil on gestational day 17 (Ponomarkov & Tomatis, 1978). Male and female progeny were then similarly exposed once per week from weaning for 16 weeks for O20 mice (dosing was stopped at 16 weeks due to toxicity, instead of occurring over the lifespan of the progeny as originally intended) or 120 weeks for C57BL mice. All surviving mice were killed at experimental week 120, although very few O20 mice survived to the end of study. For O20 mice, 6/47 female untreated controls, 0/9 female dams treated with olive oil only, 0/22 female progeny treated with olive oil only, 0/29 female dams exposed to styrene, 0/39 female progeny exposed to styrene, 7/54 male untreated controls, 0/20 male progeny treated with olive oil only, and 0/45 male progeny exposed to styrene survived until week 120. The effective number of animals used for tumour evaluation was the number of survivors in all groups at the time of the first tumour. In male O20 mice progeny, there was a significantly increased incidence of adenoma or adenocarcinoma (combined) of the lung after exposure to styrene compared with male mice progeny given olive oil only ($P < 0.01$). In female O20 mice progeny, there was a significantly increased incidence of adenoma or adenocarcinoma (combined) of the lung after exposure to styrene compared with female mice progeny given olive oil only ($P < 0.01$) and compared with untreated female controls ($P < 0.001$). There was also a significantly increased incidence of adenocarcinoma of the lung in female mice progeny after exposure to styrene compared with female mice progeny given olive oil only [$P < 0.01$]. Tumours appeared at or before week 57 in male O20 mice or week 65 in female O20 mice. For C57BL mice, 19/49 female untreated controls, 3/5 female dams given olive oil only, 4/13 female progeny given olive oil only, 4/15 female dams exposed to styrene, 12/27 female progeny exposed to styrene, 33/51 male untreated controls, 7/12 male progeny given olive oil alone, and 15/27 male progeny exposed to styrene survived until week 120. There were no significant differences in the incidence of tumours of the lung in

Table 3.1 Studies of carcinogenicity in mice exposed to styrene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, O20 (M) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, progeny), 1350 (progeny) mg/kg bw 1× on GND 17 (to dams) then 1×/wk to progeny beginning at weaning for 16 wk 54, 20, 45 7, 0, 0	*Lung* Adenoma or adenocarcinoma (combined) 34/53 (64.2%), 8/19 (42.1%), 20/23 (87.0%)* Adenocarcinoma 12/53 (22.6%), 4/19 (21.1%), 8/23 (34.8%) Adenoma 22/53 (41.5%), 4/19 (21.1%), 12/23 (52.2%)	*$P < 0.01$ vs vehicle control [NS] [NS]	Principal limitations: not chronic exposure (16 wk only); poor survival; high/toxic dose (thus stopped at 16 wk) The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed; the first lung tumour appeared at or before week 57 for all groups; tumours appeared earlier in styrene-treated male progeny (average age, 48.8 wk) compared with other groups
Full carcinogenicity Mouse, O20 (F) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, dams), 0 (vehicle, progeny), 1350 (dams), 1350 (progeny) mg/kg bw 1× on GND 17 (to dams) then 1×/wk to progeny beginning at weaning for 16 wk 47, 9, 22, 29, 39 6, 0, 0, 0, 0	*Lung* Adenoma or adenocarcinoma (combined) 25/47 (53.2%), 5/8 (62.5%), 14/21 (66.7%), 11/20 (55%), 32/32 (100%)* Adenocarcinoma 14/47 (29.8%), 4/8 (50.0%), 4/21 (19.0%), 7/20 (35.0%), 18/32 (56.2%)* Adenoma 11/47 (23.4%), 1/8 (12.5%), 10/21 (47.6%), 4/20 (20.0%), 14/32 (43.8%)	*$P < 0.01$ vs vehicle control (progeny), $P < 0.001$ vs untreated control *[$P < 0.01$ vs vehicle control (progeny)] [NS]	Principal imitations: not chronic exposure (16 wk only); compared progeny with untreated (adult) controls; poor survival; high/toxic dose (thus stopped at 16 wk); low number of animals for vehicle control female dams The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed; the first lung tumour appeared at or before week 65 for all groups; tumours appeared earlier in styrene-treated female progeny (average age, 57.8 wk) compared with the other groups

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, C57BL (M) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, progeny), 300 (progeny) mg/kg bw 1× on GND 17 (to dams) then 1×/wk to progeny beginning at weaning for life 51, 12, 27 33, 7, 15	*Lung*: tumour (unspecified) 5/47 (10.6%), 3/12 (25.0%), 1/24 (4.2%)	NS	Principal limitations: poor survival (but better than O20 strain); low starting number of animals for all treated-groups The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed
Full carcinogenicity Mouse, C57BL (F) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil (0.1 mL) 0 (untreated), 0 (vehicle, dams), 0 (vehicle, progeny), 300 (dams), 300 (progeny) mg/kg bw Once on GND 17 (to dams) then once weekly to progeny beginning at weaning for life 49, 5, 13, 15, 27 19, 3, 4, 4, 12	*Lung*: tumour (unspecified) 1/47 (2.1%), 0/5, 1/13 (7.7%), 1/12 (8.3%), 1/24 (4.2%)	NS	Principal limitations: compared progeny with untreated (adult) controls; low number of animals for vehicle control female dams; poor survival (but better than O20 strain); low starting number of animals for all treated-groups The effective number of animals (denominator) is the number of survivors at the time the first tumour was observed
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 91 wk NTP (1979a)	Gavage Styrene, > 99% Corn oil 0 (vehicle control), 150, 300 mg/kg bw, 5 d/wk for 78 wk plus 13-wk observation phase (no exposure) 20, 50, 50 20, 46, 39	*Lung* Bronchioloalveolar adenoma or carcinoma (combined) 0/20*, 6/44, 9/43** Bronchioloalveolar carcinoma 0/20, 3/44, 5/43	*P = 0.023 (Cochran–Armitage trend test) **P = 0.024 (vs control, Fisher exact test) NS	Principal limitations: smaller number of controls compared with treated groups

