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Styrene

Evaluation of the mutagenicity and carcinogenicity

To: the State Secretary for Participation and Integration
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Health Council of the Netherlands

contents

samenvatting

Werknemers kunnen tijdens het werk worden blootgesteld aan stoffen die mogelijk schadelijk zijn voor hun gezondheid. Op verzoek van het ministerie van Sociale Zaken en Werkgelegenheid (SZW) heeft de Gezondheidsraad beoordeeld of styreen mutagene en/of kanker-verwekkende eigenschappen heeft. Op basis daarvan heeft de commissie een classificatievoorstel opgesteld.

Dit advies is tot stand gekomen in de subcommissie Classificatie van carcinogene stoffen, van de commissie Gezondheid en beroepsmatige blootstelling (GBBS). Op www.gezondheidsraad.nl staat informatie over de taken van deze vaste commissie van de Gezondheidsraad.
De samenstelling van de subcommissie is te vinden achter in dit advies.

Over styreen

Styreen wordt gebruikt om polystyreen en synthetisch rubber te maken. Styreen zit in verpakkingsmateriaal en in isolatiemateriaal voor gebouwen, en in met glasvezel versterkte kunststofproducten zoals boten, containers en windmolenwieken. Beroepsmatige blootstelling aan styreen vindt plaats bij de productie van styreen en op styreen gebaseerde materialen. Blootstelling vindt voornamelijk plaats door inademing. Bij de beoordeling van styreen is ook de stof styreen-7,8-oxide meegenomen. Dit is een stof die in het lichaam ontstaat na blootstelling aan styreen.

Classificeren naar bewijskracht

Op basis van de wetenschappelijke literatuur beoordeelt de commissie of er aanwijzingen zijn dat een stof onze genen kan beschadigen (mutagene stoffen) en kankerverwekkend (carcinogeen) kan zijn voor mensen.
Als dat zo is, stelt de commissie voor om de stof in te delen in gevaren-categorieën, één die aangeeft hoe groot de bewijskracht is dat de stof mutageen is in de geslachtscellen (dat wil zeggen: erfelijk overdraagbare mutaties kan veroorzaken) en één die aangeeft hoe groot de bewijskracht is dat de stof kanker kan veroorzaken. De categorieën zijn afgeleid van EU-verordening (EG) 1272/2008.

Een classificatievoorstel zegt iets over de bewijskracht voor de schadelijke eigenschappen van een stof, maar niet over de mate waarin mensen op de werkplek een gezondheidsrisico lopen. Dat hangt namelijk af van de mate waarin mensen op hun werk worden blootgesteld aan de stof.
Daar heeft de commissie geen zicht op.

Beoordeling mutagene eigenschappen

De commissie baseert de beoordeling van de mutagene eigenschappen van styreen vooral op onderzoeken bij mensen (epidemiologische studies), en in vitro-onderzoeken. Er is ook onderzoek gedaan bij knaagdieren, maar dit heeft de commissie minder mee laten wegen omdat het metabolisme van styreen bij knaagdieren anders is dan bij mensen.
Er is een epidemiologisch onderzoek gedaan naar mogelijke mutagene effecten van styreen op de geslachtscellen van mensen. Hieruit bleek dat er onvoldoende bewijs was voor een effect. Uit andere onderzoeken bij mensen bleek dat er beperkt bewijs is dat blootstelling aan styreen verband houdt met chromosomale afwijkingen en schade aan het DNA.
Uit in vitro-onderzoeken bleek dat zowel styreen als styreen-7,8-oxide een mutageen effect hadden.

Op basis van het beperkte bewijs uit onderzoek bij mensen, in combinatie met het ondersteunende bewijs uit in vitro-onderzoeken, adviseert de commissie om styreen te classificeren als stof die ervan wordt verdacht erfelijke mutaties in de geslachtscellen van mensen te kunnen veroorzaken.

Beoordeling carcinogene eigenschappen

Het verband tussen blootstelling aan styreen en de ontwikkeling van kanker bij mensen is onderzocht in meerdere epidemiologische onderzoeken. Uit deze onderzoeken bleek een verband, maar zoals bij alle observationele studies kon invloed van bias en verstorende factoren niet worden uitgesloten. Ook onderzoeken bij knaagdieren die langdurig waren blootgesteld aan styreen of styreen-7,8-oxide leverden beperkt bewijs. In verschillende onderzoeken bij muizen werd een toename gevonden van levertumoren, zowel goedaardig als kwaadaardig.
Ook werd bij muizen een toename van longtumoren gevonden, maar dit soort tumoren beschouwt de commissie als niet relevant voor de mens. In ratten die blootgesteld waren aan styreen was een toename van borst-tumoren te zien, hoewel de betrouwbaarheid van dit onderzoek beperkt was doordat er bij de ratten ook sprake was van chronische longontsteking, wat leidde tot verhoogde sterfte.

Op basis van het beschikbare beperkte bewijs uit zowel onderzoek bij mensen als bij dieren adviseert de commissie om styreen te classificeren als stof die verondersteld wordt kankerverwekkend te zijn.

Advies aan het ministerie

De commissie adviseert om styreen:

- te classificeren als stof die ervan verdacht wordt mutageen te zijn in geslachtscellen (overeenkomend met een classificatie in categorie 2) en aan te duiden als H341 (verdacht van het veroorzaken van genetische effecten);
- te classificeren als stof die verondersteld wordt kankerverwekkend te

zijn (overeenkomend met een classificatie in categorie 1B) en aan te duiden als H350 (kan mogelijk kanker veroorzaken).

Classificatie mutagene en kankerverwekkende stoffen

In classificatievoorstellen gebruikt de Gezondheidsraad een indeling in gevarencategorieën. De categorieën zijn afgeleid van EU-verordening (EG) 1272/2008 en geven aan hoe sterk de bewijskracht is voor schadelijke effecten. De stof wordt ook gelabeld met een EU-gevarenaanduiding, die op verpakkingen kan worden gebruikt.

EU-gevarencategorieën voor mutageniteit in geslachtscellen

- Categorie 1A Stoffen waarvan bekend is dat ze erfelijke mutaties in de geslachtscellen van mensen veroorzaken (EU-gevarenaanduiding H340).
- Categorie 1B Stoffen waarvan verondersteld wordt dat ze erfelijke mutaties in de geslachtscellen van mensen veroorzaken (H340).
- Categorie 2 Verdacht van het veroorzaken van erfelijke mutaties in de geslachtscellen van mensen (H341).

EU-gevarencategorieën voor kankerverwekkende stoffen

- Categorie 1A Stoffen waarvan bekend is dat ze kankerverwekkend zijn voor mensen (H350).
- Categorie 1B Stoffen waarvan verondersteld wordt dat ze kankerverwekkend zijn voor mensen (H350).
- Categorie 2 Verdacht van het veroorzaken van kanker bij mensen (H351)

Betekenis voor de werkvloer

Werkgevers zijn op grond van de Arbowet wettelijk verplicht om gezondheids- en veiligheidsrisico’s van het werken met stoffen zoveel mogelijk te voorkomen of te beperken. Op basis van de classificatievoorstellen van de Gezondheidsraad kan de minister van SZW besluiten stoffen op te nemen in de officiële lijst van kankerverwekkende, mutagene en voor de voortplanting giftige stoffen. Op die lijst staan kankerverwekkende en mutagene stoffen in categorie 1A en 1B en voor de voortplanting giftige stoffen in categorie 1A, 1B en 2. Afhankelijk van de classificatie vraagt de wetgever de werkgever aanvullende maatregelen te nemen om de werknemer te beschermen.

executive summary

At the request of the Ministry of Social Affairs and Employment, the Health Council of the Netherlands assessed whether occupational exposure to styrene may induce mutagenic effects and/or may cause cancer. Based on the assessment, they formulated a recommendation for classification for mutagenicity and carcinogenicity. The assessment was performed by the Subcommittee on Classifying carcinogenic substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council. More information on the tasks of this committee can be found on the website www.gezondheidsraad.nl. The members of the committee are listed on the last page of the assessment.

About styrene

Styrene is used in the production of polystyrene and synthetic rubbers. It can be found in packaging and building insulation, and in fibreglass-reinforced plastic products such as boats, industrial containers and wind turbine blades. Occupational exposure to styrene occurs during the manufacturing of these products, and during the production of styrene-based materials. The primary route of exposure is inhalation.
Once absorbed into the body, styrene is extensively metabolized to styrene-7,8-oxide. This metabolite has therefore been included in this evaluation as well.

Classification based on strength of evidence

Based on the available scientific literature, the committee assesses the potential mutagenetic and carcinogenic properties of the substance in question. If there are indications for such properties, it recommends classifying the substance in two hazard categories, which represent the weight of evidence that the substance is mutagenic in germ cells, and that the substance is carcinogenic. The categories are based on the globally harmonized system criteria for assessing hazard categories, which are also used by the European Commission (EU-guideline (EG) 1272/2008).

A recommendation for classification reflects the strength of evidence for the hazardous properties of a substance, but it does not reflect the health risk for workers. The health risk is based on the level of exposure to the substance in the workplace. The committee does not have sufficient data on these exposure levels.

Evaluation of mutagenic properties

The committee based the evaluation of mutagenic properties of styrene primarily on studies in exposed individuals, and in vitro studies. Rodent

studies were also available, but the committee gave less weight to these studies because the metabolism of styrene in rodents does not fully align with its metabolism in humans. There was one epidemiological study on the effect of styrene on mutagenicity in germ cells. This study did not show sufficient evidence of a significant effect. Other studies in humans showed limited evidence of an association between exposure to styrene and chromosomal aberration and DNA damage. In vitro studies with human cells showed that both styrene and styrene-7,8-oxide had mutagenic properties.

Based on the limited evidence of mutagenicity from human studies in combination with the supporting evidence from in vitro studies, the committee recommends classifying styrene as a substance suspected to induce heritable mutations in the germ cells of humans.

Evaluation of carcinogenic properties

The relationship between human exposure to styrene and the development of cancer has been studied in several epidemiological studies. These showed evidence of carcinogenic properties of styrene, but as in all observational studies, some degree of bias and confounding could not be ruled out. Also, studies in rodents that were chronically exposed to styrene or styrene-7,8-oxide showed limited evidence of carcinogenicity. In several mouse studies an increase in hepatocellular adenomas and/or carcinomas was found. Also, an increased incidence in lung tumours was found in mice, but the committee considered these tumours not relevant to humans. In rats that were exposed to styrene, an increase in mammary tumours was found. However, the reliability of this study was limited because the rats also had chronic pneumonia, which led to increased mortality.

Overall, based on the limited evidence for carcinogenicity from human studies and animal studies, the committee recommends classifying styrene as a substance presumed to be carcinogenic to humans.

Recommendation

The committee recommends classifying styrene:

- as a substance suspected to induce heritable mutations in the germ cells of humans (which corresponds with classification in category 2), and to label styrene with H341 (suspected of causing genetic effects).
- as a substance presumed to be carcinogenic to humans (which corresponds with classification in category 1B), and to label styrene with H350 (may cause cancer).

Classification for mutagenicity and carcinogenicity

The Health Council performs classification and labelling of substances according to the guidelines of the European Union (Regulation (EC) 1272/2008).
The hazard categories described below indicate the strength of the evidence for hazardous properties of the substance. The substance is labelled using an EU Hazard statement code that can be used on packaging.

EU hazard categories for mutagenicity in germ cells

- Category 1A Known to induce heritable mutations in the germ cells of humans (H340)
- Category 1B Presumed to induce heritable mutations in the germ cells of humans (H340)
- Category 2 Suspected to induce heritable mutations in the germ cells of humans (H341)

EU hazard categories for carcinogenicity

- Category 1A Known to be carcinogenic to humans (H350)
- Category 1B Presumed to be carcinogenic to humans (H350)
- Category 2 Suspected to be carcinogenic to humans (H351)

Implications for the workplace

According to the Dutch Working Conditions Act, employers are legally required to prevent or minimize the health and safety risks of working with hazardous substances as much as possible. Based on the Health Council's recommendations for classification, the Minister of Social Affairs and Employment can decide to add substances to the official list of substances that are carcinogenic, mutagenic or toxic to reproduction. This list includes carcinogenic and mutagenic substances in categories 1A and 1B, and substances toxic to reproduction in categories 1A, 1B and 2. Depending on the classification, the government asks the employer to take additional measures to protect employees.

01
scope

1.1 Background

As a result of the Dutch regulation on registration of carcinogenic compounds that came into force on 11 October 1993, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds for their carcinogenicity.
This classification is performed by the Health Council's Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). In addition to this classification, the Health Council assesses the mutagenic properties of the substance in question, and proposes a classification on germ cell mutagenicity. The request letter can be found on the website of the Health Council.

The classification is performed according to European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP regulation is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The subcommittee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as mutagens (category 1A,1B and 2) or carcinogens (category 1A, 1B and 2).

1.2 Committee and procedure

This document comprises the recommendations for classification of styrene by the Health Council's Subcommittee on the Classification of Carcinogenic Substances, hereafter called the committee. The members of the committee are listed on the last page of this report. The recommendation is based on the evaluation of published epidemiological and animal studies concerning adverse effects with respect to mutagenicity and carcinogenicity. It addresses the hazardous properties of styrene within the occupational context. Exposure to styrene in food and subsequent health risks to consumers are outside the scope of this report.

The criteria for the classification categories are based on the Globally Harmonized System, which has been incorporated into the system and guideline used by the European Union (Regulation (EC) No 1272/2008) for the classification, labelling, and packaging of substances and mixtures (the CLP regulation).

In 2023, the Health Council published their *Guideline for the classification of carcinogenic substances*.[1] This is a guideline for recommendations on classification of mutagenic and carcinogenic substances, and the assessment of the carcinogenic mode of action. The classification systems on mutagenicity and carcinogenicity are based on a weight of evidence assessment, in which more weight is given to evidence obtained from human data than to evidence obtained from animal studies or laboratory

data. Furthermore, the weight of evidence depends on the number of reliable studies that show clear associations between exposure and the occurrence of mutagenicity or carcinogenicity. This implies that studies with significant shortcomings contribute to a lesser extent to the overall weight of evidence.

Classification for mutagenicity and carcinogenicity

Classification for mutagenicity

- Category 1A Known to induce heritable mutations in the germ cells of humans (H340)
- Category 1B Presumed to induce heritable mutations in the germ cells of humans (H340)
- Category 2 Suspected to induce heritable mutations in the germ cells of humans (H341)

EU Hazard statement codes

- H340 May cause genetic effects
- H341 Suspected of causing genetic effects

Classification for carcinogenicity

- Category 1A Known to be carcinogenic to humans (H350)
- Category 1B Presumed to be carcinogenic to humans (H350)
- Category 2 Suspected to be carcinogenic to humans (H351)
- No classification for carcinogenicity

EU Hazard statement codes

- H350 May cause cancer
- H351 Suspected of causing cancer

1.3 Data

The evaluation and recommendation of the committee are based on scientific data that are publicly available. A literature summary published by the National Institute for Public Health and the Environment (RIVM), which was prepared at the request of the Health Council, was used as a starting point for the evaluation.[2] Another important source of information was the evaluation by the International Agency for Research on Cancer (IARC)[3]. The original sources of the studies, which are mentioned in the IARC-monograph, were only evaluated by the committee when these were considered most relevant for assessing the carcinogenicity and mutagenicity of the substance in question. Additional relevant data that were missing in the RIVM report and IARC-monograph were included in this report.

