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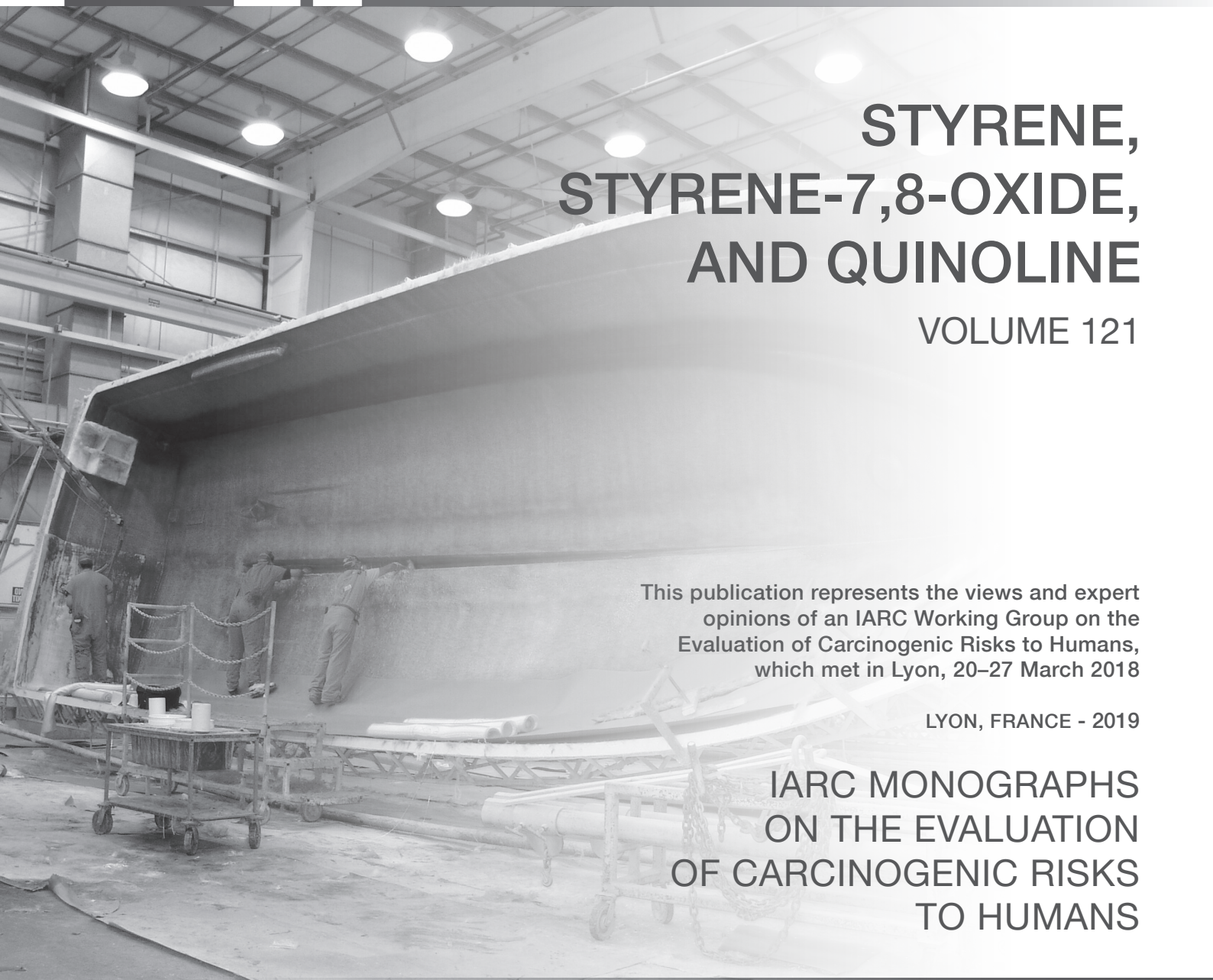

STYRENE, STYRENE-7,8-OXIDE, AND QUINOLINE

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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

reinforced plastics factory (Brugnone et al., 1993). The clearance of styrene from blood was biphasic in human volunteers (Ramsey et al., 1980).

The concentrations of styrene in blood were determined in 86 reinforced plastics workers exposed to styrene and 42 control subjects. The average styrene concentration in blood was 5.4 μM in the exposed group and 0.67 μM in the control group (Vodička et al., 2004). Blood styrene levels and other exposure markers were measured in 58 workers exposed to styrene in four fibreglass-reinforced plastics industries. A log-linear correlation (r = 0.746) was found between blood and salivary levels of styrene in exposed subjects (Bonanni et al., 2015).

Blood levels of the primary metabolite of styrene, styrene-7,8-oxide, and its metabolite styrene glycol, were measured in exposed workers (Wigaeus et al., 1983; Tornero-Velez et al., 2001). The concentrations of styrene-7,8-oxide in blood were found to be variable, dependent on exposure conditions, and generally at nanomolar levels, and the concentrations of styrene glycol in blood were generally found at low micromolar levels (Wigaeus et al., 1983).

In humans, dermal absorption was reported to be very low in occupational exposures (Limasset et al., 1999), and up to 4% using urinary styrene metabolites and exhaled styrene as markers in experimental studies (Berode et al., 1985). It was concluded that inhalation exposure leading to pulmonary absorption is the major route of absorption of styrene in exposed workers (Berode et al., 1985).

(ii) Styrene-7,8-oxide

No studies on the exclusive absorption of styrene-7,8-oxide in humans were available to the Working Group. However, in workers exposed to both styrene and styrene-7,8-oxide in the air, concentrations of styrene-7,8-oxide–albumin adducts in blood correlated with styrene-7,8-oxide exposure concentrations, indicating styrene-7,8-oxide absorption into the blood (Rappaport et al., 1996).

(b) Experimental systems

(i) Styrene

Absorption studies of styrene have been conducted in Sprague-Dawley rats (inhalation and oral route), Wistar rats (inhalation and intravenous injection), Fischer 344 rats (inhalation and dermal contact), B6C3F$_1$ mice (inhalation), and CD2F1 and NMRI mice (intraperitoneal injection) (IARC, 1994, 2002). Rodents exposed to styrene by inhalation experienced pulmonary absorption, resulting in rapid blood uptake. The uptake efficiencies of styrene in the upper respiratory tract of male CD-1 mice and male Sprague-Dawley rats were inversely related to the exposure concentration (Morris, 2000). A maximum blood concentration of 10 μg/mL (96 μM) was observed after male Fischer 344 rats were exposed to gaseous styrene at 3000 ppm by dermal absorption for 4 hours (McDougal et al., 1990). In male B6C3F$_1$ mice, the concentration of styrene in blood was 21.8 μg/mL (200 μM) and of styrene-7,8-oxide in blood was 2.25 μg/mL (20 μM) after exposure by inhalation to 500 ppm styrene for 6 hours per day, for 14 days (Mahler et al., 1999).

The concentrations of styrene-7,8-oxide in the lungs of male Sprague-Dawley rats exposed to styrene vapour using an isolated lung perfusion system, and in the lungs of male B6C3F$_1$ mice exposed to styrene vapour using an in vivo lung perfusion system, were measured over a range of exposure concentrations. After adjusting the experimental data to the species-specific in vivo conditions of lung perfusion and ventilation, the mean styrene-7,8-oxide concentrations in mouse lungs were about twice as high as those in rat lungs at equal styrene exposure concentrations of up to 410 ppm (Hofmann et al., 2006).

(ii) Styrene-7,8-oxide

Absorption studies of styrene-7,8-oxide have been conducted in Fischer 334 rats (oral route), Sprague-Dawley rats (oral route and intraperitoneal injection), $B6C3F_1$ mice (oral route and intraperitoneal injection), and CD2F1 mice (intraperitoneal injection). In CD2F1 mice given a single intraperitoneal injection of 200 mg/kg body weight (bw) of styrene-7,8-oxide, peak plasma levels were attained within 7 minutes and styrene-7,8-oxide was not detectable at 60 minutes (Bidoli et al., 1980). Oral exposure yielded poor bioavailability and variable absorption in both rats and mice, probably because of the acid-catalysed degradation of styrene-7,8-oxide (IARC, 1994). In male Fischer 344 rats exposed by inhalation to styrene (1000 ppm) or styrene-7,8-oxide (25 ppm and 50 ppm) for 6 hours per day, 5 days per week for 4 weeks, concentrations of styrene-7,8-oxide in blood of 0.37 ± 0.08 μg/g were measured. This concentration was numerically between the concentrations of styrene-7,8-oxide in the blood of rats exposed to styrene-7,8-oxide at 25 ppm and 50 ppm under the same experimental conditions (Gaté et al., 2012).

4.1.2 Distribution

(a) Humans

(i) Styrene

After exposure by inhalation, styrene is rapidly absorbed into the blood and is distributed throughout the body. Industrial workers and volunteers had styrene in their adipose tissues, and subcutaneous adipose tissue contained a higher concentration of styrene compared with blood (Wigaeus et al., 1983). It was estimated that about 8% of the styrene was retained in adipose tissues, and the half-life of styrene in adipose tissues was approximately 2.8–5.2 days (Engström et al., 1978a, b). No constant increase was observed in the mean values of urinary styrene metabolites in workers exposed over a 4-day period, suggesting that styrene does not continuously accumulate in the body (Pekari et al., 1993).

(ii) Styrene-7,8-oxide

No data on the distribution of styrene-7,8-oxide in humans were available to the Working Group.

(b) Experimental systems

(i) Styrene

The tissue distribution of styrene was determined in male Sprague-Dawley rats and CD-1 mice exposed to radiolabelled styrene at 160 ppm for 6 hours using a nose-only exposure system. Urinary excretion was the primary route of excretion in both mice and rats. Radioactivity levels were observed in many organs of both species, with nasal mucosa in both rats and mice having the highest levels. A significantly higher level of radioactivity was measured in mouse lung and nasal passages compared with rat lung and nasal passages (Boogaard et al., 2000a). The tissue distribution and time-course of styrene accumulation and loss in tissues of male CD2F1 mice given a single intraperitoneal injection of styrene at 200 mg/kg were determined. Styrene levels peaked in brain, heart, lungs, liver, kidneys, and spleen within 5–30 minutes and then declined rapidly. In perirenal fat, in which the highest concentration of styrene was measured, styrene levels peaked later (Pantarotto et al., 1980).

(ii) Styrene-7,8-oxide

The tissue distribution of styrene-7,8-oxide was determined in one study in male Sprague-Dawley rats given a single intraperitoneal injection of radiolabelled styrene-7,8-oxide at 460 μmol. The radioactivity levels were higher in liver, brain, kidney, and duodenal contents than in blood, lungs, and spinal cord (Savolainen & Vainio, 1977).

(c) Toxicokinetic and pharmacokinetic models

Multicompartment physiological-based pharmacokinetic models and toxicokinetic models have been previously described for styrene exposure (inclusive of styrene-7,8-oxide formation), and estimate styrene distribution and metabolism in humans, rats, and mice (Csanády et al., 1994; Wang et al., 1996; Tornero-Velez & Rappaport, 2001; Cohen et al., 2002; Filser et al., 2002; Sarangapani et al., 2002). Pharmacokinetic modelling of styrene-7,8-oxide concentrations in the terminal bronchioles of humans, rats, and mice predicted tissue concentrations 100-fold higher in mouse compared with human and 10-fold higher in mouse compared with rat from exposure to styrene at 0.01–10.0 ppm by inhalation (Sarangapani et al., 2002).

The physiological-based pharmacokinetic model of Csanády et al. (1994) that estimated the distribution and metabolism of styrene and styrene-7,8-oxide in humans, rats, and mice after exposure by inhalation, intravenous injection, intraperitoneal injection, and by the oral route was modified to include the parameters of enzymatic formation of styrene-7,8-oxide and styrene glycol, and of consumption of glutathione (GSH). The model simulated the distribution of styrene-7,8-oxide in both liver and lung, and the subsequent metabolism of styrene-7,8-oxide by epoxide hydrolase and glutathione-*S*-transferase (GST). The formation of styrene-7,8-oxide–haemoglobin adducts and styrene-7,8-oxide–DNA adducts in the lymphocytes of humans, rats, and mice exposed to styrene was also simulated (Csanády et al., 2003).

A population- and physiological-based pharmacokinetic model for styrene was developed using Bayesian methods to reduce the uncertainty of the partition coefficients and metabolic parameters (Jonsson & Johanson, 2002).

The physiological toxicokinetic model of Csanády et al. (1994) was used to predict the concentrations of styrene-7,8-oxide in the blood of humans and rats after oral exposure to styrene to predict genotoxic potential (Filser & Gelbke, 2016).

4.1.3 Metabolism

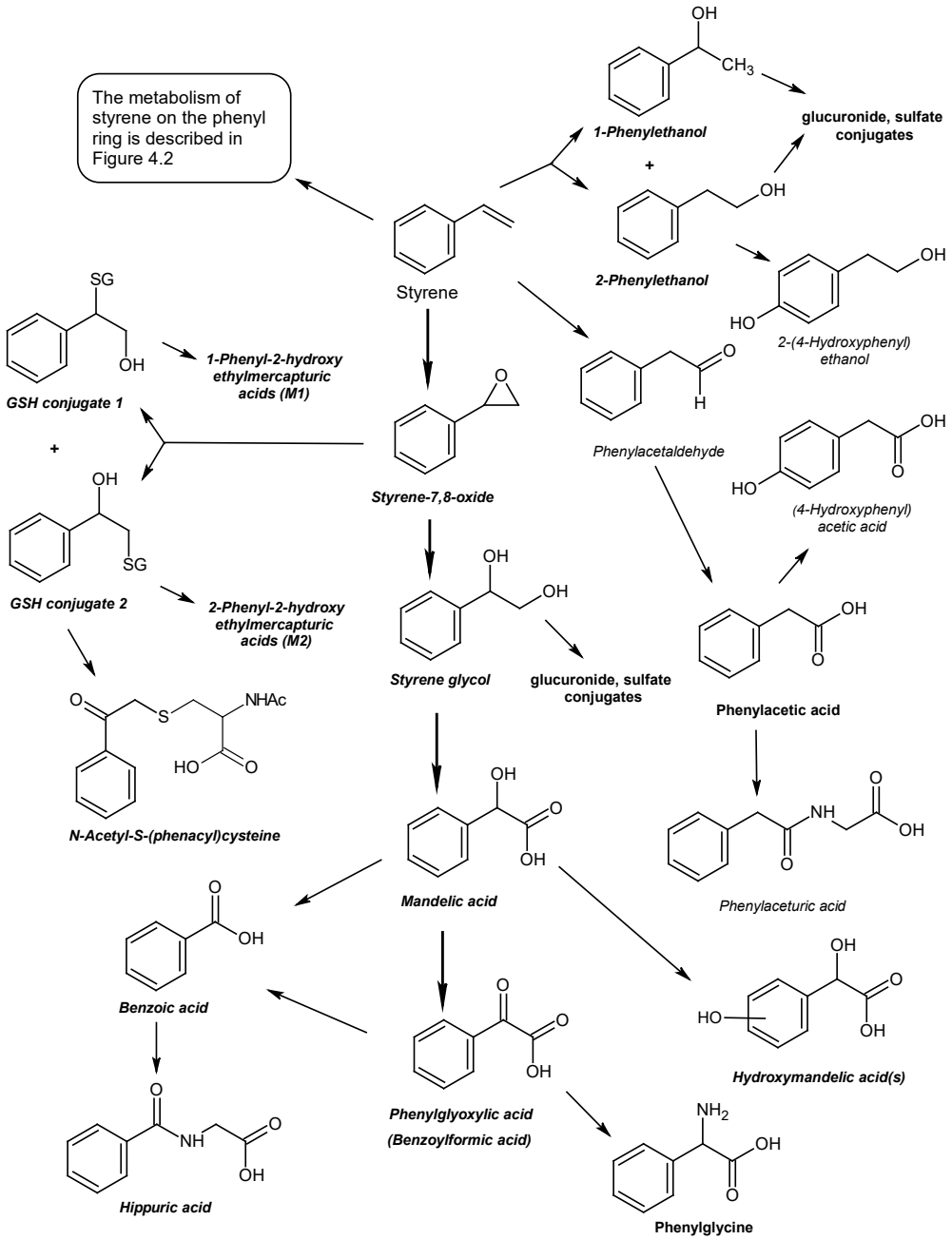
(a) Humans

See Fig. 4.1 and Fig. 4.2.

In humans, styrene is initially oxidized by cytochrome P450s (CYPs) through three distinct pathways: (i) epoxidation of the vinyl double bond, the major metabolic pathway; (ii) oxidation on the vinyl group; and (iii) oxidation on the phenyl ring. Metabolites from all three pathways have been detected in humans exposed to styrene and in experimental studies.

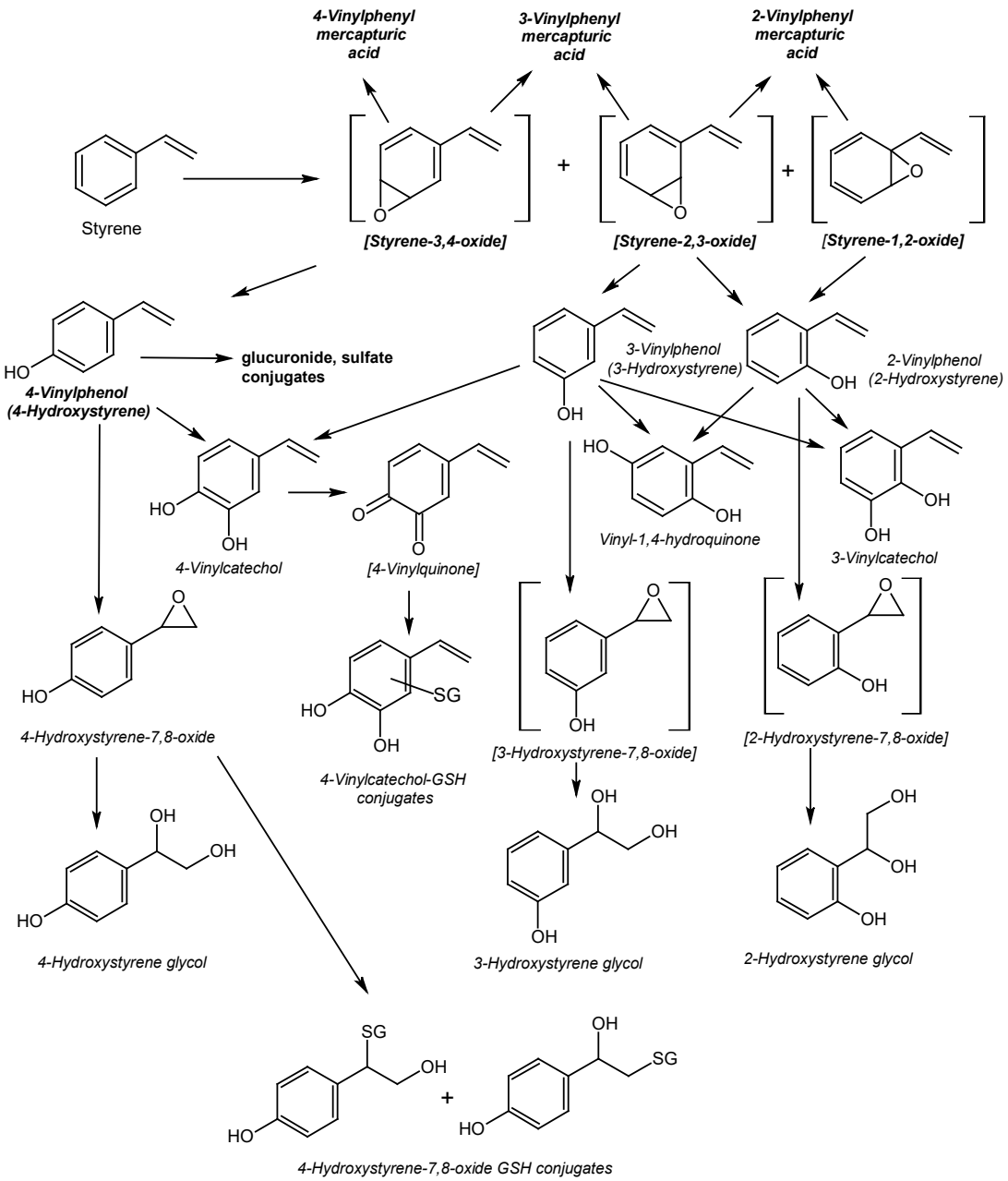
Based on in vitro studies, styrene is metabolized on the vinyl double bond to styrene-7,8-oxide by a group of human CYPs: CYP1A2, CYP2B6, CYP2C8, CYP2E1, CYP2F1, CYP3A3/3A4/3A5, and CYP4B1. CYP2B6 and CYP2E1 are found in human liver and/or lungs and CYP2F1 is found in human lungs (Nakajima et al., 1994a; Carlson, 2008). Human CYP2A13, which is preferentially expressed in the nasal mucosa, lung, and trachea (Su et al., 2000), also metabolized styrene to styrene-7,8-oxide (Fukami et al., 2008). CYP2E1 was found to play a primary role in styrene metabolism in human liver samples (Kim et al., 1997; Wenker et al., 2001a). Styrene-7,8-oxide underwent enzymatic hydration by human liver microsomal epoxide hydrolases to produce styrene glycol (phenylethylene glycol) (Oesch et al., 1974). This enzymatic process was not inhibited by the product styrene glycol (Oesch et al., 1974); however, in a racemic mixture, *R*-styrene-7,8-oxide inhibited the hydration of *S*-styrene-7,8-oxide (Wenker et al., 2000). Human liver cytosolic epoxide hydrolase can also hydrate styrene-7,8-oxide to styrene glycol, but the V_{max} was 7-fold lower compared with the microsomal form (Schladt et al., 1988). Styrene glycol was also formed by human lung microsomes (Nakajima

Fig. 4.1 Metabolism of styrene based on human and experimental studies



Metabolites in bold were found in human studies, metabolites in italics were found in experimental studies, and metabolites in both bold and italics were found in both human and experimental studies. Main pathways are indicated by thick arrows. GSH, reduced glutathione.
Adapted from Review of the Metabolic Fate of Styrene, Sumner & Fennell (1994), *Critical Reviews in Toxicology*, Taylor & Francis, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com), and Boyd et al. (1990).

Fig. 4.2 Metabolism of styrene on the phenyl ring based on human and experimental studies



Metabolites in bold were found in human studies, metabolites in italics were found in experimental studies, and metabolites in both bold and italics were found in both humans and in experimental studies. Metabolites in brackets are putative.
GS, glutathione; GSH, reduced glutathione.
Figure compiled using information from Shen et al. (2010), Zhang et al. (2011), and Linhart et al. (2012).

et al., 1994a). Styrene glycol was enzymatically conjugated to glucuronic acid by human uridine 5′-diphospho (UDP)-glucuronosyltransferases and to sulfate by human sulfotransferases, forming glucuronide and sulfate conjugates (Korn et al., 1985). Styrene-7,8-oxide can also be conjugated to GSH by GSTs to yield GSH conjugate 1 (*S*-(1-phenyl-2-hydroxyethyl)glutathione) and GSH conjugate 2 (*S*-(2-phenyl-2-hydroxyethyl) glutathione) (Pachecka et al., 1979); the human GSTs involved are GSTM1, GSTP1, and GSTT1 (Ollikainen et al., 1998; De Palma et al., 2001). These styrene-7,8-oxide–glutathione conjugates are catabolized to isomeric phenylhydroxyethylmercapturic acids (PHEMAs), *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1, also known as *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl) cysteine) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2, also known as *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine), which have been identified in the urine of exposed humans (see Section 4.1.5(a)(i)). The GSH conjugation process can also occur without enzyme involvement (Yagen et al., 1981). *N*-acetyl-*S*-(phenylacyl) cysteine is a catabolite of M2 (Manini et al., 2002). In addition to the formation of conjugates with glucuronic and sulfuric acids, styrene glycol is also metabolized to mandelic acid (MA). Depending on the sequence of oxidation (or reduction) of the α- and β-carbons of the glycol, there are several possible metabolic pathways from styrene glycol to MA. In a genetic polymorphism study of aldehyde dehydrogenase, Weng et al. (2016) proposed that styrene glycol is first metabolized by alcohol dehydrogenase to styrene glycolaldehyde (2-phenyl-2-hydroxyacetaldehyde), which is then metabolized to MA by aldehyde dehydrogenase. In humans, MA is metabolized to phenylglyoxylic acid (PGA, also known as benzoylformic acid) (Nagwekar & Kostenbauder, 1970) by alcohol dehydrogenase (Gao et al., 2009) and to *p*-hydroxymandelic acid (Pekari et al., 1993). Benzoic acid was detected in human urine after exposure to MA (Nagwekar & Kostenbauder, 1970), and is formed through a series of uncharacterized sequential oxidations. Hippuric acid (benzoylglycine), detected in human urine (Johanson et al., 2000), results from the conjugation of benzoic acid to glycine by the human glycine *N*-acyltransferases (Lino Cardenas et al., 2010). Phenylglycine was also detected in workers exposed to styrene (Manini et al., 2002; Fustinoni et al., 2008). It has been proposed that phenylglycine is formed from MA and/or PGA (Haufroid et al., 2002; Manini et al., 2002).

Another route of metabolism of styrene involves initial oxidation on the vinyl group by CYPs and then further metabolism of the observed metabolites, although the precise mechanisms of these processes are unknown. It has been proposed that the vinyl group initially undergoes both α- and β-oxidation, eventually leading to 1- and 2-phenylethanol (Cosnier et al., 2012). Racemic 1- and 2-phenylethanol were detected in the urine of workers exposed to styrene in both unconjugated forms and as glucuronide and sulfate conjugates (Korn et al., 1985, 1987). Phenylacetic acid is the oxidation product of phenylacetaldehyde (Wang et al., 2009).

In human studies, oxidation of the phenyl ring forms the putative arene oxides styrene-1,2-oxide, styrene-2,3-oxide, and styrene-3,4-oxide. Styrene-1,2-oxide and styrene-3,4-oxide can rearrange to form 2-vinylphenol (2-hydroxystyrene) and 4-vinylphenol (4-hydroxystyrene), respectively (Watabe et al., 1982). 4-Vinylphenol is conjugated to glucuronic acid and sulfate in humans, and these 4-vinylphenol conjugates represent about 0.5–1.0% of the total excretion of styrene metabolites (Manini et al., 2003). 4-Vinylphenyl mercapturic acid and traces of 2- and 3-vinylphenyl mercapturic acids were also detected in human urine, implying the formation of styrene-1,2-oxide, styrene-2,3-oxide, and styrene-3,4-oxide (Linhart et al., 2012).

Epoxidation of the vinyl group of styrene results in the formation of optically active

S- and *R*-styrene-7,8-oxides. With respect to enantiomeric selectivity, human liver microsomal epoxide hydrolase hydrated *S*-styrene-7,8-oxide 5 times faster than *R*-styrene-7,8-oxide. *R*-styrene-7,8-oxide was hydrated mainly to *R*-styrene glycol and *S*-styrene-7,8-oxide was hydrated mainly to *S*-styrene glycol. The overall activities of human styrene-7,8-oxide epoxide hydrolase varied between individuals by about 3–5-fold (Wenker et al., 2000). *R*-styrene glycol and *S*-styrene glycol were found in human blood (unconjugated and conjugated) and urine (conjugated). The maximum concentration of unconjugated *R*-styrene glycol in blood was greater than that of unconjugated *S*-styrene glycol, and the half-life of *R*-styrene glycol was longer (Wenker et al., 2001b). The ratio of the urinary stereoisomers favoured the *S*-form (or L-form as reported) (Korn et al., 1985, 1987). Both *R*- and *S*-enantiomers of MA were found in human urine at a ratio of 1:1.6 (Wenker et al., 2001b). Three mercapturic acids, degradation products of styrene-7,8-oxide–glutathione conjugates, were detected in human urine: *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1-*R*, M1-*S*) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2). M2 concentrations were higher than M1-*S* concentrations, and both were considerably greater than M1-*R* concentrations (Ghittori et al., 1997). In another study, two pairs of mercapturic acid diastereomers were identified in human urine with concentrations of M1-*R* and M2-*S* exceeding those of M1-*S* and M2-*R* (De Palma et al., 2001).

Genetic polymorphisms in styrene-metabolizing enzymes can play a role in the metabolism of styrene in humans, and urinary concentrations of the corresponding metabolites have been associated with these genetic polymorphisms. Decreased excretion levels of urinary MA and PGA, as well as decreased expression of *CYP2E1* mRNA levels, were noted in 49 subjects exposed to styrene carrying the heterozygous allele of *CYP2E1*5B* (c1/c2) when compared with subjects carrying the homozygote (c1/c1) (Prieto-Castelló et al., 2010). Of 30 workers from two fibreglass-reinforced plastics manufacturing plants, those carrying both the heterozygous alleles *CYP2E1*5B* and *CYP2E1*6* excreted lower levels of MA and PGA in their urine compared with homozygous subjects (Carbonari et al., 2015). Genotype (*CYP2E1*, *CYP2B6*, *EPHX1*, *GSTM1*, *GSTT1*, and *GSTP1* genetic polymorphisms) and lifestyle (smoking, alcohol consumption) did not significantly affect the levels of urinary MA and PGA in 73 reinforced plastics workers exposed to styrene. However, urinary MA and PGA levels were significantly decreased in a subgroup of non-smokers with the c1/c1 alleles of *CYP2E1*5B1* compared with those with the c1/c2 genotype (Ma et al., 2005). No relationships were found between genetic polymorphisms in *CYP2E1*, *EPHX1*, *GSTM1*, *GSTT1*, and *GSTP1* genes and levels of urinary MA and PGA in 75 workers in the fibreglass-reinforced plastics industry (Costa et al., 2012). In 56 workers exposed to styrene in the fibreglass-reinforced plastics manufacturing industry, the urinary concentrations of MA and PGA were significantly higher in the individuals carrying high-activity genotypes of epoxide hydrolase (*EPHX1*) compared with those carrying low-activity genotypes (Zhang et al., 2013). In 30 workers from two fibreglass-reinforced plastics manufacturing plants, individuals carrying the low-activity *EPHX1* codon 113 polymorphism exhibited reduced excretion of urinary MA and PGA compared with individuals carrying the high-activity genotype (Carbonari et al., 2015). Other studies have shown that the *EPHX1* genetic polymorphisms have no effect on urinary levels of MA and PGA in human studies (Ma et al., 2005; Costa et al., 2012). Genetic polymorphisms have been found in aldehyde dehydrogenase, and lower levels of urinary MA and PGA were observed in a group of 329 workers exposed to styrene carrying the variant inactive *ALDH2*2* allele (Weng et al., 2016). In 26 subjects experimentally exposed in a chamber to

styrene, the urinary levels of the diastereomeric PHEMAs M1 (*R*,*R*; *S*,*R*) and M2 (*R*,*R*; *S*,*R*) were significantly higher in *GSTM1*-positive subjects compared with *GSTM1*-null subjects (Haufroid et al., 2002). GSTM1-1 is the major isoenzyme catalysing GSH conjugation of styrene-7,8-oxide in humans (De Palma et al., 2001). A study of genetic polymorphisms in *EPHX1*, *GSTT1*, *GSTM1*, *GSTP1*, and *NAT2* in 95 fibreglass-reinforced plastics or polyester resins workers exposed to styrene found that *GSTM1*-null individuals produced a significantly lower level of PHEMAs compared with *GSTM1*-positive subjects (Migliore et al., 2006). In another study of 28 reinforced plastics workers exposed to low concentrations of styrene, *GSTM1*-null subjects had higher urinary concentrations of MA and PGA compared with those carrying the non-null genotype (Teixeira et al., 2004).