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 91 wk NTP (1979a)	Gavage Styrene, > 99% Corn oil 0 (vehicle control), 150, 300 mg/kg bw, 5 d/wk for 78 wk plus 13-wk observation phase (no exposure) 20, 50, 50 18, 40, 38	*Liver*: hepatocellular adenoma 0/20*, 1/44, 5/43	*P = 0.034 (Cochran–Armitage trend test)	Principal limitations: smaller number of controls compared with treated groups
Full carcinogenicity Mouse, B6C3F$_1$ (M) 6 wk 92 wk NTP (1979b)	Gavage Styrene/β-nitrostyrene solution, NR Corn oil 0 (vehicle control), 87.5, 175 mg/kg bw, 3 d/wk for 78 wk plus 14-wk observation phase (no exposure) 20, 50, 50 18, 43, 33	*Lung* Bronchioloalveolar adenoma or carcinoma (combined) 0/20, 11/49*, 2/36 Bronchioloalveolar carcinoma 0/20, 3/49, 1/36	*P = 0.016 (Fisher exact test) NS	Principal limitations: no dose–response in males (low dose effect only); purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk
Full carcinogenicity Mouse, B6C3F$_1$ (F) 6 wk 92 wk NTP (1979b)	Gavage Styrene/β-nitrostyrene solution, NR Corn oil 0 (vehicle control), 87.5, 175 mg/kg bw, 3 d/wk for 78 wk plus 14-wk observation phase (no exposure) 20, 50, 50 17, 47, 38	Any tumour type: no significant increase		Principal limitations: purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (M) 4 wk 104 wk Cruzan et al. (2001), OEHHA (2010)	Inhalation (whole-body exposure) Styrene, > 99.5% Air 0, 20, 40, 80, 160 ppm, 6 h/d, 5 d/wk 50, 50, 50, 50, 50 36, 27, 27, 37, 33	*Lung* Bronchioloalveolar adenoma 15/50*, 21/50, 35/50**, 30/50***, 33/50**	*[$P < 0.001$ (Cochran–Armitage trend-test)], **[$P < 0.001$ (Fisher exact test)], ***[$P = 0.005$ (Fisher exact test)]	Principal strengths: GLP study Male mice exposed to styrene at 80 and 160 ppm had decreased body weights over the course of the study
		Bronchioloalveolar carcinoma 4/50, 5/50, 3/50, 6/50, 7/50	NS	
		Bronchioloalveolar adenoma or carcinoma (combined) 17/50*, 24/50, 36/50**, 30/50**, 36/50**	*$P < 0.001$ (Cochran–Armitage trend test), **$P < 0.01$ (Fisher exact test)	
		All tumours Total tumours: 24, 32, 62, 62, 68	NR	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, CD-1 (F) 4 wk 98 wk Cruzan et al. (2001), OEHHA (2010)	Inhalation (whole-body exposure) Styrene, > 99.5% Air 0, 20, 40, 80, 160 ppm, 6 h/d, 5 d/wk 50, 50, 50, 50, 50 27, 32, 33, 34, 35	*Lung* Bronchioloalveolar adenoma 6/50*, 16/50**, 16/50**, 11/50, 24/50***	*[$P = 0.001$ (Cochran–Armitage trend test)], **[$P = 0.028$ (Fisher exact test)], ***[$P < 0.001$ (Fisher exact test)]	Principal strengths: GLP study Principal limitations: lower survival of controls vs treated Due to mortality in control females (23/50 mice), the surviving females were killed 6 wk earlier than originally scheduled; all four treated groups had greater survival than the controls; female mice exposed to styrene at 160 ppm had decreased body weights over the course of the study
		Bronchioloalveolar carcinoma 0/50*, 0/50, 2/50, 0/50, 7/50**	*[$P < 0.001$ (Cochran–Armitage trend test)], **[$P = 0.012$, Fisher exact test]	
		Bronchioloalveolar adenoma or carcinoma (combined) 6/50*, 16/50**, 17/50**, 11/50, 27/50***	*$P < 0.001$ (Cochran–Armitage trend test), **$P < 0.05$ (Fisher exact test, ***$P < 0.01$ (Fisher exact test)	
		All tumours Total tumours: 7, 22, 22, 14, 47	NR	
Full carcinogenicity Mouse, CD-1 (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 7, 12	*Lung* Bronchioloalveolar hyperplasia 0/67, 50/67*	*[$P < 0.0001$]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50
		Bronchioloalveolar adenoma 15/67, 14/67	[NS]	
		Bronchioloalveolar carcinoma 7/67, 17/67*	*[$P < 0.05$]	
		Bronchioloalveolar adenoma or carcinoma (combined) 21/67, 31/67	[NS]	

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, C57BL/6 (wildtype) (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 4, 18	*Lung* Bronchioloalveolar hyperplasia 0/69, 55/70* Bronchioloalveolar adenoma 3/69, 1/70 Bronchioloalveolar carcinoma 0/69, 0/70	*[$P < 0.0001$] [NS] [NS]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50; treated animals had a significantly higher survival
Full carcinogenicity Mouse, *Cyp2f2*$^{(-/-)}$ (KO) (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 21, 23	*Lung* Bronchioloalveolar hyperplasia 0/69, 0/69 Bronchioloalveolar adenoma 0/69, 0/69 Bronchioloalveolar carcinoma 2/69, 0/69	[NS] [NS] [NS]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50; KO mice with C57BL/6 background
Full carcinogenicity Mouse, *Cyp2f2*KO-*2f1*-transgenic (M) 6–7 wk 104 wk Cruzan et al. (2017)	Inhalation (whole-body exposure) Styrene, 99.95% Clean air 0, 120 ppm, 6 h/d, 5 d/wk 75, 75 9, 14	*Lung* Bronchioloalveolar hyperplasia 0/69, 0/68 Bronchioloalveolar adenoma 2/69, 1/68 Bronchioloalveolar carcinoma 1/69, 0/68 Bronchioloalveolar adenoma or carcinoma (combined) 3/69, 1/68	[NS] [NS] [NS] [NS]	Principal limitations: single dose; one sex only; lack of statistical analysis in the article Five animals killed per group after 1, 26, 26, 52, and 78 wk for histopathology and cell proliferation assessment; initial number of animals for survival analyses, 50; transgenic mice with C57BL/6 background

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence and/or multiplicity of tumours	Significance	Comments
Full carcinogenicity Mouse, A/J (F) 6–8 wk ~27 wk (20 wk after final injection) Brunnemann et al. (1992)	Intraperitoneal injection Styrene, > 99% Olive oil (0.1 mL) 0 (vehicle control), 10 µmol per mouse, 3×/wk (20 injections total; total dose, 200 µmol per mouse) 25, 25 NR	*Lung*: adenoma 1/25 (4%), 3/25 (12%) Tumour multiplicity: 0.04 ± 0.20, 0.80 ± 3.52	 NS NR	Principal limitations: not chronic exposure; non-physiological exposure route; no survival or body-weight data

bw, body weight; d, day(s); F, female; GND, gestation day; GLP, good laboratory practice; h, hour(s); KO, knockout; M, male; NR, not reported; NS, not significant; ppm, parts per million; vs, versus; wk, week(s).

C57BL mice. [The Working Group noted that the study was limited by the non-chronic exposure (16 weeks only), the poor survival, the high/toxic dose used for O20 mice (resulting in exposure stopping at week 16), the comparison of progeny with untreated (adult) controls, the low number of vehicle-control female dams, and the low starting numbers for all treated groups of C57BL mice.]

(b) Oral administration

Groups of male and female B6C3F$_1$ mice were exposed by gavage to styrene (purity, > 99%) at 0 (vehicle controls) (n = 20 per sex), 150 mg/kg bw (n = 50 per sex), or 300 mg/kg bw (n = 50 per sex) in corn oil (NTP, 1979a). Mice were treated 5 days per week for 78 weeks and then observed (no treatment) for 13 weeks (91 weeks in total). For male mice, 20/20 (100%) of the vehicle controls, 46/50 (92%) of the group given the low dose, and 39/50 (78%) of the group given the high dose survived until the end of study. For female mice, 18/20 (90%) of the vehicle controls, 40/50 (80%) of the group given the low dose, and 38/50 (76%) of the group given the high dose survived until the end of study. In males, the incidence of bronchioloalveolar adenoma or carcinoma (combined) of the lung at week 91 was significantly increased (vs vehicle control) at the highest dose (P = 0.024), and there was a significantly increased positive trend (P = 0.023). In females, there was a significantly increased positive trend (P = 0.034) for hepatocellular adenoma at week 91. [The Working Group noted the smaller number of controls compared with the numbers of treated mice.]