Data published after the last IARC evaluation were retrieved from the online databases Medline, Toxline, Chemical Abstracts, and RTECS. The last online search was performed in September 2023. The literature search was based on the following key words: Styrene; CAS No.100-42-5; Styrene-7,8-Oxide; CAS No 96-09-3; toxicity; occupational exposure; adverse health effects; dose-response relationship; hazard assessment; risk assessment; acute toxicity; chronic toxicity; genotoxicity; mutagenicity; carcinogenicity; tumourigenesis; cancer mortality. All data retrieved (i.e., data from the IARC Monograph and new data) are summarized in tables in the annexes of the present advisory report. Furthermore, available data

on styrene-7,8-oxide, the most important metabolite of styrene (see paragraph 2.2), are considered as supporting evidence for mutagenicity and carcinogenicity of styrene.

1.4 Quality and study results assessment

The committee's considerations for evaluating study quality and assessing study results are outlined in the *Guideline for the classification of carcinogenic substances*.[1]

For mutagenicity, the committee followed the quality assessment of the IARC Monograph for all the selected studies, because the considerations of IARC for determining study quality are in line with the committee's considerations. The committee did evaluate the quality of the individual studies that were not included in the RIVM report or IARC Monograph.

The results of the original studies with the outcome measures chromosomal aberration, micronuclei, aneuploidy and gene mutation were individually evaluated by the committee, as these outcome measures were considered most important for the assessment of mutagenicity. For the miscellaneous outcome measures, the committee followed the interpretation of the study results by the IARC.

For carcinogenicity, the committee followed the quality assessment of the IARC Monograph for all the included epidemiological studies. However, the committee conducted its own evaluation of the outcomes from selected key publications.

All animal carcinogenicity studies were independently assessed by the committee for both quality and results. Each study was assessed for quality by judging its relevance, reliability, and validity, to prevent less relevant or low-quality studies from contributing disproportionately to the strength of evidence.

02
general information

Information on the identification, physicochemical properties, monitoring, manufacturing and use, international classifications, and (toxico)kinetics of styrene is outlined in the RIVM document (2023) and IARC Monograph (2019).[2,3] A summary is given below.

Styrene ($C_8H_{8;}$ CAS number 100-42-5; EC/EINECS number 202-851-5) is a colourless, viscous liquid with a pungent odour. It is one of the most important monomers for polymers and copolymers that are used in a wide range of applications. Styrene polymerizes readily at room temperature in the presence of oxygen and oxidizes on exposure to light and air.

Styrene-7,8-oxide (C_8H_8O; CAS number 96-09-3; EC/EINECS number 202-476-7) is the major metabolite of styrene. It is primarily used to produce epoxy resins. Human exposure during the manufacture of styrene-7,8-oxide, or during the production or use of epoxy resins, is not well understood. 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.

2.1 Manufacture and uses

Styrene is registered under the REACH Regulation and is manufactured in and/or imported to the European Economic Area, at a total tonnage band of ≥1,000,000 to <10,000,000 tonnes.[4] The majority of styrene (90%) is produced by dehydrogenation of ethylbenzene.[5] Styrene is used by consumers, in consumer products, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing. REACH does not provide publicly available information for the current situation in the Netherlands.[2]

Styrene is primarily used as a monomer in the production of polystyrene polymers and styrene-based plastics and rubbers. This includes expandable polystyrene for packaging and building insulation, and copolymers, such as styrene-butadiene rubber or acrylonitrile-butadiene-styrene resins for the production of fibreglass-reinforced plastic products such as boats, industrial containers, and wind turbine blades.[3]
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. The primary route of exposure is inhalation.

In the Netherlands, occupational studies have mostly been performed in the fibre-reinforced plastics industry. Styrene can be a component of the polyester resin used in reinforced plastics. Fibres can be impregnated with polyester resin using a roller (hand laminating) or by spraying.
The evaporation of styrene from unsaturated polyester resin into the work environment during processing of glass fibre-reinforced plastics can result in significant exposure to styrene.

2.2 (Toxico)kinetics

This summary of the (toxico)kinetics of styrene is based on IARC Monograph (2019). For more detailed information, the committee refers to the IARC Monograph (2019).[3]

Absorption

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.

Distribution

Styrene, styrene-7,8-oxide, and styrene glycol have been measured in the blood of exposed humans. In experimental animals, styrene is widely distributed to tissues.

Metabolism

Styrene is extensively metabolized to styrene-7,8-oxide in humans and animals. Hence, external exposures to styrene encompass internal exposures to both styrene and styrene-7,8-oxide. 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. 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.

While some biological similarity was recognized between key events in mice and humans, the mode of action in mice seemed less likely to occur in humans due to quantitative differences in the metabolic capacity and qualitative differences in the type of pre-neoplastic and neoplastic lesions that occurred. It is noted that the critical role of mouse lung-specific Cyp2 F2 metabolism in mouse lung cancer caused by styrene suggests that this response is not directly comparable to the response in humans, both in terms of quality and quantity.[6,7]

Excretion

Approximately 60% of the excretion products formed from inhaled styrene come from styrene-7,8-oxide, of which the majority is eliminated via urine as mandelic acid and phenylglyoxylic acid.

2.3 Monitoring

The concentration of styrene measured in air and the concentrations of styrene and its biomarkers in urine and blood are strongly correlated.[3] Measurements of the main metabolites mandelic acid (MA) and phenylglyoxylic acid (PGA) in urine are the most commonly used biological exposure markers of exposure to styrene. Styrene itself can be

measured in alveolar air, blood, and urine, and styrene-7,8-oxide and the haemoglobin adducts of styrene-7,8-oxide can be measured in blood.

2.4 International classifications

2.4.1 European Commission

The European Commission has classified styrene as a flammable liquid and vapour (H226) that causes severe irritation to the skin (H315), eyes (H319), and upper respiratory tract (H332). Prolonged or repeated exposure to styrene is also known to cause damage to organs, including hearing organs (H372), and is suspected of damaging the unborn child (H361d).

Styrene is currently not classified for mutagenicity or carcinogenicity. Styrene-7,8-oxide is classified as harmful in contact with skin (H312), causes serious eye irritation (H319) and may cause cancer (H350; 1B).

2.4.2 IARC

IARC has re-evaluated styrene multiple times in 1994, 2002 and 2019 as new data became available over the years. The most recent re-evaluation of styrene has been conducted by IARC in 2019.[3] IARC concluded that there is limited evidence in humans for the carcinogenicity of styrene. However, they found the evidence in experimental animals to be sufficient. Overall, IARC concluded in 2019 that styrene is probably carcinogenic to humans (Group 2A). They considered styrene-7,8-oxide to be a group 2A carcinogen, based on sufficient evidence in experimental animals.[3]
It should be noted that the IARC uses a different classification scheme than the CLP criteria, with different groups, see text box.

IARC classification for carcinogenic agents (not just chemicals)

Group 1:	The agent is carcinogenic to humans
Group 2A:	The agent is probably carcinogenic to humans
Group 2B:	The agent is possibly carcinogenic to humans
Group 3:	The agent is not classifiable as to its carcinogenicity to humans
Group 4:	The agent is probably not carcinogenic to humans

2.4.3 Other countries

The United States of America has included styrene in the Report on Carcinogens (15th edition) as reasonably anticipated to be a human carcinogen.[8]

The state of California considers styrene a substance causing cancer.[9] However, styrene is currently not included in the list of substances NIOSH considers to be potential occupational carcinogens.[10]

In Germany, styrene is not included as a carcinogenic substance in the national list of CMR substances in the context of worker protection.[11]

In Australia, styrene is classified as a flammable liquid and vapour (H226), suspected of damaging the unborn child (H361d), harmful if inhaled (H332), causes damage to the hearing organs through prolonged or repeated exposure (H372), causes skin irritation (H315), causes serious eye irritation (H319), suspected of causing genetic defects (H341), may cause respiratory irritation (H335), may cause drowsiness or dizziness (H336).[12]

In Japan, styrene is classified as a flammable liquid and vapour (H226), harmful if inhaled (H332), causes skin irritation (H315), causes serious eye irritation (H319), suspected of causing genetic defects (H341), may cause cancer (H350), may damage fertility or the unborn child (H360), causes damage to central nervous system (H370), may cause respiratory irritation (H335), may cause drowsiness or dizziness (H336), causes damage to the hearing organs, central nervous system, peripheral nervous system, auditory organs, visual organs, respiratory organs and liver through prolonged or repeated exposure (H372), may be fatal if swallowed and enters airways (H304).[13]

03 mutagenicity

Mutagenicity studies focus on many different types of outcomes. The committee considers the outcome measures for chromosomal aberration, micronuclei, aneuploidy and gene mutation as most important for the assessment of mutagenicity, as these adverse effects are irreversible.

3.1 Human data

A summary of the information on the mutagenicity of styrene in epidemiological studies is presented below. An overview of the data from the IARC Monograph that were considered most important is presented in Table A1 in annex A. Two meta-analyses by Collins et al. were published after the IARC Monograph. The studies included in these meta-analyses were published before the IARC Monograph.[14,15] The committee considered individual studies included in these two meta-analyses that were not included in the IARC Monograph. One study published in Chinese was not considered by the committee as the study quality could not be determined.[16] A study published in 2020 was not considered by the committee because of co-exposure.[17] Another study has been excluded due to the use of an irrelevant control group.[18]

3.1.1 Clastogenic and aneugenic effects

Chromosomal aberration

A total of 32 studies investigated the association between styrene exposure and chromosomal aberrations. Of these studies, fifteen were seen as studies of adequate quality,[19-33] six studies had high styrene exposure concentrations[34-39] and eleven studies were of low quality and therefore disregarded.[40-50] Of the studies of adequate quality, eleven did not find a statistically significant association,[21-27,29,30,32,33] although some were limited in their sample size.[21,23,26,27,30,33] Although the study by Somorovská et al. (1999) was limited in size, it showed a dose-response relationship with the highest frequency of chromosomal aberration, expressed as frequency of aberrant cells, in the highest exposure group (3.75 ± 1.13; P<0.001), fewer chromosomal aberrations in the lower exposure group (3.27 ± 0.70; P<0.004) and still an association in the control exposure group but with fewer chromosomal aberrations (2.50 ± 0.85; P=0.0001).[28] The study of Anwar & Shamy (1995) found a statistically significant higher number of cells with chromosomal aberrations in the workers (6.06+ 4.41) compared with the controls (3.44 k 2.28) (p<0.05). However, there were no correlations found between the duration of exposure, level of urinary MA, urinary thioether level and the frequency of chromosomal aberrations or micronuclei in the exposed subjects and other (non-)work related exposures may have contributed to the findings.[31] Associations between styrene exposure and chromosomal aberrations were found by four studies with high levels of styrene exposure.[34-37]

Micronuclei

The association between styrene exposure and micronucleus induction was investigated by twenty studies.[22-24,29,31,32,37,38,41,50-60] Of these studies, thirteen were seen as studies of adequate quality,[22-24,31,32,51-57,61] five of moderate quality,[37,38,41,58,59] and one study was disregarded because of co-exposure.[50] An association was found by three studies of adequate quality.[29,32,52] Migliore et al. (2006) showed a statistically significant effect ($p<0.001$) in a fairly large study,[32] as did Vodička et al. (2004; $p=0.002$),[29] while the study of Högstedt (1984) also found a statistically significant effect ($p<0.005$) in a smaller setting.[52] Of the studies of adequate quality, nine found no statistically significant association.[22-24,31,51,53,56,57,60] The study of Yager et al. (1993) looked at the effect of styrene within the same subjects, but found no statistically significant effect.[51] In the study of Van Hummelen et al. (1994), there was a statistically significant lower frequency of micronuclei in the exposed group compared to the control group.[55]

Aneuploidy and diploidy

There was one study of adequate quality that investigated frequencies of sperm cells with aneuploidy and diploidy in individuals occupationally exposed to styrene.[62] Cytogenetic analysis 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.[62]

Gene mutation

There were five studies of adequate quality that looked at the effect of styrene on gene mutations.[63-67] None of these studies showed a convincing association.

3.1.2 Miscellaneous

There were seventeen studies that looked at DNA damage in relation to styrene exposure.[28,29,53,54,56,58-60,68-76] About half found an association[28,29,59,68-74,76] and the other half did not find a statistically significant association.[28,29,53,54,56,58,60,73,75] There were seventeen studies that looked at sister-chromatid exchange, which showed mixed results,[16,22,24,26,30,35,36,39-41,44,50,51,55-57,77] but most of these studies did not find an association.[16,22,24,26,30,36,39-41,44,55,77] There were nine studies that looked at DNA adducts in relation to styrene exposure.[58,78-85] All but one found positive associations.[58] There were two studies that found an increase in the rate of gaps.[25,26]

3.2 Animal data

A summary of the information on the mutagenicity of styrene in animal studies is presented below. Table A2 in Annex 2 presents an overview of the mutagenic data extracted from the IARC Monograph considered most

important for assessing mutagenicity. These include the available outcome measures for irreversible adverse effects (chromosomal aberration and micronuclei). Next to the available data extracted from the IARC, the committee also evaluated four additional recent studies.[86-89]

3.2.1 Clastogenic and aneugenic effects

Chromosomal aberration

In mice, styrene exposure did not cause chromosomal abnormalities. There was one inhalation study which found no chromosomal aberrations in the spleen and lung tissue of female B6C3F1 mice, and two oral studies found no chromosomal aberrations in the bone marrow of male and female CD-1 mice.[90-92] Furthermore, negative results were found for chromosomal aberrations in the bone marrow of male C57BL/6 mice after styrene exposure by intraperitoneal injection.[93]

A study on mice exposed to styrene-7,8-oxide reported chromosomal aberrations in male and female CD-1 mouse bone marrow after oral administration, and similar results were found in male CD-1 mouse bone marrow after intraperitoneal injection.[91,94] In contrast, another study found either negative or inconclusive results for chromosomal aberrations in the bone marrow, foetus and spermatocytes of BALB/c mice exposed to styrene-7,8-oxide by intraperitoneal injection.[95]

In female Fischer 344 rats exposed to styrene by inhalation, no chromosomal aberrations were observed in lymphocytes.[90] Additionally, male Fischer 344 rats and male and female Sprague-Dawley rats showed no increase of chromosomal aberrations after inhalation exposure.[96,97]

In another study, no increase in cytogenic changes was reported in the bone marrow of male Chinese hamsters after inhalation exposure.[98] Moreover, negative results for chromosomal aberrations were reported in male Chinese hamsters exposed to styrene-7,8-oxide via inhalation. However, when exposed through intraperitoneal injection, the results were equivocal for both cytogenetic tests.[99]

Micronuclei

In a study in which mice were exposed to styrene by inhalation, no increase was observed in micronucleus induction in the bone marrow of male NMRI mice, nor in the spleen and peripheral blood of female B6C3F1 mice.[90,100] An equivocal outcome for micronucleus induction was reported in the bone marrow of male NMRI mice after inhalation exposure, while weak micronucleus induction was observed in the bone marrow of male LACA Swiss mice after intraperitoneal injection, and C57BL/6 mice.[101,102]

In a study in which rats were exposed to styrene, the micronucleus assay showed no increase in micronucleus induction in the bone marrow of

female Fischer 344 rats during a 3-week inhalation study, as well as in the peripheral blood reticulocytes of male Fischer 344 rats during a 4-week inhalation study.[90,103] In a study in which rats were exposed to styrene by intraperitoneal injection, no increase was observed in micronucleus induction in the bone marrow of male Porton rats.[102]

In male Chinese hamsters exposed to styrene and styrene-7,8-oxide by intraperitoneal injection, no increase in micronucleus induction in the bone marrow was reported.[104]

3.2.2 Miscellaneous

DNA damage

In mice exposed to styrene and styrene-7,8-oxide through various routes, including inhalation and intraperitoneal injection, DNA damage was detected in various organs, including bone marrow, liver, kidney, lung, testis, and brain.[101,105-108]

In contrast, rats exposed to styrene through inhalation did not show DNA damage in lymphocytes, although an increase in DNA damage was observed in leukocytes on day 3 of the treatment, but not on day 20, in the presence of formamido pyrimidine glycosylase (Fpg).[103,109] Similarly, in a 4-week inhalation study, male Fischer 344 rats exposed to styrene-7,8-oxide through inhalation showed no increase in DNA damage in leukocytes.[103]

