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

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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

as styrene–butadiene rubber for the production of fibreglass-reinforced plastic products such as boats, industrial containers, and wind turbine blades.

The primary route of exposure in humans is inhalation. In the general population, exposure to styrene at a low concentration is widespread primarily because of its occurrence in tobacco smoke. Other sources include indoor and outdoor air pollution, and migration from styrene-based food packaging.

Occupational exposure to styrene occurs in the manufacture of fibreglass-reinforced plastic products, and in the production of styrene, polystyrene, and styrene-based plastics and rubbers. Occupational exposures are typically much higher than those measured in the general population.

Reliable and sensitive methods are available to measure styrene in environmental media and styrene biomarkers in exposed humans. The concentrations of styrene measured in air and the concentrations of styrene and its biomarkers in urine and blood are strongly correlated.

5.1.2 Styrene-7,8-oxide

Styrene-7,8-oxide is a clear liquid with a sweet odour. It is reactive and polymerizes easily. Styrene-7,8-oxide is industrially produced in many countries as a mixture of two optical isomers (enantiomers) by chemical oxidation of styrene. Styrene-7,8-oxide is used in epoxy resins and for the production of phenethyl alcohol and styrene glycol, which are intermediates in the production of many chemicals. It is formed in workplace air by the oxidation of styrene.

There are few data on human exposure to styrene-7,8-oxide. Occupational exposure has been documented in the reinforced plastics industry, where styrene-7,8-oxide co-occurs with styrene at concentrations that are typically 3 orders of magnitude lower than those of styrene.

Styrene-7,8-oxide and its albumin and haemoglobin adducts have been detected in the blood of occupationally exposed workers and of the general population.

5.2 Cancer in humans

There was a considerable body of research relating to cancers in humans available to the Working Group, mainly from cohort studies in the reinforced plastics industry (the industry with the highest exposures), the synthetic rubber industry, and the styrene monomer and polymer production industry, as well as from a series of population-based case–control studies. The stronger and more consistent evidence for cancer was found for leukaemias and, to a lesser extent, lymphomas in the reinforced plastics industry cohort studies. Only a small number of studies had sufficient power to analyse lymphoma and leukaemia subtypes; in these few studies, the stronger evidence was for AML and T-cell lymphoma, with less consistent results found for other leukaemia and lymphoma subtypes. However, effect estimates were often small with low precision (i.e., wide confidence intervals), and many different analyses were undertaken using several different exposure metrics, meaning that chance findings could have occurred. Although there was a strong signal in one large study for sinonasal adenocarcinoma, a rare cancer, this was based on only a few cases, and chance and confounding could not be discounted. Based on the internal analyses in the cohort studies and the findings from the case–control studies, there was no convincing evidence for an association with cancer of the lung. There was no convincing or consistent evidence reported for any other solid tumours in humans. Overall, the epidemiological studies provide some credible evidence that exposure to styrene causes lymphohaematopoietic malignancies in humans, but confounding, bias, or chance cannot be ruled out.

5.3 Cancer in experimental animals

5.3.1 Styrene

There were nine studies of carcinogenicity of styrene in mice: one study by gavage in males and females, five studies by inhalation (one in males and females, and four in males only), two studies of transplacental exposure followed by oral exposure by gavage in male and female pups, and one study by intraperitoneal injection in females.

In the study by gavage in B6C3F$_1$ mice, styrene significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma or carcinoma (combined) of the lung in males, and there was a significant positive trend in the incidence of hepatocellular adenoma in females. In one study of transplacental exposure followed by gavage in O20 mice, styrene significantly increased the incidence of lung carcinoma in female pups, and of lung adenoma or carcinoma (combined) in male and female pups. The other study by transplacental exposure followed by gavage in C57BL mice yielded negative results. Exposure to styrene significantly increased the incidence (with a significant positive trend) of bronchioloalveolar adenoma, and of bronchioloalveolar adenoma or carcinoma (combined) in male and female CD-1 mice in one study by inhalation; another result of this study was that exposure to styrene significantly increased the incidence (with a significant positive trend) of bronchioloalveolar carcinoma in females. Exposure to styrene also significantly increased the incidence of bronchioloalveolar carcinoma in male CD-1 mice in another study by inhalation. Three studies by inhalation, including two in genetically modified C57BL/6 mice, and the study by intraperitoneal injection all yielded negative results. Overall, exposure to styrene increased the incidence of lung tumours in the B6C3F$_1$, O20, and CD-1 strains of mice.