Groups of male and female B6C3F$_1$ mice were given a solution of styrene [purity unspecified] (~70%) and β-nitrostyrene (~30%) at a dose of 0 (vehicle controls) (n = 20 per sex), 87.5 mg/kg bw (n = 50 per sex), or 175 mg/kg bw (n = 50 per sex) by gavage in corn oil (NTP, 1979b). Doses were selected based on the concentration of β-nitrostyrene in the mixture. Mice were treated 3 days per week for 78 weeks and then observed (no treatment) for 14 weeks (92 weeks in total). For male mice, 18/20 (90%) of the vehicle controls, 43/50 (86%) of the group given the low dose, and 33/50 (66%) of the group given the high dose survived until the end of study. For female mice, 17/20 (85%) of the vehicle controls, 47/50 (94%) of the group given the low dose, and 38/50 (76%) of the group given the high dose survived until the end of study. Decreased mean body weight (compared with controls) was only observed in the female mice given the high dose. In males, the incidence of bronchioloalveolar adenoma or carcinoma (combined) at week 92 was significantly increased (vs vehicle control) for the group exposed to the lowest dose (P = 0.016). There was no significantly increased incidence of tumours in female mice. [The Working Group noted the smaller number of controls compared with the numbers of treated mice. The study was also limited by the test agent being a mixture of styrene and β-nitrostyrene, the purity of styrene in the mixture not being reported, and the unusual exposure schedule of 3 days per week. The Working Group also noted that there was no significant dose–response in males and no significant increase in males given the high dose.]

(c) Inhalation

In a good laboratory practice (GLP) study, groups of 50 male and 50 female CD-1 mice were exposed to styrene (purity, > 99.5%) via inhalation (whole-body exposure) at 0 (air control), 20, 40, 80, or 160 ppm for 6 hours per day, 5 days per week, for 104 weeks (males) or 98 weeks (females) (Cruzan et al., 2001). The percentages of male mice surviving at week 104 were 72% (control), 54% (20 ppm), 54% (40 ppm), 74% (80 ppm), and 66% (160 ppm). The corresponding percentages of female mice surviving at week 98 were 54%, 64%, 66%, 68%, and 70%. Males exposed at 80 ppm and 160 ppm exhibited significant weight loss (23% and 31%, respectively, compared with controls) at week 104. Females exposed at

160 ppm exhibited significant weight loss (15% compared with controls) at week 98. In males, the incidence of bronchioloalveolar adenoma of the lung was significantly increased (compared with controls) at week 104 with exposure to styrene at 40 ppm [$P < 0.001$], 80 ppm [$P = 0.005$], and 160 ppm [$P < 0.001$], and there was a significant positive trend [$P < 0.001$]. There was no significant increase in the incidence of bronchioloalveolar carcinoma of the lung in males, but the incidence of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased with exposure at 40, 80, and 160 ppm, with a significant positive trend (OEHHA, 2010). In females, the incidence of bronchioloalveolar adenoma of the lung at week 98 was significantly increased (compared with controls) with exposure to styrene at 20 ppm [$P = 0.028$], 40 ppm [$P = 0.028$], and 160 ppm [$P < 0.001$], with a significant positive trend [$P = 0.001$]. The incidence of bronchioloalveolar carcinoma of the lung was significantly increased (compared with controls) with exposure to the highest dose in females [$P = 0.012$], with a significant positive trend [$P < 0.001$] (Cruzan et al., 2001). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was significantly increased with exposure at 20, 40, and 160 ppm, with a significant positive trend in females (OEHHA, 2010). [The Working Group noted that the lower survival of female controls compared with treated females was a weakness of this study. Further, there were no historical control data from inhalation studies at the testing laboratory for bronchioloalveolar adenoma and carcinoma in CD-1 mice.]

Groups of 75 male CD-1, C57BL/6 wildtype, *Cyp2f2*$^{(-/-)}$ knockout, and *Cyp2f2*KO-*Cyp2f1* transgenic mice were exposed to styrene (purity, 99.95%) via inhalation (whole-body exposure) at 0 ppm (air control) or 120 ppm for 6 hours per day, 5 days per week, for 104 weeks. Five mice per group were killed after 1, 26, 26, 52, and 78 weeks for histopathological examination and cell proliferation assessment. Treated wildtype mice had a significantly higher survival compared with controls. The incidences of bronchioloalveolar hyperplasia were significantly increased in CD-1 mice [$P < 0.0001$] and wildtype mice [$P < 0.0001$] exposed to styrene (50/67 and 55/70, respectively) compared with control mice (0/67 and 0/69, respectively). Bronchioloalveolar hyperplasia was not observed in any of the knockout or transgenic mice (whether controls or mice exposed to styrene). The incidence of bronchioloalveolar adenoma or carcinoma (combined) was increased in CD-1 mice exposed to styrene (31/67) compared with control mice (22/67), but this increase was not statistically significant. There was no significant increase for the three groups of animals with a C57BL/6 background: the incidence of bronchioloalveolar adenoma or carcinoma (combined) was 1/70 (wildtype), 0/69 (knockout), and 1/68 (transgenic) for mice exposed to styrene and 3/69 (wildtype), 2/69 (knockout), and 3/69 (transgenic) for control mice. The incidence of bronchioloalveolar carcinoma was significantly increased in CD-1 mice [$P < 0.05$] exposed to styrene (17/67) compared with control mice (7/67). For the other groups, the incidences of bronchioloalveolar carcinoma were 0/70 (wildtype), 0/69 (knockout), and 0/68 (transgenic) for mice exposed to styrene and 0/69 (wildtype), 2/69 (knockout), and 1/69 (transgenic) for control mice (Cruzan et al., 2017). [The Working Group noted the use of a single dose and of males only, as well as the lack of statistical analysis in the article. There were no historical control data for bronchioloalveolar adenoma and bronchioloalveolar carcinoma in CD-1 mice.]

(d) Intraperitoneal injection

Two groups of 25 female A/J mice were given styrene (purity, > 99%) by intraperitoneal injection at doses of 0 (vehicle control) or 10 µmol per mouse in olive oil 3 times per week for a total of 20 injections (total dose, 200 µmol per mouse) (Brunnemann et al., 1992). Although there was

an increase in the percentage of mice exposed to styrene with adenomas of the lung (compared with the vehicle controls) at 20 weeks after the last injection (duration of the experiment, ~27 weeks), the difference was not statistically significant. [The Working Group noted that the study was limited by the lack of survival or body-weight data, the non-chronic exposure, and the non-physiological exposure route.]