Sister-chromatid exchange

In a study in which mice were exposed to styrene by inhalation, the sister-chromatid exchange assay showed positive results in bone marrow, liver, and alveolar macrophages of male BDF1 mice, while equivocal results were obtained in the lung, spleen, and lymphocytes of female B6C3F1 mice.[90,110] In mice exposed to styrene by intraperitoneal injection, the sister-chromatid exchange test in male LACA Swiss mouse splenocyte yielded equivocal results, while the sister-chromatid exchange test in male C57BL/6 mouse bone marrow showed negative results.[93,102]

In mice exposed to styrene-7,8-oxide by intraperitoneal injection, only the S-enantiomer tested in male CD-1 mouse bone marrow yielded positive results for sister-chromatid exchange without including gaps.[94]

In rats exposed to styrene by inhalation, positive results for the sister-chromatid exchange test in female Fischer 344 rat lymphocytes were obtained.[90] Additionally, lymphocytes in male Fisher 344 rats showed negative results after inhalation exposure with styrene, while for splenocytes of male Porton rats positive results were observed for sister-chromatid exchange after styrene exposure by intraperitoneal injection.[96,102]

An inhalation study with male Chinese hamsters exposed to styrene-7,8-oxide resulted in negative results for sister-chromatid exchange assays, whereas intraperitoneal injection yielded equivocal results.[99]

Unscheduled DNA synthesis

In female CD-1 mice exposed to styrene by inhalation, no induction of unscheduled DNA synthesis was observed in the liver.[111]

DNA adducts

In mice, DNA adducts were detected in the lung, liver, spleen and urine through various routes, while in one study no adducts were found in the lungs after styrene exposure by inhalation.[101,112-116] In rats exposed to styrene by inhalation, DNA adducts were detected in the lung and liver, while one study found equivocal results in the liver.[114,115,117]

3.2.3 Recent studies

The committee reviewed four recent additional studies: one peer-reviewed publication and three study reports, which are summarized below.[86-89]

The mutagenicity of styrene was investigated using the transgenic MutaMouse gene mutation assay (OECD TG488).[86] Styrene was orally administered at doses of 0 (corn oil; negative control), 75, 150, and 300 mg/kg/day for 28 days, and mutant frequencies were determined using the lacZ assay in the liver and lung (five male mice/group) (see Table 1).

No deaths were observed in mice treated with styrene up to the highest dose. The administration of styrene did not affect the overall conditions or weight changes. Nonetheless, the observation of significant pathological changes in the liver suggests that styrene was absorbed and reached the intended organs. No significant difference in mutant frequencies between the negative control and treated groups in the liver and lung of MutaMouse was found, except for one outlier animal at the 75 mg/kg/day dose, which was excluded from the mean value as this was considered to be a clonal mutation. The mutant frequencies were within the range of historical negative control data.

Table 1 Mutant Frequency Group Mean (× 10−6) in MutaMouse liver and lung after styrene exposure 28 days

Type of tissue	0 mg/kg/day (corn oil)	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day	Positive control (100 mg/kg/day ENU)
Liver	34.1 ± 9.4	36.3 ± 10.3#	48.7 ± 25.9	49.0 ± 11.1	109.1 ± 17.1*
Lung	33.4 ± 9.7	55.6 ± 16.4#	46.1 ± 20.1	43.7 ± 6.1	180.7 ± 35.0**

Corn oil: Control group (10 mL/kg)
ENU: Positive control (N-ethyl-N-nitrosourea, 10 mL/kg, i.p., once daily for 2 days, expression period: 10 days)
* Significant difference from the negative control (Steel's test: $p<0.05$)
** Significant difference from the negative control (Aspin-Welch's t-test: $p<0.05$)
\# This value was obtained by excluding Animal ID No.3103

In another transgenic rodent gene mutation study, the mutant frequency was determined in glandular stomach, lung, liver, and duodenum tissues obtained from Big Blue® hemizygous B6C3F1 male mice.[87] These mice were orally administered either vehicle (corn oil; group 1), or styrene at

doses of 75, 150, or 300 mg/kg daily for 28 consecutive days (groups 2 to 4, respectively), or a positive control (N-ethyl-N-nitrosourea [ENU] 40 mg/kg; Group 5) on days 1, 2, and 3. Following a further fixation period of 28 days, all animals were necropsied on day 56. There were no test substance-related clinical observations or effects on body weight, body weight gains, food consumption or organ weight. No statistically significant increase was observed in mutant frequency at the cII gene in lung, glandular stomach or duodenum of the mice treated with styrene at doses of 75, 150 or 300 mg/kg/day (see Table 2). A statistically significant increase in mutant frequency was observed in the liver at dose levels of 75 and 300 mg/kg/day, but not at 150 mg/kg/day. This increase did not show a dose-response relationship and the mean mutant frequency values from all styrene treated-groups remained within the 95% control limits of the historical vehicle control data. However, it is not clear whether the historical control data were sufficiently robust to provide a reliable distribution of negative control data. Although the OECD TG 488 criteria for a clear positive result were not met, the committee noted that the criteria were also not met for a clear negative result, and therefore no firm conclusion can be drawn on mutagenicity based on these increases in mutant frequency. Furthermore, one animal in group 1 was identified as a jackpot mutation animal yielding a high background mutant frequency in the lung and was also considered an outlier in the liver as the mutant frequency fell outside of the upper 99% historical liver control limit. This animal was therefore excluded from group 1 mean calculations and replaced by a different animal. Additionally, two more animals across each treatment group for the liver were examined to address an outlier result within the group and to help determine the biological relevance of the result.

Table 2 Mean Mutant Frequency ± SD (× 10-6) in Big Blue® C57BL/6 mice glandular stomach, lung, liver and duodenum after styrene exposure for 28 days

Type of tissue	N	0 mg/kg/day (vehicle control)	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day	Positive control (40 mg/kg/day ENU)
Glandular Stomach	5	46.0 ± 10.4	61.1 ± 23.8	60.4 ± 29.5	64.5 ± 7.9	786.6 ± 117.5**
Lung	5	69.7 ± 15.8	69.5 ± 23.5	72.0 ± 16.4	72.4 ± 25.8	174.0 ± 61.5**
Liver[a]	7	47.1 ± 8.8	62.6 ± 13.2*	51.5 ± 11.8	60.4 ± 12.8*	155.2 ± 20.5**
Duodenum	5	76.9 ± 17.0	68.3 ± 14.1	80.7 ± 34.4	88.0 ± 13.5	770.7 ± 58.6**

[a] Two additional animals across all treatment groups for liver were processed to address an outlier result in the group and to assist in establishing the biological relevance of this result.
* P≤0.05, ** P<0.001; statistically significant versus vehicle control.
ENU = N-ethyl-N-nitrosourea; SD = standard deviation.

In an oral 29-day study, 40 male B6C3F1 mice (8 per group) were administered 1 of 3 dose levels of styrene (75, 150, or 300 mg/kg/day) or the vehicle control (corn oil) daily for 29 consecutive days.[88] The animals in the positive control group received ethyl nitrosourea (ENU) daily during the initial 3 days (days 1-3), followed by ethyl methanesulfonate (EMS) for the final 3 days (days 27-29). On day 29, collected blood was used to assess Pig-a mutant frequency and micronucleus frequency. Samples from the duodenum, glandular stomach, kidneys, liver and lungs were

collected for evaluation of DNA damage using the comet assay.
No adverse clinical observations were associated with exposure to styrene. Additionally, there were no changes in body weight or body weight gain attributed to styrene exposure. Doses of styrene at 75, 150 or 300 mg/kg/day did not lead to an increase in mutagenesis, clastogenesis, or DNA damage in B6C3F1 mice liver, lung, stomach and kidney, as assessed by the mammalian erythrocyte Pig-a gene mutation assay, the mammalian erythrocyte micronucleus test and the in vivo mammalian alkaline comet assay, respectively. The committee noted that the acceptance criteria were met for the Pig-a mutant frequency and micronucleus frequency assays. However, for the comet assay, only the stomach samples fell within the historical negative control range of the test facility for this strain. The percent tail DNA values for the liver, kidney, and lung background were consistent with those found in the literature and the frozen tissue of the laboratory. The duodenum comet assay did not meet the acceptance criteria (i.e. the percentage tail DNA values measured for the vehicle and styrene groups fell outside of the laboratory's historical data and the positive control did not induce a statistically significant response) and thus the assay in duodenum was not considered valid. See Table 3, 4 and 5 for an overview of the results.

Table 3 Summary Pig-a Mutant Frequencies in Male B6C3F1 PCE and RBC after styrene exposure for 29 days

Type of tissue	N[a]	0 mg/kg/day	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day	Positive control 51.7/150 (ENU/EMS)
% PCE	6	2.83 ± 0.20	3.00 ± 0.30	3.12 ± 0.24	3.05 ± 0.27	3.12 ± 0.38
Mutant PCE per 10^6 PCE	6	1.53 ± 0.54	0.48 ± 0.45	0.33 ± 0.43	0.55 ± 0.51	167.82 ± 49.66*
Mutant RBC per 10^6 RBC	6	0.25 ± 0.16	0.38 ± 0.75	0.12 ± 0.15	0.20 ± 0.11	47.08 ± 16.08*

Abbreviations: N = number of animals; EMS = ethyl methanesulfonate; ENU = N-ethyl-N-nitrosourea, PCE = polychromatic erythrocytes; RBC = red blood cells
Values are group mean ± standard deviation
[a] Samples from the first 6 surviving animals in each group were assayed
* Statistically significant at $p<0.05$ (t-test, 1-sided)

Table 4 Summary Micronucleus (MN) Assay Results in Male B6C3F1 PCE and RBC after styrene exposure for 29 days

Type of tissue	N[a]	0 mg/kg/day	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day	Positive control 51.7/150 (ENU/EMS)
% PCE	6	1.097 ± 0.137	1.285 ± 0.184	1.251 ± 0.159	1.102 ± 0.061	1.135 ± 0.299
% MN-PCE	6	0.348 ± 0.033	0.268 ± 0.061	0.265 ± 0.063	0.303 ± 0.070	1.218 ± 0.5963*

Abbreviations: N = number of animals; EMS = ethyl methanesulfonate; ENU = *N*-ethyl-*N*-nitrosourea; MN-PCE = micronucleated PCE; PCE = polychromatic erythrocytes Values are group mean ± standard deviation
[a] Samples from the first 6 surviving animals in each group were assayed
* Statistically significant at $p<0.05$ (t-test, 1-sided)

Table 5 Summary Comet Assay Results in Male B6C3F1 duodenum, kidney, liver, lung and stomach after styrene exposure for 29 days

Type of tissue	N[a]	0 mg/kg/day (vehicle control)	75 mg/kg/day	150 mg/kg/day	300 mg/kg/day	Positive control 51.7/150 (ENU/EMS)
Duodenum % Tail DNA	6	29.11 ± 12.42	29.42 ± 15.08	26.76 ± 13.55	24.21 ± 13.21	25.14 ± 8.45
Kidney % Tail DNA	6	3.09 ± 0.68	4.97 ± 3.38	3.37 ± 0.72	3.60 ± 0.51	9.84 ± 3.03*
Liver % Tail DNA	6	2.28 ± 0.86	2.06 ± 0.61	1.98 ± 0.81	2.37 ± 0.69	14.39 ± 3.08*
Lung % Tail DNA	6	1.87 ± 0.93	2.29 ± 0.70	1.76 ± 0.57	1.96 ± 0.65	8.94 ± 5.71*
Stomach % Tail DNA	6	5.89 ± 2.39	6.64 ± 2.47	6.04 ± 2.34	6.46 ± 1.32	10.18 ± 4.86*

Abbreviations: N = number of animals; EMS = ethyl methanesulfonate; ENU = N-ethyl-N-nitrosourea Values are group mean ± standard deviation

[a] Samples from the first 6 surviving animals in each group were assayed

* Statistically significant at $p<0.05$ (t-test, 2-sided)

In another recent study, potential DNA damage was measured in glandular stomach, duodenum, lung, liver, and kidney cells of 40 male Fischer 344 rats (8 per group) exposed for up to 28 days by oral gavage to styrene at dose levels of 100, 250, 500 mg/kg/day, or the vehicle control (corn oil), using the Comet assay (OECD TG 489).[89] Administration of styrene at doses up to 500 mg/kg/day did not result in any effect on mortality, physical examinations, observations before and after dosing, body weight, or food consumption. No significant increases in the % Tail DNA compared to the respective vehicle controls were found in the styrene treated animal multi-well slides, except for the glandular stomach. However, these increases were within the historical control range.

Overall, no significant increases in DNA damage were observed in duodenum, lung, liver, and kidney in male rats after administration up to 500 mg/kg/day. The committee noted that the percentage Tail DNA in the vehicle control group was above the historical vehicle control distribution for the duodenum and kidney. See Table 6 for the complete results.

Table 6 Comet Assay Summary of Mean % Tail DNA ± S.D. in Male Fischer 344 Rats glandular stomach cells, duodenum, lung, liver, and kidney cells after stryrene exposure for 28 days

Type of tissue	N	0 mg/kg/day (corn oil)	100 mg/kg/day	250 mg/kg/day	500 mg/kg/day	Positive control (EMS 200 mg/kg/day[a])
Glandular Stomach Cells	6	1.38 ± 0.42	4.05 ± 0.45§	5.39 ± 4.03§	4.08 ± 2.94§	29.28 ± 4.15*
Duodenum Cells	6	26.14 ± 11.62	15.05 ± 14.16@	5.10 ± 2.08@	6.34 ± 2.51@	48.33 ± 12.60*
Lung Cells	6	1.07 ± 0.41	1.15 ± 0.47	1.11 ± 0.49	1.74 ± 0.43	23.53 ± 3.63*
Liver Cells	6	0.61 ± 0.44	0.45 ± 0.34	0.30 ± 0.14	0.32 ± 0.16	27.46 ± 7.00*
Kidney Cells	6	10.56 ± 2.62	12.80 ± 4.53@	5.99 ± 2.04@	4.01 ± 1.65@	34.59 ± 4.81*

S.D. = Standard Deviation

[a] Ethyl methanesulfonate (EMS), positive control for Comet assay, administered orally once daily for two consecutive days (Study Days 27 and 28). The second dose was administered 20 ± 0.5 hrs after the first dose.

* $p\leq0.05$ (Student's t-test); Statistically significant increase relative to the vehicle control

§ $p\leq0.05$ (Kruskal-Wallis, Dunnett›s test); Statistically significant increase relative to the vehicle Control

@ $p\leq0.01$ (Jonckheere's test): Statistically significant decreasing trend relative to the vehicle control, which is not considered to be biologically relevant.

3.3 In vitro data

A summary of the information on the mutagenicity of styrene in in vitro studies is presented below. An overview of the mutagenic data subtracted from the IARC Monograph considered most important can be found in Table A3 in Annex A.

Human cells

Various studies examining cytogenic effects of styrene in human whole-blood lymphocytes showed positive results for chromosomal aberrations and micronuclei, and sister-chromatid exchange, without exogenous metabolic activation systems.[17,118-127] Additionally, the comet assay detected DNA damage caused by styrene in isolated human leukocytes treated in vitro and in human skin treated in vitro in the absence of metabolic activations.[128,129]

For styrene-7,8-oxide, results were consistently positive for similar endpoints.