(b) Experimental systems

The metabolism of styrene in experimental systems is qualitatively similar to that described for humans (Section 4.1.3(a)(i)) with some quantitative differences.

In previous reviews (IARC, 1994, 2002) it was reported that, in experimental systems, the enzymatic action of Cyp catalyses the epoxidation of styrene on its vinyl group to enantiomers of styrene-7,8-oxide. Styrene-7,8-oxide can be hydrolysed by epoxide hydrolases to enantiomers of styrene glycol, or enzymatically conjugated with GSH by the GSTs to form isomeric GSH conjugates. These styrene-7,8-oxide–glutathione conjugates are further catabolized to the corresponding PHEMAs. M2 is catabolized to *N*-acetyl-*S*-(phenacyl)cysteine (Manini et al., 2002). Styrene glycol is further metabolized to MA enantiomers, and these are oxidized to PGA. Further catabolic action on MA and/or PGA eventually leads to benzoic acid and its glycine conjugate, hippuric acid. The ring oxidation of styrene by CYP forms styrene-3,4-oxide which yields 4-vinylphenol. CYP-mediated oxidation of the vinyl side chain of styrene eventually leads to 1-phenylethanol, 2-phenylethanol, and phenylacetaldehyde. Phenylacetaldehyde is a direct rat liver microsomal metabolite of styrene (Mansuy et al., 1984), and is further oxidized by aldehyde dehydrogenase to phenylacetic acid (Wang et al., 2009). The glycine conjugate of phenylacetic acid, phenylaceturic acid, was also detected in rat urine (Delbressine et al., 1980).

The Cyps involved in styrene metabolism in rat liver include Cyp2c11/6, Cyp2b1/2, Cyp1a1/2, and Cyp2e1, although in rat lung only Cyp2b1/2 is active (Nakajima et al., 1994b). Cyp2f2 and Cyp2e1 were associated with styrene metabolism in mouse lung and Cyp2e1 in mouse liver (Carlson, 1997a; Green et al., 2001a). The rate of styrene metabolism to styrene-7,8-oxide was greater in mouse lung club (Clara) cells compared with mouse type-II pneumocytes, and greater in mouse club cells compared with rat club cells. *R*-styrene-7,8-oxide was preferentially formed in mouse lung microsomes and club cells, although *S*-styrene-7,8-oxide was preferentially formed in rat lung microsomes and rat type-II pneumocytes (Hynes et al., 1999). A comparison of the rate of microsomal metabolism of styrene to styrene-7,8-oxide between species demonstrated that it was highest in mouse liver, followed by rat liver, followed by human liver (Nakajima et al., 1994a).

Male B6C3F$_1$ mice given a single intraperitoneal dose of styrene at 400 mg/kg bw metabolized the styrene to urinary 1-phenylethane diol (styrene glycol), MA, two isomeric hydroxymandelic acids (2-(4-hydroxyphenyl) ethanol and (4-hydroxyphenyl)acetic acid), and the mercapturic acids *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine and *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl)cysteine, which represented 10–15% of the given dose. PGA was a minor metabolite. Male B6C3F$_1$ mice given a single intraperitoneal injection of racemic styrene-7,8-oxide, *R*-styrene-7,8-oxide, or *S*-styrene-7,8-oxide at 150 mg/kg bw produced many of

the same metabolites as styrene. Styrene was predominantly metabolized to *S*-mandelic acid, *R*-styrene-7,8-oxide was predominantly metabolized to *R*-mandelic acid, and *S*-styrene-7,8-oxide was predominantly metabolized to *S*-mandelic acid (Linhart et al., 2000).

[The Working Group noted that there were no in vivo studies available in knockout mice or transgenic humanized mice exposed to styrene that measured the levels of styrene metabolites in the blood or in the urine. Moreover, the hepatic Cyp reductase knockout mice, epoxide hydrolase knockout mice, and transgenic humanized mice were derived from C57BL/6 mice, a strain that is insensitive to the tumorigenic effects of styrene.]

A series of in vitro metabolic studies, using liver and lung tissues taken from genetically altered mice that had metabolic enzymes related to styrene metabolism removed from their genomes, have been reported. These mice were deficient in Cyps (*Cyp2e1* knockout, *Cyp2f2* knockout), epoxide hydrolase (epoxide hydrolase knockout mice), or a hepatic Cyp reductase (Cyp reductase knockout mice). The rates of the microsomal metabolism of styrene to each enantiomer of styrene-7,8-oxide were similar using liver microsomes from *Cyp2e1* knockout mice and from wildtype controls; the rate of formation of styrene-7,8-oxide by lung microsomes from *Cyp2e1* knockout mice was lower compared with microsomes from wildtype mice. These results indicated that other Cyps in the knockout mice were capable of metabolizing styrene to styrene-7,8-oxide. Using specific inhibitors, the Cyp2e1 inhibitor diethyldithiocarbamate was the most potent towards inhibiting the formation of both enantiomers of styrene-7,8-oxide in lung and liver microsomes from both *Cyp2e1* knockout and wildtype mice, indicating that other unknown Cyps in the knockout mice were sensitive to this inhibitor (Carlson, 2003). The rates of metabolism of styrene to *R*-styrene-7,8-oxide was slightly reduced in liver microsomes from *Cyp2f2* knockout mice compared with wildtype controls, although the metabolic rates of *S*-styrene-7,8-oxide formation were similar in both *Cyp2f2* knockout mice and wildtype mice. There was a large decrease in the metabolic rate of *R*-styrene-7,8-oxide formation in the *Cyp2f2* knockout mice compared with wildtype controls, and a smaller decrease in the rate of formation of *S*-styrene-7,8-oxide in lung microsomes from both mouse strains (Carlson, 2012). It has been reported that liver and lung microsomal protein levels of Cyp1a, Cyp2a, Cyp2b, Cyp2e, and Cyp3a were similar in *Cyp2f2* knockout and wildtype mice (Li et al., 2011). The rates of liver microsomal metabolism of styrene to each enantiomer of styrene-7,8-oxide, and the enantiomeric ratios of styrene-7,8-oxides between wildtype mice and mice deficient in epoxide hydrolase, were not different. The rates of liver microsomal metabolism of styrene-7,8-oxide to both enantiomers of styrene glycol were lower in mice deficient in epoxide hydrolase than in wildtype mice (Carlson, 2010a). The liver and lung microsomal metabolism of styrene to enantiomers of styrene-7,8-oxide were compared between wildtype and hepatic Cyp reductase knockout mice. The rates of formation of each of the *R*- and *S*-enantiomers in liver microsomes decreased to greater than 96% in the hepatic Cyp reductase knockout mice. However, in lung microsomes there was a higher rate of formation of the *R*-enantiomer in hepatic Cyp reductase knockout mice compared with wildtype mice, but no difference in the formation of the *S*-enantiomer (Carlson, 2012).

In studies of male CD-1 mice, 2-vinylphenol, 3-vinylphenol (3-hydroxystyrene), 4-vinylphenol, and styrene glycol were detected in both liver and lung microsomal incubations of styrene, with the liver microsomes producing a higher rate of formation of each metabolite compared with the lung incubations. Liver microsomal incubations of styrene also produced 2-hydroxystyrene glycol, 4-hydroxystyrene glycol, and vinyl-1,4-hydroquinone. Liver microsomal incubations of the individual vinylphenols produced

the following results: 2-vinylphenol was metabolized to 2-hydroxystyrene glycol, 3-vinylcatechol, and vinyl-1,4-hydroquinone; 3-vinylphenol was metabolized to 3-hydroxystyrene glycol, 3-vinylcatechol, vinyl-1,4-hydroquinone, and 4-vinylcatechol; and 4-vinylphenol was metabolized to 4-hydroxystyrene glycol and 4-vinylcatechol. These glycols are hydration products of the intermediary hydroxystyrene-7,8-oxides. Using inhibitors, it appeared that Cyp2f2 may be more important in the metabolism of styrene to vinylphenols than Cyp2e1 in the mouse lung (Shen et al., 2010).

The rates of overall metabolism (loss of substrate) of 4-vinylphenol in lung and liver microsomes in male wildtype and *Cyp2e1* knockout mice were not different (Vogie et al., 2004).

The lung and liver microsomal metabolism of styrene to styrene glycol and 2-, 3-, and 4-vinylphenol was compared between wildtype mice and *Cyp2e1* knockout mice, and between wildtype mice and *Cyp2f2* knockout mice. *Cyp2e1* knockout mouse liver microsomes had reduced metabolic rates compared with liver microsomes from wildtype mice in producing 2-vinylphenol, 4-vinylphenol, and styrene glycol, although only the metabolic rate of 2-vinylphenol formation was reduced in the lung microsomal incubations. The order of the rates of vinylphenol metabolites formed were 2-vinylphenol > 4-vinylphenol > 3-vinylphenol in liver and lung microsomes from both *Cyp2e1* knockout and wildtype mouse strains. *Cyp2f2* knockout mouse liver microsomes produced no detectable levels of 3-vinylphenol and 4-vinylphenol, and reduced rates of formation of 2-vinylphenol and styrene glycol compared with wildtype mouse liver microsomes. *Cyp2f2* knockout mouse lung microsomes produced no detectable levels of 2-, 3-, or 4-vinylphenol, and a reduced rate of formation of styrene glycol compared with wildtype mouse lung microsomes (Shen et al., 2014).

Male CD-1 mouse lung microsomes metabolized 4-vinylphenol to 4-hydroxystyrene-7,8-oxide (4-(2-oxiranyl)-phenol) and 4-vinylcatechol, with 4-vinylcatechol predominant. Co-incubation of 4-vinylphenol with GSH and microsomes from male CD-1 mouse liver, mouse lung, male Sprague-Dawley rat lung, or human lung produced a group of 4-hydroxystyrene-7,8-oxide–glutathione conjugates. Mouse lung microsomes metabolized 4-vinylphenol at greater rates compared with rat or human lung microsomes. Co-incubation of 4-vinylphenol with GSH and mouse lung microsomes also produced 4-vinylcatechol–glutathione conjugates formed via a 4-vinylcatechol metabolite [possibly a quinone, although this metabolite has not been isolated]. 4-Hydroxystyrene-7,8-oxide–glutathione conjugates and 2-hydroxystyrene-7,8-oxide–glutathione conjugates were detected in incubations of mouse liver microsomes with *R*-styrene-7,8-oxide in the presence of GSH, suggesting an alternate metabolic pathway to vinylphenol metabolites. Using specific inhibitors, both Cyp2f2 and Cyp2e1 were implicated in the metabolism of 4-vinylphenol to 4-hydroxystyrene-7,8-oxide–glutathione conjugates (Zhang et al., 2011).

The metabolic parameters of the metabolism of styrene in isolated mitochondrial and microsomal (endoplasmic reticulum) fractions from livers of female Sprague-Dawley rats were applied to a kinetic model that incorporated Cyp2e1 levels. At low styrene concentrations (10 μM), 67% of the styrene metabolism occurred in mitochondria and 33% in the endoplasmic reticulum. At higher styrene concentrations (500 μM), the estimated styrene metabolism was 85% in the endoplasmic reticulum and 15% in the mitochondria. These results are explained by the increased metabolic efficiency of Cyp2e1 in the endoplasmic reticulum (Hartman et al., 2015).

4.1.4 Modulation of metabolic enzymes

(a) Styrene

Inhalation pre-exposure to styrene had no effect on the metabolism of styrene after subsequent styrene exposure (Wang et al., 1996). In 58 moulders and finishers exposed to styrene and acetone at four fibreglass-reinforced plastics manufacturing sites, simultaneous exposure to styrene and acetone modified styrene metabolism as evidenced by a reduction in the combined levels of urinary MA and PGA (Bonanni et al., 2015). However, the metabolism of styrene was not affected in workers exposed to styrene or human subjects experimentally co-exposed to styrene and acetone (Wigaeus et al., 1984; De Rosa et al., 1993; Apostoli et al., 1998). Multiple oral doses of ethanol temporally lowered urinary levels of MA and PGA in subjects exposed to styrene by inhalation (Cerný et al., 1990). Ethanol is a known inducer of CYP2E1 (IARC, 2012). Both single and multiple doses of ethanol shifted the excretion level versus time relationship of the urinary excretion of MA and PGA in subjects exposed to styrene by inhalation (Wilson et al., 1983; Cerný et al., 1990). Styrene metabolism in workers was not affected by occupational co-exposure to methanol and methyl acetate (Kawai et al., 1995).

(b) Styrene-7,8-oxide

No data on the modulation of metabolic enzymes by styrene-7,8-oxide in humans were available to the Working Group.

4.1.5 Excretion

(a) Humans

(i) Styrene

Micromolar levels of unmetabolized styrene were found in the urine of occupational (Ghittori et al., 1997) and experimental subjects (Johanson et al., 2000). Chamber exposure studies demonstrated that about 92% of the total absorbed dose of styrene was metabolized. Of the amount absorbed, 37% was eliminated in the urine as MA and 54% as PGA after 8 hours (Caperos et al., 1979). In another chamber exposure study, the cumulative percentage of MA and PGA excreted was 58% after 28 hours of exposure (Wigaeus et al., 1983). Several other previously discussed studies have confirmed the presence of urinary MA (Vodička et al., 1999). PGA (Wieczorek & Piotrowski, 1985), phenylethylene glycol (styrene glycol) (Korn et al., 1987), hippuric acid (Johanson et al., 2000), *p*-hydroxymandelic acid (Pekari et al., 1993), and 4-vinylphenol were excreted as a conjugate (Pfäffli et al., 1981). Urinary PHEMAs were found in workers exposed to styrene, and represent about 1% of styrene uptake in humans (De Palma et al., 2001). MA, PGA, 4-vinylphenol conjugates, and regioisomeric PHEMA levels were determined at the end of the shift in the urine of 86 reinforced plastics workers (employed in three plants) exposed to styrene, in 16 controls (maintenance workers from one of the plants), and in 26 unexposed individuals (external controls). The mean concentrations of total 4-vinylphenol conjugates were 5.64 ± 4.82 mg/g creatinine in exposed workers, with concentrations of 0.39 ± 0.39 mg/g creatinine in the plant controls and none detectable in the external controls (Vodička et al., 2004).

Phenylacetic acid and/or phenylaceturic acid accounted for less than 5% of the total excreted metabolites (Johanson et al., 2000). 1-Phenylethanol and 2-phenylethanol in both conjugated and unconjugated forms were found in human urine (Korn et al., 1985, 1987).

The urine of 10 workers exposed to styrene, sampled at the end of shift and the next morning, contained MA, PGA, phenylglycine, *N*-acetyl-*S*-(phenacyl)cysteine, the glucuronide and sulfate conjugates of 4-vinylphenol and of styrene glycol, and PHEMAs. The median concentrations at the end of shift of 4-vinylphenol glucuronide and 4-vinylphenol sulfate were 7.5 mg/g creatinine

and 6.5 mg/g creatinine, respectively (Manini et al., 2002). [The Working Group noted that no exposure data were provided.] The urine of 174 workers exposed to styrene, 26 volunteers exposed to styrene at 11.8 ppm for 8 hours, and 99 subjects not occupationally exposed to styrene was examined for levels of MA, PGA, 4-vinylphenol glucuronide, and 4-vinylphenol sulfate. The total 4-vinylphenol conjugate concentrations at the end of the shift were 1.2–3.97 mg/g creatinine (geometric mean). These metabolites comprised about 0.5–1.0% of the total excreted styrene metabolites and were eliminated following monophasic kinetics. In unexposed subjects, the background levels of total 4-vinylphenol conjugates were 0.22 mg/g creatinine (geometric mean). Smoking was related to the excretion of total 4-vinylphenol conjugates, but did not appear to be related to the excretion of MA and PGA in exposed workers (Manini et al., 2003). In a companion study, the distribution of the background levels of MA and PGA in the urine of 129 workers not exposed to styrene was log-normal across the population. No significant correlation was found between metabolite levels and sex, age, smoking, or alcohol consumption (Manini et al., 2004).

Urinary concentrations of styrene, MA, PGA, phenylglycine, 4-vinylphenol glucuronide and sulfate conjugates, and the PHEMAs M1 and M2 were determined in workers (before and after the shift) exposed to styrene and styrene-7,8-oxide (Fustinoni et al., 2008). Median exposure concentrations of styrene and styrene-7,8-oxide were 18.2 mg/m^3 and 133 µg/m^3 for reinforced plastics workers, 3.4 mg/m^3 and 12 µg/m^3 for varnish workers, and less than 0.3 mg/m^3 and less than 5 µg/m^3 for controls, respectively. The study group included 13 varnish workers and 8 reinforced plastics workers, and 22 automobile mechanics as controls. The median concentrations of urinary metabolites in the samples taken at the end of the shift were generally greater in the reinforced plastics workers compared with the varnish workers. The median concentrations of the total of MA and PGA at the end of the shift in workers exposed to styrene were 69.5–226.3 mg/g creatinine, compared with 1.17 mg/g creatinine in control subjects. The median concentrations of total 4-vinylphenol conjugates at the end of the shift in workers exposed to styrene were 1.72–3.69 mg/g creatinine, compared with 0.245 mg/g creatinine in control subjects (Fustinoni et al., 2008). Analysis of repeated measurements (four measurements per worker over 6 weeks) of the concentrations of urinary styrene and styrene metabolites in these workers indicated that within-worker variability was typically much smaller than between-worker variability for the majority of exposure metrics examined (Fustinoni et al., 2010).

The total concentrations of MA and PGA in the urine of 10 male hand-lamination workers in a reinforced plastics plant occupationally exposed to styrene were 141–1466 mg/g creatinine in the samples from the end of shift and 41.5–784.0 mg/g creatinine in the samples from the next morning. The urine samples also contained 4-vinylphenyl mercapturic acid, the degradation product of the styrene-3,4-oxide–glutathione adduct. The mean concentration of 4-vinylphenyl mercapturic acid in the samples from the end of shift was 4.59 ± 3.64 ng/mL, although in the samples from the next morning it was 2.14 ± 2.07 ng/mL. Total urinary 4-vinylphenyl mercapturic acid accounted for about 3.4×10^{-4}% of the absorbed dose of styrene (Linhart et al., 2012).

(ii) Styrene-7,8-oxide

No data on the excretion of styrene-7,8-oxide in humans were available to the Working Group.

(b) Experimental systems

(i) Styrene

The primary route of excretion in male F344 rats, male CD-1 mice, and male B6C3F$_1$ mice using nose-only exposure to radiolabelled styrene was in urine; faecal excretion was a

minor route (Sumner et al., 1997). The overall quantitative excretion of styrene and its metabolites was similar in male CD-1 mice and male Sprague-Dawley rats exposed to radiolabelled styrene at 160 ppm for 6 hours by nose-only inhalation (Boogaard et al., 2000a). Male Fischer 344 or Sprague-Dawley rats exposed to styrene by inhalation at 75 ppm and 250 ppm for 4 days excreted increased levels of MA, PGA, and hippuric acid in their urine compared with controls. After 1 day of exposure, the urinary MA and PGA concentrations of rats exposed to styrene at 250 ppm were 256 ± 55 mg/g creatinine and 672 ± 258 mg/g creatinine, respectively (Cosnier et al., 2012). Male Sprague-Dawley rats exposed by inhalation to styrene at 25–200 ppm, 6 hours per day, 5 days per week for 4 weeks, excreted MA, PGA, *N*-acetyl-*S*-(1-phenyl-2-hydroxyethyl)-L-cysteine (M1) and *N*-acetyl-*S*-(2-phenyl-2-hydroxyethyl)-L-cysteine (M2) (Truchon et al., 1990). The urine of male Sprague-Dawley rats given styrene at 1.1 mmol by intraperitoneal injection contained MA, PGA, and PHEMA (the major metabolites), and phenylglycine, *N*-acetyl-*S*-(phenacyl)cysteine, glucuronide, and sulfate conjugates of 4-vinylphenol and of styrene glycol (Manini et al., 2002). Although Bakke & Scheline (1970) found 1-phenylethanol and traces of 2-phenylethanol in the urine of rats given styrene by gavage, Manini et al. (2002) did not report the detection of the conjugates or the free forms of 1- and 2-phenylethanol.

Male B6C3F$_1$ mice given a single intraperitoneal injection of styrene at 400 mg/kg bw excreted MA, *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine, *N*-acetyl-*S*-(2-hydroxy-1-phenyl ethyl)cysteine, 1-phenylethane-1,2-diol (styrene glycol), 2-(4-hydroxyphenyl)ethanol, (4-hydroxyphenyl)acetic acid, 2-(methylthio)-2-phenylethanol, 2-(methylthio)-1-phenylethanol, and two unidentified isomeric hydroxymandelic acids (Linhart et al., 2000). The mercapturic acids were the major metabolites, followed by MA and 1-phenylethane-1,2-diol (styrene glycol) (Linhart et al., 2000). 2-Vinylphenol, 3-vinylphenol, and 4-vinylphenol were measured in the urine of male NMRI mice exposed to styrene at 600 ppm and 1200 ppm for 6 hours. Mercapturic acids related to styrene-2,3-oxide–glutathione and styrene-3,4-oxide–glutathione conjugates, that is, 2-, 3-, and 4-vinylphenylmercapturic acids, were also found in the urine at a ratio of 2:1:6. The urinary concentrations of 4-vinylphenyl mercapturic acid after exposure at 600 ppm and 1200 ppm were 0.75 ± 0.1 mg/L and 1.09 ± 0.07 mg/L, which represented 0.047% and 0.043%, respectively, of the adsorbed dose of styrene (Linhart et al., 2010).

(ii) Styrene-7,8-oxide

The urinary concentrations of MA, PGA, and hippuric acid were determined in male Fischer 344 rats exposed by inhalation to styrene-7,8-oxide at 25 ppm and 75 ppm for 4 days. After 4 days of exposure at 75 ppm, the concentrations of MA, PGA, and hippuric acid were 279 ± 92, 294 ± 118, and 1619 ± 525 mg/g creatinine, respectively (Cosnier et al., 2012). Male B6C3F$_1$ mice given a single intraperitoneal injection of styrene-7,8-oxide at 150 mg/kg bw excreted MA, *N*-acetyl-*S*-(2-hydroxy-2-phenylethyl)cysteine, *N*-acetyl-*S*-(2-hydroxy-1-phenylethyl)cysteine, 1-phenylethane-1,2-diol (styrene glycol), 2-(4-hydroxyphenyl)ethanol, (4-hydroxyphenyl) acetic acid, and two partially identified isomeric hydroxymandelic acids. Based on excretion, the mercapturic acids were the major metabolites followed by MA (Linhart et al., 2000).

4.2 Mechanisms of carcinogenesis

4.2.1 Protein adducts

Because of its electrophilicity, styrene-7,8-oxide produces stable covalent adducts not only with DNA (see Section 4.2.2) but also at the nucleophilic sites in proteins. Most relevant from this point of view are the adducts with blood protein globin because: (i) globin is easily

accessible in large amounts; (ii) a range of procedures for analysis of globin adducts is available; and (iii) globin adducts accumulate in the body during prolonged exposure in a predictable way, reflecting the lifespan of erythrocytes (~120 days in humans). The adducts with albumin have also been studied, but to a lesser extent. The three major areas of styrene-7,8-oxide–protein adducts described in this section are studies in exposed humans, in adduct characterization in human cells in vitro, and in experimental animals.

(a) Exposed humans

A limited number of studies have been undertaken to assess the styrene-7,8-oxide adduct levels in the blood proteins of reinforced plastics manufacturing workers occupationally exposed to styrene or to both styrene and styrene-7,8-oxide (Table 4.1).

The concentrations of styrene-7,8-oxide–valine in exposed workers, measured in all studies using the modified Edman degradation procedure, were mostly of the order of picomoles per gram of globin, close to the limits of detection of the gas chromatography (GC) -MS methods used. Regarding control globins from unexposed subjects, the concentrations of styrene-7,8-oxide–valine in some studies were below the limit of detection or not distinctly lower than those of exposed workers (Brenner et al., 1991; Severi et al., 1994). In other studies, the reported concentrations of 3.08 ± 3.30 pmol/g globin (Godderis et al., 2004) and 2.59 ± 0.25 pmol/g globin (Teixeira et al., 2007) were remarkably higher than that of less than 0.1 pmol/g globin reported by Vodička et al. (1999). [The Working Group noted the low variability in background levels reported by Teixeira et al. (2007).] With the exception of the study by Christakopoulos et al. (1993), no clear relationship between external exposure to styrene and the adduct levels could be established among the studies in exposed workers.

In addition, in a single relevant study on volunteers, Johanson et al. (2000) exposed four men to [$^{13}C_8$]styrene vapour at 50 ppm (213 mg/m^3) for 2 hours to attain a concentration of styrene-7,8-oxide–valine of 0.3 pmol/g globin, that is, 0.003 pmol/g per parts per million by weight (ppmh). A comparable value derived from the study of Christakopoulos et al. (1993) was 0.001 pmol/g per ppmh.

In several studies, styrene-7,8-oxide–cysteine concentrations in globin and albumin of workers exposed to known levels of styrene and styrene-7,8-oxide simultaneously were assessed using the procedure of reductive cleavage by Raney nickel followed by GC–electrochemical detector or GS-MS. No evidence was found of any exposure-related increase in globin adducts. In contrast, albumin adducts were observed to increase with exposure to either styrene or styrene-7,8-oxide. There was a stronger association with exposure to styrene-7,8-oxide than to styrene. Of the two isomeric styrene-7,8-oxide–cysteine adducts, better correlation with the concentrations of the parent compounds was observed for *S*-(2-hydroxy-1-phenylethyl)cysteine adduct, that is, the adduct in which styrene-7,8-oxide was bound to cysteine via α-carbon, and was measured as 2-phenylethanol (Yeowell-O'Connell et al., 1996). Only non-significant differences in styrene-7,8-oxide–cysteine adduct concentrations among exposed workers and controls were found by Fustinoni et al. (1998, 2008).

(b) Experimental systems in vivo

In studies in mice, the binding of intraperitoneally injected styrene or styrene-7,8-oxide to globin and to plasma proteins has been demonstrated. A linear dose–response relationship was demonstrated at all but the highest dose of styrene (0.28–4.35 mmol/kg bw) for the concentrations of styrene-7,8-oxide–valine in globin collected 3 hours later (range, 15–305 pmol/g globin) (Pauwels et al., 1996). In a separate study, styrene-7,8-oxide given at up to

Table 4.1 Styrene and styrene-7,8-oxide adducts in blood proteins of exposed workers in reinforced plastics manufacturing

Styrene or styrene-7,8-oxide concentration (mg/m³)[a]	Exposed workers			Controls		Reference
	No.	Adduct level[a]	Correlation with exposure (if $P \leq 0.05$)	No.	Adduct concentration[a]	
N-terminal valine (adduct concentration in pmol/g globin)						
Styrene: GM, 47 (3–189)	13	GM, 5.5 (2.0–15.3)		8	GM, 2.2 (0–24)	Brenner et al. (1991)
Styrene: ~300	7	28 (15–52)		3	< 13 (LOD not specified))	Christakopoulos et al. (1993)
Styrene: 31	52	< 10 (LOD)		24	< 10 (LOD)	Severi et al. (1994)
Styrene: 68 ± 49	13	1.7 ± 1.1	Styrene-7,8-oxide–valine vs styrene ($P = 0.001$) Styrene-7,8-oxide–valine vs MA ($P = 0.002$)	8	< 0.1 (LOD)	Vodička et al. (1999)
Styrene: 41 ± 41 (0–157)	44	5.23 ± 3.49 (< 1–25.52)	Styrene-7,8-oxide–valine vs styrene ($P = 0.017$)	44	3.08 ± 3.30 (< 1–13.06) (LOD, 1)	Godderis et al. (2004)
Styrene: 131 ± 16 (2–490)	57	5.98 ± 0.41	Styrene-7,8-oxide–valine vs MA+PGA ($P < 0.02$)	71	2.59 ± 0.25 (LOD, 1)	Teixeira et al. (2007)
Cysteine (adduct concentration in nmol/g protein)						
Styrene: 64.3 (0.9–235) Styrene-7,8-oxide: 0.159 (0.013–0.525)	48	1-PE-Hb: 0.084 ± 0.014 (0.02–0.45) 2-PE-Hb: 0.078 ± 0.003 (0.03–0.16) 1-PE-Alb: 0.29 ± 0.04 (0.02–1.8) 2-PE-Alb: 1.68 ± 0.12 (0.24–3.7)	2-PE-Alb vs styrene ($P = 0.017$) 2-PE-Alb vs styrene-7,8-oxide (0.010)	NR	NR	Yeowell–O'Connell et al. (1996)
Styrene: ~100	22	1-PE-Hb: 0.43 ± 0.10 (0.23–0.63) 2-PE-Hb: 5.44 ± 1.10 (3.71–7.97) 1-PE-Alb: 0.60 ± 0.49 (0.28–2.36) 2-PE-Alb: 2.84 ± 1.31 (1.02–6.79)	2-PE-Hb vs MA ($P = 0.053$) 2-PE-Alb vs MA ($P = 0.001$)	15	1-PE-Hb: 0.39 ± 0.17 (0.19–0.73) 2-PE-Hb: 5.27 ± 1.65 (1.93–8.57) 1-PE-Alb: 0.50 ± 0.23 (0.24–1.06) 2-PE-Alb: 2.74 ± 1.01 (1.17–4.91)	Fustinoni et al. (1998)

Table 4.1 (continued)

Styrene or styrene-7,8-oxide concentration (mg/m³)[a]	Exposed workers			Controls		Reference
	No.	Adduct level[a]	Correlation with exposure (if $P \leq 0.05$)	No.	Adduct concentration[a]	
Styrene: Median, 18.2 (2.3–93.4) Styrene-7,8-oxide: 0.134 (0.040–0.282) Styrene: 3.4 (0.55–16.0) Styrene-7,8-oxide: 0.012 (0.007–0.032)	8 13	1-PE-Hb: median, 0.20 (< 0.03–0.74) 2-PE-Hb: 2.31 (2.18–5.12) 1-PE-Alb: 0.23 (< 0.03–1.22) 2-PE-Alb: 5.91 (4.40–8.14) 1-PE-Hb: 0.11 (< 0.03–0.55) 2-PE-Hb: 2.80 (< 0.60–4.48) 1-PE-Alb: 0.48 (0.21–0.75) 2-PE-Alb: 6.18 (2.66–9.53)		22	1-PE-Hb: median, 0.22 (< 0.03–0.99) 2-PE-Hb: 1.96 (1.01–3.33) 1-PE-Alb: 0.19 (< 0.03–0.53) 2-PE-Alb: 3.57 (< 0.90–5.18)	Fustinoni et al. (2008)
Carboxylic acids (adduct concentration in nmol/g protein)						
Styrene: 64.3 (0.9–235) Styrene-7,8-oxide: 0.159 (0.013–0.525)	41 48	SG-Hb: 0.481 ± 0.132 (0.09–4.8) SG-Alb: 1.80 ± 0.19 (0.1–6.3)				Yeowell–O'Connell et al. (1996)

1-PE, 1-phenylethanol releasing moiety (i.e. 2-hydroxy-2-phenylethyl); 2-PE, 2-phenylethanol releasing moiety (i.e. 2-hydroxy-1-phenylethyl); Alb, albumin; GM, geometric mean; Hb, haemoglobin; LOD, limit of detection; MA, mandelic acid; NR, not reported; PGA, phenylglyoxylic acid; SG, styrene glycol releasing moiety (i.e. 2-hydroxy-2-phenylethyloxy or 2-hydroxy-1-phenylethyloxy); vs, versus.