3.1.2 Rat

See Table 3.2.

(a) Oral administration

(i) Gavage

Groups of 50 male and 50 female Fischer 344 rats were given styrene (purity, 99.7%) in corn oil by gavage at doses of 0, 1000 (medium dose), and 2000 (high dose) mg/kg bw, 5 days a week for 78 weeks, followed by a 27-week observation period. Groups of 20 male and 20 female rats received the corn oil vehicle alone (medium- and high-dose vehicle control) (NTP, 1979a). Mortality among male and female rats given the high dose was significantly higher than their respective vehicle controls. In response to this elevated and early mortality, a group given a low dose of styrene was included for each sex at experimental week 23. These dosed rats were intubated with styrene at a dose of 500 mg/kg bw for 103 weeks, followed by a 1-week observation period; a separate vehicle control group was also started for each sex for this low-dose group. Males surviving at the end of the experiment were 17/20 (low-dose vehicle control), 18/20 (medium- and high-dose vehicle control), 44/50 (low dose), 47/50 (medium dose), and 6/50 (high dose, at week 53). Corresponding numbers of surviving females were 15/20, 18/20, 46/50, 46/50, and 7/50 (at week 53). A dose-related mean body-weight decrease compared with their controls was observed in male rats, but there was no significant mean body-weight decrease in female rats when compared with their controls. [Since there was a significant accelerated mortality among rats exposed at the high dose, it is possible that the medium dose may have exceeded the maximum tolerated dose.] There were inadequate numbers of rats that survived the high dose of styrene to determine the risk of late-developing tumours; these groups were therefore excluded from the statistical analyses. There was no significant increase in the incidence of any tumour type in treated males or females. [The Working Group noted the smaller number of controls.]

Groups of 50 male and 50 female Fischer 344 rats were given a solution of styrene [purity unspecified] and β-nitrostyrene (70% and 30%, respectively) in corn oil by gavage at doses of 150 or 300 mg/kg bw for males and 75 or 150 mg/kg bw for females, 3 days per week for 79 weeks, followed by an additional observation period of 29 weeks. Groups of 20 male and 20 female rats received the corn oil vehicle alone (NTP, 1979b). Doses were selected based on the concentration of β-nitrostyrene in the mixture. There was no significant difference between the survival of male and female rats given the test solution and that of their controls. The numbers of surviving males at the end of the experiment were 16/20 (controls), 34/50 (low dose), and 31/50 (high dose); the corresponding numbers of surviving females were 12/20, 33/50, and 31/50. A decrease in mean body weight of the male rats given the high dose, compared with their controls, was observed, indicating that the doses may have approximated the maximum tolerated dose. There was no significant increase in the incidence of any tumour type in treated males or females. [The Working Group noted the smaller number of controls compared with the treated groups. The study was also limited by the test agent being a mixture of styrene and β-nitrostyrene, the lack of information about the purity of styrene in the mixture, and the unusual exposure schedule of 3 days per week.]

Table 3.2 Studies of carcinogenicity in rats exposed to styrene

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Fischer 344 (M) 6 wk 104–105 wk NTP (1979a)	Gavage Styrene, 99.7% Corn oil 0 (low-dose vehicle control), 0 (medium- and high-dose vehicle control), 500 mg/kg bw (low-dose) for 103 wk, 1000 mg/kg bw (medium-dose group) for 78 wk, and 2000 mg/kg bw (high-dose group) for 78 wk, 5 d/wk, then observation at wk 104–105 20, 20, 50, 50, 50 17, 18, 44, 47, 6	*Skin and subcutaneous tissue*: fibroma 0/20, 0/20, 3/50, 0/50 *Adrenal gland*: pheochromocytoma 2/20, 1/19, 1/48, 4/49 *Testis*: interstitial cell tumours 15/20, 19/20, 42/47, 48/50	NS NS NS	Principal limitations: inadequate numbers of surviving high-dose (2000 mg/kg bw) rats, meaning that these were excluded from the statistical analyses (only 6/50 male rats survived past week 53 to the end of the study); smaller number of controls compared with treated groups
Full carcinogenicity Rat, Fischer 344 (F) 6 wk 104–105 wk NTP (1979a)	Gavage Styrene, 99.7% Corn oil 0 (low-dose vehicle control), 0 (medium-dose vehicle control), 500 mg/kg bw (low-dose) for 103 wk, 1000 mg/kg bw (medium-dose group) for 78 wk, and 2000 mg/kg bw (high-dose group) for 78 wk, 5 d/wk, then observation at wk 104–105 20, 20, 50, 50, 50 15, 18, 46, 46, 7	*Uterus*: endometrial stromal polyp 4/18, 3/20, 9/48, 5/50	NS	Principal limitations: inadequate numbers of surviving high-dose (2000 mg/kg bw) rats, meaning that these were excluded from the statistical analyses (only 7/50 female rats survived past week 53 to the end of the study); smaller number of controls compared with treated groups
Full carcinogenicity Rat, F344 (M) 6 wk 108 wk NTP (1979b)	Gavage 70% styrene, 30% β-nitrostyrene, NR Corn oil 0, 150, 300 mg/kg bw, 3 d/wk for 79 wk, 29 wk observation 20, 50, 50 16, 34, 31	*Pituitary gland*: chromophobe adenoma 4/17*, 4/42, 1/44** *Pancreas*: islet cell adenoma 2/18*, 1/42, 0/42 *Adrenal gland*: pheochromocytoma	*P = 0.010 (trend, decrease), **P = 0.019 (decrease) *P = 0.039 (trend, decrease)	Principal limitations: purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344 (M) 6 wk 108 wk NTP (1979b) (cont.)		1/19, 3/48, 1/46 *Thyroid*: C-cell adenoma or carcinoma (combined) 0/18, 1/47, 3/41 *Testis*: interstitial cell tumours 15/19, 38/47, 39/46	NS NS NS	
Full carcinogenicity Rat, F344 (F) 6 wk 108 wk NTP (1979b)	Gavage 70% styrene, 30% β-nitrostyrene, NR Corn oil 0, 75, 150 mg/kg bw, 3 d/wk for 79 wk, 29 wk observation 20, 50, 50 12, 33, 31	*Pituitary gland*: chromophobe adenoma 5/18, 15/49, 18/44 *Mammary gland*: fibroadenoma 2/20, 5/50, 7/50 *Uterus* Adenocarcinoma, NOS 1/20, 3/48, 0/45 Endometrial stromal polyp 1/20, 9/48, 8/45	NS NS NS NS	Principal limitations: purity of styrene in mixture not reported; smaller number of controls compared with treated groups; solution contained ~70% styrene and ~30% β-nitrostyrene; doses were based on β-nitrostyrene concentration; unusual exposure schedule of 3 d/wk
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Gavage Styrene, 99.8% Olive oil 0, 50, 250 mg/kg bw, 1×/d, 4–5×/wk, 52 wk 40, 40, 40 NR	*Mammary gland* "Benign tumours" 4/40, 3/40, 4/40 "Malignant tumours" 0/40, 0/40, 0/40 *Haematopoietic and lymphoid tissues*: leukaemia 0/40, 0/40, 2/40	 [NS] [NS] [NS]	Principal strengths: adequate number of animals used, randomly allocated in groups Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT102