Mammalian cells

Two studies examining the induction of chromosomal aberrations by styrene in Chinese hamster lung cells yielded negative results in the absence of exogenous metabolic activation, while showing weakly positive results with activation.[130,131]

Styrene caused mutations at the Hprt locus in Chinese hamster lung V79 cells when an exogenous metabolic activation system was present.[132,133] In Chinese hamster ovary cells, sister-chromatid exchanges were induced when an exogenous metabolic activation system was present.[134] In contrast, sister-chromatid exchanges were induced in rat lymphocytes in the absence of exogenous metabolic activation.[135] Furthermore, a study in isolated male mouse hepatocytes showed increased DNA damage after styrene exposure, and styrene was shown to induce DNA strand breaks in rat primary hepatocytes.[136,137]

Styrene-7,8-oxide induced genetic alterations in various cells, including sister-chromatid exchanges, chromosomal aberration, micronuclei formation, and Hprt locus mutations in Chinese hamster lung V79 cells, without exogenous metabolic activation.[132,133,138-140] It also caused DNA damage in Chinese hamster lung V79 cells.[141] In mouse lymphoma L5178Y cells, mutations at the Tk locus occurred without metabolic activation, while DNA strand breaks were induced in rat primary hepatocytes and rat pheochromocytoma PC12 cells without metabolic activation.[137,142,143]

Non-mammalian cells

In Drosophila melanogaster, styrene showed mixed results, causing sex-linked recessive lethal mutations, but not leading to aneuploidy, nor induction of somatic mutations.[104,144,145] In Allium cepa, styrene caused

chromosomal aberrations.[118,119] Yeast cells also exhibited genotoxic effects from styrene, such as gene conversion and reverse mutation.[146]
When tested on various bacterial strains (e.g., Salmonella typhimurium, Escherichia coli), styrene's mutagenic potential varied, often showing negative results without metabolic activation but some positive results with metabolic activation.[147-156] One study showed that styrene exposure led to significant DNA damage in non-mammalian species, such as fish and mussels, with continued exposure over a week.[157]

Styrene-7,8-oxide was extensively tested in various bacterial strains, both with and without external metabolic activation. Positive results were consistently seen in strains detecting base substitution mutations, while strains detecting frameshift mutations mostly yielded negative results. Additionally, styrene-7,8-oxide showed positive results for sex-linked recessive lethal mutations in Drosophila melanogaster and induced chromosomal aberrations and micronuclei in Allium cepa root tip cells.[118,119,144] Notably, styrene-7,8-oxide showed positive outcomes in DNA damage tests without metabolic activation.[148,152,156,158-166]
Overall, these findings align with observations regarding the mutagenicity of styrene in similar assays.

3.4 Evidence synthesis regarding mutagenicity

Human data

Human studies showed variable results, with some demonstrating an association for various relevant endpoints (primarily cytogenetic endpoints),[28,29,31,32,34-37,52,55,59] while others did not show an association.[21-27,29,30,32,33,46,4831,55-58,60] One study that investigated the frequencies of sperm cells with aneuploidy and diploidy in individuals occupationally exposed to styrene did not provide sufficient evidence for an effect, as the study population was small.[62]

The committee is of the opinion that the positive findings for cytogenic effects, despite study limitations, suggest mutagenic properties of styrene and cannot be ignored.

The committee further considers studies investigating DNA damage (including DNA adducts) in humans as supporting evidence. Since the majority of these studies were positive,[28,29,59,68-74,69-76] the committee concludes that an interaction between styrene (or its metabolites) and DNA is possible in humans. Therefore, the committee considers the positive results for chromosomal aberration and micronuclei from human studies, in combination with the supporting evidence for DNA damage and DNA adducts, as limited evidence for mutagenicity.

Animal data

The committee concludes that the animal studies provide inadequate evidence for mutagenicity. Mice studies that investigated cytogenic effects (chromosomal aberration and micronuclei) have shown negative results,[88,90-93,95,100] equivocal results,[92] and some positive results.[91,94,93] In contrast, only negative results were found in rats,[90,96,97,102,103] while the results in hamsters were mostly negative[98,104] with some equivocal results.[99] Overall, studies on rodents exposed to styrene or styrene-7,8-oxide mainly yielded negative or inconclusive outcomes regarding cytogenetic effects.

Regarding gene mutation, three mouse studies did not show an association with styrene.[86-88] The committee placed less emphasis on the evidence for cytogenic effects and gene mutation from rodent studies compared to the evidence from human studies, because the metabolism of styrene in rodents does not fully align with its metabolism in humans.[6,7]

Furthermore, the studies that looked at reversible endpoints, such as DNA damage, DNA adduct formation, sister-chromatid exchange, and unscheduled DNA synthesis, did not show a consistent genotoxic effect. These findings were given less weight than data on cytogenetic and gene mutation endpoints.

In vitro

The committee considers the predominantly positive effects from in vitro studies as supporting evidence for mutagenicity. Effects of styrene in mammalian cells varied depending on the presence of metabolic activation, and specific responses varied across different organisms and test systems,[130-134,136,137] but for styrene-7,8-oxide the results were consistently positive for similar endpoints.[132,133,137-143] In vitro studies with human cells have consistently shown that both styrene and its metabolite, styrene-7,8-oxide, cause genotoxic effects.[118-129]

3.5 Evaluation of mutagenicity

Classification in category 1A for germ cell mutagens requires positive evidence from human epidemiological studies. There was one epidemiological study on the mutagenicity of styrene in germ cells, which did not provide sufficient evidence for an effect.[62] The committee concludes that the available data do not indicate that styrene should be classified in category 1A for germ cell mutagenicity.

A substance can be classified in category 1B if mutagenicity is observed in germ cells in mammals in vivo or in somatic cells in mammals in vivo combined with evidence indicating the potential to cause mutations in germ cells. Since there are no in vivo data on mutagenicity in germ cells for styrene, and the one germ cell mutagenicity test in styrene-7,8-oxide

was negative,[95] styrene should not be classified in category 1B for mutagenicity.

A substance can be classified in category 2 if there is evidence for mutagenicity from experiments in somatic cells in mammals in vivo or other in vivo somatic cell genotoxicity tests supported by in vitro data. However, regarding mutagenicity, the committee primarily based its assessment on in vitro studies with human cells and epidemiological studies in exposed individuals. Less weight was attributed to rodent studies and in vitro studies with cells from rodents, because the metabolism of styrene in rodents does not fully align with its metabolism in humans.[6,7] The committee concludes that the evidence from animal studies provides inadequate evidence for mutagenicity.

Based on the limited evidence for mutagenicity from human studies in combination with the supporting evidence from in vitro studies, the committee considers classification in category 2 warranted.

3.6 Recommendation on the classification for mutagenicity

The committee recommends classifying styrene as *Suspected to induce heritable mutations in the germ cells of humans*, which corresponds with category 2 for mutagenicity, and to label styrene with H341 (suspected of causing genetic effects).

04 carcinogenicity

Data on carcinogenicity have been summarized in the RIVM report[2] and the IARC Monograph.[3] An overview of the most relevant data from these reports can be found below. The committee did not find any additional or new relevant data in the literature.

4.1 Human data

There are three main cohort studies on the effects of styrene exposure with results published in multiple articles. Of these studies, two are American: one among boatbuilders in Washington State, and one nationwide study among workers in the reinforced plastics and composites industry. The third cohort is a combined cohort from different countries in Europe among workers at reinforced plastics production plants.

For each of the three cohorts, the committee selected one publication that presented the most recent and complete study results. These studies are called key publications. An overview of the carcinogenicity data in humans can be found in Table B1 in Annex B.

The boatbuilder study in Washington State is a retrospective cohort study that has resulted in several publications.[167-172] The population included in this study consisted of around 5,200 boatbuilders working at one of two boatbuilding facilities in Washington State, USA, in the period 1959-1978. Fibreglass-reinforced plastics and composites were used in the manufacture of boats, which potentially exposed workers to styrene fumes through air. Health outcomes in these workers, in particular mortality, were compared to the general population, and, by internal comparisons, between workers potentially exposed to different levels of styrene. Estimates of levels of exposure were partially based on measurements performed as part of industrial hygiene surveys and personal air sampling measurements performed on site in 1978, and further on expert opinion. Detailed job histories were available for each worker and using a job-exposure matrix approach cumulative exposures were estimated.

IARC concluded that the strengths of this study were the high concentrations of styrene exposure in general, the few competing risk factors, and the long follow-up. Limitations were the lack of individual quantitative styrene exposure and information on smoking.[3]

The committee agrees with the conclusions of IARC on the quality of the studies within this large cohort. For this cohort, the committee chose the study of Daniels as key publication as it uses the most recent mortality figures and extended the analyses by making fuller use of available employment information and exposure measurement data.[172] Daniels et al. (2020) estimated mean and median cumulative exposures at 31 and 5.7 ppm-years, respectively. Furthermore, they concluded that there was a monotonic relation between styrene exposure and risk of leukaemia (hazard ratio [HR] per 50 ppm-years 1.46, 1.04-1.97) and risk of bladder cancer (1.64, 1.14-2.33) after trimming person-time with extreme exposures.[2,172]

There are three publications within the cohort study among workers in the reinforced plastics and composites industry in the United States.[173-175] For these studies, a cohort of almost 16,000 workers working at one of 30 reinforced plastics manufacturing plants in various US states in the period 1948-1977 was formed to analyse the health effects of styrene exposure. IARC concluded that the strengths of this study were the long follow-up, the high number of cases, the high concentrations of styrene exposure, and the lack of known carcinogenic occupational co-exposures within the industry. Quantitative styrene exposure metrics were applied but information on the exposure assessment was sparse; no styrene intensity information appeared to be available for a substantial part of the exposure period, namely between 1948 and 1976, and for 27% of the cohort exposure data after 1977 were missing.[3] The committee noticed that the cohort was formed with aid of the industry. The key publication within this cohort, Collins et al. (2013), provided the most recent update with follow-up until the end of 2008. At this point, they only found significant differences with the general population for lung cancer (SMR 1.34, 1.23-1.46), but with an inverse trend with cumulative exposure.[2,175]

The six-country study on workers at reinforced plastics production plants is a study of workers in the reinforced plastics industry in Denmark, Finland, Italy, Norway, Sweden and the UK, conducted at the initiative of the IARC. Altogether, the cohort includes over 40,000 workers at one of more than 600 reinforced plastics production plants. There are four publications that report on the full cohort,[176-179] six publications that focus on the Danish cohort[180-185] and another two publications on the UK cohort.[186,187] Loomis et al. (2019) is the most recent study on this six-country cohort, and was therefore chosen as key publication. In this study, the data were re-analysed (excluding the Norwegian cohort), finding the mean level of styrene exposure to be associated with an increased risk of dying from non-Hodgkin's lymphoma (RR 2.31, 1.29-4.12 per 100 ppm), from cancer of the oesophagus (2.44, 1.11-5.36 per 100 ppm), or of the pancreas (RR 1.89, 1.17-3.09). Oesophageal cancer mortality was also associated with cumulative styrene exposure 20 years after the start of exposure (RR 1.16, 1.03-1.31).[2,179] IARC noted that the strengths of this study were the large study population of workers of small- and medium-sized companies, with expected homogeneous and high-concentration exposure to styrene, and a long and almost complete follow-up. The limitations were the lack of quantitative estimates of exposure to styrene or any information on the prevalence of smoking.[3]

In addition, there are smaller cohort studies on workers in the synthetic rubber industry, all based on North American workers in the styrene-butadiene rubber (SBR) industry. These workers were exposed to styrene at lower concentrations, but for longer times. Within these studies an elevated risk of mortality from leukaemia was found, which is in line with the results from the boatbuilder cohort. However, these results might be confounded by co-exposure by butadiene.[3,188-190] Several case-control

studies have investigated the association between workplace exposure to styrene and the risk of various cancers. Cancers of the lymphoid and haematopoietic tissues, as well as renal cell carcinoma and cancer of the lung, have received particular attention and elevated risks were observed.[3]

4.2 Animal data

A summary of the available data on the carcinogenicity of styrene and styrene-7,8-oxide is presented below. An overview of the carcinogenicity data in animals can be found in Table B2 and B3 in Annex B. Several studies have been excluded from the evaluation of carcinogenicity, including dose-range finding studies.[191] Studies by intraperitoneal- and subcutaneous injection have been excluded as this route of exposure is not considered relevant for styrene in humans.[192-194] Additionally, the studies of Maltoni and Conti et al. have been excluded from the evaluation due to poor study quality.[193,195,196]

4.2.1 Studies with styrene in mice

For the evaluation of carcinogenicity, six studies with styrene in mice were considered, of which two studies by oral gavage, two studies involving transplacental exposure followed by oral exposure by gavage in male and female pups, and two studies by inhalation.

Oral studies

A carcinogenicity study in B6C3F1 mice was performed by the National Cancer Institute (NCI).[197] Male and female mice (20 controls/sex and 50/sex/dose group) were exposed to a mixture of 70% styrene and 30% β-nitrostyrene 3 times per week for 78 weeks via oral gavage. Mice were exposed at dose levels of 0, 87.5 and 175 mg/kg bw/day. These dosages are defined in terms of the β-nitrostyrene present in the styrene solution. In males, a dose-response relation for increased mortality upon treatment was observed (P=0.007). In females, mean body weight was decreased (175 mg/kg bw) compared to control. A statistically significant increased incidence of combined lung alveolar/bronchiolar carcinoma and adenomas in low dose male mice was noticed compared to control (P=0.016), although there was no significant increase in malignant tumours. However, the high dose Fisher exact test and the Cochran-Armitage test were not significant for these neoplastic lesions. The committee observed a lack of clarity regarding the number of mice that dropped out during the study. Additionally, they noted a higher attrition rate among male mice in the high-dose group. Since the analysis only included mice that survived for a minimum of 52 weeks, the exclusion of those mice lost in the high-dose group could have potentially affected the outcome of the study.

A carcinogenicity study in B6C3F1 mice was performed by the NCI.[198] Male and female mice (20 controls/sex and 50/sex/dose group) were exposed to styrene 5 days per week for 78 weeks via oral gavage.

Mice were exposed at 0, 150 and 300 mg/kg bw/day. Mortality was increased in all dose groups in males. In females, a slight dose-related mean body weight depression was observed, but mortality was not affected. Combined alveolar/bronchiolar adenomas and carcinomas of the lung compared to the control were significantly increased in males (300 mg/kg bw, P=0.024). However, no difference was found in carcinomas. The authors noted that a large variation in occurrence of lung tumours exists in historical control data of untreated male mice and that incidence in vehicle controls was lower than expected based on these data. Hepatocellular adenomas were observed in female mice, but a statistically significant increase was only found at the highest dose (300 mg/kg bw, P=0.034). Although a noticeable trend was indicated by the Cochran-Amirage test, the comparison of individual groups to the control group was not significant.

A carcinogenicity study in O20 mice and C57 BL mice was performed by Ponomarkov et al.[199] For O20 mice, pregnant dams (29 exposed, 9 control) were given a single oral gavage administration of styrene (1350 mg/kg bw, purity: 99%) or olive oil at gestation day 17. Their offspring was treated weekly from the time of weaning for the whole lifespan with the same dose of styrene or olive oil via oral gavage. An extra control group of 54 untreated males and 47 untreated females was included. Treatment of offspring had to be suspended after 16 weeks due to severe toxicity. Preweaning mortality was higher in the styrene group compared to control. Overall mortality was high in the styrene progeny group: at 20 weeks, 50% of males and 20% of females died. Survival rates of other groups (styrene pregnancy, vehicle pregnancy, vehicle progeny) were not affected. The average age of death was lower in exposed animals (32 weeks, males; 49 weeks females) compared to controls (88 weeks, males; 85 weeks, females). There was an increased incidence in total tumour bearing animals in offspring of styrene-treated dams in males and females (no details on statistics). An increased incidence of lung adenoma and adenocarcinoma combined was observed in treated offspring of styrene-treated dams in males and females compared to the olive oil control group (P<0.01 for both). However, no details on the statistics used were provided. Lung tumours appeared earlier in the styrene-treated progeny groups (both male and female) compared to control. Additionally, the committee noted that O20 mice are sensitive for developing lung tumours.