[a] Mean ± SD (minimum to maximum) unless stated otherwise. The values represent pooled values from both non-smokers and smokers.

1.1 mmol/kg bw was bound to both globin and plasma proteins at a higher-than-proportional extent, although styrene-7,8-oxide given at up to 4.9 mmol/kg bw was bound to globin at a higher- and to plasma proteins at a lower-than-proportional extent (Byfält Nordqvist et al., 1985). In the linear components of dosage–binding relationships in both types of blood proteins, similar levels of radioactivity were found for both styrene and styrene-7,8-oxide. In other comparable studies (see paragraph below), the extent of styrene binding was much lower than that of styrene-7,8-oxide.

In a study by Rappaport et al. (1993), the concentrations of styrene-7,8-oxide–cysteine adducts in globin and albumin of rats given equimolar levels of styrene and styrene-7,8-oxide by intraperitoneal injection were about 50 times lower in both proteins after dosing with styrene. In the same paper, the in vitro reactivity of styrene-7,8-oxide with rat and human blood proteins was assessed. The values of second-order rate constants (expressed in (L/mol protein) per hour) of styrene-7,8-oxide binding (i.e. rat globin, 72; rat albumin, 63; human globin, 2.4; human albumin, 32) indicate that human globin was the least reactive among the proteins tested.

In a similar study, in both mice and rats given non-labelled and labelled styrene or styrene-7,8-oxide at up to 2.5 mmol/kg bw (Osterman-Golkar et al., 1995) by intraperitoneal injection, relationships between styrene or styrene-7,8-oxide exposure and styrene-7,8-oxide–valine adduct levels in globin were faster than linear, reflecting overload of the detoxification pathways. At low doses, styrene-7,8-oxide exposure generated 4–7 times higher concentrations of styrene-7,8-oxide–valine adduct than that for styrene exposure; at higher doses the difference was even greater. The elimination rate of total radioactivity and styrene-7,8-oxide–valine adducts from globin corresponded to the lifespan of mice erythrocytes (40 days) although carboxylic acid adducts were eliminated faster, perhaps because of hydrolysis of the ester bonds. In plasmatic proteins, initial bound radioactivity was about 25-fold higher compared with the binding to globin but its elimination rate was much faster, corresponding to the half-life of mice serum albumin (2 days).

In male Wistar rats given a single intraperitoneal injection of styrene-7,8-oxide at 0.83 mmol/kg bw, the total styrene-7,8-oxide–valine concentration in globin 24 hours after exposure was estimated as 0.72 nmol/g globin (Mráz et al., 2016b). For comparison, giving Sprague-Dawley rats an intraperitoneal injection of styrene-7,8-oxide at 1 mmol/kg bw resulted in a styrene-7,8-oxide–valine concentration of about 0.4 nmol/g globin. However, in the study by Osterman-Golkar et al. (1995), only a single diastereomer of styrene-7,8-oxide–valine was determined. In two recent studies in rats, the fate of several types of styrene-7,8-oxide–globin adducts following physiological removal of erythrocytes was investigated. In the urine of rats dosed intravenously with erythrocytes modified by styrene-7,8-oxide, styrene-7,8-oxide–valine and styrene-7,8-oxide–valine-leucine (N-terminal dipeptide of α-globin adducted by styrene-7,8-oxide) were identified as ultimate products of styrene-7,8-oxide-adducted N-terminal Val in globin, whereas the adducts with cysteine, histidine, and lysine were excreted in the form of corresponding N_{α}-acetyl derivatives (Mráz et al., 2016a). The same urinary products were also found in the urine of rats given styrene-7,8-oxide by intraperitoneal injection (Mráz et al., 2016b).

(c) Studies in vitro

In contrast to the binding of styrene-7,8-oxide to proteins, which is a straightforward chemical reaction, adduct formation from styrene is only possible in systems containing enzymes that oxidize styrene to styrene-7,8-oxide, that is, in vivo, in some cellular in vitro systems, and in subcellular microsomal fractions.

In the incubations in vitro of styrene-7,8-oxide with human or rat blood, erythrocytes,

haemoglobin, or globin, the formation of adducts was reported with cysteine, histidine, N-terminal valine, and lysine based on mass spectrometric confirmation (Hemminki, 1986; Kaur et al., 1989; Basile et al., 2002; Jágr et al., 2007). In addition, adducts derived from styrene-7,8-oxide with carboxylic acids in globin (phenylhydroxyethyl esters of aspartic and glutamic acids) were indirectly identified following mild base hydrolysis of these ester adducts to styrene glycol (Sepai et al., 1993). In some of the above studies, the concentrations of styrene-7,8-oxide adducts with particular amino acids in globin have been quantified. However, the relative proportions of all adduct types produced in globin under physiological conditions within a single experiment have not been reported. Nevertheless, by combining results from various studies, the adduct concentrations appear to decrease in the order cysteine > histidine > valine > lysine. In a study where human blood was incubated with styrene-7,8-oxide, and serum and haemoglobin were separated, digested with proteinase K, and analysed by HPLC, cysteine adducts greatly predominated both in haemoglobin and serum proteins (Hemminki, 1986). In a similar type of experiment using digestion with pronase, Jágr et al. (2007) reported histidine adducts in haemoglobin at concentrations approximately 10-fold higher than those of cysteine adducts. However, it later became obvious that the pronase digestion cleaves cysteine adducts of styrene-7,8-oxide incompletely, underestimating true concentrations by a factor of 20–30 (Mráz et al., 2016a). Histidines were found to be a dominant alkylated site in human haemoglobin when incubation was carried out with a high excess of styrene-7,8-oxide, perhaps because of the increasing number of alkylated histidine units after saturation of cysteine (Kaur et al., 1989). Detailed structural analysis of human globin adducted by styrene-7,8-oxide, based on tryptic digestion followed by HPLC-MS, revealed valine-1, histidine-20, histidine-45, histidine-50, histidine-72, and cyseine-104 as major alkylating sites in the globin α-chain, and valine-1, histidine-77, cysteine-93, histidine-97, cysteine-112, and histidine-143 as major alkylating sites in the globin β-chain (Kaur et al., 1989; Basile et al., 2002).

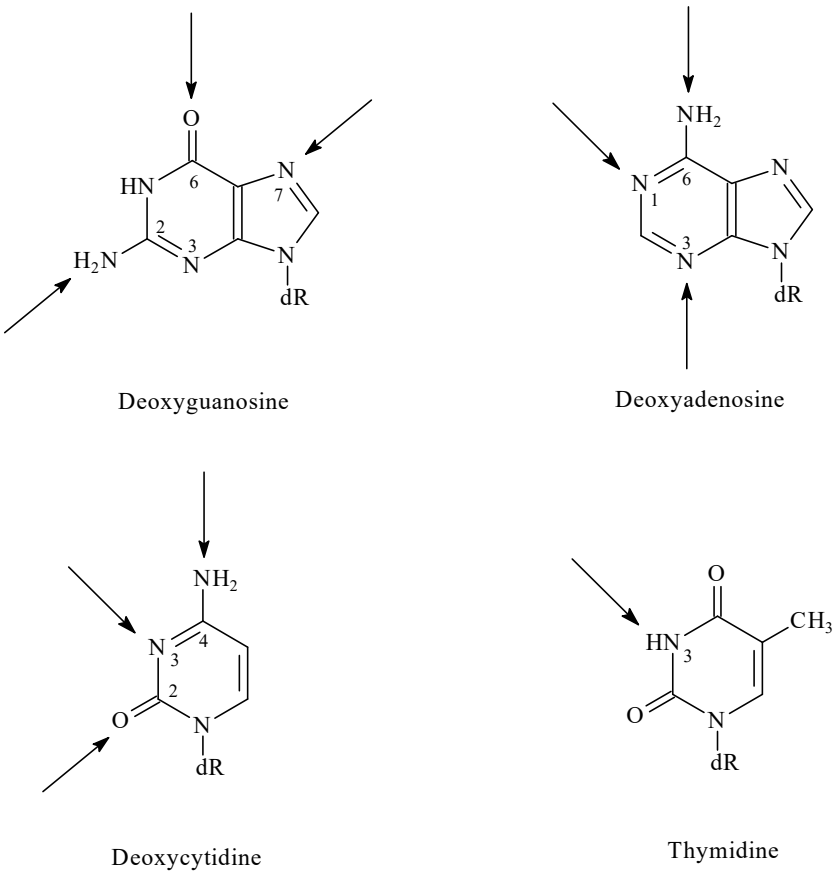
Cysteine was also found to be a dominant binding site for styrene-7,8-oxide in human albumin (Rappaport et al., 1993).

4.2.2 DNA adducts

(a) Properties

Styrene requires metabolic activation to be effectively eliminated from the body and to become genotoxic. Its principal metabolite in vivo, styrene-7,8-oxide, contributes quantitatively by far the most (> 95% in humans) to the genotoxicity of styrene; minor ring oxidation products are also shown to contribute to local toxicities, especially in the respiratory system (Vodička et al., 2006a). Styrene-7,8-oxide interacts covalently with biological macromolecules; its two electrophilic carbons, the α- and β-carbons of the epoxide moiety, attack nucleophilic sites in DNA (Fig. 4.3). Modification of the ring-nitrogens of purines and pyrimidines through the β-carbon follows the SN2 (nucleophilic substitution 2) reaction kinetics. In contrast, the epoxides that have a substituent capable of stabilizing positive charge can also react at the exocyclic amino groups through the α-carbon, the reaction following the SN1 type of mechanism (Koskinen & Plná, 2000). Table 4.2 summarizes covalent binding sites of styrene-7,8-oxide and their proportions, stabilities, and expected role in mutagenesis. *N*7-guanine is the primary target of styrene-7,8-oxide alkylation in DNA, followed by the N^2 and O^6 positions of guanine; *N*7-alkylation occurs predominantly through the β-carbon, whereas the other sites in guanine are primarily reacted through the α-carbon. As reported by Moschel et al. (1986), in O^6-guanine substitution the α-isomer can isomerize to that of β. Adenine residues are alkylated at the *N*3,

Fig. 4.3 Nucleophilic sites in DNA bases found to be covalently modified by styrene-7,8-oxide



dR, 2′-deoxyribose; SN1, nucleophilic substitution 1.
Compiled by the Working Group.

*N*1, and N^6 positions. Both α- and β-isomers of *N*1-adenine adducts are formed, the β-isomer in moderate excess (Barlow et al., 1997; Barlow & Dipple, 1998). The N^6 position is initially reacted only through the α-carbon of styrene-7,8-oxide; however, a considerable proportion of the βN^6 product arises, probably because of the Dimroth rearrangement of the β*N*1-adenine (Qian & Dipple, 1995; Barlow & Dipple, 1998). In addition, *N*1-adenine adducts undergo hydrolytic deamination under neutral conditions; the α-isomer adduct deaminates fairly readily (Barlow & Dipple, 1998). In vitro mechanisms for the formation of 2′-deoxyadenosine adducts with styrene-7,8-oxide have been summarized and compared with polycyclic aromatic hydrocarbon dihydrodiol epoxides (Kim et al., 2000). Styrene-7,8-oxide alkylates cytosine, mainly at the *N*3 position through the β-carbon and at the N^4 position through the α-carbon (Barlow & Dipple, 1999). In addition, the α-isomers of *N*3- and O^2-cytosine adducts have been identified. Interestingly, the *N*3-substituted deoxycytidine undergoes rapid hydrolytic deamination to the corresponding deoxyuridine adduct (the half-lives of the α- and β-isomer are 6 minutes and 160 minutes, respectively). O^2-cytosine adducts are also unstable, being prone to depyrimidation and interconversion between the α- and β-isomers (Koskinen et al., 2000b). Thymidine is a poor nucleophile towards styrene-7,8-oxide, and only minor alkylation at the *N*3 position has been

Table 4.2 Spectrum of styrene-7,8-oxide DNA adducts: stabilities, proportions, and expected role in mutagenesis

Base	Position	Chemical stability	Proportion and half-life in DNA[a]	Expected mutation	References
Guanine	*N*7	Depurination, ring-opening	α*N*7 29%, 51 h β*N*7 45%, 51 h	GC → TA	Hemminki & Hesso (1984); Moschel et al. (1986); Vodička et al. (1994, 2002); Koskinen et al. (2000a, 2001a)
	N^2	Stable	αN^2 3%	GC → TA	
	O^6	Unstable, α to β isomerization	~1%, 1320 h	GC → AT	
Adenine	N^6	Stable	αN^6 6% βN^6 1%	AT → GC	Savela et al. (1986); Qian & Dipple (1995); Koskinen et al. (2000a, 2001a, b); Vodička et al. (2002)
	*N*3	Depurination	α*N*3 6%, 10 h β*N*3 3%, 20 h	AT → TA	
	*N*1	Dimroth rearrangement (→ N^6 adenine), hydrolytic deamination (→ *N*1 hypoxanthine)	α*N*1 < 1%, 94 h β*N*1 2%, 450 h α*N*1-hypoxic 2%	AT → GC	
Cytosine	*N*3	Hydrolytic deamination (→ *N*3 uracil)	β*N*3-uracil 2%	CG → AT, GC → TA	Barlow & Dipple (1999); Koskinen et al. (2000b, 2001a)
	N^4	Stable	αN^4 1%	–	
	O^2	Depyrimidation, α/β interconversion	ND	–	
Thymidine	*N*3		ND	–	Savela et al. (1986); Koskinen et al. (2000a)
Guanine/ deoxyguanosine	8-OHdG	Stable	ND	GC → TA	Vettori et al. (2005)

8-OHdG, 8-hydroxy-2′-deoxyguanosine (determined in human SK-N-MC neuroblastoma cell lines); h, hour(s); ND, not determined.

[a] Relative proportions of various adducts in double-stranded DNA treated for 32 h with styrene-7,8-oxide in a buffered solution at pH 7.4, including depurinated fraction.

Source: Styrene metabolism, genotoxicity, and potential carcinogenicity. Vodička et al. (2006a), *Drug Metabolism Reviews*, reprinted by permission of the publisher (Taylor & Francis Ltd, http://www.tandfonline.com) and of Dr Vodička.

observed (Koskinen & Plná, 2000; Koskinen et al., 2000a). The phosphate group was also identified as a target of alkylation in nucleotides; however, in the dinucleotide dGpdT no styrene-7,8-oxide alkylation of the intervening phosphodiester was observed (Koskinen & Hemminki, 1999). As apparent from Table 4.2, the relative proportions of the DNA adducts depend on the time of exposure to the alkylating agent. Adducts induced by styrene-7,8-oxide at *N*7-guanine [7-(hydroxyphenylethyl)guanines] and *N*3-adenine [3-(hydroxyphenylethyl)adenines] readily depurinate and reach an apparent saturation level considerably faster than adducts formed at positions involved in base-pairing. Half-lives of depurination from double-stranded DNA are listed in Table 4.2. *N*1-adenine adducts are partially unstable in DNA, undergoing Dimroth rearrangement or hydrolytic deamination; the corresponding half-lives are listed in Table 4.2. Chemically stable styrene-7,8-oxide–DNA adducts are αN^6-adenine, αN^2-guanine, and β*N*3-uracil adducts. O^6-guanine adducts account for about 1%; the respective half-life for the β-isomer form of this adduct in double-stranded DNA has been estimated as 1320 hours (Vodička et al., 1994, 2002). The chemical reactivity of styrene-7,8-oxide with 4-(*p*-nitrobenzyl)pyridine (with similar nucleophilic character as DNA bases) was recently investigated; the reactivity is strongly affected by temperature and pH, suggesting careful

consideration of these parameters in physiological models (González-Pérez et al., 2014).

(b) Mutagenic potential

Table 4.2 illustrates the mutagenic potential of the different styrene-7,8-oxide–DNA adducts. Mutations in human cells and in experimental systems, including the Ames assay, are addressed in Sections 4.2.3(b) and 4.2.3(c), respectively; studies of mutations in exposed humans are addressed in Section 4.2.3(a).

*N*7-guanine adducts are expected to result in GC → TA and *N*3-adenine adducts in AT → TA transversions (Vodička et al., 2002, 2006a), since DNA polymerases preferentially insert an adenine opposite an apurinic site (Loeb et al., 1986). In assays for mutagenicity in cultured human T-lymphocytes treated in vitro with styrene-7,8-oxide, these mutations were found more frequently in hypoxanthine-guanine phosphoribosyl transferase (*HPRT*) mutant clones than in controls, although the predominant mutation type was AT → GC transition (Bastlová & Podlutsky, 1996). AT → TA mutations were also observed in a site-specific mutation study (carried out on oligodeoxynucleotides) in which a styrene-7,8-oxide adduct at the N^6 position of adenine was inserted in the N-*RAS* gene at codon 61 (Latham et al., 1993; Latham & Lloyd, 1994). However, the N^6-adenine adduct showed only minor miscoding potential, as it still has the ability to base-pair with thymine. Possibilities of by-passing of the N^6-adenine adduct in experimental systems were further discussed by Latham et al. (1996), but no major impact on DNA polymerization was reported (Latham et al., 2000; Hennard et al., 2001). Additional evidence suggests that major groove N^6-adenine adducts of styrene and butadiene oxides do not strongly perturb DNA structure and are not particularly mutagenic (Simeonov et al., 2000; Scholdberg et al., 2004). The *N*1-adenine or the corresponding *N*1-hypoxanthine adducts may contribute more to mutagenesis at AT-base pairs, since these adducts block a central hydrogen bonding site of adenine residues. The role of *N*3-cytosine adducts in mutagenesis may be deduced on the basis of experiments with protein extracts from mammalian cells that enzymatically repair *N*3-(2-hydroxypropyl)-dCyd, but not the corresponding uracil adduct (Plna et al., 1999). *N*3-(2-hydroxyethyl)-2′-deoxyuridine represents a mutagenic lesion, resulting in a GC → AT transition and, to a minor extent, GC → TA transversion mutations (Zhang et al., 1995). Since the β-isomer of the *N*3-uracil adduct was found in DNA treated in vitro with styrene-7,8-oxide , *N*3-uracil adducts may contribute to the GC → TA mutations, detected in the *HPRT* gene (Bastlová & Podlutsky, 1996). Alkylation at the O^6 position of guanine is also considered as a pro-mutagenic lesion, resulting in GC → AT transitions (Jansen et al., 1995); however, molecular analysis of styrene-induced mutations at the *HPRT* locus suggested that O^6-guanine adducts do not represent a major mutagenic lesion (Bastlová & Podlutsky, 1996). In an in vitro study of oligonucleotides containing N^2-guanine adducts derived from butadiene, acrolein, crotonaldehyde, and styrene, all the adducts blocked deoxycytidine triphosphate incorporation opposite them; adenine was preferentially incorporated opposite the acrolein- and crotonaldehyde-formed adducts, although thymine incorporation was preferred at the butadiene- and styrene-derived adduct sites (Zang et al., 2005).

No studies on the DNA binding potential of styrene-2,3-oxide and styrene-3,4-oxide were available to the Working Group.

(c) Exposed humans

See data reported in Table 4.3.

There were no data available to the Working Group on DNA adducts from exposure to styrene-7,8-oxide only.

In an early study, two DNA adducts, chromatographically similar to products of styrene-7,8-oxide, reacted with DNA and

Table 4.3 DNA damage and gene mutation in humans exposed to styrene

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
DNA adducts	NR	Reinforced plastics	Peripheral blood	1 exposed, 1 control	96 ppm (8 h TWA)		+	Liu et al. (1988)
DNA adducts (^{32}P-postlabelling)	NR	Hand-lamination workers (two workplaces)	Peripheral blood	10 exposed, 8 controls 13 exposed, 10 controls	370 (300–700) mg/m^3 210 mg/m^3		+	Vodička et al. (1993) See also Vodička & Hemminki (1993), Hemminki & Vodička (1995)
DNA adducts (^{32}P-postlabelling)	NR	Boat manufacturing	Peripheral blood	47 exposed	65.6 (1–235) mg/m^3		+ (one-sided $P = 0.049$ for adduct 1, one-sided $P = 0.012$ for adduct 2)	Horvath et al. (1994)
DNA adducts	Czech Republic, 1993	Hand-lamination workers	Lymphocytes Granulocytes	9 exposed, 7 controls	122 (40–225) mg/m^3	Age, smoking	+ ($P < 0.01$) – ($P > 0.05$)	Vodička et al. (1994); see also Hemminki & Vodička (1995)
• DNA damage/ SSBs • DNA adducts	Germany, 1991	Styrene production plant	Peripheral blood	25 exposed, 25 controls	0.31 (SD, 0.88) ppm (8 h TWA)	Smoking, age, sex	– ($P > 0.05$) – ($P > 0.05$)	Holz et al. (1995)
DNA adducts	NR	Reinforced plastics workers	Urine	61 exposed, 22 controls	NR (up to 286) mg/m^3	Smoking	+ ($P < 0.05$)	Mikes et al. (2010)
DNA damage/ SSBs	USA, 1988	Reinforced plastics workers	Peripheral blood	14 exposed, 9 controls	GM, 11.2 (1–44) ppm (8 h TWA)		+ ($P < 0.003$)	Brenner et al. (1991)
DNA adducts	Czech Republic, NR	Hand-lamination workers	Peripheral blood	9 exposed, 11 controls	76.2 (10–115) mg/m^3	Sex, age, smoking, alcohol, medication	+	Koskinen et al. (2001b)
DNA damage/ SSBs (comet assay)	Italy, 2003	Reinforced plastics and polyester resins workers	Peripheral blood	48 exposed, 14 controls	GM, 36.8 (1.2–115.7) ppm (8 h TWA)		+ ($P < 0.001$)	Buschini et al. (2003)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs (comet assay) • Oxidative damage to DNA/ comet assay version (Fpg and EndoIII) • Chromosomal aberrations	Czech Republic, 1999	Reinforced plastics workers	Peripheral blood	17 high concentration, 12 medium concentration, 19 controls 44 exposed, 19 controls	199.1 (SD, 101.6) mg/m^3 55.0 (SD, 22.9) mg/m^3 199.1 (SD, 101.6) mg/m^3 55.0 (SD, 22.9) mg/m^3 101.2 (SD, 102.4) mg/m^3	Smoking	(+) ($P < 0.001$) – ($P > 0.05$) + ($P < 0.001$)	Somorovská et al. (1999); see also Vodička et al. (2001b)
DNA damage/ SSBs	Egypt, 2002	Reinforced plastics workers	Peripheral blood	26 exposed, 26 controls	Median, 130 (90–170) mg/g creatinine MA in urine Median, 110 (88–150) mg/g creatinine PGA in urine	Smoking, sex	+ ($P < 0.01$)	Shamy et al. (2002)
DNA damage/ SSBs (comet assay)	Spain, 2002	Reinforced plastics workers	Peripheral blood	14 exposed, 30 controls	< 20 ppm (8 h TWA; converted from MA in urine)		+ ($P < 0.01$)	Laffon et al. (2002a)
• DNA damage/ SSBs (comet assay) • Oxidative damage to DNA (8-OHdG)	Thailand, 2011	Reinforced plastics workers	Peripheral blood	50 exposed, 40 controls	17.0 (0.3–66.53) ppm (8 h TWA)	Smoking, alcohol	+ ($P < 0.05$) + ($P < 0.05$)	Wongvijitsuk et al. (2011)
DNA damage/ SSBs	Sweden, 1993	Plastics factory	Peripheral blood	17 exposed	7.0 (0.04–20.0) ppm (8 h TWA)		+ ($P = 0.03$)	Walles et al. (1993)
DNA damage/ SSBs (comet assay)	Portugal, 2012	Reinforced plastics workers	Peripheral blood	67 exposed, 68 controls	30.4 (0.5–114.0) ppm (8 h TWA)	Smoking, age, sex	– ($P = 0.058$)	Costa et al. (2012)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs (comet assay) • Chromosomal aberrations • Base excision repair capacity of oxidative damage to DNA • Oxidative DNA damage/comet assay version (EndoIII) • Micronuclei	Czech Republic, 2004	Reinforced plastics workers	Peripheral blood	86 exposed, 42 controls	81.3 (SD, 56.3) mg/m^3		– ($P > 0.05$) – + ($P < 0.001$) – ($P > 0.05$) (+) ($P = 0.002$)	Vodička et al. (2004)
• DNA damage/ SSBs (comet assay) • Oxidative damage to DNA/ comet assay version (Endo III) • DNA repair capacity • Micronuclei	Czech Republic, 2010	Reinforced plastics workers	Peripheral blood	60 exposed, 37 controls 60 exposed, 37 controls 60 exposed, 37 controls 62 exposed, 50 controls	50.3 (0–238) mg/m^3	Smoking, age, sex Smoking, age, sex Smoking, age, sex Smoking	– ($P < 0.001$) – ($P > 0.05$) + ($P = 0.005$) – ($P > 0.05$)	Hanova et al. (2010)
• DNA damage/ SSBs (comet assay) • Micronuclei in blood and nasal mucosa	Belgium, 2000–2001	Reinforced plastics industries	Peripheral blood Peripheral blood, nasal mucosa	37 exposed, 44 controls 38 exposed, 41 controls (blood); 23 exposed, 17 controls (nasal mucosa)	9.5 (SD, 9.6) ppm (converted from urine)	Smoking, age Smoking, alcohol consumption, age	– ($P = 0.878$) + ($P < 0.05$)	Godderis et al. (2004)
DNA damage/ SSBs (comet assay)	Italy, 2002	Reinforced plastics workers	Sperm	46 exposed, 27 controls	Median, 173.6 (5.8–1428.7) mg/g creatinine MA in urine	Age, smoking	+ ($P < 0.001$)	Migliore et al. (2002)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs, DSBs (comet assay) • Oxidative damage to DNA/ comet assay (version Fpg and EndoIII)	Italy, 2009	Reinforced plastics workers	Peripheral blood	34 exposed, 29 controls	46.74 (9.86–106.10) ppm (8 h TWA)	Smoking, sex, age	+ ($P < 0.0001$) – ($P > 0.05$)	Fracasso et al. (2009)
Oxidative damage to DNA, 8-OHdG	Germany, 1997	Boat builders	Peripheral blood	17 exposed, 67 controls	NR	Smoking, age	+ ($P < 0.001$)	Marczynski et al. (1997a)
• Oxidative damage to DNA, 8-oxo-dGuo • Oxidative damage to DNA: U-8-oxodGuo, U-8-oxoGuo, U-8-oxoGua	Italy, 2009	Reinforced plastics workers	Peripheral blood Urine	60 exposed, 50 controls	107.4 mg/m^3 66.7 mg/m^3	Smoking, age	– ($P = 0.002$) +/– ($P = 0.24$) ($P = 0.008$) ($P = 0.74$)	Manini et al. (2009)
Gene mutation/ GPA in vivo somatic cell mutation assay	Berkeley, USA, 1993	Boat manufacturing and maintenance workers	Peripheral blood	15 high concentration 22 low concentration	32 ppm 1.2 ppm (8 h TWA)		– ($P = 0.028$)	Compton-Quintana et al. (1993)
Gene mutation/ GPA in vivo somatic cell mutation assay	Finland, 1996	Reinforced plastics workers	Peripheral blood	47 exposed, 47 controls	37 (6–114) ppm (8 h TWA)	Age, smoking, sex	– ($P = 0.058$)	Bigbee et al. (1996)
Gene mutation/ *HPRT* MF	Czechia, 1999	Reinforced plastics workers	Peripheral blood	19 exposed, 19 controls	101.2 (SD, 102.4) mg/m^3		+/– ($P > 0.05$)	Vodička et al. (2001b)

Table 4.3 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• DNA damage/ SSBs (comet assay) • O^6 guanine adducts • N-terminal valine of haemoglobin • Gene mutation/ *HPRT* MF	Czechia, 1995	Reinforced plastics workers	Peripheral blood	13 exposed, 13 controls	68.0 (15–156) mg/m^3	Smoking	(+) ($P < 0.001$) (+) ($P < 0.001$) (+) ($P < 0.001$) (+) ($P = 0.039$)	Vodička et al. (1999)
Gene mutation/ *HPRT* MF	Czechia, 1993–1994	Hand-lamination workers	Peripheral blood	9 exposed, 15 controls	91 (25–250) mg/m^3	Smoking	– ($P = 0.021$)	Vodička et al. (1995)

8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; 8-oxoGuo, 8-oxo-7,8-dihydroguanosine; DSB, double-strand break; EndoIII, endonuclease III; Fpg, formamido pyrimidine glycosylase; GM, geometric mean; GPA, glycophorin A; h, hour(s); *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; MA, mandelic acid; MF, mutation frequency; NR, not reported; PGA, phenylglyoxylic acid; ppm, parts per million; SD, standard deviation; SSB, single-strand break; TWA, time-weighted average; U-, urinary.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+), positive result in a study of limited quality.

deoxyguanosine monophosphate in vitro were detected by ^{32}P-postlabelling analysis in DNA isolated from lymphocytes of a worker exposed to styrene (Liu et al., 1988). There followed reports of styrene–DNA adducts in the leukocytes of 23 lamination workers, characterized as O^6-guanine adducts at a level of about 5 adducts per 10^8 nucleotides (Vodička & Hemminki, 1993; Vodička et al., 1993). Background levels in unexposed controls (n = 8) were at less than 1 adduct per 10^8 nucleotides. [The Working Group noted that these may not have been styrene-derived adducts because they were distinct from the standard (Hemminki & Vodička, 1995).]

In a study of 47 boat makers exposed to styrene, 2 DNA adducts were detected in blood mononuclear cells by ^{32}P-postlabelling analysis (Horvath et al., 1994). Adduct 1 was identified as N^2-(2-hydroxy-1-phenylethyl)-2′-deoxyguanosine-3′,5′-bisphosphate, for which a standard was available, although adduct 2 was not characterized. Adduct 1 was detected at a level of 0.6–102.1 per 10^8 nucleotides, and adduct 2 at 0.1–70.9 adducts per 10^8 nucleotides.

In a study of the same population, involving multiple sampling of 48 healthy workers at a boat manufacturing factory exposed to both styrene and styrene-7,8-oxide, adducts 1 and 2 were again detected; the correlation between the concentrations of the adducts and of the styrene exposure was observed to be at or near the 0.05 level of statistical significance (Rappaport et al., 1996). Adduct concentrations were more highly correlated with styrene exposure among non-smokers (n = 22) than among smokers (n = 26).

In a study of 9 lamination workers, ^{32}P-postlabelling analysis was used to detect styrene–DNA adducts formed at the O^6 position of guanine in blood cells (Vodička et al., 1994). Adduct levels in lymphocytes, but not granulocytes, were significantly higher in workers than in 7 unexposed controls (clerical workers at the same factory): 5.4 adducts per 10^8 nucleotides versus 1.0 adducts per 10^8 nucleotides. After 2 weeks of vacation, adduct levels in the same workers were not significantly diminished. After an additional 1 month of work, adduct concentrations remained similar.

The results of these three sampling times, plus a fourth sampling about 6 months later, were reported in a subsequent study (Vodička et al., 1995). Adduct concentrations in lymphocytes were very similar at all four time points, and significantly higher in exposed workers than in controls.