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk old Lifetime Conti et al. (1988)	Gavage Styrene, 99.8% Olive oil 0, 50, 250 mg/kg bw, 1×/d, 4–5×/wk, 52 wk 40, 40, 40 NR	*Mammary gland* "Benign and malignant tumours" 24/40, 30/40, 15/40 "Malignant tumours" 5/40, 6/40, 5/40 *Haematopoietic and lymphoid tissues*: leukaemia 1/40, 3/40, 0/40	[NS] [NS] [NS]	Principal strengths: adequate number of animals used, randomly allocated in groups Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported a higher mortality rate in high-dose females; Experiment BT102
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov & Tomatis (1978)	Gavage (of pregnant females) Styrene, 99% Olive oil 0, 1350 mg/kg bw, 1×/wk 10, 21 8, 10	*Stomach*: tumours 0/10, 1/20 *Mammary gland*: tumours 3/10, 6/20 *Uterus*: carcinoma 0/10, 3/20 *Pituitary gland*: adenoma 0/10, 3/20 *Ovary*: tumours 0/10, 1/20	NS NS NS NS NS	Principal strengths: the duration of observation was adequate Animals were dams of male and female offspring of transplacental exposure/gavage experiment (see experiment below)
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov & Tomatis (1978) (cont.)				
Full carcinogenicity Rat, BDIV (M) GND 17 120 wk Ponomarkov & Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil 0, 500 mg/kg bw, 1×/wk beginning at weaning for life 36, 73 14, 8	*Nerve trigeminus*: neurinoma 0/32, 1/54 *Heart*: neurinoma 0/32, 1/54	NS NS	Principal strengths: adequate number of animals used; duration of exposure and observation was adequate Animals studied were offspring from dams treated with 0 or 1350 mg/kg bw of styrene dissolved in olive oil by gavage 1× on day 17 of gestation (see experiment above)

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov &Tomatis (1978)	Transplacental exposure/gavage Styrene, 99% Olive oil 0, 500 mg/kg bw, 1×/wk beginning at weaning for life 39, 71 18, 20	*Stomach*: tumours 1/35, 2/68 *Liver*: tumours 0/35, 1/68	NS NS	Principal strengths: adequate number of animals used; duration of exposure and observation was adequate Animals studied were offspring from dams treated with 0 or 1350 mg/kg bw of styrene dissolved in olive oil by gavage 1× on day 17 of gestation (see experiment above)
Full carcinogenicity Rat, Charles River COBS (SD) BR (M) 50 d 2 yr Beliles et al. (1985)	Drinking-water Styrene, 98.9% Drinking-water 0, 125, 250 ppm [mg/L] ad libitum 76, 50, 50 42, 27, 31	*Mammary gland*: tumours 1/65, 0/23, 0/40	NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation Principal limitations: dosing was limited by the solubility of styrene in water; styrene intake was calculated from the concentration, water consumption, and body weight as being 7.7 and 14 mg/kg bw per day in males
Full carcinogenicity Rat, Charles River COBS (SD) BR (F) 50 d 2 yr Beliles et al. (1985)	Drinking-water Styrene, 98.9% Drinking-water 0, 125, 250 ppm [mg/L] ad libitum 106, 70, 70 60, 40, 44	*Mammary gland*: tumours 54/96, 20/30, 45/60	NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation Principal limitations: dosing was limited by the solubility of styrene in water; styrene intake was calculated from the concentration, water consumption, and body weight as being 12 and 21 mg/kg bw per day in females
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Inhalation (whole-body exposure) Styrene, 99.8% Air flow 0, 25, 50, 100, 200, 300 ppm, 4 h/d, 5 d/wk for 52 wk 60, 30, 30, 30, 30, 30 NR	*Mammary gland* "Benign and malignant tumours (combined)" 8/60, 6/30, 3/30, 6/30, 4/30, 5/30 "Malignant tumours" 1/60, 1/30, 1/30, 0/30, 1/30, 0/30	[NS] [NS]	Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT101

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Inhalation (whole-body exposure) Styrene, 99.8% Air flow 0, 25, 50, 100, 200, 300 ppm, 4 h/d, 5 d/wk for 52 wk 60, 30, 30, 30, 30, 30 NR	*Mammary gland* "Benign and malignant tumours (combined)" 34/60*, 24/30**, 21/30, 23/30, 24/30**, 25/30** "Malignant tumours" 6/60*, 6/30, 4/30, 9/30**, 12/30**, 9/30**	 *[$P < 0.05$, Cochran–Armitage trend test], **[$P < 0.05$, Fisher exact test] *[$P \leq 0.013$, Cochran–Armitage trend-test], **[$P < 0.05$, Fisher exact test]	Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT101
Full carcinogenicity Rat, CD (Sprague-Dawley) (M) NR (received at age 4 wk) 104 wk Cruzan et al. (1998)	Inhalation (whole-body exposure) Styrene, > 99.5% Vapour (air flow) 0, 50, 200, 500, 1000 ppm, 6 h/d for 5 d/wk 60, 60, 60, 60, 60 NR	*Mammary gland*: adenocarcinoma 0/60, 0/60, 0/60, 1/60, 0/60	NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation; adequate schedule of exposure; GLP study

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, CD (Sprague-Dawley) (F) NR (received at age 4 wk) 104 wk Cruzan et al. (1998)	Inhalation (whole-body exposure) Styrene, > 99.5% Vapour (air flow) 0, 50, 200, 500, 1000 ppm, 6 h/d for 5 d/wk 60, 60, 60, 60, 60 29, 28, 29, 40, 49	*Mammary gland* Adenocarcinoma 20/60*, 13/60, 9/60**, 5/60***, 2/60**** Fibroadenoma 21/60, 16/60, 13/60, 18/60, 17/60	 *[$P < 0.001$ (trend) (decrease)] **[$P = 0.032$ (decrease)] ***[$P = 0.001$ (decrease)] ****[$P < 0.001$ (decrease)] NS	Principal strengths: adequate number of animals used; adequate duration of exposure and observation; adequate schedule of exposure; GLP study
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Intraperitoneal injection Styrene, 99.8% Olive oil 0, 50 mg, 4× (with 2-mo interval) over 8 mo 40, 40 NR	*Mammary gland* "Benign tumours" 1/40, 6/40 "Malignant tumours" 0/40, 0/40	 [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment and low dose; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT103
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Intraperitoneal injection Styrene, 99.8% Olive oil 0, 50 mg, 4× (with 2-mo interval) over 8 mo 40, 40 NR	*Mammary gland* "Benign and malignant tumours (combined)" 24/40, 25/40 "Malignant tumours" 7/40, 6/40	 [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment and low dose; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT103

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Subcutaneous injection Styrene, 99.8% Olive oil 0, 50 mg (1×) 40, 40 NR	*Mammary gland* "Benign and malignant tumours (combined)" 8/40, 3/40 "Malignant tumours" 1/40, 0/40 *Adrenal gland*: pheochromocytoma 0/40, 1/40	[NS] [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT104
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Subcutaneous injection Styrene, 99.8% Olive oil 0, 50 mg (1×) 40, 40 NR	*Mammary gland* "Benign and malignant tumours (combined)" 24/40, 25/40 "Malignant tumours" 7/40, 6/40 *Adrenal gland*: pheochromocytoma 0/40, 3/40	[NS] [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: no mortality data were included in this study; statistical analysis was not conducted by the authors; non-physiological exposure route; short duration of treatment; limited reporting The authors reported no significant difference in survival related to treatment; Experiment BT104

bw, body weight; d, day(s); F, female; GLP, good laboratory practice; GND, gestation day; M, male; mo, month(s); NOS, not otherwise specified; NR, not reported; NS, not significant; ppm, parts per million; wk, week(s).