For C57 BL mice, pregnant dams (15 exposed, 5 control) were given a single oral gavage administration of styrene (300 mg/kg bw, purity: 99%) or olive oil at gestation day 17. Their offspring was treated weekly from the time of weaning for the whole lifespan with the same dose of styrene or olive oil via oral gavage. An extra control group of 51 untreated males and 49 untreated females was included. Litter size, preweaning mortality, offspring mortality and body weight did not differ between the groups. An increased incidence in tumour-bearing females receiving a single dose of styrene during pregnancy was observed. This was due to an increased

incidence of lymphomas which was not statistically significant. There was an increased incidence in hepatocellular carcinoma or adenoma in treated males. However, no details on statistics were reported.

Inhalation studies

In a GLP study, CD-1 mice (70/sex/group) were exposed to styrene vapour (whole body) at concentrations of 0, 20, 40, 80, or 160 ppm for 6h/day during 5 days/week for 104 weeks (males) or 98 weeks (females).[200]

Styrene did not impact the survival in male mice. The remaining exposed females had a slightly higher survival rate than the control group.

An increase in the total number of tumour-bearing mice was observed in females exposed to 40 ppm and 160 ppm compared to the control group (both $P<0.05$). An increased tumour incidence was predominantly seen in the lung. In males, there was an increased incidence of bronchioloalveolar adenomas at 40 ppm, 80 ppm, and 160 ppm (all $P<0.05$). In females, an increased incidence of bronchioloalveolar adenomas was observed at 20 ppm and 40 ppm (both $P<0.05$), as well as an increased incidence of bronchioloalveolar carcinomas at 160 ppm ($P<0.05$). Non-neoplastic lesions in male and female CD1-mice are briefly summarized in Table B2, Annex B. It should be noted that no historical control data were available from inhalation studies conducted at the testing laboratory for bronchioloalveolar adenoma and carcinoma in CD-1 mice.

A follow-up study was conducted in which 55 males were exposed to styrene.[200] No effects in the lung were observed. In the 40 ppm, there were slight changes in the olfactory epithelium. In the 80 ppm group, single-cell necrosis occurred in the olfactory epithelium. After 2, 4 and 7 exposures, there was an increase in degree of lesions and changes in the Bowman's glands. After 40 or 65 exposures, more pronounced atrophy and disorganization leading to respiratory metaplasia was seen.

In another inhalation study (whole-body exposure), groups of 75 male CD-1, C57BL/6 wildtype (WT), *Cyp2f2*$_{(-/-)}$ knockout (KO), and *Cyp2f2*KO-*Cyp2f1* transgenic (TG) mice were exposed to styrene at 0 ppm or 120 ppm for 6 hours per day, 5 days per week, for a duration of 104 weeks.[201] Treated wildtype mice showed significantly higher survival rates compared to the control group. The incidence of bronchioloalveolar carcinoma was significantly increased in CD-1 mice [$P<0.05$] exposed to styrene (17/67) compared with the CD-1 control mice (7/67). There was no statistically significant increase in tumours in the genetically modified mice (KO, TG). No statistically significant increase in lung adenomas or adenocarcinomas was observed in the other strains of mice. CD-1, WT and KO mice exposed to styrene weighed less than controls (2-13%; 2-10%; up to 7% respectively). Mean body weights in exposed CD-1, WT and KO mice were statistically significantly lower compared to controls at multiple time points. Non-neoplastic lesions observed in the four strains of mice are briefly summarized in Table B2, Annex B.

4.2.2 Studies with styrene in rats

For the evaluation of carcinogenicity, six studies with styrene in male and/or female rats were considered: three studies by oral gavage, two studies by inhalation, and one study involving transplacental exposure followed by oral exposure by gavage in male and female pups.

Oral studies

A carcinogenicity study in Fischer 344 rats was performed by the NCI.[197] Male and female rats (20 controls/sex and 50/sex/dose group) were exposed to a mixture of 70% styrene and 30% β-nitrostyrene 3 times per week via oral gavage for a duration of 79 weeks. Males were exposed at dose levels of 0, 150 or 300 mg/kg bw/day and females at dose levels of 0, 75 and 150 mg/kg bw/day. Tumour incidences were statistically analysed with a Fisher exact test (one-tailed). Survival was not affected by styrene. Mean body weight was decreased in male rats (300 mg/kg bw) compared to control. There were no significant effects on tumour incidences.

A carcinogenicity study in Fischer 344 rats was performed by the NCI.[198] Male and female rats (20 controls/sex and 50/sex/dose group) were exposed to styrene 5 days per week via oral gavage. Rats were exposed at dose levels of 0, 1,000 and 2,000 mg/kg bw/day for 78 weeks and 0 and 500 mg/kg bw/day for 103 weeks. The 500 mg/kg bw group and extra control group were added later due to excessive mortality in the high dose groups. Tumour incidences were statistically analysed with a Fisher exact test (one-tailed). Mortality was significantly higher in high-dose male and female rats compared to control (both $P<0.001$). A slight dose-related mean body weight depression was observed in males. There was no significant increase in tumour incidences.

A chronic toxicity and reproduction study was performed by Beliles et al.[202] In the chronic toxicity part of the study, male (76 controls and 50/exposure group) and female (106 controls and 70/exposure group) Charles River COBS (SD) BR rats were continuously exposed to styrene (purity: 98.9%) orally for two years via drinking water at concentrations of 0, 125 and 250 ppm. Survival of both male and female rats was not affected by styrene exposure. A decrease in terminal body weight and increased relative brain weight was observed in females (250 ppm).
Water consumption was decreased in both males and females (125 ppm and 250 ppm) and a dose-response relationship was established.
There were no reported treatment-related increased incidences of non-neoplastic lesions or neoplastic lesions.

A carcinogenicity study in BD IV rats was performed by Ponomarkov et al.[199] Pregnant dams (21 exposed, 10 control) were given a single oral administration of styrene (1350 mg/kg bw, purity: 99%) or olive oil via gavage at gestation day 17. Their offspring was treated from the time of weaning weekly for the whole lifespan with 500 mg/kg bw styrene or olive

oil via oral gavage. Details of statistical analysis were not reported. Preweaning mortality of the offspring of styrene-treated females given a single administration of styrene during pregnancy was higher compared to the offspring of olive-oil treated dams. There were no other differences in survival or body weight. A non-significant increased incidence was observed in tumour-bearing females receiving a single styrene administration during pregnancy.

Inhalation studies

Jersey et al. performed a carcinogenicity study in 1978. This study was not published and data were summarized by the NTP based on information retrieved from secondary sources in which the study was reviewed.[203] The NTP also performed a Cochran-Armitage exact trend test on tumour incidences. Sprague-Dawley rats (7-8 weeks old) were exposed to styrene (purity 99.5%) via inhalation at concentrations of 0, 600 or 1000 ppm (corresponding to 0, 2556 or 4260 mg/m3 conform the CLP-guidance). Each group consisted of 96/97 males and 96 females, and they were exposed for 5 days/week until 50% mortality was reached at 18.3 (females) or 20.7 (males) months. Initially, the high-dose group was exposed to 1200 ppm styrene, but due to excessive toxicity, this was reduced to 1000 ppm after 2 months. Survival was lower in males than in females. The committee noted that others (McConnell and Swenberg, 1994) state that the presence of chronic murine pneumonia caused excessive mortality in control and exposed males.

In females, the incidence of mammary adenocarcinoma was increased at 600 ppm compared to control, but not when compared to historical controls. The P-value for trend was 0.002. A statistically significant increased incidence of combined lymphosarcomas and leukaemia was observed in females compared to incidences in historical controls, but not when compared to the concurrent controls. The P-value for trend was 0.035. However, the committee agrees with McConnell and Swenberg, 1994 that the reliability of this study was limited by the presence of chronic murine pneumonia.

A chronic toxicity/oncogenicity study was performed by Cruzan et al.[204] Rats (70/sex/group) were exposed to styrene at 0, 50, 200, 500, or 1000 ppm for 104 weeks. The exposure was performed by inhalation (whole body) of styrene vapour 6h/day 5 days/week for 104 weeks (520 exposures). During week 61, eight males in the 1000 ppm group and six males in the 500 ppm group received a massive dermal exposure of styrene due to a technical problem. All died or were sacrificed and were not included in the analysis. There were no further effects on survival of male rats.
A dose-related increase in survival of female rats was noticed.
No statistically significant treatment-related increase of the number of animals bearing tumours was observed in males and females.
A treatment-related decrease was noted in pituitary adenomas in females.
Additionally, a treatment-related decrease in mammary adenocarcinomas

in females was noted as well as a treatment-related decrease in mammary fibroadenomas in females.

4.2.3 Studies with styrene-7,8-oxide in mice

For the evaluation of carcinogenicity, one study by oral gavage with styrene-7,8-oxide in male and female mice was considered.[205]

Oral studies

B6C3F1 mice (52/sex/group) were treated with styrene-7,8-oxide via oral gavage at concentrations of 0 (vehicle), 375 mg/kg bw and 750 mg/kg bw, 3 times per week for 104 weeks (Lijinsky, 1986).[205] Styrene-7,8-oxide was dissolved in corn oil (purity 96.6%) and the authors noted that 3.3% of the solution consisted of benzaldehyde, benzene and one other unspecified compound. Fisher's exact tests and Cochran-Armitage tests were performed, but it is not clear to what data these were applied. Survival of animals (750 mg/kg bw) was lower compared to control; half of the group died by 60 weeks. Weight gain was reduced in males and females (375 and 750 mg/kg bw) compared to control and weight loss was observed in males (375 and 750 mg/kg bw) after 75 weeks (no details). Some non-neoplastic lesions occurred, although their incidences were not reported, as summarized in Table B3, Annex B. Increased incidences in combined liver carcinomas and adenomas were observed in males, which were statistically significantly different from the controls in the 375 mg/kg group ($P<0.001$). Increased incidences of papillomas (in males and females), carcinomas (in males), and the combination of both (in males and females) were observed in the forestomach, which were statistically significantly different from controls at doses of 375 and 750 mg/kg bw ($P<0.001$). There was a decreased incidence of malignant lymphoma and leukaemia in females (750 mg/kg bw, $P=0.01$).

4.2.4 Studies with styrene-7,8-oxide in rats

For the evaluation of carcinogenicity, two studies with styrene-7,8-oxide in rats were considered, of which one study by gavage in males and females,[205] and one study involving transplacental exposure followed by oral exposure by gavage in male and female pups.[205,206]

Oral studies

F344 rats (52/sex/group) were treated with styrene-7,8-oxide via oral gavage at concentrations of 0 (vehicle), 275 mg/kg bw and 550 mg/kg bw, 3 times per week for 104 weeks.[205] Styrene-7,8-oxide was dissolved in corn oil (purity 96.6%) and the authors noted that 3.3% of the solution consisted of benzaldehyde, benzene and one other unspecified compound. Survival and weight gain of animals in the 550 mg/kg bw group was reduced compared to control. A small weight loss was observed in males (550 mg/kg bw) after 75 weeks (no details reported). Increased incidence of combined carcinomas and papillomas in the forestomach was observed in treated males and females, which was

statistically significantly different from controls in the males at 275 mg/kg (P<0.001). This styrene-related increased incidence in forestomach tumours was also confirmed by an increased incidence of hyperplasia in the forestomach. Because some of the rats given the high dose died relatively early with neoplasms attributable to the treatment, the incidences of some of the common spontaneous neoplasms, such as islet cell adenomas and/or carcinomas of the pancreas, mammary fibroadenomas, neoplastic nodules of the liver in females, and endometrial stromal polyps, were lower in the treated animals than in the controls. There was a decreased incidence of leukaemia in males and females (both 550 mg/kg bw) compared to control, which was, according to the study authors, considered less likely due to the early deaths.

A carcinogenicity study in BDIV rats was performed by Ponomarkov et al.[206] Pregnant dams (14 exposed, 14 control) were given a single oral administration of styrene-7,8-oxide (200 mg/kg bw, purity: 97%) or olive oil at gestation day 17. Their offspring was treated with 96 weekly doses of styrene-7,8-oxide (100-150 mg/kg bw) or olive oil from week 4 of age (weaning) until termination of the experiment at 120 weeks. Styrene-7,8-oxide was administrated via oral gavage. Litter size, preweaning mortality, offspring mortality and body weights did not differ between the groups. No carcinogenic effects were observed in the pregnant dams except that the incidence in tumour-bearing pregnant dams was decreased compared to the control group (31% for styrene-7,8-oxide and 57% in controls). In treated offspring, the percentage of tumour-bearing animals was 77% (females) and 52% (males) versus 58% (females) and 20% (males) in the control group. An increased incidence in several types of forestomach tumours was observed in treated offspring. The incidence of carcinomas in situ and early carcinomas or carcinomas increased significantly in both males and females, ranging from P<0.0001 to P<0.04. The number of papillomas was increased in males only (P<0.003). Early changes in squamous epithelium were frequently observed in styrene-7,8-oxide groups. Other increased tumour incidences were not statistically significant.

4.3 Evidence synthesis regarding carcinogenicity

Human data

Several large and well-performed epidemiological studies are available. The committee considers the evidence from the boatbuilder study in Washington State and the European cohort as most relevant as they present dose-response relationships within exposed workers only. This reduces the impact of bias. The study of Daniels et al. (2020) showed an elevated risk for leukaemia and bladder cancer[172] and the study of Loomis et al. (2019) showed an elevated risk for non-Hodgkin-lymphoma, oesophageal and pancreatic cancer.[179] Although bias and confounding cannot be excluded, the committee concludes that overall there is limited evidence of carcinogenicity from human studies.

Animal data

In several studies with mice, exposure to styrene led to an increased incidence of lung tumours in B6C3F1, O20, and CD-1 mice.[197,198,199,200,201] In these studies, the increased incidence in lung tumours consisted of adenomas (benign) or a combination of adenomas and carcinomas (malignant). The committee carefully evaluated the evidence of the increased incidence of these tumours, taking into account the crucial role of mouse lung-specific Cyp2 F2 metabolism in the carcinogenicity induced by styrene. This indicates that this tumour response is not relevant, either qualitatively or quantitatively, to humans.[6,7]

Forestomach carcinogenicity was observed in male mice after styrene-7,8-oxide exposure.[205] However, the committee considers forestomach tumours not relevant to humans, based on the WOE decision criteria for assessing the relevance of forestomach tumours in human cancer risk assessment.[207,208]

In some mouse strains, oral exposure to styrene and to styrene-7,8-oxide has been associated with an increased incidence of hepatocellular adenomas and/or carcinomas.[198,199,205] Significantly increased hepato-cellular adenomas were found in female B6C3F1 mice after styrene exposure only at the highest dose,[198] and increased hepatocellular carcinomas/adenomas were found in C57 BL male mice, but without reported statistics.[199] Exposure to styrene-7,8-oxide led to increased combined hepatocellular carcinomas and adenomas in male B6C3F1 mice.[205]

Taking into account the limitations of the styrene studies, and treating the data from styrene-7,8-oxide studies only as supportive evidence, the committee concludes that the findings of hepatocellular adenomas and/or carcinomas in mice provide limited evidence of carcinogenicity.

In rats, the effects of styrene exposure on tumour incidence varied depending on the route of exposure. Inhalation exposure was associated with an increased risk of certain tumours in females, including combined lymphosarcomas/leukaemia and mammary adenocarcinoma.[203] The incidence of lymphosarcomas/leukaemia was increased compared to historical control data rather than concurrent controls. The mammary adenocarcinomas were statistically significant only in the low dose group, but not when compared to the historical control data. The committee notes that the reliability of this study is limited by the presence of chronic murine pneumonia, so less weight has been given to the evidence from this study.[203] On the other hand, another inhalation study showed a decrease in mammary adenocarcinomas and fibroadenomas in females.[204] Other routes of exposure to styrene, such as oral gavage or drinking water, did not show a significant increase in tumour incidence.[197,198,202,199]

After styrene-7,8-oxide exposure in male and female rats, an increased incidence of tumours of the forestomach was found.[205,206] As mentioned before, the committee considers these tumours not relevant to humans based on the WOE decision criteria for assessing the relevance of forestomach tumors in human cancer risk assessment.[207,208] A decreased risk of leukaemia incidence after styrene-7,8-exposure was also observed.[205]

The committee concludes that the evidence for mammary adenocarcinomas in rats, despite the limited study quality, provides limited evidence for carcinogenicity.