Similar concentrations of styrene–O^6-guanine adducts were again reported in exposed workers, higher than in controls in all consecutive samplings, in a subsequent 3-year study of lamination workers (Vodička et al., 1999),

The concentrations of several uncharacterized DNA adducts were not elevated in the peripheral leukocytes of 25 healthy workers at a styrene production plant, reportedly exposed to low concentrations of styrene (73–3540 µg/m^3), relative to those in 25 healthy unexposed controls matched for age and sex (Holz et al., 1995). [The Working Group noted that no authentic standards were used, in direct contrast to other studies.]

In a study of 61 hand-lamination workers at four reinforced plastics plants, urine samples were collected from 58 men and 3 women at the end of the work shift (Mikes et al., 2010). Ten workers also provided a sample the next morning. Using MA measurements as an indicator of exposure concentrations, 28 samples at the end of the shift (68–1145 mg/g creatinine) and all 10 of the samples from the next morning were analysed for N3-adenine adducts of styrene [3-(2-hydroxy-1-phenylethyl)adenine (N3αA) and 3-(2-hydroxy-2-phenylethyl)adenine (N3βA)] by mass spectrometry. As controls, samples from non-smoking volunteers (17 men and 5 women) were analysed. The adducts were detected in 7 out of 9 samples with MA concentrations greater than 400 mg/g creatinine, and in 6 out of 19 samples with MA concentrations less

than 400 mg/g creatinine. *N*3αA and *N*3βA were also detected in 9 and 3 samples, respectively, of the 22 control samples. The concentrations of the two adducts at the end of the shift were 2.8 ± 1.6 pg/mL in the group exposed to a high concentration of styrene (n = 9), 1.8 ± 1.3 pg/mL in the group exposed to a low concentration of styrene (n = 19), and 1.5 ± 1.3 pg/mL in the unexposed controls (n = 22).

A method combining high-performance liquid chromatography (HPLC) and ^{32}P-postlabelling that was developed to detect *N*1-adenine DNA adducts in workers exposed to styrene had a detection limit of 0.4 adducts per 10^8 nucleotides (Koskinen et al., 2001b). Using this method, adducts were detected in 3 out of 9 exposed workers (mean level, 0.79 ± 0.14 adducts per 10^9 nucleotides), but not in any of the unexposed control subjects (n = 11).

(d) Human cells in vitro

See also data reported below, in Table 4.4.

A dose-dependent increase in both *N*7-guanine and alkali-labile lesions in human embryonic lung cells treated with styrene-7,8-oxide was demonstrated. The level of *N*7-guanine adducts was 3-fold higher after treatment with styrene-7,8-oxide at 100 µM for 3 hours compared with the treatment for 18 hours, whereas alkali-labile lesions continued to increase in a concentration-dependent manner. The differences in *N*7-guanine adduct levels following 3-hour and 18-hour treatments could be ascribed to the conversion of *N*7-guanines into abasic sites, either spontaneously or as a result of DNA repair processes (Vodička et al., 1996).

Treatment of whole blood with styrene-7,8-oxide at a concentration of 40 µM, followed by DNA isolation and analysis by HPLC–mass spectrometry (MS) by electrospray ionization, led to the detection of adducts formed at *N*7 of guanine (Pauwels & Veulemans, 1998). Similarly, styrene-derived *N*7-guanine adducts were detected by ^{32}P-postlabelling analysis in human embryonal lung cells treated in vitro with styrene-7,8-oxide (Vodička et al., 1996).

In peripheral blood lymphocytes, styrene-7,8-oxide treatment resulted in a dose-dependent decrease in cell survival and an increase in O^6-guanine adducts in DNA, alkali-labile lesions, and *HPRT* mutations, whereas higher concentrations induced pronounced cytotoxic effects. The levels of O^6-guanine [O^6-(2-hydroxy-1-phenylethyl)guanine] adducts in DNA in treated cells correlated with styrene-7,8-oxide concentrations (4 adducts per 10^8 nucleotides were detected at the highest concentration of styrene-7,8-oxide). O^6-guanine adducts in DNA were still detectable in peripheral blood lymphocytes cultured for 6–8 days after treatment, suggesting slow removal of these adducts. Although O^6-guanine adducts in DNA correlated strongly with alkali-labile lesions in DNA comet assay, no correlation was found between DNA adducts and *HPRT* mutant frequencies (Bastlová et al., 1995). A concentration-related increase of diastereomeric *N*7β-guanine adducts was observed in peripheral blood lymphocytes by using an optimized technique including HPLC and ^{32}P-postlabelling. *N*7-guanine adducts were found at a 150-fold excess compared with O^6-guanine adducts; N^2-guanine adducts have also been detected (Vodička et al., 2002).

(e) Experimental systems

The potential of styrene and styrene-7,8-oxide to induce DNA adducts in experimental animals was initially studied using radiolabelled styrene or styrene-7,8-oxide (Cantoreggi & Lutz, 1992, 1993; Lutz et al., 1993), and reviewed by Phillips & Farmer (1994). Comparisons between animal studies are difficult because of varying experimental designs (rats and mice, various routes of exposure, and doses varying over several orders of magnitude). As described in Table 4.5 the following DNA adducts induced by styrene have been identified in experimental animals: *N*7-guanine, O^6-guanine, and *N*1-adenine

Table 4.4 Genetic and related effects of styrene and styrene-7,8-oxide in human cells in vitro

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Styrene						
DNA strand breaks (comet assay)	Blood mononuclear leukocytes	+	NT	5 mM [521 µg/mL]		Laffon et al. (2003b)
Other (comet assay)	Human skin in vitro	+	NT	10 000 ppm	Positive dose–response relationship	Costa et al. (2006)
Chromosomal aberrations, micronuclei	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.03% (v/v)	Single dose	Linnainmaa et al. (1978a)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.5 mM [52 µg/mL]		Pohlová et al. (1984)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes and isolated lymphocytes)	+	NT	1 mM [104 µg/mL]		Jantunen et al. (1986)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	2 mM [208 µg/mL]	Positive dose–response relationship	Norppa et al. (1983)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	10 µM [1 µg/mL]	Positive dose–response relationship	Chakrabarti et al. (1993)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.5 mM [52 µg/mL]		Lee & Norppa (1995)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	+	0.5 mM [52 µg/mL]	Concurrent lack of GSTM1/GSTT1 increase in genotoxicity of styrene; dose–response with two doses	Bernardini et al. (2002)
Styrene-7,8-oxide						
DNA adducts (*N*7-guanine adducts, ^{32}P-postlabelling); DNA strand breaks (comet assay)	Human embryonal lung fibroblasts	+		10 µM [1.2 µg/mL]		Vodička et al. (1996)

Table 4.4 (continued)

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA adducts (*N*7-guanine adducts, HPLC EMS)	Human whole blood (lymphocytes)	+		40 µM [5 µg/mL]		Pauwels & Veulemans (1998)
DNA adducts (O^6-guanine adducts, ^{32}P-postlabelling); *HPRT* locus mutations	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	+		0.2 mM [24 µg/mL]		Bastlová et al. (1995), Bastlová & Podlutsky (1996)
DNA strand breaks (comet assay)	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	+		50 µM [6 µg/mL]		Bastlová et al. (1995)
DNA strand breaks (comet assay)	Isolated human lymphocytes	+		50 µM [6 µg/mL]	Endonuclease III sensitive sites were induced at 100 µM	Köhlerová & Stětina (2003)
DNA strand breaks (comet assay)	Human lymphocytes (whole-blood lymphocytes)	+		0.025 mM [3 µg/mL]		Speit et al. (2012)
DNA strand breaks (comet assay)	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	+		0.01 mM [1.2 µg/mL]		Bausinger & Speit (2014)
DNA strand breaks (comet assay)	Human lymphocytes (isolated lymphocytes)	+		0.05 mM [6 µg/mL]		Cemeli et al. (2009)
DNA strand breaks (comet assay)	Human blood mononuclear leukocytes	+		0.1 mM [12 µg/mL]		Laffon et al. (2002b)
DNA strand breaks (comet assay)	Human peripheral blood mononuclear cells	+		0.1 mM [12 µg/mL]		Godderis et al. (2004)
DNA strand breaks (comet assay)	Human peripheral blood mononuclear cells	+		0.05 mM [6 µg/mL]		Fabiani et al. (2012)
DNA strand breaks (comet assay)	Human testicular cells	+		0.1 mM [12 µg/mL]		Bjørge et al. (1996)
DNA strand breaks (DNA fragmentation PFGE)	Whole-blood cells	+		0.06 µmole, 7 mL of blood [8.6 µM, 1 µg/mL]		Marczynski et al. (1997b)

Table 4.4 (continued)

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
HPRT locus	Human lymphoblastoid cell lines	+		0.6 mM [72 µg/mL]	GSTM1 reduces mutagenicity and toxicity of styrene-7,8-oxide	Shield & Sanderson (2004)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes)	+		0.1 mM [12 µg/mL]	Dose–response with two doses, including gaps	Fabry et al. (1978)
Chromosomal aberrations, micronuclei	Human lymphocytes (whole-blood lymphocytes)	+		0.008% (v/v)	Single dose	Linnainmaa et al. (1978a)
Chromosomal aberrations	Human lymphocytes (whole-blood lymphocytes)	+		0.05 mM [6 µg/mL]		Pohlová et al. (1984)
Micronuclei	Human lymphocytes (whole-blood lymphocytes)	+		0.6 mM [72 µg/mL]		Speit et al. (2012)
Micronuclei	Human peripheral blood lymphocytes	+		100 µM [12 µg/mL]		Laffon et al. (2001b)
Micronuclei	Human lymphocytes (isolated from whole blood)	+		50 µM [6 µg/mL]		Laffon et al. (2003a)
Micronuclei	Human peripheral blood mononuclear cells	+		0.1 mM [12 µg/mL]		Godderis et al. (2006)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+		0.15 mM [18 µg/mL]		Norppa et al. (1983)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+		0.005 mM [0.6 µg/mL]		Pohlová et al. (1984)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	100 µM [12 µg/mL]		Chakrabarti et al. (1997)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	50 µM [6 µg/mL]		Ollikainen et al. (1998)

Table 4.4 (continued)

End-point	Tissue, cell line (if specified)	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.1 mM [12 µg/mL]	Single dose	Zhang et al. (1993)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.05 mM [6 µg/mL]	Dose–response with two doses	Lee & Norppa (1995)
Sister-chromatid exchange	Human lymphocytes (whole-blood lymphocytes)	+	NT	0.05 mM [0.6 µg/mL]	Dose–response with two doses; no change by *GSTM1* null genotype	Uusküla et al. (1995)
Sister-chromatid exchange	Human peripheral blood lymphocytes	+	NT	50 µM [6 µg/mL]		Laffon et al. (2001b)

EMS, electrospray mass spectrometry; GSTM1, glutathione *S*-transferase mu1; GSTT1, glutathione *S*-transferase θ1; HIC, highest ineffective concentration; HPLC, high-performance liquid chromatography; *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; LEC, lowest effective concentration; NT, not tested; PFGE, pulsed-field gel electrophoresis; v/v, volume/volume.

[a] +, positive; the level of significance was set at $P < 0.05$ in all cases.

Table 4.5 Studies of DNA adducts in experimental animals exposed to styrene

End-point	Species, strain (sex)	Tissue	Result (number of adducts/dNp)[a]	Route, dosing regimen	Reference
*N*7-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine *O*^{6}-(2-hydroxy-1-phenylethyl)guanine	Mouse, NMRI (M)	Liver, spleen, lungs	+ (up to 6.3/10^7 dNp) + (up to 3.8/10^7 dNp)	Intraperitoneal injection, 0–4.35 mmol/kg bw [457 mg/kg bw], 3 h	Pauwels et al. (1996)
O^6-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine	Rat, NR	Liver	± (7/10^7 dNp)	Inhalation, 1000 ppm [4300 mg/m^3], 2 yr	Otteneder et al. (1999)
7-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine Unidentified adducts	Rat, Sprague-Dawley Mouse, CD-1	Lungs, liver	+ (1–3/10^8 dNp) + (up to 9/10^8 dNp)	Inhalation, 160 ppm [700 mg/m^3], 6 h	Boogaard et al. (2000a,b)
7-(2-hydroxy-2-phenylethyl)guanine 1-(2-hydroxy-2-phenylethyl)adenine	Mouse, NMRI (M)	Lungs	+ (up to 23/10^8 dNp) + (up to 0.6/10^8 dNp)	Inhalation, 750 and 1500 mg/m^3 [176 and 352 ppm] for 1, 3, 7, and 21 d for 6 h, 5 d/wk	Vodička et al. (2001a)
O^6-(2-hydroxy-1-phenylethyl and 2-hydroxy-2-phenylethyl)guanine	Mouse, CD-1	Lungs	– (1/10^7 dNp, i.e. detection limit)	Inhalation, 40 and 60 ppm [172 and 688 mg/m^3], 6 h/d, 5 d/wk, 2 wk	Otteneder et al. (2002)
3-(2-hydroxy-1-phenylethyl)adenine (*N*3αA), 3-(2-hydroxy-2- phenylethyl) adenine (*N*3βA) 7-(2-hydroxy-1-phenylethyl)guanine (*N*7αG), 7-(2-hydroxy-2-phenylethyl) guanine (*N*7βG)	Mouse, NMRI (M)	Urine	+ Dose-dependent urinary excretion, *N*3αA and *N*3βA up to 0.8 × 10^{-5}% of the absorbed dose + *N*7αG and *N*7βG amounted to 1.4 × 10^{-5}% of the absorbed dose	Inhalation, 0, 600, and 1200 mg/m^3 [0, 141, and 282 ppm], 6 h/d, 10 d	Mikes et al. (2009)

bw, body weight; d, day(s); dNp, deoxynucleotide; h, hour(s); M, male; NR, not reported; ppm, parts per million; wk, week(s); yr, year(s).

[a] +, positive result; –, negative result.

adducts (Pauwels et al., 1996; Otteneder et al., 1999, 2002; Boogaard et al., 2000b; Vodička et al., 2001a). More recently, *N*3αA and *N*3βA have been detected in the urine of NMRI mice after exposure to styrene by inhalation (Mikes et al., 2009). However, some unidentified styrene-related alkylation products were also reported (Boogaard et al., 2000b). The main stable adducts identified in vitro (αN^2-guanine, αN^6-adenine, and β*N*3-uracil) have not been demonstrated to be formed in experimental animals. A single intraperitoneal injection of styrene to NMRI mice resulted in a dose-related increase in both *N*7-guanine and O^6-guanine adducts. The highest concentrations of DNA adducts were found in the lungs, followed by liver and spleen (Pauwels et al., 1996; reviewed by Vodička et al., 2006a). By using a modified ^{32}P-postlabelling method, O^6-guanine adducts were detected at the concentration of 7 adducts per 10^7 nucleotides in the liver of rats exposed by inhalation to styrene at 1000 ppm (4300 mg/m^3) for 2 years (Otteneder et al., 1999). The formation of the O^6-guanine adduct in the lung DNA of CD-1 mice exposed by inhalation to styrene at 172 mg/m^3 and 688 mg/m^3 for 6 hours per day, 5 days per week for 2 weeks, was studied by the same authors; the adduct concentration was below the limit of detection of 1 adduct per 10^7 nucleotides (Otteneder et al., 2002). *N*7-guanine (β-isomers) and *N*1-adenine adducts in lung and liver DNA of mice exposed by inhalation to styrene at concentrations of 750 mg/m^3 and 1500 mg/m^3 for 1, 3, 7, and 21 days (Table 4.5) were investigated (Vodička et al., 2001a). In the lungs, β*N*7-guanine adducts were 40-fold more abundant than β*N*1-adenine adducts. Both adducts correlated strongly with exposure parameters, particularly with styrene concentration in blood. The N^2-guanine and β*N*3-uracil adducts were below the limit of detection (Vodička et al., 2002). A quantitative comparison of *N*7-guanine adducts excreted in urine with those in lungs (after correction for depurination) revealed that persisting *N*7-guanine DNA adducts in lungs amounted to about 0.5% of the *N*7-guanine adducts in urine. The total styrene-specific *N*7-guanine alkylation accounted for about 1.0×10^{-5}% of the total styrene uptake, whereas *N*1-adenine alkylation was still considerably lower (Vodička et al., 2006b). Boogaard et al. (2000a, b) found higher alkylation levels in the livers than in the lungs of rats and mice after inhalation of ^{14}C-styrene at 160 ppm [700 mg/m^3] for 6 hours (Boogaard et al., 2000a, b); the quantitative data on the two isomeric *N*7-styrene-7,8-oxide–guanine adducts in mouse lungs were in concordance with contemporary studies (reviewed by Vodička et al., 2006a, b). In mice exposed to styrene by inhalation, excretion of *N*3-adenine adducts (*N*3αA and *N*3βA) in the urine amounted to nearly 0.8×10^{-5}% of the absorbed dose, and urinary *N*7-guanine adducts 7-(2-hydroxy-1-phenylethyl)guanine (*N*7αG) and 7-(2-hydroxy-2-phenylethyl)guanine (*N*7βG) amounted to nearly 1.4×10^{-5}% of the dose. The excretion of both *N*3-adenine and *N*7-guanine adducts ceased shortly after the end of the exposure period as a result of their rapid depurination from the DNA (Mikes et al., 2009).

4.2.3 Other genetic effects

(a) Exposed humans

See Table 4.3 and Table 4.6.

(i) DNA damage (comet assay)

Most of the available epidemiological studies detected DNA damage by alkaline comet assay.

Buschini et al. (2003) evaluated DNA damage by comet assay in 48 workers exposed to styrene at 36.8 ± 0.7 ppm and in 14 unexposed controls. The levels of DNA damage were significantly higher ($P < 0.001$) in the groups exposed to styrene compared with the controls. [The Working Group noted the low number of individuals in the control group.] In another study, lymphocytes from 29 hand-lamination workers and 19

Table 4.6 Cytogenetic effects in humans exposed to styrene

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
Micronuclei	Italy, NR	Reinforced plastics production	Peripheral blood	92 exposed, 98 controls	37.1 (2–535) mg/m^3; 300.0 (10.2–1856) mg/g creatinine MA+PGA in urine	Smoking, sex, age	+ ($P < 0.001$)	Migliore et al. (2006)
• Micronuclei • Sister-chromatid exchanges	USA, before 1993	Reinforced plastic boat manufacturing facility	Peripheral blood	48 exposed	64.2 (0.88–235.35) mg/m^3 (8 h TWA)	Smoking, sex, age	– ($P > 0.05$) + ($P < 0.05$)	Yager et al. (1993)
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges	China, 1992	Fibreglass-reinforced plastics factories	Peripheral blood	83	129.3 (48.3–223.9) mg/m^3		+ + –	Huang (1992)
Chromosomal aberrations	Milan, Italy, 1985–1986	Factory A, reinforced plastic laminates and insulating polymers; Factory B, small plastic boats manufacture	Peripheral blood	Factory A, 32 exposed; Factory B, 8 exposed, 40 controls	A, 123–249 (up to 1978), 1.7–17.0 (after 1978) mg/m^3; B, 41–198 (after 1978) mg/m^3	Smoking, age, other exposures to mutagenic chemicals	+/– (Factory A, $P < 0.001$; Factory B, $P < 0.05$)	Forni et al. (1988)
Micronuclei	Sweden, 1983	Reinforced plastics and polyester resins workers	Peripheral blood	38 exposed, 20 controls	13 (1–40) ppm (8 h TWA)	Sex	+ ($P = 0.005$)	Högstedt (1984)
Chromosomal aberrations	Egypt (El Oboor City), before 2013	Reinforced plastics production	Peripheral blood	40 exposed, 50 controls	1117 (SD, 64.52) µg/L in blood 246 (SD, 21.60) µmol/L MA in urine	Smoking, sex, socioeconomic status, age	+ ($P < 0.001$)	Helal & Elshafy (2013)
• Chromosomal aberrations • Sister-chromatid exchanges	Italy, 1983	Reinforced plastics industries (six plants)	Peripheral blood	25 exposed, 22 controls	NR (30–400) mg/m^3	Age, sex, smoking	+ ($P < 0.005$ for all 6 plants) + ($P < 0.005$ for 2 plants, $P < 0.05$ for 2 plants, $P > 0.05$ for 2 plants)	Camurri et al. (1983)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Sister-chromatid exchanges	Sweden, 1978	Factory making boats from fibreglass-reinforced plastics	Peripheral blood	36 exposed, 37 controls 20 exposed, 21 controls	Low concentration, 137 (6–283) mg/m^3; high concentration, 1204 (710–1589) mg/m^3	Age, sex	+ ($P < 0.001$) +/– ($P < 0.05$)	Andersson et al. (1980)
• Chromosomal aberrations • Micronuclei	Italy, 1990	Reinforced plastics production factories (Group 1, fibreglass tanks; Group 2, small boat production)	Peripheral blood	Group 1, 7 exposed, 7 controls; Group 2, 12 exposed, 12 controls	Group 1, NR (21–100); Group 2, NR (112–435) mg/m^3 Group 1, 186 (46–345); Group 2 725 (423–1325) mg/g creatinine MA in urine	Smoking, age, sex	+ ($P < 0.05$) – ($P > 0.05$)	Tomanin et al. (1992)
• Chromosomal aberrations • Micronuclei	Sweden, 1980	Fibreglass-reinforced polyester factory	Peripheral blood	15 exposed, 13 controls 12 exposed, 12 controls	24 ppm NR (< 2) mmol/L MA in urine	Sex, smoking	– ($P > 0.05$) + (one-sided $P = 0.00017$)	Nordenson & Beckman (1984)
Chromosomal aberrations	Germany, NR	Boat manufacturing	Peripheral blood	14 exposed, 7 controls	< 100 mg/m^3 35 (1.5–211) µg/L styrene in blood	Smoking	+/–	Oberheitmann et al. (2001)
Chromosomal aberrations	Czechia, NR	Laminators of various kinds of sport utensils, boats, and containers	Peripheral blood	11 exposed, 11 controls	253 (118–582) mg/m^3 NR (214–711) µL/mmol creatinine MA NR (50–175) µL/mmol creatinine PGA	Smoking, sex, alcohol consumption, drug intake, X-ray examination, rtg. therapy	–	Jablonická et al. (1988)
• Chromosomal aberrations • Sister-chromatid exchanges • Micronuclei	Finland, before 1991	Reinforced plastics production	Peripheral blood	109 exposed, 54 controls 70 exposed, 31 controls 50 exposed, 37 controls	Laminators, 43 (5–182) ppm Others, 11 (1–133) ppm (8 h TWA); laminators, 2.2 (SD, 2.4) nmol/L MA+PGA in urine	Age, smoking	– ($P > 0.05$) – –	Sorsa et al. (1991)
• Chromosomal aberrations • Micronuclei	Sweden, 1985–1986	Reinforced plastics production	Peripheral blood	11 exposed, 14 controls 20 exposed, 22 controls	43–221 mg/m^3, 4–551 mg/m^3 (1974–1986); 128 (< 6–317) mmol/mol creatinine, MA+PGA in urine (in 1985)	Smoking, age	– ($P > 0.5$) – ($P > 0.5$)	Hagmar et al. (1989)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges	Finland, 1987	Reinforced plastics workers	Peripheral blood	21 exposed, 21 controls	98 (34–263) mg/m^3; 1.6 (< LOD–7) mmol/L MA in urine	Smoking, sex	– – –	Mäki-Paakkanen (1987)
• Chromosomal aberrations • Gaps	Czechia, before 1985	Two polystyrene plants: A, food vessel manufacturing; B, boat manufacturing	Peripheral blood	A, 36 exposed, 19 controls; B, 22 exposed, 22 controls	A, 70–150 (5.6–982.8) mg/m^3; B, ~200 (39–548) mg/m^3	Smoking, acute viral diseases, sex, drug intake The above plus X-ray examinations, alcohol	– +	Pohlová & Srám (1985)
• Chromosomal aberrations • Gaps • Sister-chromatid exchanges	Norway, before 1984	Reinforced plastics production	Peripheral blood	(i) 11 exposed, (ii) 7 exposed; 9 controls	(i) 7.5 (2–13) ppm; (ii) 22.3 (14–44) ppm	Smoking, sex, age	– ($P > 0.1$) + ($P = 0.0002$) –	Hansteen et al. (1984)
Chromosomal aberrations	Germany, 1975	Polystyrene production plant	Peripheral blood	12 exposed, 12 controls	GM, 0.23 (0.02–46.92) ppm; NR (< 10–100) mg/L MA in urine	Age, sex, smoking, drug intake, acute viral diseases, X-ray examinations, vaccinations	–	Thiess & Fleig (1978)
• Chromosomal aberrations • Sister-chromatid exchanges	Italy, Viareggio, 1988–1990	Fibre-reinforced plastic boat factory	Peripheral blood	(i) 23 low concentration; (ii) 23 high concentration, 51 controls	(i) NR, 2–120; (ii) NR, 86–1389 mg/m^3	NR	(+) ($P < 0.01$) +/– ($P < 0.05$)	Artuso et al. (1995)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges	Finland, before 1991	Reinforced plastics production in a plant manufacturing containers	Peripheral blood	17 exposed, 17 controls	300 (NR) mg/m^3 (based on ACGIH conversion); 9.4 (< 1–21.5) mmol/L MA in urine	Age, sex, smoking, viral infections, vaccinations, other exposures to mutagenic chemicals, alcohol consumption, drug intake	+/– (one-sided $P < 0.02$) – –	Mäki-Paakkanen et al. (1991)
Chromosomal aberrations	Finland, 1977	Plants manufacturing polyester plastic products	Peripheral blood	10 exposed, 5 controls	NR	Sex	(+)	Meretoja et al. (1977)
Sister-chromatid exchanges	NR, before 1990	Fibre-reinforced plastic boat factories	Peripheral blood	20 exposed, 20 controls	Smokers, 209.8 (7.5–570.8) mg/m^3; non-smokers, 230.1 (25.3–564.1) mg/m^3 Smokers, 275 (SD, 241) mg/g creatinine MA in urine; non-smokers: 323 (SD, 224) mg/g creatinine MA in urine	Smoking, alcohol, age, coffee	(–)	Kelsey et al. (1990)
Chromosomal aberrations	Poland, before 1983	Laminated styrene plates production	Peripheral blood	37 exposed, 2 controls	NR (< 100) mg/m^3		+/–	Dolmierski et al. (1983)
• Chromosomal aberrations • Sister-chromatid exchanges	Finland, 1976–1977	Reinforced plastics production, two plants	Peripheral blood	16 exposed, 6 controls	569.8 (55–3257) mg/g creatinine MA in urine in 1976 329.3 (53–1646) mg/g creatinine MA in urine in 1977	Smoking	+ ($P < 0.001$) – ($P > 0.05$)	Meretoja et al. (1978)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
Chromosomal aberrations	Finland, NR	Three plants: (i) styrene manufacturing; (ii) polystyrene production; (iii) unsaturated polyester resins processing	Peripheral blood	(i) 5 exposed; (ii) 12 exposed; (iii) 14 exposed, 20 controls	(i) NR (19–40) mg/L MA in urine; (ii) NR (< 5–100) mg/L MA in urine; (iii) NR (102 to > 1500) mg/L MA in urine		+/–	Fleig & Thiess (1978)
Chromosomal aberrations	Lithuania, before 1993	Chemical plant	Capillary blood	109 exposed, 64 controls	NR (< 1.9 ppm) in year before sampling	Sex, smoking	(+) ($P < 0.01$)	Mierauskiene et al. (1993)
Chromosomal aberrations	Lithuania, (i) 1983–1984; (ii) 1985–1986	Two plants: (i) carpet production; (ii) plastics production	Peripheral blood	(i) 79 exposed; (ii) 97 exposed, 90 controls	(i) NR (0.13–1.4) mg/m^3; (ii) NR (4.4–6.2) mg/m^3	Smoking, age	(+) ($P < 0.0001$)	Lazutka et al. (1999)
Aneuploidy and diploidy	Italy, Tuscany, before 2002	Reinforced plastics production	Semen	18 out of 46 exposed, 13 out of 27 controls	292.5 (20.8–947.8) mg/g creatinine MA in urine	Smoking, age, alcohol consumption	+/– ($P > 0.05$)	Naccarati et al. (2003)
• Chromosomal aberrations • Sister-chromatid exchanges	Japan, before 1983	Boat manufacturing	Peripheral blood	18 exposed, 6 controls	40–50 (NR) ppm	Smoking, age, sex	+/– +/–	Watanabe et al. (1983)
Chromosomal aberrations (four basic classes)	Czech Republic, 1989?	Workers occupationally exposed to styrene	Peripheral blood	13 women exposed, 6 women controls	225 (83–366) mg/m^3	Sex	+	Smejkalová et al. (1989)
Chromosomal aberrations	Sweden, 1977	Plant manufacturing polyester resin boats	Peripheral blood	6 exposed, 6 controls	115 (50–400) mg/m^3	Sex, age, smoking	(+) ($P = 0.001$)	Högstedt et al. (1979)

Table 4.6 (continued)

End-point	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Reference
• Chromosomal aberrations • Micronuclei • Sister-chromatid exchanges • Gene mutation/ *HPRT* MF	Germany, 1990	Container and board manufacturing (plus dichloromethane exposure)	Peripheral blood	46 exposed, 23 controls 46 exposed, 22 controls 46 exposed, 23 controls 45 exposed, 5 of 23 controls	70 (0–598) mg/m^3 (8 h TWA)	Smoking, age, sex	(+) ($P < 0.0001$) (+) ($P < 0.0001$) (+) ($P < 0.0001$) (–)	Tates et. al (1994)

ACGIH, American Conference of Governmental Industrial Hygienists; *HPRT*, hypoxanthine-guanine phosphoribosyl transferase; LOD, limit of detection; MA, mandelic acid; MF, mutation frequency; NR, not reported; PGA, phenylglyoxylic acid; SD, standard deviation; TWA, time-weighted average

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+)/(–), positive/negative result in a study of limited quality.

unexposed controls were assayed for alkali-labile lesions (Somorovská et al., 1999). DNA damage was measured by comet assay, and was observed to be increased in workers exposed to styrene compared with controls ($P < 0.001$); the increase was also correlated with years of exposure. Shamy et al. (2002) similarly reported an increase in DNA damage in 26 reinforced plastics workers compared with 26 controls. Laffon et al. (2002a) reported a significant increase in DNA damage in a group of 14 workers exposed to styrene at less than 20 ppm [< 84.4 mg/m^3] compared with 30 controls ($P < 0.01$). Wongvijitsuk et al. (2011) studied 50 workers exposed to styrene in fibreglass-reinforced plastics production at levels below the American Conference of Governmental Industrial Hygienists limit of 20 ppm and 40 unexposed control subjects. Workers were stratified into three exposure groups: group I workers were exposed at less than 10 ppm (< 42.20 mg/m^3); group II at 10–20 ppm (42.20–84.40 mg/m^3); and group III at more than 20 ppm (> 84.4 mg/m^3). Urinary MA and PGA concentrations were observed to increase in relation to increasing levels of environmental styrene exposure. DNA strand breaks in peripheral leukocytes were higher in the exposed workers ($P < 0.05$). Walles et al. (1993) carried out a study on male workers exposed to styrene at 0.04–20.0 ppm [0.17–85.0 mg/m^3] in a Swedish plastics factory. The single-strand breaks in leukocytes were observed to be increased at the end of the shift, but not before a shift or the next morning. No control subjects were studied. Brenner et al. (1991) reported a significant increase of DNA damage in 14 subjects exposed to styrene at 11.2 ± 0.9 ppm compared with 9 controls ($P < 0.003$); however, the study did not control for smoking habits [and the number of subjects studied was small].