There were nine studies of the carcinogenicity of styrene in male and/or female rats.

In one lifetime inhalation study in male and female Sprague-Dawley rats exposed to styrene for 1 year, there was a significant increase (with a significant positive trend) in the incidences of malignant tumours of the mammary gland and of benign or malignant tumours (combined) of the mammary gland in females. In a 2-year inhalation study in male and female Sprague-Dawley rats, there was a significant dose-dependent decrease in the incidence of mammary gland adenocarcinoma in females. There was no significant increase in the incidence of any tumour type in three studies by gavage in males or females, in one study by transplacental exposure followed by gavage in male and female pups, in one study by drinking-water, in one study by intraperitoneal injection, and in one study by subcutaneous injection in males and females.

5.3.2 Styrene-7,8-oxide

There were three studies of the carcinogenicity of styrene-7,8-oxide in mice: one study by gavage in males and females, and two studies by skin application. In the study by gavage, styrene-7,8-oxide significantly increased the incidences (with a significant positive trend) of squamous cell papilloma, squamous cell carcinoma, and squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach in males and females; exposure to styrene-7,8-oxide also significantly increased the incidence (with a significant positive trend) of hepatocellular adenoma or carcinoma (combined) in males. Both studies by skin application were inadequate for the evaluation.

There were three studies of the carcinogenicity of styrene-7,8-oxide in rats: two studies by gavage in males and females and one study by transplacental exposure followed by gavage in male and female pups. Exposure to styrene-7,8-oxide significantly increased the incidences

(with a significant positive trend) of squamous cell papilloma and squamous cell carcinoma of the forestomach in males and females in both studies by gavage, and of squamous cell papilloma or squamous cell carcinoma (combined) of the forestomach in males and females in one study by gavage; exposure to styrene-7,8-oxide also significantly increased the incidence (with a significant positive trend) of benign or malignant (combined) tumours of the mammary gland in males in one study by gavage. In the study by transplacental exposure followed by gavage in male and female pups, styrene-7,8-oxide caused a significantly increased incidence of forestomach papilloma in males and of forestomach carcinoma in males and females.

5.4 Mechanistic and other relevant data

In humans, styrene is absorbed after inhalation (the major route), skin contact, or ingestion, after which styrene is rapidly absorbed into the blood and has been shown to distribute to adipose tissue. In experimental animals, styrene is widely distributed to tissues. In both humans and experimental systems, styrene is metabolized mainly by CYP2E1, CYP2F, CYP2A13, and CYP2B to enantiomers of styrene-7,8-oxide, which are further metabolized by epoxide hydrolase to styrene glycol. Styrene, styrene-7,8-oxide, and styrene glycol have been measured in the blood of exposed humans. Approximately 60% of the excretion products formed from inhaled styrene come from styrene-7,8-oxide, the majority eliminated via urine as mandelic acid and phenylglyoxylic acid. The rates of metabolism of styrene to styrene-7,8-oxide were higher in microsomes from mouse lung compared with rat lung, and much higher compared with human lung. There are genetic polymorphisms in human cytochrome P450s, glutathione S-transferases,

aldehyde dehydrogenase, and epoxide hydrolase that modulate excretion levels of metabolites.