4.4 Evaluation of carcinogenicity

Classification of a substance in category 1A requires sufficient evidence from epidemiological studies to support the existence of a causal relationship between human exposure and the development of cancer. Overall, the committee concludes that there is limited evidence of carcinogenicity from human studies, and bias and confounding cannot be excluded. Therefore, category 1A is not applicable.

Classification in category 1B (presumed to be carcinogenic to humans) requires a marked increase in the number of malignant tumours, in at least two experimental animal species, or in a single species in two or more independent studies. From neither mice nor rat studies, firm conclusions can be drawn by the committee regarding the carcinogenicity of styrene. However, the findings on hepatocellular adenomas and/or carcinomas in mice in different studies, and the findings on mammary adenocarcinomas in rats do provide limited evidence for carcinogenicity. Therefore, the committee considers classification category 1B warranted based on the combination of the limited evidence for carcinogenicity from human studies and animal studies.

4.5 Recommendation on the classification for carcinogenicity

The committee recommends classifying styrene as *Presumed to be carcinogenic to humans*, which corresponds with classification in category 1B with H350 (may cause cancer).

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annexes

A1 Summary table of mutagenicity in humans after styrene exposure

Table A1.1 Chromosomal aberration in humans after styrene exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Migliore et al. (2006)	Italy	Fibreglass-reinforced plastic manufacturing	Peripheral blood	72 exposed 89 controls	37.1 (2-535) mg/m3; 300.0 (10.2-1856) mg/g creatinine MA+PGA in urine	Smoking, sex, age	- No significant differences between the workers and controls for total and chromatid-type CAs; controls had significantly higher chromosome-type CA than the exposed group	Adequate study size
Forni et al. (1988)	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/m3; B, 41-198 (after 1978) mg/ m3	Smoking, age, other exposures to mutagenic chemicals	+/- (Factory A, P<0.001; Factory B, P<0.05)	No clear results. No overall effect, only with high dosage (nested case-control)
Oberheitmann et al. (2001)	Germany, NR	Boat manufacturing	Peripheral blood	14 exposed, 7 controls	<100 mg/m3 35 (1.5-211) µg/L styrene in blood	Smoking	+/-	Small study. Elevated, but not statistically significant effect
Jablonická et al. (1988)	Czechia, NR	Laminators of various kinds of sport utensils, boats, and containers	Peripheral blood	11 exposed, 11 controls	253 (118-582) mg/m3 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	-	Small study. No evidence for association

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Sorsa et al. (1991)	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)	Study report not accessible.
Hagmar et al. (1989)	Sweden, 1985-1986	Reinforced plastics production	Peripheral blood	11 exposed, 14 controls 20 exposed, 22 controls	43-221 mg/m3, 4-551 mg/m3 (1974-1986); 128 (<6-317) mmol/mol creatinine, MA+PGA in urine (in 1985)	Smoking, age	- (P>0.5)	Small study. No statistically significant effect
Mäki- Paakkanen (1987)	Finland, 1987	Reinforced plastics workers	Peripheral blood	21 exposed, 21 controls	98 (34-263) mg/m3; 1.6 (<LOD-7) mmol/L MA in urine	Smoking, sex	-	
Pohlová & Srám (1985)	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/ m3; B, ~200 (39-548) mg/ m3	Smoking, acute viral diseases, sex, drug intake The above plus X-ray examinations, alcohol	-	
Hansteen et al. (1984)	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)	Small study. No association
Thiess et al. (1980)	Germany, 1978	Pilot plant and laboratory	Peripheral blood	24 exposed 24 controls	58.1 (0.1-178) ppm (pilot plant); 6.0 (1.0-11.5) ppm (laboratory); urinary MA ranged from 0-320 mg/L		- (P>0.05)	
Thiess & Fleig (1978)	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	-	Small study. No association

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Anwar & Shamy (1995)	Egypt	Reinforced plastics production	Peripheral blood	18 exposed 18 controls	Urinary MA 328.44 ± 266.21 (mg/g creatinine)	Matched on sex, age and smoking habits Analyses only in non-smokers	+ (P<0.05)	No correlations were found between the duration of exposure, level of urinary MA, urinary thioether level and the frequency of CAs or MN in the exposed subjects, other (non-)work related exposures may have contributed to the findings.
Biró et al (2002)	Hungary	Oil refinery	Peripheral blood	10 exposed 25 controls	No exposure data presented	All individuals were interviewed for smoking and drinking habits, age, medications, medical and work histories. No matching for sex or smoking	- (P>0.05)	Small study. No statistically significant association. Smoking may explain the results observed
Somorovská et al. (1999); see also Vodička et al. (2001b)	Czech Republic, 1999	Reinforced plastics workers	Peripheral blood	17 high concentration (I), 12 medium concentration (II), 15 low concentration (III), 19 controls	I: 199.1 (SD, 101.6) mg/m3 II: 55.0 (SD, 22.9) mg/m3 III: 27.3.1 (SD, 25.1) mg/m3	Smoking	I:+ (P<0.001) II:+ (P<0.004) III:+ (P=0.0001) Frequency I: 3.75 ± 1.13 II: 3.27 ± 0.70 III: 2.50± 0.85	Small study. Effects in three different exposure groups with highest frequency of CA in high exposure group.
Vodička et al. (2004)	Czech Republic, 2004	Reinforced plastics workers	Peripheral blood	86 exposed, 42 controls	81.3 (SD, 56.3) mg/m3		-	Adequate size. No association. Low exposure, but comparable to Somorovská et al. (1999)
Helal & Elshafy (2013)	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)	High level exposure
Camurri et al. (1983)	Italy, 1983	Reinforced plastics industries (six plants)	Peripheral blood	25 exposed, 22 controls	NR (30-400) mg/m3	Age, sex, smoking	+ (P<0.005 for all 6 plants)	High level exposure
Andersson et al. (1980)	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/m3; high concentration, 1204 (710-1589) mg/m3	Age, sex	+ (P<0.001)	High level exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Tomanin et al. (1992)	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/m3 Group 1, 186 (46-345); Group 2 725 (423-1325) mg/g creatinine MA in urine	Smoking, age, sex	II:+ (P<0.05) I: -	High level exposure. Less clear results
Nordenson & Beckman (1984)	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)	High level exposure.
Watanabe et al. (1983)	Japan, before 1983	Boat manufacturing	Peripheral blood	18 exposed, 6 controls	40-50 (NR) ppm	Smoking, age, sex	+/-	High level exposure
Artuso et al. (1995)	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/m3	NR	(+) (P<0.01)	Disregarded
Mäki- Paakkanen et al. (1991)	Finland, before 1991	Reinforced plastics production in a plant manufacturing containers	Peripheral blood	17 exposed, 17 controls	300 (NR) mg/m3 (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)	Disregarded
Meretoja et al. (1977)	Finland, 1977	Plants manufacturing polyester plastic products	Peripheral blood	10 exposed, 5 controls	NR	Sex	(+)	Disregarded. Small study, no dose-response relationship
Dolmierski et al. (1983)	Poland, before 1983	Laminated styrene plates production	Peripheral blood	37 exposed, 2 controls	NR (<100) mg/m3		+/-	Disregarded. Small control group and lack of control for confounders
Meretoja et al. (1978)	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)	Disregarded

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Fleig & Thiess (1978)	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		+/-	Disregard. Small study, lack of control for confounders. Suggestion of a dose-response relationship.
Smejkalová et al. (1989)	Czech Republic	Workers occupationally exposed to styrene	Peripheral blood	13 women exposed, 6 women controls	225 (83-366) mg/m3	Sex	+	Disregarded. Small study
Högstedt et al. (1979)	Sweden, 1977	Plant manufacturing polyester resin boats	Peripheral blood	6 exposed, 6 controls	115 (50-400) mg/m3	Sex, age, smoking	(+) (P=0.001)	Disregarded. Small study
Mierauskiene et al. (1993)	Lithuania, before 1993	Chemical plant	Capillary blood	109 exposed, 64 controls	NR (<1.9 ppm) in year before sampling	Sex, smoking	(+) (P<0.01)	Disregarded
Lazutka et al. (1999)	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/m3; (ii) NR (4.4-6.2) mg/m3	Smoking, age	(+) (P<0.0001)	Disregarded
Tates et al. (1994)	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/m3 (8 h TWA)	Smoking, age, sex	(+) (P<0.0001)	Disregarded. Co-exposure

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

Table A1.2 Micronuclei in humans after styrene exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Migliore et al. (2006)	Italy, NR	Reinforced plastics production	Peripheral blood	92 exposed, 98 controls	37.1 (2-535) mg/m3; 300.0 (10.2-1856) mg/g creatinine MA+PGA in urine	Smoking, sex, age	+ (P<0.001)	Adequate study size with statistically significant association
Costa et al. (2012)	Portugal	Fibreglass-reinforced plastic production	Peripheral blood	75 exposed 77 controls	mean 30.4 ppm mean MA+ PGA 443 ± 44 mg/g creatinine		- (P>0.05)	Study population believed to overlap with that of Teixeira et al. (2010) but includes data for both smokers and non-smokers.
Teixeira et al. (2010)	Portugal, NR	Reinforced plastics	Peripheral blood	52 exposed 54 controls	Mean workplace air 29.9 p.p.m. Mean mandelic acid+PGA 419 ± 52 mg/g creatine	Matched for sex, age, smoking, lifestyle habits	- (P>0.05)	Study among non-smokers Study population is believed to overlap with that of Costa et al. 2012
Teixeira et al. (2004)	Portugal, NR	Reinforced plastics	Peripheral blood	28 exposed 28 controls	Mean workplace air 27 ± 5 p.p.m. Mean mandelic acid 401 ± 73 mg/g creatine	Stratified by sex. No differences between smoke status were observed	- (P>0.05)	Small study
Yager et al. (1993)	USA, before 1993	Reinforced plastic boat manufacturing facility	Peripheral blood	48 exposed	64.2 (0.88-235.35) mg/m3 (8 h TWA)	Smoking, sex, age	- (P>0.05)	No association. Subjects are own control (before/after exposure)
Högstedt (1984)	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)	Positive association. Small study.
Sorsa et al. (1991)	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	-	No association. High exposure
Hagmar et al. (1989)	Sweden, 1985-1986	Reinforced plastics production	Peripheral blood	11 exposed, 14 controls 20 exposed, 22 controls	43-221 mg/m3, 4-551 mg/m3 (1974-1986); 128 (<6-317) mmol/mol creatinine, MA+PGA in urine (in 1985)	Smoking, age	- (P>0.5)	No association. Small study.

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Mäki-Paakkanen (1987)	Finland, 1987	Reinforced plastics workers	Peripheral blood	21 exposed, 21 controls	98 (34-263) mg/m3; 1.6 (<LOD-7) mmol/L MA in urine	Smoking, sex	-	No association. Small study.
Vodička et al. (2004)	Czech Republic, 2004	Reinforced plastics workers	Peripheral blood	86 exposed, 42 controls	81.3 (SD, 56.3) mg/m3		+/- (P=0.002)	
Hanova et al. (2010)	Czech Republic, 2010	Reinforced plastics workers	Peripheral blood	62 exposed, 50 controls	50.3 (0-238) mg/m3	Smoking	- (P>0.05)	No association
Godderis et al. (2004)	Belgium, 2000-2001	Reinforced plastics industries	Peripheral blood, nasal mucosa	38 exposed, 41 controls (blood); 23 exposed, 17 controls (nasal mucosa)	9.5 (SD, 9.6) ppm (converted from urine)	Smoking, alcohol consumption, age	+ (P<0.05)	
Anwar & Shamy (1995)	Egypt	Reinforced plastics production	Peripheral blood	18 exposed 18 controls	Urinary MA 328.44 ± 266.21 (mg/g creatinine)	Matched on sex, age and smoking habits Analyses only in non-smokers	- (P>0.05)	No association
Tomanin et al. (1992)	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/m3 Group 1, 186 (46-345); Group 2 725 (423- 1325) mg/g creatinine MA in urine	Smoking, age, sex	- (P>0.05)	High Level exposure. No statistically significant association in both groups
Nordenson & Beckman (1984)	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	+ (one-sided P=0.00017)	Effect was seen in the group of 12 exposed workers.
Van Hummelen et al. (1994)	Belgium	Fibreglass-reinforced polyester factory	Peripheral blood	52 exposed 24 controls	31.0 ± 22.4 (mg/m^3) in air 102 ± 99 (mg/g creatinine) mandelic acid in urine	Stratified by smoke status	- (P>0.05)	Controls had statistically higher MN frequencies than the workers. Exposure was rather low and the number of working years was small

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Laffon et al. (2002)	Spain, NR	Reinforced plastics production	Peripheral blood	14 exposed 30 controls	Extrapolated means, 16.76-19.33 ppm; 313-352 mg/g creatinine MA in urine.	Age, smoking	Overall (+) (p≤0.01) Stratified by age statistically non-significant Stratified by smoking only statistically significant in exposed smokers	Authors note that it was not possible to determine that the genotoxic effects detected were caused only by styrene; also noted the small number of subjects and a large impact of age on MN frequency in both controls and workers.
Holz et al. (1995)	Germany, 1991	Styrene production plant	Peripheral blood	25 exposed, 25 controls	0.31 (SD, 0.88) ppm (8h TWA)	Smoking, age, sex	Overall - (P>0.05) Kinetochore micronuclei+ (p=0.007)	
Mäki-Paakkanen et al. (1991)	Finland, before 1991	Reinforced plastics production in a plant manufacturing containers	Peripheral blood	17 exposed, 17 controls	300 (NR) mg/m3 (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	-	Small study
Tates et al. (1994)	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/m3 (8h TWA)	Smoking, age, sex	(+) (P<0.0001)	Disregarded. Co-exposure, styrene secondary exposure

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

Table A1.3 Aneuploidy and diploidy in humans after styrene exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Comments committee
Naccarati et al. (2003)	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)	No overall association, positive association among exposed non-smokers (n=6)

[a] +/-, equivocal (variable response in several experiments within an adequate study).

Tabel A1.4 Gene mutation in humans after styrene exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Comments committee
Compton-Quintana et al. (1993)	Berkeley, USA, 1993	Boat manufacturing and maintenance workers	Peripheral blood	15 high concentration 22 low concentration	32 ppm 1.2 ppm (8h TWA)		+ (*P*=0.028)	Association effected by smoking.
Bigbee et al. (1996)	Finland, 1996	Reinforced plastics workers	Peripheral blood	47 exposed, 47 controls	37 (6-114) ppm (8h TWA)	Age, smoking, sex	- (*P*=0.058) + (p=0.036)	No overall association. Statistically significant association among highly exposed.
Vodička et al. (2001b)	Czechia, 1999	Reinforced plastics workers	Peripheral blood	19 exposed, 19 controls	101.2 (SD, 102.4) mg/m3		+/- (*P*>0.05)	Two outliers in exposed group.
Vodička et al. (1999)	Czechia, 1995	Reinforced plastics workers	Peripheral blood	13 exposed, 13 controls	68.0 (15-156) mg/m3	Smoking	(+) (*P*=0.039)	
Vodička et al. (1995)	Czechia, 1993- 1994	Hand-lamination workers	Peripheral blood	9 exposed, 15 controls	91 (25-250) mg/m3	Smoking	+ (*P*=0.021)	No association compared to factory controls, but association found compared to laboratory controls.