However, these positive results were not confirmed in other publications. Costa et al. (2012) reported no effect of exposure to styrene at the high concentration of 30.4 ppm [~130 mg/m^3] (8-hour time-weighted average, TWA) on DNA damage in a group of 67 reinforced plastics workers when compared with 68 controls subjects. Teixeira et al. (2010) reported the same result in a population of 46 workers exposed to styrene at 29.9 ppm [127 mg/m^3] compared with 47 controls. Similarly, other studies of exposure at different concentrations did not support these findings. Hanova et al. (2010) did not find significant differences in the levels of DNA damage measured by comet assay in a group of 60 workers exposed to styrene at 50.3 mg/m^3 compared with that of 37 control individuals. Vodička et al. (2004) did not find significant differences in the levels of DNA damage between 86 reinforced plastics workers exposed at 81.3 mg/m^3 compared with 42 controls. Similarly, Godderis et al. (2004) reported no differences in DNA damage between 37 workers exposed to styrene at 9.5 ppm [40 mg/m^3] and 44 controls.

In another study, DNA damage was evaluated in germ cells (sperm) in 46 reinforced plastics workers exposed to styrene (median value of concentration of MA at the end of the work shift: 173.6 mg/g creatinine); the study reported a significant increase in sperm DNA damage when compared with 27 unexposed individuals (Migliore et al., 2002).

(ii) Oxidative damage to DNA

Fracasso et al. (2009) evaluated oxidative damage to DNA in the enzyme-modified version of the comet assay (sites sensitive to formamido pyrimidine glycosylase (Fpg) and endonuclease III) in lymphocytes from 34 reinforced plastics workers exposed at 46.74 ppm and 29 unexposed controls, and Somorovská et al. (1999) evaluated 29 exposed workers and 19 controls. Neither study found any effect of occupational exposure in reinforced plastics workers. Using only endonuclease III in the comet assay, other studies (e.g. Hanova et al., 2010) reported no significant differences in the levels of oxidative damage to

DNA in workers exposed to styrene compared with control subjects.

8-Hydroxy-2′-deoxyguanosine (8-OHdG) was increased in leukocyte DNA in 17 boat builders exposed to styrene compared with 67 age-matched controls with no prior occupational exposure to styrene (Marczynski et al., 1997a). Among the exposed workers, the 11 workers with more than 10 years of exposure had a higher level of 8-OHdG than the 6 workers with less than 10 years of exposure, although the difference was not statistically significant ($P = 0.05$). [The Working Group noted the small number of subjects exposed to styrene in the study.]

In the study of Wongvijitsuk et al. (2011) mentioned in the previous section, 8-OHdG levels in peripheral leukocytes were higher in the exposed workers compared with controls ($P < 0.05$).

In a study of a group of 60 reinforced plastics workers and 50 controls (Manini et al., 2009), lower concentrations of leukocyte 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) were observed in exposed workers compared with controls; however, significantly higher levels of urinary 8-oxo-7,8-dihydroguanosine (8-oxoGuo) were observed in exposed workers compared with controls.

(iii) Gene mutations

Since the previous IARC evaluation of styrene (IARC, 1994), no further data have become available on glycophorin A variant frequency in erythrocytes (Compton-Quintana et al., 1993; Bigbee et al., 1996). Concerning the induction of mutation in the *HPRT* locus in lymphocytes of reinforced plastics workers exposed to styrene at 101.2 mg/m^3, Vodička et al. (2001b) reported a slight increase of *HPRT* mutant frequencies in exposed workers compared with controls, but the observed difference was not significant. Other studies were not informative because of their small number of subjects or other limitations (Tates et al., 1994; Vodička et al., 1995, 1999).

(iv) Chromosomal damage

More than 30 studies investigated whether exposure to styrene had effects on chromosomal damage in humans (see Table 4.6). No studies were found that addressed the main styrene metabolite, styrene-7,8-oxide. In most of the studies considered, environmental and/or biological monitoring exposure data are available. In the discussion that follows, the studies are briefly described in terms of study size, design, the presence of controls and the criteria for their selection, the presence of and adjustment for confounders, the concordance of different end-points tested, and the exposure levels at which the effects were observed. The statistical analyses of the data and the levels of significance of the results are also considered.

Several studies of adequate size and design reported positive effects on chromosomal end-points. Several of these encompassed relatively low concentrations of exposure (< 100 ppm). A significantly higher frequency of micronucleated binucleated cells (13.8 ± 0.5% vs 9.2 ± 0.4%; $P < 0.001$) was observed in peripheral blood lymphocytes of 92 reinforced plastics production workers exposed to styrene compared with 98 controls (Migliore et al., 2006). Workers were exposed to styrene at 37.1 ± 3.9 mg/m^3 [9.0 ± 0.9 ppm], producing levels of excretion of 300 ± 338.2 mg/g creatinine of MA and PGA in urine. In *GSTM1*-null workers exposed to styrene, a significantly lower concentration of PHEMAs in urine and a higher frequency of micronucleated lymphocytes were observed. In another study, the lymphocytes from 44 hand-lamination workers exposed to styrene, discussed in Section 4.2.3(a)(i) and (ii) above (Somorovská et al., 1999; see Table 4.3), showed a significantly increased frequency of chromosomal aberrations. Yager et al. (1993) published the results of a study of 48 reinforced plastics production workers (approximately 50% men and 50% women); micronucleated cells and

sister-chromatid exchanges were determined in peripheral lymphocytes 4 times at intervals of 3 months for a 1-year period. Individual levels of exposure ranged from 0.88 to 235.35 mg/m^3 of styrene (mean, 64.2 ± 71.5 mg/m^3) and exhaled styrene concentrations ranged from 0 to 7.16 mg/m^3 (mean, 1.65 ± 1.82 mg/m^3). Individual mean sister-chromatid exchanges increased with exposure to styrene and with cigarette smoking. Multivariate analysis showed that smoking contributed about 62% and exposure to styrene contributed 25% to variability, although no variation was observed in micronucleus frequencies. The study also showed significant inter-individual differences in micronucleus frequencies associated with sex, with the highest levels observed in women. Huang (1992) studied a group of 83 Chinese workers exposed to styrene in reinforced plastics production, showing that exposure to styrene at a concentration of 129.3 mg/m^3 (range, 48.3–223.9 mg/m^3) resulted in an increase in the frequencies of chromosomal aberrations and micronuclei, but had little influence on the frequency of sister-chromatid exchange. Forni et al. (1988) studied a group of 40 workers at a medium-sized reinforced plastics manufacturing plant, with a history of exposure to high concentrations but with low concentrations of exposure at the time of the study (1978, 32 subjects, plant A), and a modern boat manufacturing plant with good environmental hygiene conditions (8 subjects, plant B). The study included 40 unexposed controls matched for age, sex, and smoking. Plant A workers had a significantly higher rate of total abnormal metaphases excluding gaps and chromosome-type unstable aberrations, with a significantly different distribution of chromosome-type unstable aberrations and asymmetric chromosome exchanges. Plant B workers showed a significantly higher frequency of chromatid-type aberrations, but did not have significantly increased gaps. The study failed to show any correlation between exposure indices and chromosomal aberration rates. However, in the subjects who had been exposed to the highest cumulative concentrations of styrene, higher rates of chromosome-type aberrations or dicentric and ring chromosomes were observed. Högstedt (1984) carried out a study to determine a new method for the assessment of cytogenetic damage in humans; frequencies of micronuclei and chromosomal aberrations were measured in 38 male reinforced plastics production workers exposed to styrene at 13 ppm (range, 1–40 ppm) and in 20 unexposed controls. The study revealed a statistically significant effect of styrene ($P = 0.005$) and of smoking ($P = 0.014$) on micronuclei in lymphocytes. Age and smoking was also correlated with these changes, but all these factors combined explained only 12–24% of the total variance.

Three studies involving exposure to styrene at higher concentrations also reported positive results, with weaker evidence. In particular, a statistically significant difference in gap, iso-gap, break, iso-break, centromere separation, deletion, and total aberrations was reported in an Egyptian study of 40 male reinforced plastics production workers exposed to styrene (blood styrene concentration, 1117 ± 64.52 µg/L; MA in urine, 246 ± 21.60 µmol/L) and 50 unexposed healthy controls (blood styrene level, 0.24 ± 0.15 µg/L; MA in urine, 4.20 µmol/L) matched with the exposed group for sex, socioeconomic status, and smoking habits (Helal & Elshafy, 2013). Camurri et al. (1983) studied a group of 25 workers exposed to styrene in six reinforced plastics production industries characterized by different concentrations of exposure (30–400 mg/m^3). A control group was selected for each plant, up to a total of 22 subjects. Comparisons were made between exposed workers and controls within each plant and for the entire group of workers. Styrene airborne concentrations were measured during winter and summer and individual doses were measured by the determination of the concentrations of MA and PGA in the urine of the

workers. Evaluation of chromosomal aberrations and sister-chromatid exchanges revealed an increased frequency of chromosomal aberrations in the exposed workers, mainly of the chromatid type. In addition, sister-chromatid exchanges were higher in the workers at four out of the six plants, at which airborne styrene concentrations of 30–250 mg/m^3 were measured. Sister-chromatid exchanges were only observed in groups exposed to styrene at more than 200 mg/m^3, but no linear regression between sister-chromatid exchanges and urinary levels of styrene metabolites was observed, and there was no significant correlation between either smoking habit or years of exposure and chromosomal aberrations or sister-chromatid exchanges in the exposed workers. During 1973–1978, Andersson et al. (1980) studied a group of 36 reinforced plastics production workers exposed to styrene and unexposed controls employed in the same factory matched for age and sex. Environmental styrene concentrations measured over a 3-year period allowed identification of two exposure groups: a low-concentration group with mean levels of exposure of 137 mg/m^3, and a high-concentration group with mean levels of exposure of 1204 mg/m^3. The study revealed a statistically significant increase in chromosomal aberrations ($P < 0.001$) compared with controls ($n = 37$). A slight increase in sister-chromatid exchange was also observed in the exposed group ($n = 20$) compared with controls ($n = 21$), with no difference between the groups exposed to the high and low concentrations.

Similar but less clear results were reported in three smaller studies. In the study on reinforced plastics production workers by Tomanin et al. (1992), two groups were studied. Group 1 comprised 7 subjects exposed to styrene at 21–100 mg/m^3 (urinary MA, 46–345 mg/g creatinine) while engaged in the production of fibreglass tanks over a period of 9.0 ± 7.3 years (range, 1–19 years); Group 2 comprised 12 subjects exposed to styrene at 112–435 mg/m^3 (urinary MA, 423–1325 mg/g creatine) while engaged in the production of fibreglass boats over a period of 7.2 ± 4.7 years (range, 1.5–15.0 years). Control groups ($n = 7$ and $n = 12$, respectively) were not occupationally exposed to genotoxicants and were matched for sex, age, and smoking habits. A significant increase in the percentage of aberrant cells and total aberrations was only observed in Group B, although micronuclei frequencies were not significantly increased in either group. No correlation between length of exposure and frequency of chromosomal aberrations or micronuclei in exposed subjects were found. In the Nordenson & Beckman (1984) study, micronuclei in lymphocytes were significantly increased in 12 men exposed to styrene in a factory where fibreglass-reinforced polyester was used, compared with 12 controls. The average styrene concentration was 24 ppm, and the levels of urinary MA were less than 2 mmol/L. No significant increase in the rates of chromosomal aberrations (gaps and breaks) was found in peripheral lymphocytes, but the rate of micronucleus formation was significantly increased in the exposed group. An earlier study by Watanabe et al. (1983) investigated 18 workers exposed to styrene at concentrations of 40–50 ppm in reinforced plastics production (boat manufacturing) and 6 unexposed controls. The study showed only a marginal increase in chromosomal aberrations in the exposed workers compared with controls. A slight but statistically significant correlation between the rate of sister-chromatid exchange and the concentration of exposure was reported.

In 10 studies of adequate size and design, no effects of styrene on chromosomal end-points were observed, including in subjects exposed at more than 100 ppm. In the study by Hanova et al. (2010; see Table 4.3), micronuclei were assessed in 62 subjects exposed to styrene and 50 control individuals, but were not observed to increase with styrene exposure. In the study by Vodička et al. (2004) (see Table 4.3), cytogenetic markers were investigated in 86 lamination workers

exposed to styrene and 42 control individuals, but did not increase with styrene exposure. In a study of a group of 14 workers exposed to styrene at less than 100 mg/m^3 while engaged in the production of fibreglass boats, Oberheitmann et al. (2001) did not observe any statistically significant difference in the rate of exchange-type aberrations between the exposed workers and the 7 unexposed controls. No differences in chromosomal aberrations in the peripheral lymphocytes of exposed workers were observed in 11 women exposed to styrene at 253 mg/m^3 (mean) while performing various kinds of laminating (sport utensils, boats, and containers) compared with 11 unexposed controls matched for age, social habits, and living and working environment (Jablonická et al., 1988). Sorsa et al. (1991) did not find an increase in the frequency of sister-chromatid exchanges, chromosomal aberrations, or micronuclei in peripheral lymphocytes in subjects in 32 productive settlements exposed to styrene compared with unexposed controls. Similarly, no increase in micronuclei frequencies or size ratios, irrespective of proliferative stimulation with phytohaemagglutinin or pokeweed mitogen, or in chromosomal aberrations, was reported in a study of workers engaged in the production of reinforced plastics in Sweden (Hagmar et al., 1989). Micronuclei were assessed in 18 operators and 2 supervisors exposed to styrene and 22 unexposed controls, and chromosomal aberrations were assessed in 11 exposed and 14 control subjects. The mean levels of styrene exposure for the period 1974–1986 were 43–221 mg/m^3 (range, 4–551 mg/m^3) and the concentration of urinary MA and PGA in 1985 was 128 mmol/mol creatinine (range, < 6–317 mmol/mol creatinine). No increase was detected in the frequency of any of the cytogenetic end-points studied (sister-chromatid exchange, micronuclei, and other chromosomal aberrations) in a group of 21 workers exposed to styrene at 98 mg/m^3 (range, 34–263 mg/m^3) in reinforced plastics production compared with 21 subjects working mainly in office jobs, matched according to sex and smoking habits (Mäki-Paakkanen, 1987). Further, no correlations between the number of aberrations, micronuclei, or sister-chromatid exchanges and the extent or duration of exposure to styrene could be detected. Pohlová & Srám (1985) did not report an effect on chromosomal aberrations in exposed workers, but an increase was found in the rate of gaps in two groups of exposed workers in two production plants (A and B). In plant A, at which workers were engaged in polystyrene production, there were 36 exposed workers (27 men and 9 women) and 19 unexposed controls (15 men and 4 women). In plant B, at which workers were engaged in reinforced plastics production, there were 22 exposed workers (7 men and 15 women) and 22 unexposed controls (13 men and 9 women). Airborne concentrations of styrene were 5.6–982.8 mg/m^3 (plant A) and 39–548 mg/m^3 (plant B). Hansteen et al. (1984) reported no increase in chromosomal aberrations or sister-chromatid exchange, but did find a significant (P = 0.0002) increase in gaps in two groups of subjects (n = 11 and 7) exposed to styrene in the production of reinforced plastics compared with unexposed office employees (n = 9) matched for sex, age group, and smoking habits. Thiess et al. (1980) failed to identify a statistically significant increase in chromosomal aberration rates in a study of 24 workers exposed to styrene at concentrations of 6 ppm (in an annexed laboratory) to 58.1 ppm (in a pilot polystyrene processing plant) for 4–27 years compared with 24 unexposed controls [number not clearly reported]. Likewise, in a previous study Thiess & Fleig (1978) considered a group of 12 subjects extracted from a larger group of 93 subjects engaged in the synthesis of styrene from ethylbenzene, 10 with 19–39 years of possible exposure and 2 with high concentrations of MA in urine, without finding any increase in chromosomal aberrations. Levels of exposure were of the order of fractions of parts per million for most of

the workers under study. Different levels of exposure were considered, but no control group was included in the study.

Other available studies were limited either by their small sample size or by other critical factors. Artuso et al. (1995) carried out a study on two groups each of 23 workers exposed to styrene in fibreglass-reinforced plastic boat production, one group at a low concentration of 2–120 mg/m^3 and the other at a high concentration of 86–1389 mg/m^3, and 51 matched controls living in the same area but not exposed to styrene. Through statistical multivariate analysis, an increase in aberrations were found in the low-concentration group and in the high-concentration group. The incidence of sister-chromatid exchanges showed a dose–response association. However, X-ray irradiations were more frequent in controls, smokers more frequent in the high-concentration group, and chromosome aberrations were evaluated after 72 instead of 48 hours. [The Working Group noted the limitations of the study, although the consistency among indicators and the evidence of an exposure–response suggest that the changes observed were at least partially attributed to styrene exposure.] Mäki-Paakkanen et al. (1991) studied the frequency of chromosomal aberrations, micronuclei, and sister-chromatid exchanges in 17 workers exposed to styrene in the production of plastic containers and 17 unexposed controls. The concentrations of exposure, estimated through the determination of MA in urine, were of the order of 300 mg/m^3. Chromosomal aberrations were significantly higher in the exposed workers, without a significant correlation with levels of exposure, although the frequencies of micronuclei and sister-chromatid exchanges did not differ between the two groups. [The Working Group noted the inconsistency of the results obtained by different end-points, and the lack of a clear dose–response correlation.] Meretoja et al. (1977) studied 10 male workers exposed to styrene in three plastics manufacturers and 5 unexposed controls; frequency of chromosomal aberrations of 11–26% (vs 3% in controls), and a higher frequency of micronuclei and cells connected with nuclear bridges, were measured in the peripheral lymphocytes of the exposed workers. However, no correlation between styrene exposure, estimated through the determination of MA concentration in urine, and the incidence of chromosomal aberrations was found. [The Working Group noted the small number of subjects and the absence of a dose–response relationship.] Kelsey et al. (1990) studied a group of 20 workers exposed to styrene in the construction of boats in two factories and a control group comprising 20 unexposed workers from the same small companies. Exposure was measured through determination of styrene concentration in environmental and exhaled air, and by urinalysis of styrene metabolites. Mean airborne styrene concentrations of 209.8 mg/m^3 and 230.1 mg/m^3 and mean MA concentrations of 275 mg/g creatinine and 323 mg/g creatinine were measured for smokers and non-smokers, respectively. The frequency of sister-chromatid exchange did not differ between exposed and unexposed workers, although smoking significantly induced sister-chromatid exchange in these workers. [The Working Group noted that the main limitation of this study was that controls might have experienced exposure to styrene previously.] Smejkalová et al. (1989) evaluated the number of chromosomal aberrations in peripheral lymphocytes of 13 women occupationally exposed to styrene and 6 paired controls. The average styrene airborne concentration was 225 ± 89 mg/m^3. The number of chromosomal aberrations in the exposed group was significantly higher than in controls, but the study groups were small. Dolmierski et al. (1983) conducted a study on 37 workers engaged in the production of laminated styrene plates and 2 controls. They found chromosomal aberrations in 6.8% of cells in the exposed subjects. [The Working Group noted that the main limitations of the study were the small control group

(n = 2), the apparently younger age of controls compared with exposed subjects (22–28 years), and the apparent lack of control for confounders.] Meretoja et al. (1978) published the results of a study conducted during 1976–1977 on a group of 16 workers exposed to styrene in two reinforced plastics production plants and a control group of 6 unexposed subjects. Occupational histories and smoking habits were recorded for both exposed and unexposed subjects. Workers were exposed to styrene at 200–300 ppm (occasionally), and the concentrations of MA in urine were measured as 55–3257 mg/g creatinine in 1976 and 53–1646 mg/g creatinine in 1977. There was a significant increase in chromosomal aberrations confirmed 1 year later, although the frequency of sister-chromatid exchange was not increased. In a study conducted in Sweden, Högstedt et al. (1979) considered a group of 6 reinforced plastics production workers (3 smokers) exposed to styrene at 50–400 mg/m^3 and 6 unexposed controls (3 smokers) matched for sex, age, and smoking. The results showed a significant increase in the frequency of chromosomal aberrations in the lymphocytes of exposed workers compared with controls (5.2 per 100 cells). [The Working Group noted that the study was limited by the small number of subjects in both groups.] Fleig & Thiess (1978) studied three groups of workers engaged in styrene manufacturing (5 men), polystyrene production (12 men), and unsaturated polyester resins production (14 workers from three different plants, 6 + 3 +5), where each group was matched with 20 controls, and found an increase in chromosomal aberrations only in the polyester resins production group. [The Working Group noted that the limitations of the study were the small sizes of the study groups, the lack of control for confounders, and unclear criteria for the selection of controls; however, the evidence of effects only in the group of workers exposed to the highest concentration, together with the absence of effects in the group exposed to the lowest concentration, may suggest the existence of a dose–response relationship.]

Three other studies were limited by co-exposure to other genotoxicants. The study of Tates et al. (1994; see Table 4.6) investigated a group of 46 workers exposed to styrene and dichloromethane (used to clean machines) engaged in the production of containers and boards and 23 paired unexposed controls. Workers were exposed to styrene at 17 ppm [70 mg/m^3] and dichloromethane at 31 ppm (TWA values). The study showed statistically significant increases in the frequencies of chromosomal aberrations with or without gaps ($P < 0.0001$), of aberrant cells (gaps always included), micronuclei, and sister-chromatid exchange ($P < 0.0001$; one-sided). Both duration and intensity of exposure were not correlated with genetic effects, but the TWA value for dichloromethane was positively correlated with the frequencies of chromosomal aberrations (with gaps) and aberrant cells. [The Working Group noted the contemporary presence of two genotoxicants; moreover, a correlation between dose and effect was observed only for dichloromethane and not for styrene.] In a study based in Lithuania, Mierauskiene et al. (1993) studied a group of 109 workers (68 women and 41 men; 38 smokers) exposed to styrene, formaldehyde, and phenol (concentrations not measured) for a duration of 1–25 years and 64 unexposed controls (25 women and 39 men; 16 smokers), mostly students and clerks (mean age, 37.4 years; range, 18–60 years). The frequency of aberrant cells in occupationally exposed workers was significantly higher than in controls. In addition, an increased frequency of breaks (both chromosome and chromatid) was observed, but not exchanges. Also in Lithuania, Lazutka et al. (1999) conducted a study on several environmental risk factors of different groups, including 38 men and 41 women engaged in the production of carpets, and 34 men and 63 women employed at a plastics production plant. Each group was compared with 90 unexposed controls (26 women and 64 men;

27 smokers and 63 non-smokers) approximately matched by age. Exposure to other chemical substances was observed in both groups (phenol and formaldehyde). An increased frequency of chromosomal aberrations was observed in the lymphocytes of exposed workers in both groups.

One study reported on chromosomal end-points in semen samples. Naccarati et al. (2003) studied chromosomal spermatozoa aberrations in 18 workers exposed to styrene in the production of fibreglass-reinforced plastics and in 13 paired controls. The median value of MA urine concentration in these workers was 292.5 mg/g creatinine (range, 20.8–947.8 mg/g creatinine). A cytogenetic analysis by fluorescence in situ hybridization conducted on semen samples did not show a statistically significant difference in the incidence of aneuploidy and diploidy between the group of 18 exposed workers and the 13 unexposed controls. The only statistically significant finding was an excess of nullisomy in the exposed non-smokers (Naccarati et al., 2003).

(v) DNA repair

Using the ^{32}P-postlabelling technique, Vodička et al. (1994) assessed the persistence of O^6-guanine DNA adducts in lamination workers exposed to styrene. Although lymphocyte adduct concentrations were higher in laminators compared with controls (see Section 4.2.2(c)), no decrease was noted between concentrations before and after a 2-week vacation, indicating that the repair process was slow. Oberheitmann et al. (2001) used the challenge assay, a cytogenetic approach to measure the repair competence, to assess DNA repair in lamination workers exposed to styrene. Exchange-type aberrations per 100 metaphases after X-ray challenge were 13.26 in 2 historical controls and 16.19 in 14 exposed laminators ($P < 0.038$). Among the exposed group, the challenge response was significantly correlated with the cumulative lifetime exposure to styrene ($P < 0.015$) but not with the current exposure as measured in blood, which was suggested to indicate the interference of long-term exposure with DNA repair.

Vodička et al. (2004) investigated cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in 86 lamination workers exposed to styrene and in 42 control individuals. A negative correlation between all exposure parameters and single-strand breaks (see Section 4.2.3(a)(i)), and a positive correlation between exposure parameters and DNA repair rates, were reported. Occupational exposure to styrene was studied in 34 workers employed in the production of fibreglass-reinforced plastic sheets compared with 29 unexposed healthy controls (Fracasso et al., 2009). A decrease in DNA repair activity compared with controls was observed.

Repair polymorphisms and gene expression in workers exposed to styrene

The Working Group noted that the results described in the paragraphs that follow were from a small number of individuals and that some results are not consistent. Godderis et al. (2004) studied 44 workers exposed to styrene (calculated average concentration, 9.5 ppm) and 44 matched controls to examine the influence of polymorphisms on genes encoding biotransformation, and of DNA repair enzymes on the levels of N-terminal haemoglobin adducts and on genotoxicity biomarkers. In the group of 88 individuals in total, higher frequencies of micronucleated mononucleated lymphocytes were found in individuals possessing the *XRCC3 Met*^{241} allele. Individuals with the *XRCC1 Gln*^{399} allele showed higher frequencies of micronucleated mononucleated lymphocytes and micronucleated binucleated lymphocytes. The same study population was used to investigate more polymorphisms, but no new associations were reported for subjects exposed to styrene (Mateuca et al., 2008). Kuricova et al. (2005) analysed 1-(2-hydroxy-phenylethyl)adenine

(1-styrene-7,8-oxide–adenine) DNA adducts, DNA single-strand breaks, chromosomal aberrations, mutant frequency at the *HPRT* gene, and immune parameters (relating to haematological and humoral immunity) in human subjects exposed to styrene (n = 48) and controls (n = 24). Results were correlated with genetic polymorphisms in DNA repair genes (xeroderma pigmentosum *XPD*, exon 23; *XPG*, exon 15; *XPC*, exon 15; *XRCC1*, exon 10; and *XRCC3*, exon 7) and cell cycle gene cyclin D1. The polymorphism in exon 23 of the *XPD* gene modulated levels of chromosomal and DNA damage and *HPRT* mutation frequency, and moderately affected DNA adduct levels. The highest levels of biomarkers were associated with the wildtype homozygous AA genotype. The exposed individuals with the wildtype GG genotype for the *XRCC1* gene exhibited the lowest frequencies of chromosomal aberrations compared with those with an A allele ($P < 0.05$). The same team also showed that, in 24 lamination workers occupationally exposed to styrene and 15 unexposed controls, DNA repair capacity was significantly lower in individuals with variant *Gln/Gln* genotype in *XRCC1 Arg399Gln* than in those with heterozygous *Arg/Gln* and wildtype *Arg/Arg* genotypes (Slyskova et al., 2007). Significantly lower repair capacity was also found in individuals with the wildtype *Lys/Lys* genotype in *XPC* at locus *Lys939Gln* compared with those homozygous for the *Gln/Gln* variant genotype.

Vodička and colleagues investigated 60 workers exposed to styrene and 50 unexposed clerks for mRNA expression levels of the human 8-oxoguanine DNA *N*-glycosylase (*hOGG1*) gene, and the role of the *hOGG1* polymorphism *Ser326Cys* (Manini et al., 2009). Subjects bearing the (*hOGG1*) *Ser/Ser* genotype showed lower levels of 8-oxodGuo in leukocytes than those with at least one variant *Cys* allele. Workers showed higher levels of *hOGG1* expression compared with controls ($P < 0.0005$).

In subsequent studies, Vodička and colleagues studied the modulation of DNA repair capacity and mRNA expression levels of *XRCC1*, *hOGG1*, and *XPC* genes in workers exposed to styrene (Hanova et al., 2010). The study assessed the associations between DNA strand breaks, micronuclei, DNA repair capacity, and mRNA expression in *XRCC1*, *hOGG1*, and *XPC* genes in 71 workers exposed to styrene and in 51 control individuals. The mRNA expression levels of *XRCC1*, *hOGG1*, and *XPC* were negatively correlated with styrene concentrations in the blood and in workplace air ($P < 0.001$), and positively correlated with strand breaks ($P < 0.001$). In a related study on the same worker population, the team studied the relationship between DNA damage, DNA repair rates, and mRNA expression levels of cell cycle genes *TP53*, $p21^{CDKN1A}$, *BCL2*, and *BAX* (Hanova et al., 2011). The results showed negative correlations between mRNA expression of *TP53*, *BCL2*, and *BAX* ($P < 0.001$ for all parameters) and styrene exposure, although a positive correlation with $p21^{CDKN1A}$ expression and exposure was recorded ($P = 0.001$). DNA strand breaks and sites sensitive to endonuclease III increased with increasing mRNA levels of *TP53* ($P < 0.001$ for both) and *BCL2* ($P = 0.038$ and $P = 0.002$, respectively), and decreased with increasing mRNA levels of $p21^{CDKN1A}$ ($P < 0.001$ and $P = 0.007$, respectively). In a study of 50 fibreglass-reinforced plastics workers exposed to styrene at less than 10 ppm, 10–20 ppm, and more than 20 ppm, and 40 control subjects (Wongvijitsuk et al., 2011), the expression of *CYP2E1*, *hOGG1*, and *XRCC1* in all groups exposed to styrene was higher than that of the control group ($P < 0.05$).

(b) Human cells in vitro

See Table 4.4.

(i) Styrene

Styrene-induced DNA damage was detected by comet assay in isolated human leukocytes treated in vitro (Laffon et al., 2003b) and in

human skin treated in vitro (Costa et al., 2006) without metabolic activations. Cytogenetic effects were analysed in human whole-blood lymphocytes treated in vitro without metabolic activations, including chromosomal aberrations (Linnainmaa et al., 1978a, b; Pohlová et al., 1984; Jantunen et al., 1986), micronucleus formation (Linnainmaa et al., 1978a), and sister-chromatid exchange (Norppa & Vainio, 1983a, b; Norppa et al., 1983; Chakrabarti et al., 1993; Lee & Norppa, 1995; Bernardini et al., 2002). Chromosomal aberration was also analysed in human isolated lymphocytes treated in vitro without metabolic activation systems (Jantunen et al., 1986). These various reports showed positive results without exogenous metabolic activation systems. Activation, probably resulting from the conversion of styrene to styrene-7,8-oxide, was attributed to erythrocytes present in the cultures (Norppa & Vainio, 1983a; Norppa et al., 1983).