Groups of 40 male and 40 female Sprague-Dawley rats were exposed by gavage to styrene (purity, 99.8%) at 0, 50, or 250 mg/kg bw in olive oil for 4–5 days per week for 52 weeks, and then observed until death. There was no significant increase in the incidence of any tumour type in treated males or females (Conti et al., 1988). [The Working Group noted the limited reporting of this study and the 1-year duration of treatment.]

(ii) Transplacental exposure and oral administration (by gavage)

Ponomarkov & Tomatis (1978) reported on a group of 21 pregnant BDIV rats that were given styrene (purity, 99%) at a dose of 1350 mg/kg bw in olive oil by a single gastric intubation on day 17 of gestation. A control group of 10 pregnant rats were given olive oil alone. There was a slight treatment-related increase in neonatal mortality. Groups of 73 male and 71 female progeny of dams that were given styrene were also exposed to styrene at 500 mg/kg bw in olive oil by gastric intubation once per week from weaning for up to 120 weeks, at which point the experiment was terminated. Control groups of 36 male and 39 female offspring rats were given olive oil alone. There was no treatment-related effect on body weight or survival. At the time of observation of the first tumour, 10 control and 20 treated dams, 32 male control and 54 treated male offspring, and 35 female control and 68 treated female offspring were still alive. Non-neoplastic stomach lesions [morphology and incidence unspecified, probably glandular] were reported in rats who were exposed to styrene. There was no significant treatment-related increase in tumour incidence at any site.

(iii) Drinking-water

In a chronic toxicity and three-generation reproduction study, two groups of 50 male and two groups of 70 female Charles River COBS (SD) BR rats (F_0 generation rats) were continuously exposed to styrene (purity, 98.9%) in drinking-water at nominal doses of 125 or 250 ppm for 2 years (chronic study). Groups of 76 male and 106 female rats were observed as vehicle controls. Males (10–15) and females (20–30) from each group in the chronic study were mated to produce F_1 pups (reproductive toxicity study) 90 days after the start of the experiment. At weaning of the litters, the F_0 parents were returned to the chronic toxicity study. At 52 weeks, 10 F_0 rats per sex and group were killed. There was no significant difference between the survival and food consumption of rats exposed to styrene in drinking-water and those of their controls. The numbers of surviving males at the end of the experiment were 42/76 (control), 27/50 (low dose), and 31/50 (high dose); the corresponding numbers of surviving females were 60/106, 40/70, and 44/70. There was a decrease in mean body weight in female rats given the high dose. No treatment-related increase in the incidence of any type of tumour was observed (Beliles et al., 1985). [The Working Group noted the adequate number of animals used and the adequate duration of exposure and observation.]

(b) Inhalation

Conti et al. (1988) reported on groups of 30 male and 30 female Sprague-Dawley rats that were exposed to styrene (purity, 99.8%) by whole-body inhalation at doses of 25, 50, 100, 200, or 300 ppm for 4 hours per day, 5 days a week, for 52 weeks followed by lifetime observation; groups of 60 male and 60 female rats served as control groups. There was no significant difference in survival between the groups exposed to styrene by inhalation and controls. There was no relevant body weight difference between exposed groups and controls. The incidence of benign and malignant (combined) mammary tumours was significantly higher in exposed female rats than in controls: 34/60 (controls), 24/30 (25 ppm), 21/30 (50 ppm), 23/30 (100 ppm), 24/30 (200 ppm), and 25/30 (300 ppm) [$P < 0.05$, trend test; $P < 0.05$ for groups exposed at 25, 200, and 300 ppm vs

controls]. The incidence of malignant mammary tumours was significantly increased in treated females than in controls: 6/60 (controls), 6/30 (25 ppm), 4/30 (50 ppm), 9/30 (100 ppm), 12/30 (200 ppm), and 9/30 (300 ppm) [$P \leq 0.013$, trend test; $P < 0.05$ for groups exposed at 100, 200, and 300 ppm vs controls]. There was no significant increase in the incidence of benign and/or malignant mammary tumours in males. [The Working Group noted the 1-year duration of treatment and incomplete reporting of the study.]

In a GLP study, Cruzan et al. (1998) reported on groups of 60 male and 60 female CD (Sprague-Dawley) rats that were exposed to air containing styrene vapour (purity, > 99.5%) at doses of 0 (control), 50, 200, 500, and 1000 ppm by whole-body inhalation, 6 hours per day, 5 days a week, for 104 weeks. During week 61, a technical problem which resulted in liquid styrene dripping into the exposure chambers at a discrete location meant that eight males in the group exposed at 1000 ppm and six males in the group exposed at 500 ppm experienced important dermal exposure of styrene; all the rats died or were killed within the 2 weeks that followed, and these were excluded from the mortality or tumour incidence analysis. Styrene had no effect on survival in males, but a dose-related increase in survival of females exposed at 500 or 1000 ppm was observed. Males exposed at 500 or 1000 ppm gained less weight than the controls during the first year, and maintained the difference during the second year. Females exposed at 200, 500, or 1000 ppm gained less weight during the first year, and those exposed at 500 or 1000 ppm continued to gain less weight during months 13–18. From week 91 to termination of the study, females exposed at 500 ppm weighed less than those exposed at 1000 ppm. Exposure to styrene did not cause treatment-related increases in the incidence of any tumour type in males or females. A significant dose-dependent decrease in the incidence of mammary gland adenocarcinoma was reported in females. [The Working Group noted that the number of animals used, the duration of exposure and observation, and the schedule of exposure were all adequate.]

(c) Intraperitoneal injection

Groups of 40 male and 40 female Sprague-Dawley rats were given styrene (purity, 99.8%) by intraperitoneal injection at 50 mg per animal in olive oil, 4 times, at 2-month intervals (total dose, 200 mg). Control groups of 40 male and 40 female rats were given olive oil alone. The study was terminated when the last rat died. There was no significant increase in the incidence of any tumour type (Conti et al., 1988). [The Working Group noted the incomplete reporting of the data, short duration of treatment, and low total dose.]

(d) Subcutaneous injection

Groups of 40 male and 40 female Sprague-Dawley rats were given a single subcutaneous injection of 50 mg styrene (purity, 99.8%) per animal in olive oil. Control groups of 40 male and 40 female rats were given olive oil alone. The study was terminated when the last rat died. There was no significant increase in the incidence of any tumour type (Conti et al., 1988). [The Working Group noted the limited reporting of the study and the short duration of the treatment.]