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

A2 Summary tables of mutagenicity in animals after styrene and styrene-7,8-oxide exposure

Table A2.1 Chromosomal aberration in animals after styrene exposure

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Kligerman et al. (1993)	Rat, F344 (F)	Lymphocytes	500 ppm	Inhalation, 6 h/d, 14 d	-
Sinha et al. (1983)	Rat, Sprague- Dawley (M, F)	Bone marrow	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 1 yr	-
Preston & Abernethy (1993)	Rat, F344 (M)	Peripheral blood lymphocytes	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	-
Kligerman et al. (1993)	Mouse B6C3F_1 (F)	Lung, spleen	500 ppm	Inhalation, 6 h/d, 14 d	-
Loprieno et al. (1978)	Mouse, CD1 (M, F)	Bone marrow	1000 mg/kg	Gavage, single dose (1×), 24 h after treatment	-
Sbrana et al. (1983)	Mouse, CD-1 (M)	Bone marrow	200x70, 500x4 mg/kg	Oral, 4 or 70 mg/kg per day	-
Sharief et al. (1986)	Mouse, C57BL/6 (M)	Bone marrow	1000 mg/kg bw	Intraperitoneal injection, BrdU-labelled M1 cells 16 h after BrdU implantation	-
Norppa et al. (1980)	Hamster, Chinese (M)	Bone marrow	300 ppm	Inhalation, 6 h/d, 5 d/wk, 4 d or 3 wk	-

[a] -, negative. The level of significance was set at P<0.05 in all cases.

Table A2.2 Chromosomal aberration in animals after styrene-7,8-oxide exposure

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Fabry et al. (1978)	Mouse, BALB/c (M)	Fetus	250 mg/kg bw	Intraperitoneal injection, mate after 1-3 wk	-
Fabry et al. (1978)	Mouse, BALB/c (M)	Spermatocytes	250 mg/kg bw	Intraperitoneal injection, 2 mo after treatment	-
Fabry et al. (1978)	Mouse, BALB/c (M)	Bone marrow	250 mg/kg bw	Intraperitoneal injection, 1-13 d	-
Loprieno et al. (1978)	Mouse, CD1 (M, F)	Bone marrow	50 mg/kg bw	Gavage, 1×, 24 h after treatment	+
Sinsheimer et al. (1993)	Mouse, CD-1 (M)	Bone marrow	Enantiomer (*S*- or *R*-) 100 mg/kg bw	Intraperitoneal injection, 24 h after treatment	+
Norppa et al. (1979)	Hamster, Chinese (M)	Bone marrow	100 ppm	Inhalation, 9 h	-
Norppa et al. (1979)	Hamster, Chinese (M)	Bone marrow	500 mg/kg bw	Intraperitoneal injection, 24 h after treatment	(+)

[a] +, positive; -, negative; (+), positive result in a study of limited quality. The level of significance was set at *P*<0.05 in all cases.

Table A2.3 Micronuclei in animals after styrene exposure

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Gaté et al. (2012)	Rat, F344 (M)	Leukocytes. peripheral blood reticulocytes	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	-
Kligerman et al. (1993)	Rat, F344 (F)	Lymphocytes	500 ppm	Inhalation, 6 h/d, 14 d	-
Simula & Priestly (1992)	Rat, Porton (M)	Bone marrow (PCE)	3000 mg/kg	Intraperitoneal injection, 48 h after treatment	-
Kligerman et al. (1993)	Mouse B6C3F1 (F)	Lung, spleen	500 ppm	Inhalation, 6 h/d, 14 d	-
Vodička et al. (2001a)	Mouse, NMRI (M)	Bone marrow	1500 mg/m3	Inhalation, 5 h/d, 7 d/wk, 1-21 d	+/-
Engelhardt et al. (2003)	Mouse, NMRI (NR)	Bone marrow (PCE)	1500 mg/m3	Inhalation, 6 h/d, 1-21 d	-
Simula & Priestly (1992)	Mouse, LACA Swiss (M)	Bone marrow (PCE)	600 mg/kg	Intraperitoneal injection, 48 h after treatment	+
Norppa (1981)	Mouse, C57BL/6 (M)	Bone marrow (PCE)	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment	+
Penttilä et al. (1980)	Hamster, Chinese (M)	Bone marrow	1000 mg/kg bw	Intraperitoneal injection, 30 h after treatment	-

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

Table A2.4 Micronuclei in animals after styrene-7,8-oxide exposure

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Gaté et al. (2012)	Rat, F344 (M)	Leukocytes	75 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	-
Penttilä et al. (1980)	Hamster, Chinese (M)	Bone marrow	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment	-

[a] -, negative. The level of significance was set at $P<0.05$ in all cases.

A3 Summary table of mutagenicity studies in vitro with styrene and styrene-7,8-oxide

Table A3.1 Chromosomal aberration in in vitro human cells after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Comments committee
Linnainmaa et al. (1978a)	Human lymphocytes (whole-blood lymphocytes)	0.03% (v/v)	+	Lack of concurrent cytotoxicity data and inappropriate endpoint scoring
Pohlová et al. (1984)	Human lymphocytes (whole-blood lymphocytes)	0.5 mM [52 µg/mL]	+	Cytotoxicity data is missing
Jantunen et al. (1986)	Human lymphocytes (whole-blood lymphocytes and isolated lymphocytes)	1 mM [104 µg/mL]	+	
Norppa et al. (1983)	Human lymphocytes (whole-blood lymphocytes)	2 mM [208 µg/mL]	+	

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

Table A3.2 Chromosomal aberration in in vitro human cells after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Comments committee
Fabry et al. (1978)	Human lymphocytes (whole-blood lymphocytes)	0.1 mM [12 µg/mL]	+	Cytotoxicity data is missing
Linnainmaa et al. (1978a)	Human lymphocytes (whole-blood lymphocytes)	0.008% (v/v)	+	Cytotoxicity data is missing
Pohlová et al. (1984)	Human lymphocytes (whole-blood lymphocytes)	0.05 mM [6 µg/mL]	+	Cytotoxicity data is missing

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

Table A3.3 Micronuclei in in vitro human cells after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)
Linnainmaa et al.(1978a)	Human lymphocytes (whole-blood lymphocytes)	0.03% (v/v)	+
Ladeira et al. (2020)	Human lymphocytes (whole-blood lymphocytes)	124 ppm	- No metabolic activation + With metabolic activation

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

Table A3.4 Micronuclei in in vitro human cells after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Comments committee
Linnainmaa et al. (1978a)	Human lymphocytes (whole-blood lymphocytes)	0.008% (v/v)	+	
Speit et al. (2012)	Human lymphocytes (whole-blood lymphocytes)	0.6 mM [72 µg/mL]	+	
Laffon et al. (2001b)	Human peripheral blood lymphocytes	100 µM [12 µg/mL]	+	
Laffon et al. (2003a)	Human lymphocytes (isolated from whole blood)	50 µM [6 µg/mL]	+	
Godderis et al. (2006)	Human peripheral blood mononuclear cells	0.1 mM [12 µg/mL]	+	Cytotoxicity data is missing

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

Table A3.5 Gene mutation in in vitro human cells after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Comments committee
Bastlová et al. (1995), Bastlová & Podlutsky (1996)	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	0.2 mM [24 µg/mL]	+, Hprt locus	Inadequacies in the number of cells treated as well as the cell survival following treatment

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

Table A3.6 Chromosomal aberration in in vitro mammal cells after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)	Comments committee
Ishidate & Yoshikawa (1980)	Chinese hamster, lung cells	100 µg/mL	-	(+)	Limitations on data reported
Matsuoka et al. (1979)	Chinese hamster, lung cells	250 µg/mL	-	(+)	Lack of concurrent measure of cytotoxicity

[a] -, negative. The level of significance was set at P<0.05 in all cases.

[b] (+), positive result in a study of limited quality. The level of significance was set at *P*<0.05 in all cases.

Table A3.7 Chromosomal aberration in in vitro mammal cells after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results (with metabolic activation)
Turchi et al. (1981)	Chinese hamster, lung V79	90 µg/mL	+	

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

Table A3.8 Micronuclei in in vitro mammal cells after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results (with metabolic activation)
Turchi et al. (1981)	Chinese hamster, lung V79	90 µg/mL	+	

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

Table A3.9 Gene mutation in in vitro mammal cells after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
Loprieno et al. (1976)	Chinese hamster, lung V79	1771 µg/mL	-	
Beije & Jenssen (1982)	Chinese hamster, lung V79	6250 µg/mL	-	+

[a] -, negative. The level of significance was set at P<0.05 in all cases.
[b] +, positive. The level of significance was set at P<0.05 in all cases.

Table A3.10 Gene mutation in in vitro mammal cells after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a] (no metabolic activation)	Results (with metabolic activation)
Amacher & Turner (1982)	Mouse, L5178 lymphoma cells	13.80 µg/mL	Tk locus	+	-
Loprieno et al. (1976)	Chinese hamster, lung V79	1020 µg/mL	Hprt locus	+	
Loprieno et al. (1978)	Chinese hamster, lung V80	504 µg/mL	Hprt locus	+	
Beije & Jenssen (1982)	Chinese hamster, lung V79	240 µg/mL	Hprt locus	+	-

[a] +, positive. The level of significance was set at P<0.05 in all cases.
[b] -, negative. The level of significance was set at P<0.05 in all cases.

Table A3.11 Chromosomal aberration in non-mammalian experimental system after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results (with metabolic activation)
Linnainmaa et al. (1978a, b)	Plant Allium cepa	0.01%, 90 µg/mL	+	

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

Table A3.12 Chromosomal aberration in non-mammalian experimental system after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results (with metabolic activation)
Linnainmaa et al. (1978a, b)	Plant Allium cepa	0.05% [500 µg/mL]	+	

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

Table A3.13 Aneuploidy in non-mammalian experimental system after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results (with metabolic activation)
Penttilä et al. (1980)	Drosophila melanogaster null	500 µg/mL, feed	-	

[a] -, negative. The level of significance was set at P<0.05 in all cases.

Table A3.14 Gene mutation in non-mammalian experimental systems after styrene exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
Donner et al. (1979)	Drosophila melanogaster	182 µg/mL, feed	Sex-linked recessive lethal mutations	+	
Rodriguez-Arnaiz (1998)	Drosophila melanogaster	1040 µg/mL, feed	Somatic mutation	-	
Del Carratore et al. (1983)	Saccharomyces cerevisiae D7	104 µg/mL	Gene conversion	+	
Paolini et al. (1988)	Saccharomyces cerevisiae D7	12.5 mM [1300 µg/mL]	Gene conversion, mitotic crossing over, reverse mutation		- - - (Liver S9 from mice given 1 injection of chemical inducers (phenobarbital and β-naphthoflavone)
Paolini et al. (1988)	Saccharomyces cerevisiae D7	12.5 mM [1300 µg/mL]	Gene conversion, mitotic crossing over, reverse mutation		- - - (Liver S9 from mice given 2 injections of inducers (phenobarbital and β-naphthoflavone) 4 or 5 weeks apart)
Loprieno et al. (1976)	Saccharomyces pombe P1	10 400 µg/mL	Forward mutation	-	-
Bauer et al. (1980)	Saccharomyces pombe P1	2080 µg/mL	Forward mutation		-

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
Del Carratore et al. (1983)	Saccharomyces cerevisiae D7	104 µg/mL	Reverse mutation	+	
Vainio et al. (1976)	Salmonella typhimurium TA100	52 µg/mL	Reverse mutation	(+)	(+)
De Meester et al. (1977)	Salmonella typhimurium TA100, TA1537, TA1538, and TA98	100 µmol/plate [5200 µg/mL]	Reverse mutation	-	-
Stoltz & Whitey (1977)	Salmonella typhimurium TA100, TA1535, TA1537, TA1538, and TA98	500 µg/mL	Reverse mutation	-	-
Watabe et al. (1978)	Salmonella typhimurium TA100, TA1535, TA1537, TA1538, and TA98	250 µg/mL	Reverse mutation		-
Busk (1979)	Salmonella typhimurium TA100, TA1535, TA1537, TA1538, and TA98	104 µg/mL	Reverse mutation	-	-
De Flora (1979)	Salmonella typhimurium TA100, TA1535, TA1538, and TA98	250 µg/mL	Reverse mutation	-	-
Florin et al. (1980)	Salmonella typhimurium TA100, TA1535, TA1537, and TA98	312 µg/mL	Reverse mutation	-	-
De Meester et al. (1981)	Salmonella typhimurium TA100	1000 µg/mL	Reverse mutation	-	+
Brams et al. (1987)	Salmonella typhimurium TA100 and TA98	500 µg/mL	Reverse mutation	-	-
De Meester et al. (1981)	Salmonella typhimurium TA1530	0.02 µg/mL	Reverse mutation	+	+
Vainio et al. (1976)	Salmonella typhimurium TA1535	0.5 µg/mL	Reverse mutation	-	+
De Meester et al. (1977)	Salmonella typhimurium TA1535	52 µg/mL	Reverse mutation	-	+
Poncelet et al. (1980)	Salmonella typhimurium TA1535	521 µg/mL	Reverse mutation	NT	+
De Meester et al. (1981)	Salmonella typhimurium TA1535	1000 µg/mL	Reverse mutation	-	+
Vainio et al. (1976)	Salmonella typhimurium TA1537, TA1538, and TA98	52 µg/mL	Reverse mutation	-	-
De Meester et al. (1981)	Salmonella typhimurium TA1537, TA1538, and TA98	1000 µg/mL	Reverse mutation	-	-
Zeiger et al. (1988)	Salmonella typhimurium TA97, TA98, TA100, TA1535, and TA1537	1666 µg/plate	Reverse mutation	-	-

[a] +, positive; -, negative;+/-, equivocal (variable response in several experiments within an adequate study); (+), positive/negative in a study of limited quality (e.g. only a single dose tested; data or methods not fully reported); the level of significance was set at P<0.05 in all cases.

[b] +, positive; -, negative;+/-, equivocal (variable response in several experiments within an adequate study); (+), positive/negative in a study of limited quality (e.g. only a single dose tested; data or methods not fully reported); the level of significance was set at P<0.05 in all cases.