(ii) Styrene-7,8-oxide

Using the comet assay, DNA damage induced by styrene-7,8-oxide was detected in isolated human lymphocytes (Köhlerová & Stětina, 2003; Speit et al., 2012; Bausinger & Speit, 2014), in whole-blood lymphocytes (Cemeli et al., 2009; see also Section 4.2.6(b)), and in human peripheral blood mononuclear cells (mononuclear leukocytes) (Bastlová et al., 1995; Laffon et al., 2002b;Godderis et al., 2004; Fabiani et al., 2012; Bausinger & Speit, 2014) treated in vitro. The sites sensitive to endonuclease III, corresponding to apurinic sites, were also shown to be induced (Köhlerová & Stětina, 2003). Induction of DNA strand breaks in human testicular germ cells treated with styrene-7,8-oxide in vitro was reported using the alkaline elution assay (Bjørge et al., 1996). Induction of DNA strand breaks was also reported in whole-blood cells in vitro using pulsed-field gel electrophoresis (Marczynski et al., 1997b). 7-Alkylguanine adducts of styrene-7,8-oxide and DNA single-strand breaks were determined by ^{32}P-postlabelling and DNA alkaline unwinding assay in human embryonal lung fibroblasts, respectively (Vodička et al., 1996). There was a concentration-dependent increase of both 7-alkylguanine adducts and DNA strand breaks.

As noted above in Section 4.2.2(b), the induction of gene mutations in human lymphocytes in vitro was reported at the *HPRT* locus (Bastlová et al., 1995; Bastlová & Podlutsky, 1996). A *GSTM1*-positive human recombinant cell line (FB7) showed lower mutation frequency at the *HPRT* locus than its parent *GSTM1*-negative cell line (WIL2NS) after treatment with styrene-7,8-oxide (Shield & Sanderson, 2004).

Chromosomal damage was analysed in human whole-blood lymphocyte cultures treated in vitro for chromosomal aberrations (Fabry et al., 1978; Linnainmaa et al., 1978a; Pohlová et al., 1984), micronuclei (Linnainmaa et al., 1978a; Speit et al., 2012), and sister-chromatid exchanges (Norppa & Vainio, 1983b; Norppa et al., 1983; Pohlová et al., 1984; Zhang et al., 1993; Lee & Norppa, 1995; Uusküla et al., 1995; Chakrabarti et al., 1997; Ollikainen et al., 1998). The frequency of micronucleus formation and sister-chromatid exchange was also analysed in isolated human mononuclear leukocytes (Laffon et al., 2001b, 2003a). Micronucleus induction was also analysed in human peripheral blood mononuclear cells (Godderis et al., 2006); results were consistently positive.

(c) Experimental systems

(i) Styrene: in vivo

See Table 4.7.

Rats

The comet assay was negative in leukocytes of male Fischer 344 rats (Gaté et al., 2012). However, a significant increase in DNA damage was observed on the 3rd, but not the 20th, day of treatment in the presence of Fpg, an enzyme able to recognize and excise DNA at the level of some oxidized DNA bases.

Table 4.7 Genetic and related effects of styrene and styrene-7,8-oxide in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Styrene							
Oxidative damage to DNA (comet assay with Fpg sensitive sites)	Rat, F344 (M)	Leukocytes	+/–	75 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay assessed at 3rd and 20th days; in the presence of Fpg, positive result was observed at 3rd but not at 20th day	Gaté et al. (2012)
DNA strand breaks, micronuclei	Rat, F344 (M)	Leukocytes. peripheral blood reticulocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay and micronuclei evaluated at the 3rd and 20th day of exposure	Gaté et al. (2012)
DNA strand breaks (comet assay), chromosomal aberrations, micronuclei	Rat, F344 (F)	Lymphocytes	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
Chromosomal aberrations	Rat, Sprague-Dawley (M, F)	Bone marrow	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 1 yr		Sinha et al. (1983)
Chromosomal aberrations, sister-chromatid exchange	Rat, F344 (M)	Peripheral blood lymphocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk		Preston & Abernethy (1993)
Micronuclei	Rat, Porton (M)	Bone marrow (PCE)	–	3000 mg/kg	Intraperitoneal injection, 48 h after treatment		Simula & Priestly (1992)
Sister-chromatid exchange	Rat, F344 (F)	Lymphocytes	(+)	125 ppm	Inhalation, 6 h/d, 14 d	Small increase, although statistically significant	Kligerman et al. (1993)
Unscheduled DNA synthesis	Mouse CD-1 (F)	Liver	–	250 ppm	Inhalation, 6 hr		Clay (2004)
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Lymphocytes, liver, kidney, bone marrow	+	250 mg/kg	Intraperitoneal injection, 4 h after treatment	LED is 350 mg/kg bw for bone marrow	Vaghef & Hellman (1998)
DNA strand breaks (DNA unwinding)	Mouse NMRI (M)	Kidney, liver, lung, testis, brain	+	8.3 mmol/kg bw	Intraperitoneal injection, NR		Solveig Walles & Orsén (1983)
DNA adducts (^{32}P-postlabelling)	Mouse, NMRI (M)	Lung	+	750 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	*N*7-guanine and *N*1-adenine adducts were analysed	Vodička et al. (2001a)

Table 4.7 (continued)

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (comet assay without EndoIII) and oxidative damage to DNA (comet with EndoIII)	Mouse, NMRI (M)	Lymphocytes, bone marrow	+/–	750 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	Positive in bone marrow for EndoIII-sensitive sites after 21 d	Vodička et al. (2001a)
DNA strand breaks (comet assay without EndoIII) and oxidative damage to DNA (comet with EndoIII)	Mouse, NMRI (M)	Liver	–	1500 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	Negative results with and without EndoIII	Vodička et al. (2001a)
Chromosomal aberrations, micronuclei	Mouse B6C3F$_1$ (F)	Lung, spleen	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
Chromosomal aberrations	Mouse, CD1 (M, F)	Bone marrow	–	1000 mg/kg	Gavage, single dose (1×), 24 h after treatment		Loprieno et al. (1978)
Chromosomal aberrations	Mouse, CD-1 (M)	Bone marrow	–	200 × 70, 500 × 4 mg/kg	Oral, 4 or 70 mg/kg per day		Sbrana et al. (1983)
Chromosomal aberrations, sister-chromatid exchange	Mouse, C57BL/6 (M)	Bone marrow	–	1000 mg/kg bw	Intraperitoneal injection, BrdU-labelled M1 cells 16 h after BrdU implantation		Sharief et al. (1986)
Micronuclei	Mouse, NMRI (M)	Bone marrow	+/–	1500 mg/m^3	Inhalation, 5 h/d, 7 d/wk, 1–21 d	2-fold higher than in control after 7 d (but not 21 d) of exposure	Vodička et al. (2001a)
Micronuclei	Mouse, NMRI (NR)	Bone marrow (PCE)	–	1500 mg/m^3	Inhalation, 6 h/d, 1–21 d		Engelhardt et al. (2003)
Micronuclei	Mouse, LACA Swiss (M)	Bone marrow (PCE)	+	600 mg/kg	Intraperitoneal injection, 48 h after treatment		Simula & Priestly (1992)
Micronuclei	Mouse, C57BL/6 (M)	Bone marrow (PCE)	+	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Norppa (1981)

Table 4.7 (continued)

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Sister-chromatid exchange	Mouse, BDF1 (M)	Bone marrow, liver, alveolar macrophages	+	387 ppm	Inhalation, 6 h/d, 4 d	Partial hepatectomy for liver regeneration	Conner et al. (1980)
Sister-chromatid exchange	Mouse, $B6C3F_1$ (F)	Lung, spleen, lymphocytes	(+)	125 ppm	Inhalation, 6 h/d, 14 d	Small increase, although statistically significant	Kligerman et al. (1993)
Sister-chromatid exchange	Mouse, LACA Swiss (M)	Splenocytes	(+)	45 mg/kg	Intraperitoneal injection, 24 h after treatment +43 h culture	Small increase, although statistically significant	Simula & Priestly (1992)
Chromosomal aberrations	Hamster, Chinese (M)	Bone marrow	–	300 ppm	Inhalation, 6 h/d, 5 d/wk, 4 d or 3 wk		Norppa et al. (1980)
Micronuclei	Hamster, Chinese (M)	Bone marrow	–	1000 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Penttilä et al. (1980)
Styrene-7,8-oxide							
DNA strand breaks (comet assay), micronuclei	Rat, F344 (M)	Leukocytes	–	75 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Evaluated at 3rd and 20th day; comet assay results negative with and without Fpg	Gaté et al. (2012)
DNA adducts (covalent binding to DNA (HPLC))	Rat, CD (M)	Forestomach	+	1.3 mg/kg bw	Gavage, 4 h after treatment		Lutz et al. (1993)
DNA strand breaks (comet assay)	Mouse, ddY (M)	Stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	+	400 mg/kg bw	Intraperitoneal injection, 3 h after treatment		Tsuda et al. (2000)
DNA strand breaks (comet assay)	Mouse, CD-1 (M)	Liver, kidney, lung, spleen, bone marrow	+	400 mg/kg bw	Intraperitoneal injection, 3 h and 24 h after treatment		Sasaki et al. (1997)
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Lymphocytes, liver, kidney, bone marrow	+	100 mg/kg bw	Intraperitoneal injection, 4 h after treatment	LED is 150 mg/kg bw for bone marrow	Vaghef & Hellman (1998)

Table 4.7 (continued)

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks (DNA unwinding)	Mouse, NMRI (M)	Kidney, liver, lung, testis, brain	+	5.3 mmol/kg bw [637 mg/kg bw]	Intraperitoneal injection, 5×/wk, 4 wk		Solveig Walles & Orsén (1983)
Dominant lethal test (dominant lethal)	Mouse, BALB/c (M)	Fetus	–	250 mg/kg bw	Intraperitoneal injection, mate after 1–3 wk	No positive control	Fabry et al. (1978)
Chromosomal aberrations	Mouse, BALB/c (M)	Spermatocytes	–	250 mg/kg bw	Intraperitoneal injection, 2 mo after treatment	No positive control	Fabry et al. (1978)
Chromosomal aberrations, micronuclei	Mouse, BALB/c (M)	Bone marrow	–	250 mg/kg bw	Intraperitoneal injection, 1–13 d (chromosomal aberration) or 30 d (micronuclei) after treatment	No positive control	Fabry et al. (1978)
Chromosomal aberrations	Mouse, CD1 (M, F)	Bone marrow	+	50 mg/kg bw	Gavage, 1×, 24 h after treatment		Loprieno et al. (1978)
Chromosomal aberrations, sister-chromatid exchanges	Mouse, CD-1 (M)	Bone marrow	+	Enantiomer (*S*- or *R*-) 100 mg/kg bw	Intraperitoneal injection, 24 h after treatment (chromosomal aberration)	Positive only for *S*-enantiomer	Sinsheimer et al. (1993)
Chromosomal aberrations, sister-chromatid exchanges	Hamster, Chinese (M)	Bone marrow	–	100 ppm	Inhalation, 9 h		Norppa et al. (1979)
Chromosomal aberrations, sister-chromatid exchanges	Hamster, Chinese (M)	Bone marrow	(+)	500 mg/kg bw	Intraperitoneal injection, 24 h after treatment	Single dose, positive only for dead animals	Norppa et al. (1979)
Micronuclei	Hamster, Chinese (M)	Bone marrow	–	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Penttilä et al. (1980)

BrdU, bromodeoxyuridine; bw, body weight; d, day(s); EndoIII, endonuclease III; F, female; h, hour(s); HIC, highest ineffective concentration; HPLC, high-performance liquid chromatography; LEC, lowest effective concentration; LED, lowest effective dose; M, male; mo, month(s); NR, not reported; NT, not tested; PCE, polychromatic erythrocytes; ppm, parts per million; wk, week(s); yr, year(s).

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+)/(–), positive/negative result in a study of limited quality; the level of significance was set at $P < 0.05$ in all cases.

Styrene did not induce DNA damage as determined by the comet assay, chromosomal aberrations, or sister-chromatid exchanges in female Fischer 344 rat lymphocytes (Kligerman et al., 1993).

Negative results for chromosomal aberrations were also reported in male Fischer 344 rats (Preston & Abernethy, 1993) and in bone marrow of male and female Sprague-Dawley rats (Sinha et al., 1983).

Micronucleus assay results were negative in the 3-week inhalation study in bone marrow of female Fischer 344 rats (Kligerman et al., 1993) and in the 4-week inhalation study in peripheral blood reticulocytes of male Fischer 344 rats (Gaté et al., 2012).

A negative result for sister-chromatid exchange was obtained in lymphocytes of male rats (Preston & Abernethy, 1993). A positive result was obtained for sister-chromatid exchange in splenocytes of the male Porton rat (Simula & Priestly, 1992).

Overall, results were negative or weakly positive for chromosomal damage in rats after exposure to styrene.

Mice

No data were available to the Working Group in transgenic models.

Styrene did not induce unscheduled DNA synthesis in female CD-1 mouse liver (Clay, 2004).

DNA damage induced by styrene was detected by comet assay in female C57BL/6 mouse lymphocytes, liver, kidney, and bone marrow (Vaghef & Hellman, 1998). DNA strand breaks in kidney, liver, lung, testis, and brain of male NMRI mice were detected by the DNA unwinding assay (Solveig Walles & Orsén, 1983).

DNA damage induced by styrene was detected by the comet assay in bone marrow of male NMRI mice (with an increase in sites sensitive to endonuclease III), although no induction was observed in liver (Vodička et al., 2001a). DNA adducts were detected in lung.

Negative results for chromosomal aberrations were reported in the lung and spleen of female B6C3F$_1$ mice (Kligerman et al., 1993). Negative results for chromosomal aberrations were reported in male and in female CD-1 mouse bone marrow (Loprieno et al., 1978; Sbrana et al., 1983).

Results for micronucleus induction were negative in the bone marrow of male NMRI mice (Engelhardt et al., 2003), and in the spleen and peripheral blood of female B6C3F$_1$ mice (Kligerman et al., 1993). An equivocal result for micronucleus induction was reported in male NMRI mouse bone marrow (Vodička et al., 2001a). A weak micronucleus induction was reported in bone marrow of male LACA Swiss mice (Simula & Priestly, 1992) and C57BL/6 mice (Norppa, 1981).

In the assay for sister-chromatid exchange, positive results were obtained in bone marrow, liver, and alveolar macrophages of male BDF1 mice (Conner et al., 1980). Equivocal results were obtained in the lung, spleen, and lymphocytes of female B6C3F$_1$ mice (Kligerman et al., 1993). In the assay for sister-chromatid exchange, an equivocal result was reported in male LACA Swiss mouse splenocytes (Simula & Priestly, 1992) and a negative result was obtained in male C57BL/6 mouse bone marrow (Sharief et al., 1986).

Overall, results for chromosomal damage induced by styrene were negative or weak in mouse cells in vivo.

Hamsters

After exposure by inhalation (Norppa et al., 1980) or intraperitoneal injection (Penttilä et al., 1980), negative results for cytogenetic changes were reported in the bone marrow of male Chinese hamster.

All results were negative for chromosomal damage induced by styrene in hamster.

(ii) Styrene-7,8-oxide: in vivo

Rats

Results were negative in leukocytes as measured by comet assay, with and without Fpg, and were negative for micronucleus induction in peripheral blood reticulocytes when analysed during a 4-week inhalation study in male Fischer 344 rat (Gaté et al., 2012).

Mice

DNA strand breaks were detected by the DNA unwinding assay in kidney, liver, lung, testis, and brain of male NMRI mice (Solveig Walles & Orsén, 1983). DNA damage induced by styrene-7,8-oxide was detected by comet assay in all organs tested in male CD-1 mice (liver, kidney, lung, spleen, and bone marrow) (Sasaki et al., 1997) and in male ddY mice (stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow) (Tsuda et al., 2000). DNA damage was detectable in multiple organs after exposure to styrene-7,8-oxide by intraperitoneal injection.

A positive result for chromosomal aberration was reported in male and female CD-1 mouse bone marrow (Loprieno et al., 1978). A negative or equivocal result for chromosomal aberration was reported in the bone marrow and spermatocytes of BALB/c mice (Fabry et al., 1978). The bone marrow micronucleus test was negative in BALB/c mice (Fabry et al., 1978). A negative result for the mouse dominant lethal test was also reported (Fabry et al., 1978).

Enantiomers of styrene-7,8-oxide were tested by an assay for sister-chromatid exchange in male CD-1 mouse bone marrow; only the *S*-enantiomer gave a positive result without including gaps (Sinsheimer et al., 1993).

[The Working Group noted the possibility that only the *S*-enantiomer has the potential to induce chromosomal damages, but there was only one study.]

Hamsters

In male Chinese hamsters exposed to styrene-7,8-oxide by inhalation, the results of assays for sister-chromatid exchange and chromosomal aberration were negative; however, in male Chinese hamsters exposed by intraperitoneal injection, the results were equivocal for both cytogenetic tests (Norppa et al., 1979).

The bone marrow micronucleus test was negative in Chinese hamster after intraperitoneal injection (Penttilä et al., 1980).

Overall, no positive result was obtained in hamsters.

(iii) Styrene: in vitro

The majority of genotoxicity testing for styrene in non-human mammalian in vitro systems and in non-mammalian systems is reported in *IARC Monographs* Volume 60 (IARC, 1994). Styrene has been studied for its effects on DNA strand breaks, mutagenesis and chromosomal aberrations, and sister-chromatid exchanges, primarily in rodent cell lines, *Drosophila melanogaster*, yeast, and *Escherichia coli*. These results are summarized below and in Supplemental Table S2 and Supplemental Table S3.

In a study in non-human mammalian cells using the comet assay, exposure to styrene at 2.5 mM for 2 hours significantly increased DNA damage in isolated hepatocytes obtained from male Swiss albino mice (Fontaine et al., 2004). This effect was attenuated when cells were pre-incubated with SKF-525A, a broad inhibitor of CYP enzymes.

In earlier studies, styrene induced DNA strand breaks in rat primary hepatocytes in an alkaline elution assay (Sina et al., 1983). Styrene induced mutations at the *Hprt* locus in Chinese hamster lung V79 cells with, but not without, exogenous metabolic activation system (Loprieno et al., 1976; Beije & Jenssen, 1982). Two studies on the induction of chromosomal aberrations in Chinese hamster lung cells reported negative results without exogenous metabolic

activation and weakly positive results with exogenous metabolic activation (Matsuoka et al., 1979; Ishidate & Yoshikawa, 1980). Sister-chromatid exchanges were induced in Chinese hamster ovary cells with, but not without, exogenous metabolic activation system (de Raat, 1978), whereas sister-chromatid exchanges were induced in rat lymphocytes without exogenous metabolic activation (Norppa et al., 1985).

In non-mammalian systems, styrene (0.2 mg/L in seawater) significantly increased DNA damage in cells from the peripheral blood of fish (*Symphodus melops*) and the haemolymph of mussels (*Mytilus edulis*) after 7 days of continuous exposure (Mamaca et al., 2005).

Styrene was positive for sex-linked recessive lethal mutations and negative for aneuploidy in *D. melanogaster* (Donner et al., 1979), whereas Penttilä et al. (1980) reported negative results for aneuploidy. Rodriguez-Arnaiz (1998) reported negative results for induction of somatic mutations by styrene in *D. melanogaster*; however, in the same study styrene was positive in two strains of flies that had higher levels of CYP activity.

Styrene induced chromosomal aberrations in the root tip cells of *Allium cepa* (Linnainmaa et al., 1978a,b).

Styrene was genotoxic for several end-points in yeast, including gene conversion and reverse mutation (Del Carratore et al., 1983). It was also shown that enhancing the metabolic activity of mouse liver S9 by treating animals twice (4–5 weeks between injections) with phenobarbital and β-naphthoflavone increased the genotoxicity of styrene in *Saccharomyces cerevisiae* D7 in assays for mitotic gene conversion, mitotic crossing-over, and point reverse mutation (Paolini et al., 1988).

Styrene was tested in various *Salmonella typhimurium* strains (TA98, TA100, TA1530, TA1535, TA1537, and TA1538) by several research groups. Styrene was reported negative in the absence of exogenous metabolic activation in eight tests for TA100, and negative in the presence of exogenous metabolic activation in seven studies and positive in one (de Meester et al., 1981). In a ninth test, styrene was reported as weakly mutagenic in the presence and absence of exogenous metabolic activation; however, concern was expressed about the results because of toxicity (Vainio et al., 1976). In one study, positive results were reported for TA1530 in the presence and absence of exogenous metabolic activation (de Meester et al., 1981). [The Working Group noted that the authors reported that the result in the absence of metabolic activation is a false-positive result because of contamination with a volatile genotoxicant, possibly styrene-7,8-oxide, from neighbouring plates that had been incubated with styrene and S9 (de Meester et al., 1981).] Of nine tests reported for TA1535, all were negative in the absence of exogenous metabolic activation and four were positive with exogenous metabolic activation (Vainio et al., 1976; de Meester et al., 1977, 1981; Poncelet et al., 1980). Styrene was negative in TA98, TA1537, and TA1538 with or without exogenous metabolic activation (Vainio et al., 1976; de Meester et al., 1977, 1981; Stoltz & Whitey, 1977; Watabe et al., 1978; Busk, 1979; Florin et al., 1980). In studies conducted by the National Toxicology Program, styrene was tested in TA97, TA98, TA100, TA1535, and TA1537 with and without exogenous metabolic activation; negative results were found (Zeiger et al., 1988). Styrene was also negative in an *E. coli* SOS chromotest for DNA damage in the absence of exogenous metabolic activation (Brams et al., 1987). [The Working Group noted that, overall, styrene was mutagenic with exogenous metabolic activation in strains that detect base-pair substitutions (TA100, TA1530, and TA1535), but not in strains that detect frameshift mutations (TA98, TA1537, and TA1538). Overall, styrene is not mutagenic in the Ames assay in the absence of exogenous metabolic activation.]

(iv) Styrene-7,8-oxide: in vitro

The majority of genotoxicity testing for styrene-7,8-oxide in non-human mammalian in vitro systems and in non-mammalian systems was reported in *IARC Monographs* Volume 60 (IARC, 1994). Similar to styrene, styrene-7,8-oxide has been studied for effects on DNA strand breaks, mutagenesis, chromosomal aberrations, and sister-chromatid exchanges primarily in rodent cell lines, *D. melanogaster*, and yeast. These results are included in Supplemental Table S2 and Supplemental Table S3.

Styrene-7,8-oxide induced sister-chromatid exchanges, micronuclei, and mutations at the *Hprt* locus in Chinese hamster lung V79 cells (Loprieno et al., 1976, 1978; Beije & Jenssen, 1982; Nishi et al., 1984), and induced sister-chromatid exchanges in Chinese hamster ovary cells, in the absence of exogenous metabolic activation (de Raat, 1978). DNA damage was also induced by 50 µM of styrene-7,8-oxide in Chinese hamster lung V79 cells as measured by alkaline elution assay (Oesch et al., 2000); however, it was shown that 200 µM of styrene-7,8-oxide was required to produce the same amount of DNA damage in Chinese hamster lung V79 cells engineered to express human microsomal epoxide hydrolase at levels comparable to that of human liver cells (Oesch et al., 2000). Styrene-7,8-oxide also induced mutations at the *Tk* locus in mouse lymphoma L5178Y cells without exogenous metabolic activation; however, the result was negative when styrene-7,8-oxide was tested with uninduced rat liver S9 (Amacher & Turner, 1982). Styrene-7,8-oxide induced DNA strand breaks in rat primary hepatocytes (Sina et al., 1983) and rat pheochromocytoma PC12 cells (without exogenous metabolic activation) (Dypbukt et al., 1992) in alkaline elution assays.

Styrene-7,8-oxide was positive for sex-linked recessive lethal mutations in *D. melanogaster* (Donner et al., 1979).

Styrene-7,8-oxide induced chromosomal aberrations and micronuclei in the root tip cells of *Allium cepa* (Linnainmaa et al., 1978a,b).

Styrene-7,8-oxide was tested in various *S. typhimurium* strains (TA97, TA98, TA100, TA104, TA1530, TA1535, TA1537, TA1538, TA4001, and TA4006) and in *E. coli* WP2 *uvrA* by several research groups. Positive results were obtained without the need for exogenous metabolic activation in TA97, TA100, TA104, TA1530, TA1535, TA4001, TA4006, and *E. coli* WP2 *uvrA*, all of which detect base substitution mutations. Styrene-7,8-oxide was also mutagenic in all of these strains with exogenous metabolic activation except for TA104, TA4001, TA4006, and *E. coli* WP2 *uvrA*, which were not tested under these conditions (Sugiura et al., 1978; Sugiura & Goto, 1981; Einistö et al., 1993). Nearly all testing performed with styrene-7,8-oxide in TA98, TA1537, and TA1538, which detects frameshift mutations, produced negative results in the absence or presence of metabolic activation (e.g. Wade et al., 1978; Watabe et al., 1978; de Meester et al., 1981); however, a single study that used TA97, which also detects frameshift mutations, reported a positive result without metabolic activation (Brams et al., 1987). Styrene-7,8-oxide was positive in TA100 and negative in TA98 with or without exogenous metabolic activation in studies conducted by the National Toxicology Program (Zeiger et al., 1992). Styrene-7,8-oxide was also positive in *S. typhimurium* and *E. coli* SOS chromotests for DNA damage without exogenous metabolic activation (Głośnicka & Dziadziuszko, 1986; Nakamura et al., 1987; von der Hude et al., 1990). More recently, the mutagenic potency of styrene-7,8-oxide was reduced in TA100 by using S9 prepared from rats treated with organic sulfur compounds obtained from garlic and onions to enhance epoxide hydrolase activity (Guyonnet et al., 2001). [The Working Group noted that, overall, the Ames assay results for styrene-7,8-oxide are largely in agreement with the observation that styrene was mutagenic

in strains that detect base substitutions when an exogenous metabolic activation system was used.]

4.2.4 Alteration of cell proliferation or cell death

(a) Exposed humans

(i) Styrene

Exposure to styrene among 18 fibreglass-reinforced plastics workers was associated with significantly reduced rates of cell proliferation (bromodeoxyuridine (BrdU) incorporation) in their cultured lymphocytes compared with 6 unexposed control subjects (Watanabe et al., 1983). Exposure to styrene in two groups of hand laminators (27 workers) suppressed the proliferative responses of isolated cultured lymphocytes stimulated by the mitogen concanavalin A compared with 19 control workers (Somorovská et al., 1999) (see Section 4.2.7(a)).

(ii) Styrene-7,8-oxide

No data on the alteration of cell proliferation or cell death in humans by styrene-7,8-oxide were available to the Working Group.

(b) Human cells in vitro

(i) Styrene

The treatment of whole-blood cultures from 12 donors with 10–200 μM of styrene increased the length of the cell cycle in a dose-related manner (Chakrabarti et al., 1993).

(ii) Styrene-7,8-oxide

[The Working Group noted the small number of donors as a limitation of the available studies.] In whole-blood cultures from four donors treated with styrene-7,8-oxide at 50 μM and 200 μM, there was large inter-individual variation in the expression of *p53*, *p21*, *bcl-2*, and *bax* genes in lymphocytes. Apoptosis was increased in cells from two of the donors at either 50 μM and/or 200 μM of styrene-7,8-oxide. The cytokinesis block proliferation index decreased in lymphocytes from all four donors at 200 μM of styrene-7,8-oxide, suggesting a delay in the cell cycle (Laffon et al., 2001a).

Styrene-7,8-oxide (10–200 μM) reduced the proliferative rate index (BrdU incorporation) in isolated cultured human peripheral leukocytes from four donors; the extent of the reductions were dependent on the donor. Similar results with styrene-7,8-oxide were found using cytokinesis block proliferation indices of binucleated cells with micronuclei (Laffon et al., 2001b).

Styrene-7,8-oxide (100 μM) significantly reduced the replication index (BrdU incorporation) in human whole-blood lymphocyte cultures from two donors; however, there was no linear relationship between the replication index and the duration of exposure (Chakrabarti et al., 1997).

In another study, styrene-7,8-oxide (100 μM) significantly reduced the replication index (BrdU incorporation) in human whole-blood lymphocyte cultures from two donors from 1.65 to 1.30, with a simultaneous 20% reduction in cell viability (Zhang et al., 1993).

(c) Experimental systems

(i) Styrene, styrene-7,8-oxide, and other styrene metabolites: in vivo

After exposure by inhalation of male and female CD-1 mice to styrene at 150–200 ppm for 6 hours per day, 5 days per week, cell proliferation (BrdU incorporation) in lung Clara cells was increased after 2 weeks (by up to 3.6-fold) and 5 weeks (by 1.3-fold) of exposure. No changes in cell proliferation in Clara cells were observed after 13 weeks of exposure, and no increases in cell proliferation were noted in type-II pneumocytes or in hepatocytes.

A dose-related increase in cell proliferation (BrdU incorporation) was seen in the terminal and large bronchioles of male and female CD-1 mice exposed by inhalation to styrene at 40 ppm

or 160 ppm for 6 hours per day for 5 days. The increased cell replication was not present after a 2-day break in exposure, but reoccurred in the large bronchioles when the mice were exposed for a further 5 days for 6 hours per day. Similar effects were seen in mice given styrene at 10, 100, or 200 mg/kg by oral gavage every day for 5 days. Increases in cell proliferation were seen in the terminal bronchioles in mice given styrene at 100 mg/kg and 200 mg/kg, but not at 10 mg/kg (Green et al., 2001b).

Cell proliferation (Ki-67 staining) was increased compared with controls in the terminal bronchioles of male CD-1 mice (3.9-fold) and male C57BL/6 wildtype mice (5.7-fold) exposed by inhalation to styrene at 120 ppm for 6 hours per day after 1 week, but not after 26, 52, or 72 weeks, of treatment. No increases in cell proliferation were detected in the terminal bronchioles of male *Cyp2f2*$^{(-/-)}$ mice or male *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1/CYP2A13/CYP2B6* transgene under the same experimental conditions. The cell proliferation results in these strains of mice mirror the results of the chronic bioassays of styrene under the same experimental conditions (Cruzan et al., 2017). Compared with their respective vehicle controls, significantly increased hyperplasia of the terminal bronchioles was seen in male CD-1 and male C57BL/6 wildtype mice given styrene between weeks 78 and 104, although epithelial degeneration was also induced in C57BL/6 mice for 26 weeks but largely resolved within 52 weeks of exposure. No responses were observed in the vehicle control or treated male *Cyp2f2*$^{(-/-)}$ mice or male *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1/CYP2A13/CYP2B6* transgene (Cruzan et al., 2017). Cell proliferation (BrdU incorporation) was not altered in the terminal bronchioles of male *Cyp2f2*$^{(-/-)}$ mice given styrene at 400 mg/kg bw once per day for 5 days by gavage, although cell proliferation in the terminal bronchioles of male C57BL/6 wildtype control mice dosed under the same conditions increased 14.2-fold. Intraperitoneal injection of *R*-styrene-7,8-oxide or *S*-styrene-7,8-oxide at 200 mg/kg per day for 5 days in male and female *Cyp2f2*$^{(-/-)}$ mice and C57BL/6 wildtype control mice elicited similar responses as observed in studies where styrene was given by intraperitoneal injection. The observation that the substantial cell proliferation responses in each sex of wildtype mice treated with either enantiomer of styrene-7,8-oxide were not present in *Cyp2f2*$^{(-/-)}$ mice of the corresponding sex treated with the same agents indicates that styrene-7,8-oxide requires CYP2F2 to further metabolize it to a metabolite or to metabolites that induce cell proliferation (Cruzan et al., 2012).