Regarding the key characteristics of carcinogens, there is *strong* evidence that styrene is metabolically activated in animals and in exposed humans to an electrophile, styrene-7,8-oxide. Styrene-7,8-oxide is electrophilic and reacts directly with DNA to form adducts mainly at $N7$-guanine, followed by the N^2 and O^6 positions of guanine, as well as sites in adenine, cytosine, and thymine. In several rodent studies, styrene exposure by inhalation or intraperitoneal injection resulted in styrene-7,8-oxide–DNA adducts found in several tissues (e.g. liver and lung) and in mouse urine. In various human cells in vitro, DNA adduct formation was demonstrated after exposure to styrene or styrene-7,8-oxide. Several studies detected DNA adducts derived from styrene-7,8-oxide in the peripheral blood cells of workers exposed to styrene, at levels significantly higher than in unexposed controls.

Styrene-7,8-oxide reacts directly with globin and albumin to form various amino acid adducts. Adducts with valine and cysteine in globin, and with cysteine in albumin, were detected in some studies of workers exposed to styrene or to styrene and styrene-7,8-oxide.

There is *strong* evidence that both styrene and styrene-7,8-oxide are genotoxic, and this mechanism can also operate in humans. Styrene-7,8-oxide–DNA adducts are found in the blood and urine of workers exposed to styrene, although results of studies on other aspects of genotoxicity are mixed. In workers exposed to styrene, the majority (but not all) of the several available studies showed increased levels of DNA damage as measured by the comet assay. However, results were negative in studies using the comet assay to assess oxidative damage to DNA, studies measuring 8-hydroxy-2′-deoxyguanosine in DNA were inconsistent, and, in the few studies on gene mutation, no clear relationship was found with occupational exposure to styrene. Of the more than 30 studies available on chromosomal

end-points in blood cells in exposed humans, six studies of adequate size and design reported positive effects with good concordance among different indicators (in some cases, < 100 ppm). Several other studies with design limitations (e.g. small size) also reported positive results. A number of studies of adequate size and design did not report changes in chromosomal end-points. The remaining studies were less informative as a result of their small sample size or confounding co-exposures.

In human cells in vitro, styrene and styrene-7,8-oxide were consistently genotoxic. Cytogenetic effects, analysed by sister-chromatid exchange, chromosomal aberration, and micronucleus formation, mainly in whole-blood lymphocyte cultures, were consistently positive. Styrene-7,8-oxide induced *HPRT* gene mutations in human lymphocytes in two studies. Overall, results were negative or equivocal for cytogenetic effects in rodents exposed to styrene or styrene-7,8-oxide, although positive results for DNA damage (e.g. comet assay) were obtained in multiple tissues in several studies. In various non-human experimental systems (non-human mammalian cells in vitro, *Drosophila melanogaster*, yeast, bacteria, and plants), styrene or styrene-7,8-oxide was consistently positive across a variety of end-points (DNA damage, gene mutation, chromosomal aberration, micronucleus formation, and sister-chromatid exchange).

Inconsistent results from several studies investigating the possible influence of occupational exposure to styrene on DNA repair, or on the expression levels of DNA repair genes, mean that the evidence is *weak* that styrene alters DNA repair. DNA repair was measured by the comet assay in lymphocytes from exposed workers and from control individuals challenged ex vivo.

There is *strong* evidence that both styrene and styrene-7,8-oxide alter cell proliferation. There were a few studies in humans, in each of which styrene reduced cell proliferation in cultured lymphocytes in vivo and in vitro.

Styrene-7,8-oxide induced cell proliferation in rat forestomach in two studies. In several studies, styrene induced cell proliferation in lung and liver in multiple strains of mice. In mouse lung, cell proliferation induced by styrene or styrene-7,8-oxide was dependent on the presence of CYP2F2. A mechanism has been proposed for the induction of mouse lung tumours that involves the metabolism of styrene to 4-vinyl-phenol by CYP2F2, cytotoxicity in club (Clara) cells, and regenerative epithelial proliferation in the terminal bronchioles. Cytotoxicity, lung cell proliferation, and bronchial hyperplasia were induced in CD-1 and C57BL/6 mouse strains; however, only the CD-1 mice developed lung tumours. Furthermore, lung cell proliferation did not persist beyond a short-term period, even with continuous exposure. Cytotoxicity, lung cell proliferation, bronchial hyperplasia, and lung tumour incidence were not increased in C57BL/6 *Cyp2f2*[(–/–)] mice, or in a humanized C57BL/6 strain, exposed to styrene. No in vivo metabolism data were available in the C57BL/6 mouse strains. The mechanistic events for mouse lung tumour induction by styrene in CD-1, B6C3F$_1$, and O20 mice are therefore not yet established.