3.2 Styrene-7,8-oxide

See Table 3.3.

3.2.1 Mouse

(a) Oral administration

Groups of 52 male and 52 female B6C3F$_1$ mice were given styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified amounts of benzaldehyde and benzene) at a dose of 0 (control), 375, or 750 mg/kg bw in corn oil by gavage 3 times per week, for 104 weeks. All

Table 3.3 Studies of carcinogenicity in experimental animals exposed to styrene-7,8-oxide

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 7 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 375, 750 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 42, 34, 6	*Forestomach* Squamous cell papilloma 2/51*, 22/51**, 8/52***	*[$P < 0.001$ (trend)], **[$P < 0.001$], ***[$P < 0.05$]	Principal strengths: adequate number of animals used Survival read from figure, marked reduction at high dose; body weight and body-weight gain reduction in treated males
		Squamous cell carcinoma 0/51*, 16/51**, 15/52**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	
		Squamous cell papilloma or carcinoma (combined) 2/51*, 37/51**, 21/52**	*[$P < 0.001$ (trend)], **$P < 0.001$	
		Liver: hepatocellular adenoma or carcinoma (combined) 12/51*, 28/51**, 15/52	*[$P = 0.002$ (trend)], **$P < 0.001$	
Full carcinogenicity Mouse, B6C3F$_1$ (F) 7 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 375, 750 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 35, 33, 18	*Forestomach* Squamous cell papilloma 0/51*, 14/50**, 17/51**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	Principal strengths: adequate number of animals used Survival read from figure, marked reduction at high dose; body-weight gain reduction in treated females
		Squamous cell carcinoma 0/51*, 10/50**, 3/51	*[$P < 0.001$ (trend)], **[$P < 0.001$]	
		Squamous cell papilloma or carcinoma (combined) 0/51*, 24/50**, 20/51**	*[$P < 0.001$ (trend)], **$P < 0.001$	
		Liver: hepatocellular adenoma or carcinoma (combined) 7/51, 4/50, 9/51	NS	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (M) 13 wk Lifetime Conti et al. (1988)	Gavage Styrene-7,8-oxide, NR Olive oil 0, 50, 250 mg/kg bw, 4–5×/wk for 52 wk 40, 40, 40 NR	*Forestomach* Squamous cell papilloma and acanthoma 0/40*, 3/40, 9/40** Squamous cell carcinoma 0/40*, 11/40**, 30/40** Squamous cell carcinoma (in situ) 0/40*, 6/40**, 18/40*** Squamous cell carcinoma (invasive) 0/40*, 5/40**, 12/40*** *Adrenal gland*: pheochromocytoma 2/40, 4/40, 6/40 *Mammary gland* Benign tumours 1/40*, 0/40, 10/40** Malignant tumours 0/40, 0/40, 0/40	 *[$P = 0.002$ (trend)], **[$P < 0.05$] *[$P < 0.001$ (trend)], **[$P < 0.01$] *[$P < 0.001$ (trend)], **[$P < 0.05$], ***[$P < 0.01$] *[$P < 0.001$ (trend)], **[$P < 0.05$], ***[$P < 0.01$] [NS] *[$P < 0.001$ (trend)], **[$P < 0.01$] [NS]	Principal strengths: adequate number of animals used Principal limitations: limited reporting Slight increase in mortality rate was observed in treated males; Experiment BT105

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, Sprague-Dawley (F) 13 wk Lifetime Conti et al. (1988)	Gavage Styrene-7,8-oxide, NR Olive oil 0, 50, 250 mg/kg bw, 4–5×/wk for 52 wk 40, 40, 40 NR	*Forestomach* Squamous cell papilloma and acanthoma 0/40, 3/40, 5/40* Squamous cell carcinoma 0/40*, 8/40**, 33/40** Squamous cell carcinoma (in situ) 0/40*, 7/40**, 19/40** Squamous cell carcinoma (invasive) 0/40*, 1/40, 14/40** *Adrenal gland*: pheochromocytoma 1/40, 2/40, 0/40 *Mammary gland* Benign and malignant tumours 4/40, 7/40, 9/40 Malignant tumours 1/40, 0/40, 1/40	*[$P < 0.05$] *[$P < 0.001$ (trend)], **[$P < 0.01$] *[$P < 0.001$ (trend)], **[$P < 0.01$] *[$P < 0.001$ (trend)], **[$P < 0.01$] [NS] [NS] [NS]	Principal strengths: adequate number of animals used Principal limitations: limited reporting Experiment BT105
Full carcinogenicity Rat, F344/N (M) 9 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 275, 550 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 29, 33, 12	*Forestomach* Squamous cell papilloma 1/52*, 23/52**, 18/51** Squamous cell carcinoma 0/52*, 35/52**, 43/51**	*[$P < 0.001$ (trend)], **[$P < 0.001$] *[$P < 0.001$ (trend)], **[$P < 0.001$]	Principal strengths: adequate number of animals used Survival read from figure; marked reduction of survival and body-weight gain at high dose

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 9 wk 107–108 wk Lijinsky (1986) (cont.)		Squamous cell papilloma or carcinoma (combined) 1/52*, 50/52**, 50/51**	*[$P < 0.001$ (trend)], **[$P < 0.001$]	
Full carcinogenicity Rat, F344/N (F) 9 wk 107–108 wk Lijinsky (1986)	Gavage Styrene-7,8-oxide, 96.6% Corn oil 0, 275, 550 mg/kg bw, 3×/wk for 104 wk 52, 52, 52 38, 39, 17	*Forestomach* Squamous cell papilloma 0/52*, 21/52**, 24/52** Squamous cell carcinoma 0/52*, 32/52**, 36/52** Squamous cell papilloma or carcinoma (combined) 0/52*, 46/52**, 50/52**	*[$P < 0.001$ (trend)], **[$P < 0.001$] *[$P < 0.001$ (trend)], **[$P < 0.001$] *[$P < 0.001$ (trend)], **[$P < 0.001$]	Principal strengths: adequate number of animals used Survival read from figure; marked reduction of survival and body-weight gain at high dose
Full carcinogenicity Rat, BDIV (M) GND 17 120 wk Ponomarkov et al. (1984)	Transplacental exposure and oral (gavage) administration Styrene-7,8-oxide, 97% Olive oil 0, 100–150 mg/kg bw, 1×/wk at age 4 wk until experimental wk 120 49, 43 49 (when first tumour observed), 42 (when first tumour observed)	*Forestomach* Papilloma 0/49, 7/42* Carcinoma (in situ) 0/49, 4/42* Carcinoma 0/49, 10/42*	*$P < 0.003$ *$P < 0.04$ *$P < 0.0002$	Principal strengths: adequate number of animals used Principal limitations: age of dams NR Dams were given a single dose of olive oil (control) or 200 mg/kg bw styrene-7,8-oxide on day 17 of gestation