Exposure of male B6C3F$_1$ mice to styrene by inhalation at 500 ppm for 6 hours per day produced large increases in hepatic cell proliferation (BrdU incorporation) after 1, 6, and 14 days of exposure (Mahler et al., 1999). In male or female CD rats exposed by inhalation to styrene at 500–1500 ppm for 6 hours per day, 5 days per week, no increase in cell proliferation was observed in hepatocytes in the liver or in alveolar and bronchiolar cells in the lungs after 2, 5, or 13 weeks of exposure (Cruzan et al., 1997). In male Sprague-Dawley CD rats exposed to styrene by inhalation at 500 ppm for 6 hours per day for up to 5 days, there was no increase in cell proliferation in any region of the lungs at any time point (Green et al., 2001b).

Cell proliferation was increased in the saccus caecus, midregion, and prefundic regions of the forestomach of male Fischer 344 rats given styene-7,8-oxide at 137, 275, and 550 mg/kg bw by gavage 3 times per week for 4 weeks. No dose–response was evident (Cantoreggi et al., 1993). Increases in cell proliferation (^{3}H-thymidine incorporation) in the forestomach of male F344 rats were detected after both a single dose of styrene-7,8-oxide at 800 mg/kg bw by gavage and after nine doses. Increases in dose-related cell proliferation were detected after a single dose of styrene-7,8-oxide at 50–250 mg/kg bw, with

the cell proliferation response plateauing at doses of less than 800 mg/kg bw (Dalbey et al., 1996). Some of these doses were used in previously reported cancer studies of styrene-7,8-oxide using the same route of administration.

Styrene, *R*-styrene-7,8-oxide, or *S*-styrene-7,8-oxide given to C57BL/6 control mice of both sexes at 200 mg/kg bw per day for 5 days by intraperitoneal injection increased cell proliferation in the terminal bronchioles (Cruzan et al., 2012, 2013). Comparable exposure did not increase cell proliferation in male and female *Cyp2f2*$^{(-/-)}$ mice (Cruzan et al., 2012) or in male and female *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene (verified for functional CYP2A13 and CYP2F1 activities by in vitro studies) (Cruzan et al., 2013). In male and female CD-1 mice given 4-vinylphenol at 60 mg/kg bw and 105 mg/kg bw per day for 5 days by intraperitoneal injection, a 3.8-fold to 7.6-fold (higher dose) dose-dependent increased cell proliferation in the terminal bronchioles was observed. The equivalent treatment of C57BL/6 wildtype mice yielded a 8.9-fold to 9.4-fold (higher dose) dose-dependent increased cell proliferation, but not for *Cyp2f2*$^{(-/-)}$ mice. Treatment with 4-vinylphenol at 105 mg/kg bw per day for 5 days by intraperitoneal injection increased cell proliferation in the terminal bronchioles of male and female *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene by 2.4- to 3.1-fold compared with transgenic mice used as vehicle controls, and in male and female C57BL/6 wildtype transgenic control mice by 4.8- to 6.6-fold compared with vehicle controls. [The Working Group noted that 4-vinylphenol, but not styrene or *R*- or *S*-styrene-7,8-oxide, induced cell proliferation in humanized transgenic mice.]

In 3-day studies of female Crl Icr: CD1 mice given styrene or styrene-7,8-oxide at 100 mg/kg bw per day by intraperitoneal injection, increased cell proliferation in the large and/or medium bronchi, terminal bronchioles, and alveoli was observed. Treatment with 4-vinylphenol at 20 mg/kg bw or 35 mg/kg bw per day increased cell proliferation in the large and/or medium bronchi and terminal bronchioles, whereas phenylacetaldehyde at 100 mg/kg bw per day decreased cell proliferation in the large and/or medium bronchi and terminal bronchioles and increased cell proliferation in the alveoli. Phenylacetic acid at 100 mg/kg bw per day decreased cell proliferation in terminal bronchioles and increased cell proliferation in the alveoli. At lower doses of 35 mg/kg bw per day, phenylacetaldehyde had no effect and phenylacetic acid decreased cell proliferation in the large and/or medium bronchi and terminal bronchioles. Treatment with 1-phenylethanol, 2-phenylethanol, or acetophenone at 100 mg/kg bw per day produced either no effects or marginal effects on cell proliferation. Styrene-7,8-oxide, phenylacetaldehyde, or phenylacetic acid at 100 mg/kg bw per day produced some increases in apoptosis that were not statistically significant (Kaufmann et al., 2005).

A single intraperitoneal injection of styrene at 600 mg/kg bw or *R*-styrene-7,8-oxide at 300 mg/kg bw significantly increased the ratio of bax/bcl-2 based on RNA and protein expression in Clara cells isolated from male CD-1 mice at various time points. Treatment with either racemic styrene-7,8-oxide, *S*-styrene-7,8-oxide, or 4-vinylphenol at 300 mg/kg bw was without effect. A small increase in caspase 3 activity was measured in Clara cells from male CD-1 mice exposed to *R*-styrene-7,8-oxide at 300 mg/kg bw by intraperitoneal injection, although no changes in caspase 8 activity was detected (Harvilchuck et al., 2009).

(ii) Styrene, styrene-7,8-oxide, and other styrene metabolites: in vitro

Styrene-7,8-oxide (0.8–1.0 mM) induced significant apoptosis, mediated by caspase 3 activation, in Norway rat adrenal pheochromocytoma PC12 cells in culture. Styrene-7,8-oxide also reduced Bcl-2 protein levels and increased

Bax protein levels, decreasing the Bcl-2/Bax ratio (Boccellino et al., 2003).

Styrene (1 nM to 1 mM) had no effect on C57BL/6 female mouse splenic T-lymphocyte or B-lymphocyte proliferation induced by the mitogens concanavalin A or lipopolysaccharide (LPS) (Poirier et al., 2002).

An increase in apoptosis as measured by caspase 3 activity was induced by styrene-7,8-oxide (75 μM) in C3H/An mouse fibroblast L929 cells in culture (Brockmann et al., 2006).

4.2.5 Receptor-mediated effects

Multiple studies in workers exposed to styrene have reported increases in serum prolactin levels. Serum prolactin levels were increased in 53 glass-reinforced plastics workers (33 men, $P < 0.01$; 20 women, $P < 0.05$) compared with 60 unexposed industrial workers comparable in age, sex, and smoking and drinking habits (Bergamaschi et al., 1996), as well as in 30 female glass-reinforced plastics workers compared with 30 age-matched female factory workers living in the same area, but not exposed to styrene or other industrial chemicals (Mutti et al., 1984). Serum prolactin levels were positively correlated with urinary styrene metabolite levels (MA and PGA) (Mutti et al., 1984). Plasma prolactin levels were increased in 46 male glass-reinforced plastics workers ($P < 0.001$) compared with 30 male blue-collar workers in local industries with no history of chemical exposures (Bergamaschi et al., 1997). In a study with repeated measurements of serum prolactin taken about 1 year apart over the course of 2–3 years in a cohort of glass-reinforced plastics workers from several different facilities (173 men, 33 women), a 2-fold increase in serum prolactin was associated with every 10-fold increase in blood styrene concentration (Luderer et al., 2004). Prolactin release from the anterior pituitary gland can be inhibited by dopamine secreted by the tuberoinfundibular dopaminergic system in the hypothalamus, although prolactin release can be stimulated by thyrotrophin-releasing hormone (TRH). A challenge dose of TRH was given to 16 female workers exposed to styrene and 16 sex- and age-matched controls; 15 of the 16 women exposed to styrene responded with abnormally high serum prolactin levels, compared with an abnormal serum prolactin response in only 1 of 16 controls (Arfini et al., 1987). When two of the women in the group exposed to styrene with abnormally high serum prolactin responses were removed from styrene exposure for 2 months, serum prolactin responses were within the normal range after TRH challenge (Arfini et al., 1987). [The Working Group noted that this is consistent with styrene inhibiting dopamine release by tuberoinfundibular dopaminergic system neurons, as suggested by Mutti et al. (1984).]

Workers exposed to styrene also had higher serum levels of growth hormone and lower levels of thyroid-stimulating hormone (TSH) than the unexposed controls, although no differences were seen for follicle-stimulating hormone or luteinizing hormone (Mutti et al., 1984). In 38 male glass-reinforced plastics workers exposed to styrene, no significant differences were observed in thyroid volume, serum levels of TSH, free thyroxine (FT4), or free triiodothyronine (FT3) compared with 123 unexposed male workers. Among workers exposed to styrene, urinary styrene metabolite levels were correlated positively with serum FT4 levels, as well as with the ratio of serum FT4 to serum FT3, although a negative correlation was observed between urinary styrene metabolite levels and serum FT3 levels; no correlation was observed between urinary styrene metabolite levels and serum TSH levels (Santini et al., 2008). Among workers exposed to styrene, increasing duration (years) of styrene exposure was correlated positively with thyroid volume; no correlation was observed between duration of styrene exposure and urinary styrene metabolite levels. [The Working Group noted the

small number of workers exposed to styrene in this study.]

No relevant data from human in vitro studies, or from experimental animal studies, were available to the Working Group.

4.2.6 Oxidative stress

Studies on oxidative damage to DNA are discussed in Section 4.2.3(a).

(a) Exposed humans

No additional studies were available to the Working Group.

(b) Human cells in vitro

(i) Styrene

Chakrabarti et al. (1993) incubated human whole-blood lymphocytes and isolated lymphocytes obtained from 10 healthy male non-smokers and non-consumers of alcohol with no recent exposure to radiation or pharmaceuticals with styrene (0–200 μM) for 72 hours. At non-cytotoxic concentrations of styrene, dose-dependent depletion of GSH (measured as total non-protein sulfhydryls) and concentration-dependent increases in malondialdehyde (MDA, measured as thiobarbituric acid-reactive substances), a marker of lipid peroxidation, occurred in both the whole-blood lymphocyte and isolated lymphocyte cultures.

In vitro exposure of human abdominal skin to styrene vapour for 8 hours decreased the concentration of GSH as well as GST, superoxide dismutase (SOD), and catalase activities in a dose-dependent manner, while increasing MDA and carbonyl compounds derived from protein peroxidation, markers of oxidative damage (Costa et al., 2006); as noted in Section 4.2.3(b)(i), this study also reported DNA damage (as measured by the comet assay).

In a series of studies with human A549 lung bronchioloalveolar epithelial carcinoma cells exposed to styrene vapour in a multicell chamber culture system, non-cytotoxic concentrations of styrene were found to increase concentrations of intracellular reactive oxygen species (ROS, as measured by 2,7′-dichlorofluorescein diacetate), induce several responses associated with oxidative stress, and activate the redox-sensitive transcription factors NF-κB and p38 MAPK (Röder-Stolinski et al., 2008; Mörbt et al., 2009; Mögel et al., 2011). Specific responses indicative of oxidative stress included an initial increase in GSH concentrations after 1 hour of exposure followed by depletion of GSH with longer exposure, and increases in *GSTP1* mRNA expression and GSTP1 and hemoxygenase-1 protein concentrations. These effects were abrogated in the presence of *N*-acetylcysteine (NAC). Additional responses indicative of oxidative stress included increases in concentrations of SOD1, biliverdin reductase A, DJ-1, Clic1, transaldolase 1 (TALDO1), 6-phosphogluconate dehydrogenase, COX2, prostaglandin E_2 (PGE_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and a decrease in peroxiredoxin 4 protein. NAC was shown to abrogate increased concentrations of COX2, PGE_2, and $PGF_{2\alpha}$ induced by styrene, although increasing NF-κB gene expression and NF-κB phosphorylation.

(ii) Styrene-7,8-oxide

Treatment of blood samples from nine healthy unexposed individuals with styrene-7,8-oxide reduced high-molecular-weight DNA fragments and increased low-molecular-weight DNA fragments in leukocytes from seven of the individuals (Marczynski et al., 2000). Reductions in leukocyte high-molecular-weight DNA fragments were also observed in these samples after treatment with hydrogen peroxide. [Although DNA fragmentation may result from oxidative DNA damage, the Working Group noted that increased DNA fragmentation is not a specific marker of oxidative stress.] In the study by Cemeli et al. (2009) (see Section 4.2.3(b)(ii)), the addition of catalase during treatment with

styrene-7,8-oxide did not significantly affect the level of DNA damage induced.

Treatment of human neuroblastoma 3K-H-MC cells with styrene-7,8-oxide increased lipid peroxidation (MDA, measured as thiobarbituric acid-reactive substances) after 12 hours, concomitant with an increase in mitochondrial dysfunction, measured as a decrease in mitochondrial Ca^{2+} capacity (Daré et al., 2004). The addition of the superoxide scavenger manganese(III) tetrakis(4-benzoic acid) porphyrin reduced the degree of mitochondrial dysfunction and cell death induced by styrene-7,8-oxide in these cells at 12, 14, and 16 hours; MDA levels were not assessed.

(c) Experimental systems

(i) Styrene: in vivo

Subchronic exposure to styrene via inhalation decreased the levels of reduced or total GSH (including all non-protein sulfhydryls) in rat liver, lung, and brain when evaluated within a few hours after the last exposure (reviewed in Vainio et al., 1979; Elovaara et al., 1990; Coccini et al., 1996; Coccini et al., 1997; NTP, 2008; NRC, 2014), similar to observations after acute exposures via intraperitoneal injection (Srivastava et al., 1983; Coccini et al., 1997); however, no effects on tissue GSH levels were reported when evaluated 24 hours or more after the last exposure (Katoh et al., 1989; Coccini et al., 1997). In terms of duration of effect, the concentrations of GSH in liver and lung of rats exposed to styrene decreased the most severely in the first 4 weeks of an 11-week exposure (Vainio et al., 1979). Decreased GSH has also been reported in fetal livers isolated from Wistar dams given styrene orally (Srivastava et al., 1992). In a detailed evaluation of the depletion of tissue GSH with time in male Wistar rats, liver concentrations of both GSH and oxidized glutathione (GSSG) decreased rapidly after exposure by repeated intraperitoneal injection with styrene, remained depleted until 12 hours after exposure, recovered to overshoot control levels after 24 hours, and finally decreased back to control levels after 40 hours (Katoh et al., 1989).

Although tissue GSH concentrations were affected in a time- and dose-dependent manner, lipid peroxidation (LPO) was not increased in the brain, liver, or lung from male Sprague-Dawley rats after subchronic inhalation exposure to styrene (Coccini et al., 1996, 1997). A dose-responsive increase in markers of oxidative stress (decreased ratio of ferric:ferrous iron, total thiol molecules, and increased protein carbonyls) was observed in the plasma and livers of male Wistar rats subchronically exposed via gavage, with increased concentrations of ROS and LPO products observed at sequentially higher doses (Niaz et al., 2017a, b). [The Working Group noted that all the identified studies evaluated ROS concentrations using dichlorofluorescein, and recognized the significant limitations of using this compound as a measure of oxidative stress (Rota et al., 1999; Bonini et al., 2006).] In a separate study, LPO concentrations were elevated in serum from both male and female Wistar rats subchronically exposed to styrene via oral gavage (El-Ziney et al., 2016). [The Working Group noted the 1000-fold disparity between the dose reported by El-Ziney et al. (2016) and other studies evaluating LPO levels in rats.] However, LPO was not elevated in the brain or lung of male Sprague-Dawley or Wistar rats after acute to subchronic exposure by intraperitoneal injection (Srivastava et al., 1983; Katoh et al., 1989; Coccini et al., 1997). Other studies reported LPO induction in the liver of male rats after styrene exposure via intraperitoneal injection in the absence of decreased concentrations of tissue GSH (Katoh et al., 1989; Hirasawa et al., 2007). [The Working Group noted the tissues in these studies were harvested more than 24 hours after the last dose, a time point at which tissue GSH concentrations would have normalized.] In male rat livers evaluated within 3 hours of the last

intraperitoneal dose, increased LPO concentrations were only observed after styrene exposures that decreased tissue GSH concentrations by more than 50% (Srivastava et al., 1983).

Consistent with this relationship between sufficient depletion of tissue GSH and increased measures of lipid oxidation, daily supplementation with NAC almost abrogated the ototoxicity observed after subchronic oral gavage exposure to styrene, as determined by histological evaluation of cochlear cells and hearing loss in Long-Evans rats (Yang et al., 2009). Hepatotoxicity induced by acute exposure could not be abrogated by NAC pre-treatment in C57BL/6 mice (Morgan et al., 1997), although GSH depletion induced by buthionine sulfoximine (BSO) greatly enhanced hepatotoxicity after a single oral dose in dYY mice (Mizutani et al., 1994) or intraperitoneal injection in non-Swiss albino (NSA) mice, but elicited a protective effect on pneumotoxicity (Gadberry et al., 1996).

Dose-responsive decreases in total GSH concentrations were also observed in the livers of mice repeatedly exposed to styrene via inhalation; the degree of GSH depletion was correlated with concentrations of styrene-7,8-oxide in blood, and differences with sex (greater correlation for females than males) and strain sensitivity (in order of greatest correlation, B6C3F$_1$ ≥ DBA/2 > Swiss) have been reported (Morgan et al., 1993, 1995). GSH, GSSG, and/or total GSH concentrations were decreased in the lungs of female CD-1 mice after inhalation exposure (Gamer et al., 2004), as well as in the lungs, livers, and plasma of male CD-1 and NSA mice after acute exposure via intraperitoneal injection, with a time-course describing depletion, recovery, and overshoot in liver concentrations similar to that described at the beginning of this section in rats (Turner et al., 2005; Carlson et al., 2006; Carlson, 2010b). [The Working Group noted that the concentrations of GSH and GSSG followed a similar time-course of decrease and resurgence in rats and mice, indicating that this effect is primarily due to conjugation to styrene or metabolites, and not oxidation of GSH to GSSG.] Acute exposure to 4-vinylphenol by intraperitoneal injection also decreased GSH or total GSH concentrations in the liver and lung of male or female CD-1 mice, albeit to a lesser extent than styrene, and was followed by a more rapid recovery (Kaufmann et al., 2005; Turner et al., 2005). In club (Clara) cells isolated from male CD-1 mice exposed via intraperitoneal injection, 4-vinylphenol increased total GSH concentrations after 3 hours (unlike styrene), which rebounded in a manner similar to styrene after 12 hours (Harvilchuck & Carlson, 2006).

LPO levels fluctuated in the lungs of female CD-1 mice exposed via inhalation for up to 4 weeks (Gamer et al., 2004), and increased transiently in the livers but not lungs of male CD-1 mice exposed to styrene via intraperitoneal injection, but no effects were observed in either tissue after exposure to 4-vinylphenol (Carlson et al., 2006). Although production of ROS in lung homogenates of female CD-1 mice did not clearly increase after inhalation exposure for up to 4 weeks (Gamer et al., 2004), ROS concentrations increased in club cells isolated from male CD-1 mice exposed to styrene via intraperitoneal injection, but not for 4-vinylphenol exposure (Harvilchuck et al., 2009). Lung activity of cellular antioxidant enzymes such as SOD were generally unaffected (Gamer et al., 2004; Harvilchuck et al., 2009), although lung catalase activity decreased after 4 weeks of inhalation exposure (Gamer et al., 2004). Lung mRNA and concentrations of club cell secretory protein associated with inflammatory lung disease (CC10 or uteroglobin) fluctuated over time, although concentrations of another club cell product (surfactant protein A) were unaffected (Harvilchuck et al., 2008).

(ii) Styrene-7,8-oxide: in vivo

As for styrene, styrene-7,8-oxide (racemic mixture) given by repeated intraperitoneal injection rapidly decreased levels of both GSH and GSSG in male Wistar rat liver. GSH and GSSG remained depleted for up to 12 hours, recovered to overshoot control levels after 24 hours, and finally decreased back to control levels after 40 hours (Katoh et al., 1989). Styrene-7,8-oxide induced a greater effect than styrene on liver GSH and GSSG, in addition to depleting brain GSH (Katoh et al., 1989), and dramatically decreased GSH concentrations in the lungs and livers of male Sprague-Dawley rats when evaluated within a few hours of exposure via intraperitoneal injection (Coccini et al., 1997). LPO induction has been reported in the absence of decreased tissue GSH concentrations in the liver of male Wistar rats after subchronic exposure to styrene-7,8-oxide via intraperitoneal injection (Katoh et al., 1989). [The Working Group noted that these tissues were harvested more than 24 hours after the last dose, when tissue GSH concentrations would have normalized (see discussion for styrene in Section 4.2.6(c)(i) above).] No change in LPO levels were observed in the brain.

As summarized in the National Research Council review (National Research Council, 2014), racemic styrene-7,8-oxide and *R*-styrene-7,8-oxide decreased GSH or total GSH concentrations in the liver and lung of male and female CD-1 mice to a greater extent than styrene after acute exposure via intraperitoneal injection, but followed a more abbreviated time-course (i.e. recovery by 6–12 hours; Turner et al., 2005; Carlson et al., 2006). *R*-styrene-7,8-oxide but not *S*-styrene-7,8-oxide depleted total GSH from the bronchioloalveolar lavage fluid (BALF) and plasma in male NSA mice (Carlson, 2010b). Comparable results were reported in club cells isolated from CD-1 mice similarly exposed, although *S*-styrene-7,8-oxide treatment induced a greater recovery in total GSH concentrations after 12 hours compared with *R*-styrene-7,8-oxide (Harvilchuck & Carlson, 2006). No changes in LPO levels were observed in the livers or lungs of male CD-1 mice acutely exposed to *R*-styrene-7,8-oxide or *S*-styrene-7,8-oxide via intraperitoneal injection (Carlson et al., 2006). ROS production increased in club cells isolated from male CD-1 mice exposed to *R*-styrene-7,8-oxide, *S*-styrene-7,8-oxide, and racemic styrene-7,8-oxide via intraperitoneal injection when isolated within 3 hours of treatment (but not at later time-points), although SOD activity was only induced by *R*-styrene-7,8-oxide (Harvilchuck et al., 2009). Lung mRNA and concentrations of club cell secretory protein associated with inflammatory lung disease (CC10, or uteroglobin) fluctuated in a time-dependent manner after exposure to *R*-styrene-7,8-oxide and racemic styrene-7,8-oxide, but not *S*-styrene-7,8-oxide, although levels of surfactant protein A were unaffected by any isomer (Harvilchuck et al., 2008).

Although pre-treatment of male NSA mice with either NAC or GSH was unable to protect against most of the lung toxicity resulting from acute exposure to *R*-styrene-7,8-oxide via intraperitoneal injection, liver toxicity was significantly attenuated (Meszka-Jordan et al., 2009). BSO-induced GSH depletion elicited a protective effect on pneumotoxicity after a single intraperitoneal injection of styrene-7,8-oxide without any induction of hepatotoxicity in NSA mice (Gadberry et al., 1996); however, BSO pre-treatment potentiated hepatotoxicity after a single oral dose of styrene-7,8-oxide dose in dYY mice (Mizutani et al., 1994).

(iii) Styrene: in vitro

LPO decreased when exogenous GSH was added to rat liver homogenates treated with styrene (Srivastava et al., 1983). Increased production of ROS was observed in primary mouse club cells after treatment with styrene but not after treatment with 4-vinylphenol (Harvilchuck et

al., 2009), despite the greater depletion of total GSH after 4-vinylphenol exposure compared with styrene exposure (Harvilchuck & Carlson, 2006).

(iv) Styrene-7,8-oxide: in vitro

Murine embryonic fibroblasts (MEFs) from *Nrf2*$^{(-/-)}$ mice, which contained 20% of the GSH content and lower Gclc, Gclm, and GST subunit protein levels compared with MEFs from *Nrf2*$^{(+/+)}$ mice, were more sensitive to cytotoxicity induced by styrene-7,8-oxide compared with MEFs from *Nrf2*$^{(+/+)}$ mice (Higgins & Hayes, 2011). Furthermore, *Nrf2*$^{(-/-)}$ MEFs were not protected by pre-treatment with sulforaphane, which induced *Gcl*, *Gst*, and *Nqo1* mRNA expression, increased cellular GSH levels by more than 50% in *Nrf2*$^{(+/+)}$ MEFs, and attenuated cytotoxicity induced by styrene-7,8-oxide (Higgins & Hayes, 2011). As observed with styrene, increased levels of ROS were reported in primary mouse club cells after treatment with several styrene-7,8-oxide isoforms (*R*-, *S*-, and racemic styrene-7,8-oxide; Harvilchuck et al., 2009), although *S*-styrene-7,8-oxide was the least effective in decreasing total GSH (Harvilchuck & Carlson, 2006).

4.2.7 Immunosuppression

(a) Exposed humans

No studies on functional changes in the immune system of exposed humans were available to the Working Group.

Multiple studies of workers exposed to styrene have reported changes in peripheral blood leukocytes. [The Working Group noted that different panels of markers were evaluated across studies; three showed an increase in peripheral blood monocytes, but a fourth did not.] Hagmar et al. (1989) reported a 30% increase in the number of peripheral blood monocytes in 20 glass-reinforced plastics workers compared with controls. Increases in peripheral blood monocytes were observed in two other studies: a study of 221 glass-reinforced plastics workers by Stengel et al. (1990), in which the increase remained significant after adjusting for age, sex, tobacco use, and alcohol consumption; and a study of glass-reinforced plastics workers in a hand-lamination plant (Somorovská et al., 1999; Tulinská et al., 2000), in which the increase remained significant after adjusting for age, sex, and smoking status. In a study of 22 male glass-reinforced plastics workers and 27 healthy age-matched unexposed males, no difference in the percentage of peripheral blood CD14+ monocytes was observed; however, lower plasma levels of soluble human leukocyte antigen G (soluble HLA-G or sHLA-G), an anti-inflammatory substance secreted by CD14+ monocytes, and plasma interleukin (IL) 10, the primary inducer of sHLA-G production by monocytes, were present in workers exposed to styrene compared with unexposed controls (Rizzo et al., 2009). Reduced production of IL-10 and sHLA-G was also observed in peripheral blood monocytes isolated from workers exposed to styrene and stimulated with LPS compared with LPS-stimulated isolated monocytes from unexposed controls (Rizzo et al., 2009).

In the study by Mutti et al. (1992) of 32 glass-reinforced plastics workers exposed to styrene and 19 unexposed controls, a decrease in T helper (CD4+) lymphocytes and an increase in T CD8+ (suppressor or cytotoxic) lymphocytes were reported in workers exposed to styrene compared with controls, and a decreased CD4+:CD8+ ratio (0.92) was reported in workers exposed to higher concentrations of styrene (> 50 ppm; 8-hour TWA) compared with workers exposed to lower concentrations (1.37) and unexposed controls (1.43). In a group of 71 glass-reinforced plastics workers, decreases in total T (CD3+) lymphocytes, T helper (CD4+) lymphocytes, T (CD4+45+) cells with suppressor and/or inducer function, and the T helper (CD4+):T suppressor or cytotoxic (CD8+) lymphocyte ratio were observed compared with 65 blue-collar worker controls (Bergamaschi et al., 1995),

with dose-dependent decreases observed with increasing levels of urinary styrene metabolites.

Tulinská et al. (2000) observed a dose-dependent decrease in the percentage of large granular lymphocytes with increasing styrene exposure, measured as either blood styrene or exhaled styrene. In the study by Mutti et al. (1992), an increase in natural killer (NK) T-cells in workers exposed to styrene compared with controls, dose-dependent with increasing levels of urinary styrene metabolites, was observed. Similarly, Bergamaschi et al. (1995) observed a dose-dependent increase in NK T-cell phenotypes (CD56+, CD56+16+, CD56+16–) with increasing urinary styrene metabolites. Exposure to styrene was associated with reduced lytic activity of NK T-cells isolated from a subset of 14 workers compared with unexposed controls (Bergamaschi et al., 1995). Reduced NK cell lytic activity is a sign of immunosuppression.

An increase in B (CD19+) lymphocytes in workers exposed to styrene compared with controls was observed in the study by Bergamaschi et al. (1995), but not in the study by Mutti et al. (1992).

Bergamaschi et al. (1995) observed a dose-dependent increase in lymphocytes expressing the activation markers DR+ and CD25+ (the IL-2 receptor) with increasing urinary styrene metabolites in workers exposed to styrene. In the study of glass-reinforced plastics workers exposed to styrene in a hand-lamination plant, Somorovská et al. (1999) observed increased expression of several adhesion molecules, which are often associated with activation, on lymphocytes (CD54, CD49d, CD62L, CD18, and CD11b) and monocytes (CD54, CD49d, and CD11a) compared with controls. Further analyses of this study, focusing on workers exposed to higher concentrations of styrene, were reported by Tulinská et al. (2000) and Jahnová et al. (2002). Similar increases in adhesion molecule expression were evident in the lymphocytes (CD54, CD49d, CD62L, CD18, and CD11b), monocytes (CD54, CD49d, CD62L, CD18, CD11a, and CD11b), and granulocytes (CD54, CD49d, CD62L, CD11a, and CD11b) of the workers exposed to the highest concentrations (Jahnová et al., 2002). CD54, also known as intercellular adhesion molecule 1, is responsible for cell-to-cell contact and adhesion to endothelial cells and to fibrinogen, and is expressed at higher concentrations on activated cells. A decrease in soluble CD54 was observed in the workers exposed to the highest concentrations compared with controls. CD49d, or VLA-4, is a very late activation antigen that mediates the adhesion of lymphocytes, monocytes, and eosinophils, and is a ligand of vascular cell adhesion molecule-1 expressed on endothelial cells. The adhesion molecules CD62L and CD11b are known as L-selectin and the C3bi fragment of complement, respectively. Tulinská et al. (2000) found higher levels of both the C3 and the C4 components of complement, which are acute phase reactants involved in inflammatory responses, in workers exposed to styrene compared with controls. The levels of the C3 component were positively correlated with duration of styrene exposure. [The Working Group noted that expression of some of these activation markers is associated with immunosuppression or anti-inflammatory actions; others are associated with inflammation, and some have been associated with both immunosuppression and inflammation.]

As noted above (see Section 4.2.4(a)), in a small study of 18 glass-reinforced plastics workers and 6 unexposed controls, it was reported that the proliferation of cultured lymphocytes isolated from workers exposed to styrene was significantly reduced compared with controls (Watanabe et al., 1983). In the larger study by Somorovská et al. (1999), a dose-dependent decrease in the proliferative response of T lymphocytes stimulated by the mitogen concanavalin A was observed in cells isolated from workers exposed to styrene compared with those of unexposed controls.

Governa et al. (1994) reported that chemotaxis was impaired in polymorphonuclear leukocytes of workers exposed to styrene (n = 21), as measured ex vivo following a chemotactic stimulus, and that the chemotaxic indices of the workers improved after a 3-week period with no styrene exposure.

(b) Human cells in vitro

Governa et al. (1994) reported that in vitro styrene treatment of polymorphonuclear leukocytes isolated from healthy unexposed controls decreased chemotaxis in a dose-dependent manner following a chemotactic stimulus.

As noted in Section 4.2.4(b), styrene-7,8-oxide treatment of mitogen-stimulated lymphocytes cultured from healthy non-smokers decreased indices of cell proliferation (Laffon et al., 2001b).

(c) Experimental systems

(i) Styrene: in vivo

As reviewed in NRC (2014), styrene appears to suppress several components of innate immunity (e.g. decreased monocytic and NK cell activity) as well as stimulate some elements of adaptive immunity (e.g. enhancing cytokine production and delayed-type hypersensitivity; see Section 4.2.8(c)). Male UF rats subchronically exposed to styrene via oral gavage failed to effectively resolve subsequent infection by a hookworm parasite compared with controls who successfully resolved the infection, providing evidence of functional immunosuppression (Dogra et al., 1992). In male Sprague-Dawley rats, subchronic exposure via inhalation was associated with effects on bone marrow progenitor cells, including an accumulation of immature erythroblasts, along with decreases in more mature erythroblasts, promyelocytes, and myelocytes (reviewed in Nano et al., 2000; NTP, 2008). Alterations to bone marrow erythropoietic cell populations were also observed after repeated exposure via intraperitoneal injection (Nano et al., 2000). After subchronic inhalation exposure, peripheral blood neutrophil populations appeared immature and diminished in number, coinciding with lymphocyte numbers, before increasing after 3 weeks of recovery (Nano et al., 2000). In another study, acute exposure by inhalation decreased total leukocyte counts (Brondeau et al., 1990), consistent with transient leukocytopenia (NRC, 2014).