There is *strong* evidence that styrene modulates receptor-mediated effects, and that these effects occur in humans, based on studies of increased serum prolactin. Exposure to styrene increased serum prolactin levels in four studies of workers in the reinforced plastics industry, including one study that made repeated measurements over the course of 2–3 years. For styrene-7,8-oxide, no data were available.

There is *moderate* evidence that styrene induces oxidative stress. No data other than on oxidative damage to DNA were available in exposed humans. In human cells in vitro, non-cytotoxic levels of styrene induced various measures of oxidative stress, including oxidation of lipids and proteins. Some responses were abrogated by *N*-acetylcysteine. In the lungs and livers of mice and rats, styrene increased lipid

peroxidation at concentrations that sufficiently depleted tissue glutathione levels, but results with *N*-acetylcysteine or buthionine sulfoximine pre-treatment were not supportive. For styrene-7,8-oxide, the available studies covered disparate end-points; the evidence that styrene-7,8-oxide induces oxidative stress is therefore *weak*.

There is *moderate* evidence that both styrene and styrene-7,8-oxide induce immunosuppression. Single studies of workers exposed to styrene reported the impairment of various measures of innate immune function ex vivo, in addition to the inhibition of lymphocyte proliferation. Several studies of workers exposed to styrene reported alterations in peripheral blood leukocyte populations. In several studies in rodents, each of which evaluated different end-points, subchronic exposure to styrene inhibited resolution of infection, and affected bone marrow progenitor cell populations, peripheral leukocyte populations, and/or splenic cellularity. For styrene-7,8-oxide, no in vivo data were available. In studies in human whole-blood cells conducted in vitro, and in mouse or rat lymphocytes, proliferation was inhibited. Murine natural killer cell lytic activity was decreased in a dose-responsive manner, and interferon response to viral infection was inhibited in murine embryonic fibroblasts.

There is *weak* evidence that styrene induces chronic inflammation. In multiple studies of workers exposed to styrene, alterations in immune cell populations consistent with pro-inflammatory responses were observed. In human lung carcinoma cells in vitro, non-cytotoxic concentrations of styrene induced a number of inflammatory responses. Inflammation was not consistently increased after long-term exposure in numerous studies in mice and rats. In several studies in mice, each of which evaluated different end-points, styrene stimulated different allergic or adaptive immune responses after short-term exposure. Data are sparse for styrene-7,8-oxide;

the evidence that styrene-7,8-oxide induces chronic inflammation is *weak*.

Respiratory disease, haematological effects, altered liver function, and neurotoxicity have been reported in exposed workers. In rats and mice, styrene given by various exposure routes induced respiratory tract toxicity and hepatotoxicity. Although fewer data are available for styrene-7,8-oxide, the effects reported are similar; in addition, forestomach irritation was reported in rats after chronic oral exposure.

Results were largely null or negative in the Toxicity Forecaster and Toxicity Testing in the 21st Century high-throughput testing programmes of the United States government, and high-content gene expression studies were uninformative.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of styrene. Positive associations have been observed between exposure to styrene and lymphohaematopoietic malignancies.

There is *inadequate evidence* in humans for the carcinogenicity of styrene-7,8-oxide.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of styrene.

There is *sufficient evidence* in experimental animals for the carcinogenicity of styrene-7,8-oxide.

6.3 Overall evaluation

Styrene is *probably carcinogenic to humans (Group 2A)*.