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, BDIV (F) GND 17 120 wk Ponomarkov et al. (1984)	Transplacental exposure and oral (gavage) administration Styrene-7,8-oxide, 97% Olive oil 0, 100–150 mg/kg bw, 1×/wk at age 4 wk until experimental wk 120 55, 62 55 (when first tumour observed), 60 (when first tumour observed)	*Forestomach* Papilloma 2/55, 2/60 Carcinoma (in situ) 0/55, 6/60* Carcinoma 1/55, 16/60*	 NS *$P < 0.02$ *$P < 0.0001$	Principal strengths: adequate number of animals used Principal limitations: age of dams NR Dams were given a single dose of olive oil (control) or 200 mg/kg bw styrene-7,8-oxide on day 17 of gestation

bw, body weight; F, female; GND, gestation day; M, male; NR, not reported; NS, not significant; wk, week(s).

surviving animals were killed 3–4 weeks after the last dose. There was a marked reduction in the survival of male and female mice given the high dose, and body-weight gains were reduced in all mice exposed to styrene-7,8-oxide. Exposure resulted in a significant increase (with a significant positive trend) in the incidence of squamous cell papilloma of the forestomach at both low and high doses in males and females, and a significant increase (with a significant positive trend) in the incidence of squamous cell carcinoma of the forestomach in males at both low and high doses and in females at the low dose. The incidences of squamous cell papilloma or carcinoma (combined) of the forestomach were significantly increased (with a significant positive trend) at both low and high doses in males and females. A significant increase in the incidence (with a significant positive trend) of hepatocellular adenoma or carcinoma (combined) in males given the low dose was observed (Lijinsky, 1986).

(b) Skin application

Two groups of 40 C3H mice [sex unspecified] were given styrene-7,8-oxide [purity unspecified] by skin application of a dose of 5% or 10% in acetone [volume unspecified] on the clipped dorsal skin 3 times per week for life. Of the mice given the low dose, 17 survived over 24 months. Of the mice given the high dose, 18 survived to 12 months but only 2 of these mice survived to 17 months. No skin tumours were observed (Weil et al., 1963). [The Working Group noted the limited reporting of study details and the lack of controls, and concluded that the study was inadequate for the evaluation of the carcinogenicity of styrene-7,8-oxide.]

A group of 30 male Swiss ICR/Ha mice was given three applications per week of styrene-7,8-oxide [purified, but purity unspecified] for life on the clipped dorsal skin at a dose of 100 mg per application of a 10% solution in benzene. The median survival time was 431 days. Three of the 30 mice developed skin tumours (papillomas or cancers), one of which was a cancer that was probably a squamous cell carcinoma. Of the 150 controls treated with benzene only, 11 developed skin tumours (papillomas or cancers), one of which was a cancer that was probably a squamous cell carcinoma (Van Duuren et al., 1963). [The Working Group noted the carcinogenicity of the vehicle in experimental animals by other routes of exposure, and concluded that the study was inadequate for the evaluation of the carcinogenicity of styrene-7,8-oxide.]

3.2.2 Rat

(a) Oral administration

Groups of 40 male and 40 female Sprague-Dawley rats were given styrene-7,8-oxide [purity unspecified] at a dose of 0 (control), 50, or 250 mg/kg bw in olive oil by gavage for 4–5 days per week for 52 weeks, and then observed until they died. A slight increase in mortality rate was observed in treated males but not in treated females, and the last death occurred 156 weeks after the start of the experiment. The exposure was not observed to have any effect on body weight in both sexes. The treatment resulted in significantly increased incidences of squamous cell papilloma and acanthoma of the forestomach in males given the high dose and in females given the high dose, of squamous cell carcinoma of the forestomach (with a significant positive trend) in both groups of treated males and females, and of benign mammary tumours in males given the high dose. The incidences of acanthosis and dysplasia of the forestomach epithelium in males and females were related to treatment. No significant increase in the incidence of tumours at other sites was observed (Maltoni et al., 1979; Conti et al., 1988). [The Working Group noted the 1-year duration of treatment and the limited reporting of study details.]

Groups of 52 male and 52 female Fischer 344/N rats were given styrene-7,8-oxide (purity, 96.6%; two of the three impurities were unspecified

amounts of benzaldehyde and benzene) at a dose of 0 (control), 275, or 550 mg/kg bw in corn oil by gavage 3 times per week for 104 weeks. The experiment was terminated at 107–108 weeks. Survival and body-weight gain were reduced in males and females given the high dose. Treatment resulted in a significant increase (with a significant positive trend) in the incidence of squamous cell papilloma of the forestomach in treated males and females and in the incidence (with a significant positive trend) of squamous cell carcinoma of the forestomach in treated males and females. The incidences of squamous cell papilloma or carcinoma (combined) of the forestomach were also significantly increased in males and females, with a significant positive trend. There was a significant increase in the incidence of forestomach hyperplasia in treated males and females. No significant increase in the incidence of tumours at other sites was found (Lijinsky, 1986).

(b) Transplacental exposure and oral administration

A group of 14 pregnant BDIV inbred rats [age, unspecified] were given a single dose of styrene-7,8-oxide (purity, 97%) at 200 mg/kg bw in olive oil by gavage on day 17 of gestation. At 4 weeks of age, their offspring (43 males and 62 females) were given styrene-7,8-oxide (purity, 97%) at a dose of 100–150 mg/kg bw in olive oil by gavage once a week. The study was terminated at 120 weeks, to give estimated total doses of 5.0 g for males and 2.5 g for females. Control groups of 49 male and 55 female rats with no prenatal exposure were given olive oil only. At the time of appearance of the first tumour, 42 male and 60 female progeny that had been treated with styrene-7,8-oxide were still alive. In treated male progeny, the incidences of forestomach papilloma and forestomach carcinoma were significantly increased; in female progeny, the incidence of forestomach carcinoma was significantly increased. Hyperplasia, dysplasia, and hyperkeratosis of the forestomach were also reported in treated rats. There was no significant increase in the incidence of tumours at other sites in treated males and females (Ponomarkov et al., 1984).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Styrene is extensively metabolized to styrene-7,8-oxide in humans and in experimental systems. As a result, external exposures to styrene engender internal exposures to both styrene and styrene-7,8-oxide. The absorption, distribution, metabolism, and excretion of styrene and styrene-7,8-oxide in humans have been previously reviewed (IARC, 1994, 2002; NTP, 2016a, b).

4.1.1 Absorption

(a) Humans

(i) Styrene

Styrene is absorbed by inhalation, dermal contact, or ingestion through consumption of food (Cohen et al., 2002). The predominant route in occupational settings is inhalation. A substantial number of studies in humans exposed to styrene have been conducted using occupational cohorts or volunteers exposed in inhalation chambers or by mask exposures. The results of these previously reviewed studies (IARC, 1994, 2002; NTP, 2016a, b) demonstrate that styrene is found in the blood of those exposed. The average pulmonary uptake of styrene under experimental conditions ranged from 63% to 68% (Wigaeus et al., 1984; Löf et al., 1986). An average concentration of styrene in blood of 15.3 μM has been reported in workers exposed to styrene in a