As for rats, the immune system of male Swiss mice was less effective in responding to infection; increased mortality from both viral and malarial parasite infection after subchronic exposure to styrene via oral gavage (Dogra et al., 1992), at doses associated with decreased spleen cellularity, was reported (reviewed in Dogra et al., 1989; NRC, 2014). Of note, peritoneal exudate macrophage cellular attachment (rosette formation) and phagocytosis was inhibited (Dogra et al., 1989). Subchronic inhalation exposure has also been reported to selectively suppress bone marrow haematopoiesis in female C57BL/6 and DBA/2 F1 hybrids; both early and late bone marrow erythroid progenitor populations decreased in a dose-responsive manner and, although pluripotent stem cell populations were unaffected, blood lymphocyte numbers also decreased (Seidel et al., 1990).

(ii) Styrene-7,8-oxide: in vivo

No in vivo data in experimental systems were available to the Working Group.

(iii) Styrene: in vitro

Inhibition of NK cell-mediated destruction of allogenic tumour cells was observed at 500 µM or more (Grayson & Gill, 1986). A few studies evaluated the effect of direct styrene addition to murine splenocyte suspensions ex vivo; no significant cytotoxicity, proliferation, or impact on phytomitogen-induced blast formation at doses of up to 1000 µM for 1–3 hours, or 250 µM for up to a few days, have been reported (Sharma

et al., 1981; Grayson & Gill, 1986; Poirier et al., 2002).

(iv) Styrene-7,8-oxide: in vitro

Treatment with styrene-7,8-oxide inhibited NK cell activity at lower doses than with styrene (e.g. ≥ 200 μM) with no effect on cytotoxic T lymphocyte activity, although the inhibition was transient and was abrogated by the addition of exogenous GSH (reviewed in Grayson & Gill, 1986; NRC, 2014). However, low concentrations of styrene-7,8-oxide (0.05 μM) decreased the production of type-I interferons by 90% in MEFs after inoculation with Newcastle disease virus (Barnes et al., 1981), consistent with studies reporting decreased clearing responses in rodent infection models after in vivo exposure to styrene. Low concentrations of styrene-7,8-oxide have also been reported to inhibit phytomitogen-induced blast formation in both rat (Snyder & Valle, 1991) and mouse splenocytes (Tomar et al., 1991), although another study reported similar inhibition only at high concentrations (500 μM; Grayson & Gill, 1986). Of note, two studies reported the stimulation of splenocyte blast formation at low micromolar concentrations (Sharma et al., 1981; Tomar et al., 1991), suggesting a biphasic response to treatment with styrene-7,8-oxide.

4.2.8 Chronic inflammation

(a) Exposed humans

As described in Section 4.2.7 on immunosuppression, studies of workers exposed to styrene have reported changes in immune cells that are consistent with a proinflammatory response, such as changes in the balance of peripheral blood leukocyte subsets (Hagmar et al., 1989; Stengel et al., 1990; Mutti et al., 1992; Bergamaschi et al., 1995; Somorovská et al., 1999; Tulinská et al., 2000), increased expression of activation markers on lymphocytes (Bergamaschi et al., 1995; Somorovská et al., 1999; Tulinská et al., 2000; Jahnová et al., 2002), lower serum levels of anti-inflammatory molecules such as soluble HLA-G and IL-10, and lower production of soluble HLA-G and IL-10 in LPS-stimulated monocytes isolated from workers exposed to styrene (Rizzo et al., 2009).

(b) Human cells in vitro

(i) Styrene

Increases in the release of the proinflammatory chemotactic monocyte chemoattractant protein 1, which activates monocytes, lymphocytes, mast cells, eosinophils, and basophils, and the proinflammatory molecules IL-6 and IL-8, were observed in human A549 lung bronchioloalveolar epithelial carcinoma cells exposed to non-cytotoxic concentrations of styrene vapour (Fischäder et al., 2008). As described in Section 4.2.6 on oxidative stress, other studies conducted in this in vitro lung cell model suggest that styrene is able to induce inflammation in the lung airways though a mechanism that involves the generation of ROS, oxidative stress, and activation of the NF-κB pathway (Röder-Stolinski et al., 2008; Mörbt et al., 2009; Mögel et al., 2011). Findings supportive of the inflammatory effect of styrene in A549 cells include increased phosphorylation of NF-κB associated with increased expression of monocyte chemoattractant protein 1 (Röder-Stolinski et al., 2008), upregulation of moesin and annexin A1 and downregulation of heat shock protein B1 (Mörbt et al., 2009), increased levels of COX-2 protein and activity, and increased release of PGE_2 and $PGF_{2\alpha}$ (Mögel et al., 2011).

(ii) Styrene-7,8-oxide

Treatment of polymorphonuclear leukocytes isolated from six individuals with either the *R*- or *S*-enantiomers of styrene-7,8-oxide or the racemic mixture stimulated the release of the TH1 cytokines interferon-γ (IFN-γ) and IL-12, which can lead to increased inflammation (Merker et al., 2006). Variability in the response

to styrene-7,8-oxide was observed between the six individuals.

(c) Experimental systems

(i) Styrene

As discussed in NRC (2014), styrene may affect the polarization or recruitment of leukocytes and thereby stimulate some elements of the adaptive immune system, including allergic sensitization and type-IV hypersensitivity. Subchronic exposure via inhalation increased total lung protein concentrations associated with leukocytic infiltration and airway obturation in guinea-pigs (Petrova et al., 1992). Exposure by subchronic oral gavage induced moderate parenchymal congestion and scattered islet degeneration in the pancreas in guinea-pigs, associated with a dose-responsive decrease in serum insulin levels, as well as moderate pancreatic inflammation in male albino mice; responses in male albino rats were limited (reviewed in Khanna et al., 1994; NTP, 2008). In an assay designed to evaluate acute inflammatory responses in the dermis of male Wistar rats after dermal exposure to a styrene solution (unoccluded), mast cell degranulation and microvascular leakage were increased, and determined to be partially operating via a neurogenic (e.g. tachykinin NK1 receptor) mechanism (Futamura et al., 2009). Severe liver degeneration and/or hepatocellular necrosis was observed in female B6C3F$_1$ mice after subchronic exposure to styrene via inhalation; although this hepatocellular injury resolved within 10 days of exposure, residual chronic inflammation remained. Liver pathology and residual inflammation were largely absent in male B6C3F$_1$ mice and in both sexes of Swiss mice (Morgan et al., 1995), consistent with liver GSH depletion sensitive to mouse sex and strain (described in Section 4.2.6(c)(i) above).

Styrene also exacerbated ovalbumin-induced allergic asthma in female BALB/c mice after acute inhalation exposure, augmenting BALF levels of the TH2 cytokines IL-4, IL-5, and IL-13, and increasing both ovalbumin-specific and total serum IgE levels, as well as BALF eosinophilia, lung inflammation, and goblet cell hyperplasia (reviewed in Ban et al., 2006; NRC, 2014). A modifying (or adjuvant) effect on the immune response was also observed on the plaque-forming cell (PFC) response of female BALB/c mouse lung-associated lymph node (LALN) cells and splenocytes ex vivo, after acute styrene exposure via inhalation and sensitization to sheep red blood cells in vivo. A dose-dependent increase in IFN-γ production was observed in splenocytes without any effect on PFC response, whereas LALN lymphocyte IFN-γ production followed an inverse dose–response (i.e. highest IFN-γ production at exposure to the lowest concentrations of styrene), and the LALN PFC response increased after exposure to styrene at the highest concentration (300 ppm; Ban et al., 2003). Together, this suggests that styrene exposure may differentially impact antigen-presenting cell and lymphocyte interactions at the portal of entry (LANL) versus systemically (spleen) (NRC, 2014). In male Swiss mice acutely exposed to styrene by oral gavage, the splenocyte PFC response was inhibited in a dose-responsive manner and the total serum Ig titre decreased (Dogra et al., 1989). Low doses of styrene stimulated male CD-1 or Swiss mouse basal splenocyte mitogenesis ex vivo after acute to subchronic exposure via oral gavage in vivo, and also increased the proliferation induced by a variety of phytomitogens; however, the highest dose evaluated (50 mg/kg per day) appeared to have a more variable effect, consistent with the inhibition of the PFC response noted above (reviewed in Sharma et al., 1981; Dogra et al., 1989; NRC, 2014). The severity of type-IV hypersensitivity was also enhanced, with a diffuse and marked infiltration of mononuclear cells, suggesting that cellular immunity was stimulated along with lymphocyte proliferation (Dogra et al., 1989).

No in vitro data in experimental systems on styrene were available to the Working Group.

(ii) Styrene-7,8-oxide

No in vivo or in vitro data in experimental systems were available to the Working Group.

4.3 Other adverse effects

4.3.1 Styrene

Respiratory effects in humans resulting from occupational exposure to styrene at concentrations of more than 20 ppm include chronic bronchitis, asthma, and pneumonia, and are associated with alterations in hepatic clearance of bilirubin and in serum alanine and aspartate transaminase activities, suggesting altered liver function in humans (reviewed in NTP, 2008). The effects of acute exposure in both humans and experimental systems include irritation of the eyes, skin, and respiratory tract; longer exposures in experimental systems are also associated with toxicity in the liver, kidney, and pancreas (NTP, 2008). Effects on the haematopoietic system in workers exposed to styrene have also been described (NTP, 2008), including an increase in mean corpuscular volume and a decrease in mean corpuscular haemoglobin concentrations, associated with increasing levels of urinary styrene metabolites (Stengel et al., 1990). Although neurological effects in humans have been reported at inhalation exposures of 100 ppm or more, specific effects on memory, colour vision, reaction time, and postural stability have consistently been reported as a result of long-term occupational exposures as low as 20 ppm, although the association between occupational styrene exposure and ototoxicity is still uncertain (for further discussion, see Lawton et al. (2006) and NTP (2008)). Notably, hearing loss and impaired learning have also been observed in rats (Hoet & Lison, 2008; Yang et al., 2009).

No studies have evaluated the long-term effects of styrene exposure on children, despite the potential for exposure during the early life stage via breast milk (ATSDR, 2010).

Pneumotoxicity in experimental systems was commonly evaluated by measuring BALF cellularity, protein levels, and lactate dehydrogenase activity, with histological tissue evaluation reported less frequently. In mice, pneumotoxicity involving the upper and lower respiratory tracts was reported after short-term to subchronic exposures via inhalation, oral gavage, and/or intraperitoneal injection at 50 ppm or more, with the club cell as the main site of bioactivation, toxicity, and proliferation in the lung (reviewed in NTP, 2008). Nasal toxicity (extending down to the trachea) has also been consistently observed in rats after subchronic exposures via inhalation (Cruzan et al., 1997; reviewed in NTP, 2008), although reports of diffuse pneumotoxicity involving the tracheal, bronchiolar, and/or alveolar epithelium have been less consistent (Coccini et al., 1997; Green et al., 2001b).

Hepatotoxicity in experimental systems was commonly evaluated by measuring serum sorbitol dehydrogenase activity, with histological tissue evaluation reported less frequently. In both mice and rats, short-term to subchronic exposure was also associated with hepatotoxicity including degeneration, hepatocellular necrosis, and steatosis, although liver toxicity was observed less frequently after subchronic or longer exposures (Mahler et al., 1999; reviewed in NTP, 2008; ATSDR, 2010). Acute inhalation or intraperitoneal injection of styrene induced hepatotoxicity preceding pneumotoxicity in NSA mice (Gadberry et al., 1996), and CD-1 mice were more resistant to these effects than NSA or C57BL/6 mice (Carlson, 1997b; Sumner et al., 1997). Hepatotoxicity was decreased in *Cyp2e1*$^{(-/-)}$ or *Cyp2f2*$^{(-/-)}$ mice exposed via a single intraperitoneal injection and, although pneumotoxicity was similar between the wild-type and knockout mice (Carlson, 2004), pneumotoxicity

was not observed in *Cyp2f2*$^{(-/-)}$ mice (Carlson, 2012). Of note, 4-vinylphenol causes pneumotoxicity in mice expressing *Cyp2f2* and in *Cyp2f2*$^{(-/-)}$ knockout mice expressing human *CYP2F1*/*CYP2B6*/*CYP2A13*, induces hepatotoxicity in both mice and rats, and has been reported as a more potent toxicant than either styrene or styrene-7,8-oxide in some studies (reviewed in NTP, 2008; Cruzan et al., 2013) or similarly potent in others (Carlson, 2011).

4.3.2 Styrene-7,8-oxide

Although fewer data on exposure to styrene-7,8-oxide are available, the effects reported are qualitatively similar to those observed after exposure to styrene. Chronic oral exposure via gavage increased the incidence of basal cell hyperplasia and/or hyperkeratosis in the forestomach of both sexes of mice and rats (Lijinsky, 1986; Conti et al., 1988).

Pneumotoxicity, involving both the upper and lower respiratory tract, as well as hepatotoxicity, has been observed in mice after acute exposure (Carlson, 2011). Although the *R*-isomer was observed to be more potent than the *S*-isomer in inducing hepatotoxicity, the *R*- and *S*-enantiomers generally elicited comparable pneumotoxicity in mice expressing *Cyp2f2* (reviewed in Gadberry et al., 1996; NTP, 2008; Cruzan et al., 2012). Hepatotoxicity and pneumotoxicity in C57BL/6 mice exposed to styrene-7,8-oxide were similar to that observed in *Cyp2e1*$^{(-/-)}$ or *Cyp2f2*$^{(-/-)}$ mice as described in the previous section for exposure to styrene (Carlson, 2004, 2012).

4.4 Data relevant to comparisons across agents and end-points

4.4.1 High-content gene expression studies

(a) Humans

No data in exposed humans were available to the Working Group.

In human TK6 lymphoblastoid cells in vitro, styrene (5 mM) in the presence of S9 and styrene-7,8-oxide (0.5 mM) without S9 resulted in 297 differentially expressed genes (DEGs) in common (no significant effects were observed at lower doses), involving gene ontology categories describing purine and pyrimidine transport, and protein complex assembly (Godderis et al., 2012). The addition of S9 fraction alone resulted in 885 DEGs (Godderis et al., 2012). The styrene concentrations investigated (≤ 5.0 mM) did not decrease the viability of human TK6 cells (Godderis et al., 2012), although 0.8 mM styrene was previously reported to induce a small but significant increase in necrotic cell death in human primary cord blood mononucleated cells (Diodovich et al., 2004; NTP, 2008).

An increase in the expression ratio of Bcl-2 family members (e.g. $BclX_{S/L}$) to Bax, as well as fluctuations in c-Myc, c-Fos, and/or c-Jun expression levels, was observed in human primary cord blood mononucleated cells after exposure to styrene at 0.8 mM (Diodovich et al., 2004), and in human HepG2 liver carcinoma cells after exposure to styrene-7,8-oxide at 0.2 mM, but not to styrene at 1 mM (Diodovich et al., 2006). Interestingly, TGFβ2 and/or TGFβR3 concentrations increased after exposure to styrene and styrene-7,8-oxide in HepG2 cells, but decreased in primary human hepatocytes. Of note, neither styrene nor styrene-7,8-oxide induced CYP1A2 or CYP2E1 expression in HepG2 cells (Diodovich et al., 2006).

(b) Experimental systems

(i) Styrene

Andersen et al. (2017) conducted gene expression studies on mice previously reported by Cruzan et al. (2017), exposed via inhalation to styrene for up to 26 weeks. After 1 day of exposure, DEGs were identified using whole-genome gene expression profiling in the lungs of male C57BL/6 (parental strain) mice exposed to styrene at 5 ppm or more; up to 155 DEGs were reported in *Cyp2f2*$^{(-/-)}$ mice or in *Cyp2f2*$^{(-/-)}$ mice containing a human *CYP2F1*/*CYP2A13*/*CYP2B6* transgene, consistent with no effect on lung histopathology in the knockout and transgenic mice. After 1–5 days of exposure, pathways induced in the lungs of the parental strain mice included cell cycle and mitotic regulation, DNA repair including the ATM/ATR pathway, circadian gene expression, and cholesterol and fatty acid biosynthesis; TGFβ signalling and various cytokine and immune response pathways were induced only after 1 day, although insulin and insulin-like growth factor receptors were downregulated. Few significantly affected DEGs persisted at 4 weeks, and no differences were seen at 26 weeks. As noted above in Section 4.2.4, proliferation (determined by Ki-67 and/or BrdU labelling indices) was transiently increased in the bronchiolar epithelium, whereas hyperplasia remained elevated in the terminal bronchioles after 26 weeks of exposure. Using information-dependent enrichment analysis rank-ordering (a less restrictive analysis than the combination of magnitude of effect and statistical false discovery rate, used to identify DEGs) after 4 weeks (but not 26 weeks), expression of cellular energetic pathways and circadian genes increased, although decreases were observed in peroxisome proliferator-activated receptor α gene expression, immune system, and heat stress pathways. Various transcription factors were up- or downregulated in the parental strain mice, and some dose–response trends in transcription factor expression were also observed in the knockout and transgenic mice. Andersen et al. (2017) reported 50–1000% more DEGs after the 1-day exposures performed in 2013 compared with those performed under similar exposure conditions in 2016; furthermore, the authors observed unusually large numbers of genes with sufficient magnitude of effect that failed to satisfy their statistical criteria. [The Working Group noted the high variability in gene expression levels across groups of similarly exposed mice. The Working Group further noted the questionable relevance of the study to the carcinogenic process because of the absence of styrene-induced lung tumorigenesis in parental C57BL/6 mice (Cruzan et al., 2017), a strain resistant to chemically induced lung tumorigenesis (Malkinson, 1989).]

In Sprague-Dawley rats orally exposed to styrene for 5 days, of the 120 genes evaluated in testes only 5 genes, including peroxiredoxin-1, were upregulated, although clusterin expression was decreased in a dose- and tissue-specific manner; clusterin expression was unchanged in heart, liver, lung, or kidneys (Han et al., 2007).

No data from in vitro studies in experimental systems were available to the Working Group.

(ii) Styrene-7,8-oxide

No data from studies on styrene-7,8-oxide exposure in experimental systems were available to the Working Group.

4.4.2 High-throughput screening studies

High-throughput screening data from the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programs of United States science agencies (Kavlock et al., 2012; Tice et al., 2013) were considered in the assessment of the chemicals in Volume 121, similarly to past *IARC Monographs*, as described by Chiu et al. (2018). To date, a systematic analysis of responses from over 9000 chemicals have been

evaluated as part of the ToxCast and Tox21 efforts, across a series of 1192 assay end-points measured using seven high-throughput assay technology platforms (Judson et al., 2016; EPA, 2017a). The resulting concentration–response models and bioactivity determinations have been publicly released via the Interactive Chemical Safety for Sustainability (iCSS) ToxCast Dashboard (EPA, 2017a). Summary matrix files, the ToxCast data analysis pipeline R package, and connected database (invitrodb_v2) are also available (EPA, 2017b; Filer et al., 2017). The ToxCast data analysis pipeline R package and associated database enables access to all of the concentration–response data, the underlying automated decision logic and methods, concentration–response model outputs, bioactivity determinations, and bioactivity caution flags (Filer et al., 2017). Although styrene was only evaluated in the Tox21 assays, styrene-7,8-oxide and quinoline were evaluated in both ToxCast and Tox21 assays. Among other styrene metabolites of interest, styrene glycol and 2-phenylethanol, but not 4-vinylphenol, were evaluated in Tox21 assays along with a limited subset of ToxCast assays.

One limitation of the high-throughput evaluation of these chemicals is that small-molecular-weight compounds (approximately < 150 Da), such as styrene, styrene-7,8-oxide, and quinoline, may have low affinity for biomolecular interactions because of limited free energy for binding (Hopkins et al., 2004). In vitro screening at the concentrations used in the ToxCast and Tox21 test batteries (generally ≤ 200 µM and ≤ 100 µM, respectively) may therefore be insufficient to detect molecular receptor-type interactions, which are commonly evaluated in these test systems. As a volatile organic compound, styrene has a fairly high vapour pressure (867 Pa at 25 °C; see Section 1) that could lead to the loss of sample during storage and/or testing and therefore failure to reach effective active concentrations. Other compounds considered in Volume 121 have lower vapour pressures (styrene-7,8-oxide, 40 Pa at 20 °C; quinolone, 11 Pa at 25 °C; see Section 1, Monograph 2). In addition, styrene may polymerize in solution, which would decrease the effective concentration of bioactive monomeric agent available; however, this can be prevented by the inclusion of a polymerization inhibitor (see Section 1). Solubility may limit the maximum testable dose and, of the Volume 121 compounds, quinoline is the most soluble in water (predicted value, 0.047 mol/L; EPA, 2017c), with styrene-7,8-oxide (0.025 mol/L) and styrene (0.0030 mol/L) less soluble (EPA, 2017c). In addition, compounds with a shorter half-life because of chemical or biological reactivity, such as styrene-7,8-oxide, may degrade substantially within the exposure window, increasing uncertainty when interpreting null or negative results. Finally, some of the in vitro assays either fully lacked (e.g. biochemical NovaScreen or NVS assays) or had uncharacterized xenobiotic metabolism capacity, which may have limited the evaluation of effects to those elicited by the compound itself.

The Tox21 and ToxCast in vitro assays were selected to cover a broad range of potential toxicity mechanisms and are not specifically focused on carcinogenesis; the Working Groups of *IARC Monographs* Volumes 112 and 113 therefore mapped the 821 assay end-points available at that time to the key characteristics of known human carcinogens (Smith et al., 2016), resulting in 265 assay end-points mapped to 6 out of the 10 key characteristics as described by Chiu et al. (2018). Considering only these 6 key characteristics, different assay sets were probed for each of the Volume 121 agents: the assay end-points evaluated and bioactivity determinations or "hit calls" relating to the 6 key characteristics for the compounds of interest are included as supplemental material to the present volume (Annex 1) and are summarized in Table 4.8. A brief description of the assay coverage pertaining specifically to all Volume 121 agents is provided below (for more discussion, see Chiu et al., 2018).

Table 4.8 Summary of evidence of key characteristics of carcinogens from high-throughput testing

Key characteristic	Styrene	Styrene-7,8-oxide	Styrene glycol	2-Phenylethanol	Quinoline
1. Is electrophilic or can be metabolically activated	0 out of 1 assay[a]	0 out of 11 assays	0 out of 2 assays	0 out of 2 assays	0 out of 11 assays
2. Is genotoxic	NA	NA	NA	NA	NA
3. Alters DNA repair or causes genomic instability	NA	NA	NA	NA	NA
4. Induces epigenetic alterations	NT	0 out of 10 assays	0 out of 4 assays	0 out of 4 assays	0 out of 10 assays
5. Induces oxidative stress	0 out of 3 assays	0 out of 16 assays	0 out of 7 assays	0 out of 7 assays	1[b] (0) out of 16 assays
6. Induces chronic inflammation	0 out of 1 assay	0 out of 45 assays	0 out of 2 assays	0 out of 2 assays	0 out of 45 assays
7. Is immunosuppressive	NA	NA	NA	NA	NA
8. Modulates receptor-mediated effects	1 out of 20 assays	4 out of 92 assays	0 out of 89 assays	1 out of 89 assays	1 out of 92 assays
9. Causes immortalization	NA	NA	NA	NA	NA
10. Alters cell proliferation/death or nutrient supply	1[b] (0) out of 19 assays	0 out of 62 assays	0 out of 25 assays	0 out of 25 assays	1[b] (0) out of 60 assays
Total number of assays mapped to key characteristics	44	236	129	129	234

NA, no assays in ToxCast and/or Tox21 were determined applicable to the evaluation of the indicated key characteristic; NT, not tested.

[a] Indicates the number of positive results out of the number of assays mapped to key characteristics of carcinogens, as described by Chiu et al. (2018).

[b] Indicates an active call in an assay (i.e. "hit") which was determined to be most likely a false-positive artefact upon review of the assay parameters and dose–response data by the Working Group [the number following in "()" reflects the true number of biological hits in the opinion of the Working Group].

[The Working Group noted that these assays are not considered to comprehensively cover the full spectrum of relevant biological activity for any of the key characteristics.]

1. *Is electrophilic or can be metabolically activated*: 10 cell-free CYP biochemical activity assays, and 1 assay end-point evaluating CYP19A1 activation in MCF-7 cells; no assay end-point for electrophilicity.
2. *Induces epigenetic alterations*: 11 primarily biochemical assays targeting histone deacetylases, sirtuins, and other enzymes modifying chromatin, as well as cellular transcription factor assays.
3. *Induces oxidative stress*: 17 cellular assays targeting nuclear factor-like 2 and/or the antioxidant responsive element, other stress-related transcription factors, and upregulation of matrix-metalloproteinases.
4. *Induces chronic inflammation*: 45 assays in co-cultured primary human cells evaluating expression of cellular adhesion molecules, cytokines, and NF-κB.
5. *Modulates receptor-mediated effects*: 92 nuclear receptor assays, for example for transactivation, receptor dimerization, and nuclear translocation, in cells or cell-free systems.
6. *Alters cell proliferation, cell death, or nutrient supply*: 65 assays evaluating various measures of cytotoxicity or proliferation in human cells and/or developing zebrafish larvae.

Brief summaries follow for each chemical evaluated in Volume 121, or identified metabolites evaluated in these technology platforms.

Styrene (Chem. Abstr. Serv. Reg. No. 100-42-5): Styrene was inactive in all but 2 of the 44 Tox21 assay end-points mapped to the key characteristics of carcinogens (styrene was not evaluated in the ToxCast assay battery). It displayed borderline activity in a single estrogen receptor (ER) α agonist assay (TOX21_ERa_LUC_BG1_Agonist) with a calculated concentration for half-maximal activity (AC50) of 149 μM; no other measures of ERα activity were evaluated. The only other detected activity was in a single cytotoxicity end-point (TOX21_P53_BLA_p2_viability), but all other 18 assays mapped to the same key characteristic were negative, including 4 others in similar assay conditions. [The Working Group considered this to be a false-positive response because activity was only detected at the lowest dose (0.001 μM), the calculated AC50 was orders of magnitude lower than this dose (i.e. 0.000 067 2 μM), and no bioactivity was reported in any other assay mapped to key characteristic 10.] The tested samples passed chemical quality control (QC) (purity, > 90%), although one sample had a caution indicating a lower-than-expected concentration (NIH, 2017).

Styrene-7,8-oxide (Chem. Abstr. Serv. Reg. No. 96-09-3): Styrene-7,8-oxide was inactive in all but 4 of the 236 ToxCast and Tox21 assay end-points mapped to the key characteristics of carcinogens. Styrene-7,8-oxide was active in an assay measuring aryl hydrocarbon receptor (AhR) activation in human cells (TOX21_AhR_LUC_Agonist) with an AC50 of 49.9 μM, and also induced transcription of PXR, a xenobiotic receptor that recognizes a diverse range of chemicals (ATG_PXRE_CIS_UP), with an AC50 of 33.2 μM, both mapped to key characteristic 8. However, styrene-7,8-oxide lacked bioactivity in a separate cell-based assay for AhR activity (ATG_Ahr_CIS_up) and in a biochemical receptor–ligand binding assay for PXR (NVS_NR_hPXR). Styrene-7,8-oxide also demonstrated activity in two other biochemical receptor–ligand binding assays, one for human progesterone (NVS_NR_hPR) and one for androgen receptor (AR) binding (NVS_NR_hAR), with AC50s of 3.44 μM and 8.36 μM, respectively; both also mapped to key characteristic 8. No activity was detected in cell-free assays with orthologous receptor isoforms (bovine progesterone receptor (PR), chimpanzee AR, or rat AR), or in numerous assays for AR

activity in human cells also mapped to key characteristic 8. Sample analysis was still under way. The QC grade was not determined; one sample had a caution indicating that the concentration may have been lower than expected, and another had a purity of 75–90% (NIH, 2017).

Styrene glycol (Chem. Abstr. Serv. Reg. No. 93-56-1): No bioactivity was found in the 129 tested Tox21 assays and subset of ToxCast assay end-points mapped to the key characteristics of carcinogens. The chemical QC information was not available for the Tox21 chemical library sample, as analysis was still under way (NIH, 2017).

2-Phenylethanol (Chem. Abstr. Serv. Reg. No. 60-12-8): 2-Phenylethanol was only active in 1 of the 129 tested Tox21 assays and subset of ToxCast assay end-points mapped to the key characteristics of carcinogens. A dose-responsive increase in the assay measuring hERβ-fragment protein-binding (OT_ER_ERbERb_1440) was observed, with an AC50 of 13.2 μM mapped to key characteristic 8. Although 2-phenylethanol lacked bioactivity in at least 10 other assays for ER binding or activity across various technology platforms, including both cell-based and biochemical receptor–ligand binding assays, the majority of these were selective for ERα versus ERβ. Although one sample had a purity of more than 90%, the chemical QC information was not available for the remaining three Tox21 chemical library samples as analysis was still under way (NIH, 2017).

Quinoline (Chem. Abstr. Serv. Reg. No. 91-22-5): Quinoline was inactive in all but 3 of the 234 ToxCast and Tox21 assay end-points mapped to the key characteristics of carcinogens. Quinoline was active in an assay measuring AhR activation (ATG_Ahr_CIS_up) with an AC50 of 42.8 μM mapped to key characteristic 8, but was not positive in a different cell-based assay for AhR activity from another technology platform (TOX21_AhR_LUC_Agonist). Activity was also detected in a cell-based assay end-point measuring changes in transcription of heat shock factor 1 (Tox21_HSE_BLA_agonist_ratio) with an AC50 of 75.6 μM and mapped to key characteristic 5, as well as an end-point following cellular adenosine triphosphate content as a measure of cytotoxicity (Tox21_VDR_BLA_Agonist_viability) with an AC50 of 67.8 μM and mapped to key characteristic 10. However, quinoline was not active in any other assay mapped to either key characteristic 5 or 10, despite significant assay coverage. [The Working Group considered it likely that these two responses were false positives because: (i) activity was only detected at the highest dose administered (~100 μM), which was qualitatively similar to that observed in the background assay performed (TOX21_HSE_BLA_agonist_ch2) for this assay platform; (ii) activity was quantitatively similar to that observed in the background assay, which had an AC50 of 72.9 μM; and (iii) there was an absence of activity in any other assay end-points mapped to key characteristics 5 or 10.] The chemical QC grade was not determined, but both samples had purities of more than 90% (NIH, 2017).

5. Summary of Data Reported

5.1 Exposure data

5.1.1 Styrene

Styrene is a colourless volatile liquid with an aromatic odour. It polymerizes easily in the presence of oxygen and oxidizes by air and light; in commercial styrene products a stabilizer, e.g. 4-*tert*-butylcatechol, may therefore be added. Styrene is a high production volume chemical, the production of which has increased in recent years (mainly in East Asia).

Styrene is primarily used as a monomer in the production of polystyrene polymers, including expandable polystyrene for packaging and building insulation, and copolymers, such