Table A3.15 Gene mutation in non-mammalian experimental systems after styrene-7,8-oxide exposure

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a](no metabolic activation)	Results[b] (with metabolic activation)
Donner et al. (1979)	Drosophila melanogaster	1000 µg/mL, inhalation	Sex-linked recessive lethal mutations	+	NA
Loprieno et al. (1976)	Saccharomyces cerevisiae	1200 µg/mL	Gene conversion	+	NT
Loprieno et al. (1976)	Schizosaccharomyces pombe	600 µg/mL	Forward mutation	+	NT
Voogd et al. (1981)	Klebsiella pneumoniae	120 µg/mL	Forward mutation	+	NT
Milvy & Garro (1976)	Salmonella typhimurium TA100	200 µg/mL	Reverse mutation	+	NT
Vainio et al. (1976)	Salmonella typhimurium TA100 and TA1535	0.6 µg/mL	Reverse mutation	+	+
De Meester et al. (1977)	Salmonella typhimurium TA100	60 µg/mL	Reverse mutation	+	+
Watabe et al. (1978)	Salmonella typhimurium TA100	250 µg/mL	Reverse mutation	+	NT
Busk (1979)	Salmonella typhimurium TA100	120 µg/mL	Reverse mutation	+	+
Yoshikawa et al. (1980)	Salmonella typhimurium TA100	240 µg/mL	Reverse mutation	+	+
De Flora (1979)	Salmonella typhimurium TA100 and TA1535	NR	Reverse mutation	+	+
Sugiura & Goto (1981)	Salmonella typhimurium TA100	144 µg/mL	Reverse mutation	+	NT
Turchi et al. (1981)	Salmonella typhimurium TA100	120 µg/mL	Mutation	+	NT
Pagano et al. (1982)	Salmonella typhimurium TA100	48 µg/mL	Reverse mutation	+	NT
Glatt et al. (1983)	Salmonella typhimurium TA100	60 µg/mL	Reverse mutation	+	NT
Hughes et al. (1987)	Salmonella typhimurium TA100	500 µg/mL	Reverse mutation	+	+
Einistö et al. (1993)	Salmonella typhimurium TA100	60 µg/mL	Reverse mutation	+	NT
Sinsheimer et al. (1993)	Salmonella typhimurium TA100	120 µg/mL	Reverse mutation	+	NT
Brams et al. (1987)	Salmonella typhimurium TA100	300 µg/mL	Reverse mutation	+	NT
De Meester et al. (1981)	Salmonella typhimurium TA100, TA1530, and TA1535	768 µg/mL	Reverse mutation	+	+
Zeiger et al. (1992)	Salmonella typhimurium TA104	100 µg/plate	Reverse mutation	+	+
Guyonnet et al. (2001)	Salmonella typhimurium TA100	1200 µg/plate	Reverse mutation	+	+
Einistö et al. (1993)	Salmonella typhimurium TA104	120 µg/mL	Reverse mutation	+	NT
Milvy & Garro (1976)	Salmonella typhimurium TA1535, TA1537, TA1538, and TA98	5000 µg/mL	Reverse mutation	+	NT
Vainio et al. (1976)	Salmonella typhimurium TA1535	0.60 µg/mL	Reverse mutation	+	+
De Meester et al. (1977)	Salmonella typhimurium TA1535	24 µg/mL	Reverse mutation	+	+
Stoltz & Whitey (1977)	Salmonella typhimurium TA1535	125 µg/mL	Reverse mutation	+	+
Loprieno et al. (1978)	Salmonella typhimurium TA1535	60 µg/mL	Reverse mutation	+	+
Wade et al. (1978)	Salmonella typhimurium TA1535	250 µg/mL	Reverse mutation	(+)	NT
Watabe et al. (1978)	Salmonella typhimurium TA1535	50 µg/mL	Reverse mutation	+	NT

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a](no metabolic activation)	Results[b] (with metabolic activation)
Busk (1979)	Salmonella typhimurium TA1535	60 µg/mL	Reverse mutation	+	+
El-Tantawy & Hammock (1980)	Salmonella typhimurium TA1535	60 µg/mL	Reverse mutation	+	NT
De Flora (1981)	Salmonella typhimurium TA1535	NR	Reverse mutation	+	+
De Meester et al. (1981)	Salmonella typhimurium TA1535	768 µg/mL	Reverse mutation	+	+
Milvy & Garro (1976)	Salmonella typhimurium TA1537, TA1538, and TA98	5000 µg/mL	Reverse mutation	-	NT
Vainio et al. (1976)	Salmonella typhimurium TA1537	600 µg/mL	Reverse mutation	-	-
De Meester et al. (1977)	Salmonella typhimurium TA1537	6000 µg/mL	Reverse mutation	-	-
Wade et al. (1978)	Salmonella typhimurium TA1537 and TA98	NR	Reverse mutation	-	NT
Watabe et al. (1978)	Salmonella typhimurium TA1537	250 µg/mL	Reverse mutation	(+)	NT
El-Tantawy & Hammock (1980)	Salmonella typhimurium TA1537 and TA98	500 µg/mL	Reverse mutation	-	NT
De Meester et al. (1981)	Salmonella typhimurium TA1537	1150 µg/mL	Reverse mutation	-	-
Vainio et al. (1976)	Salmonella typhimurium TA1538	6 µg/mL	Reverse mutation	-	+
De Meester et al. (1977)	Salmonella typhimurium TA1538 and TA98	6000 µg/mL	Reverse mutation	-	-
Watabe et al. (1978)	Salmonella typhimurium TA1538 and TA98	250 µg/mL	Reverse mutation	-	NT
De Flora (1981)	Salmonella typhimurium TA1537, TA1538, and TA98	NR	Reverse mutation	-	-
De Meester et al. (1981)	Salmonella typhimurium TA1538 and TA98	1150 µg/mL	Reverse mutation	-	-
Vainio et al. (1976)	Salmonella typhimurium TA98	600 µg/mL	Reverse mutation	-	-
Ueno et al. (1978)	Salmonella typhimurium TA98	250 µg/mL	Reverse mutation	-	-
Zeiger et al. (1992)	Salmonella typhimurium TA98	3333 µg/plate	Reverse mutation	-	-
Brams et al. (1987)	Salmonella typhimurium TA97	300 µg/mL	Reverse mutation	+	NT
Einistö et al. (1993)	Salmonella typhimurium TA4001	240 µg/mL	Reverse mutation	+	NT
Einistö et al. (1993)	Salmonella typhimurium TA4006	960 µg/mL	Reverse mutation	(+)	NT
Sugiura et al. (1978)	Escherichia coli WP2 uvrA	720 µg/mL	Reverse mutation	+	NT
Sugiura & Goto (1981)	Escherichia coli WP2 uvrA	480 µg/mL	Reverse mutation	+	NT

[a] +, positive; -, negative;+/-, equivocal (variable response in several experiments within an adequate study); (+), positive/negative in a study of limited quality (e.g. only a single dose tested; data or methods not fully reported); the level of significance was set at P<0.05 in all cases.

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

B1 Summary table of carcinogenicity in humans after styrene exposure

Table B1.1 Boat builders study in Washington State, USA.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Bertke et al. (2021),[171] Daniels et al. (2020),[172] Bertke et al. (2018),[170] Ruder et al. (2017),[169] Ruder et al. (2016),[168] Ruder et al. (2004),[209] Okun et al. (1985),[167] • Retrospective cohort study • Washington State, USA • Boat building Follow-up: Job information 1959-1978 Health outcomes until end 2016 for the last study Censoring: Left censoring: 1959 (use of styrene started in 1957) Right censoring: end 1978 for exposure and work histories Inclusion criteria: employed ≥1 day in glass fibre-reinforced plastic and composites boat manufacturing between 1959 and 1978.	Cumulative exposures were based on job histories, industrial hygiene surveys, and personal air sampling measurements (n=399) and general area air-sampling performed on site in 1978 • Jobs divided into 5 exposure groups, but for most analyses divided into high exposure versus low exposure • Time-weighted average (TWA) exposure over an 8 hour workday. For high exposure jobs mean TWA 42.5 ppm/day (range 12-85 ppm) at plant A and 71.7 ppm /day (10-183 ppm) at plant B. Low exposure estimated at 5 ppm/day • Job histories and demographic data were extracted from company personnel records • Classification of jobs based on level of styrene exposure as evaluated based on in-depth industrial hygiene surveys • Cumulative exposure calculated with life table analysis system	Health outcome: Vital status and causes of death Health assessment: obtained from Social Security Administration and the National Death Index (NDI). Causes of deaths after 1979 obtained from NDI Plus. For death prior to 1979, death certificates obtained from state vital statistics offices and coded by a certified nosologist, according to ICD codes of the ICD version in effect at time of death. For cancer incidence, see specific studies	See results of the selected key publication Daniels et al. (2020)	• Exposure misclassification possible: • No information on exposure before or after leaving job, nor on potential exposure outside job (or other work). Lack of job information after 1978 may have led to underestimation of exposure (with bias towards the null) • No information on lifestyle related factors, in particular smoking and alcohol • No information on other exposures at this job, such as fibreglass, solvents, wood dust, or wood finishing agents • No information on hospitalisation • Left truncation in 1959, but use of styrene in plants started only in 1957 • Work-history records did not indicate specific job titles, with a large range of exposures among jobs classified as high exposure. Therefore, misclassification of exposure not to be excluded • Strong evidence for Healthy Worker Survivor Bias (HWSB)	• Job exposure was also possible to acetone (TWA 50.6 ppm plat A and 54.3 ppm plant B), fibrous glass (not measured), and at much lower concentrations (no quantitative data) to glycols, anhydrides, cobalt hapthenate, and methyl ethyl ketone peroxide or benzoyl peroxide, in the high exposure departments; in other departments exposure was possible to wood dusts, paints, ergonomic stress, and solvents such as toluene, xylenes, and naphtas, and isocyanates. These exposures were not assessed, but mentioned as being possible at the job • Information on exposure of cohort members since 1978 not available. In 1978 at time of job data collection 772 workers were still employed

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Study population: 5,163 boatbuilders working at one of two boat building facilities in Kelso (plant A) and Bellingham (plant B), Washington, USA. Reference population: general population in the state Washington Number of exposed and non-exposed; total amount of person-years;	Statistical analyses: Mostly calculation of standardised mortality ratios (SMR), both for overall mortality and cause-specific (cancer) deaths, and 95% CI's based on Poisson distribution				
Daniels et al. (2020),[172] See general information above Study population: 5,163 workers (after removal of 38 workers with inadequate information), 87% male and 93% Caucasian. Of those, 1958 working directly with styrene Reference population: General US population. Censoring: Date last observed or December 31, 2016; Exposure person-time truncated at 1 October 1978 plus ten-year lag for workers still employed in 1978 Follow-up: Through December 31, 2016 including extended job-exposure matrix	See further general information above for exposure assessment. For this study exposure assessment extended to a job-exposure matrix describing individual cumulative exposure as continuous variable reflecting changes in exposure potential over time: • Exposure scientists blinded to case status • Work history based on job titles and department assignments and linked to exposure levels • Exposure levels measured as described above (general information) • Individual jobs and departments categorised into similar exposure groups by plant based on expert judgement (19 for plant A, 13 for plant B) • Individual cumulative exposure calculated in ppm-years by summing product of exposure (group-specific mean styrene	Health outcomes: All-cause mortality and leukaemia (ICD10 C91-C95) incidence, evaluated as hazard ratios (HRs) exposed versus reference population (HR) Health assessment: See general information above for health assessment Vital status derived from National Death Index (NDI), Social Security Administration, Internal Revenue Service, Washington State Department of Motor Vehicles and a case location service. Data for reference population obtained from Centers for Disease Control and Prevention Wonder Database (1999-2017) with 5-year age groups, races and sexes combined	Total person-years at risk 201,951 (175,930 with truncation) HRs for cancers per 50 ppm-years (95% CI)), lagged 10 years, loglinear models, without SES adjustment, whole cohort, • Smoking-related solid cancers 0.97 (0.87-1.06) • Digestive tract (overall) 0.98 (0.81-1.12) • Oesophagus 1.00 (0.52-1.30) • Stomach 0.06 (Not calculable-1.64) • Intestine 1.06 (0.68-1.28) • Biliary liver gall bladder 1.07 (0.78-1.29) • Pancreas 0.84 (0.43-1.13) • Respiratory (overall) 0.87 (0.71-1.02) • Lung 0.87 (0.70-1.02) • Urinary tract (overall) 1.18 (0.97-1.37) • Kidney 1.12 (0.80-1.37) • Bladder and other urinary 1.27 (0.95-1.61)	See also general information above • Healthy worker effect not assessed • Those working directly with styrene on average worked shorter (1.18 years versus 1.85 years) • Cumulative exposures (unlagged) were highly positively skewed (mean 31 versus median 5.7 ppm-years). This might have detracted from validity of model • As above, no information on lifestyle-related factors. Potential effect of smoking was explored by considering smoking-associated cancers: no association observed • To avoid overestimation of risk at higher exposures, the linear slope between 0-50 ppm-years was used for risk projection. This might have resulted in underestimation of effect size	Compared to previous studies, this one used more detailed employment records and exposure assessment • 46 workers (<1%) lost to follow-up • Average age at end of follow-up 68 years and average length of employment <2 years, with 68% employed <1 year • The unit of 50 ppm-years the HRs were expressed in was based on the NIOSH Recommended Exposure Limit • SES was not included in previous studies. Here it was approximated by category of first job held, related to an occupational prestige score (range 0-100) • Analyses were performed using the NIOSH LTAS.NET life table analysis system • Relatively small study (low statistical power)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	airborne concentrations) and duration spent in each group Statistical analysis: *Cox proportional hazards regression* • Hazard ratios (HRs) per) expressed as per 50 ppm-years with zero exposure as reference; risk-sets matched on race, gender, birth data (5 years margin), and employment duration (<1 year versus ≥1 years). Timescale was age • Exposure-response relation modelled with restricted cubic splines, and full and trimmed loglinear models • Exposure lagged 10 years • Only outcomes with at least 10 deaths modelled • Models adjusted for attained age, sex, race, 5-year birth cohort, employment duration. In sensitivity analysis also adjustment for socioeconomic status (SES). • 95% CIs based on profile likelihood *Working lifetime leukemia risks estimation* • Done with a hypothetical model using the derived leukemia HR and a few assumptions (see further). Risk expressed as styrene concentration causing one extra leukemia case per 10,000 workers exposed ver a working lifetime.		• Lymphatic and haematopoietic (overall) 1.19 (0.99-1.37) • Non-Hodgkin 1.10 (0.58-1.51) • Multiple myeloma 1.18 (0.80-1.56) • Leukemia 1.21 (0.93-1.49) • Myeloid leukemia 1.33 (0.86-1.83) Same as above with SES adjustment Only minor differences *Analyses restricted to exposure <500 ppm-years* Of note (without SES adjustment): • Urinary tract overall 1.43 (1.11-1.79) • Bladder and other urinary 1.64 (1.14-2.33) • Lymphatic and haemopoietic cancers overall 1.37 (1.09-1.69) • Leukemia 1.46 (1.04-1.97) (no cases in persons with cumulative exposure ≥500 ppm-years) Restricted cubic spline models at 50 ppm-years (95% CI): • Urinary 2.39 (1.92-3.25) • Kidney 2.39 (1.92-3.83) • Bladder 6.20 (3.93-11.83) • Lymphatic and haematopoietic 4.32 (3.00-6.56) • Non-Hodgkin 0.01 (Not calculable-3.52) • Multiple myeloma 34 (14.08-96.94)	• In general: this study strongly depended on modelling and underlying assumptions • To account for mortality from competing sources life table analysis was used, under assumption that relative risk is independent of age. Assumption might be incorrect • Further modelling assumption was that increased leukemia risk is persistent, proportional to cumulative exposure, and without a threshold. • Even though more detailed exposure assessment was attempted, bias due to measurement uncertainty and exposure misclassification cannot be ruled out	

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	Subgroup analyses: • Outcomes in a major category with indication of positive exposure-response association • Separate analysis restricted to male baseline mortality and incidence rates • Separate analysis in those with exposure <500 ppm-years Latency analysis: • Models without exposure lag • Grid search over a range of lags (2-40 years) • Time since last exposure among cases, using restricted cubic splines Sensitivity analysis: • Leukemia models without person-time truncation		• Leukemia 4.10 (2.88-7.29) • Myeloid leukemia 11.67 (6.31-30.76) Furthermore, these models showed much higher risks at low exposures than did loglinear models Sensitivity analyses: • Model estimates without lag similar to those with 10-year lag • Leukemia findings not appreciably different when person-time for active workers after 1978 included Latency analysis: • Best-fitted lags >10 years for all cancers; longest lags for non-Hodgkin and multiple myelomas (both 40 years), shortest for kidney cancer (33 years) • Median time since last exposure (TSLE) ranged from 28 years (kidney cancer) to 35 years (multiple myeloma) Risk projection: Estimate of leukemia risk under 10-year lag with trimmed data: linear slope 0.0088 per ppm-year, corresponding to extra risk of 1/10,000 for a 45-year continuous exposure to 0.05 ppm styrene (sex-averaged rate) or 0.03 ppm (male only rates)		

Table B1.2 Six-country study on workers at reinforced plastics production plants.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Loomis et al. (2019),[179] Christensen et al. (2017), Christensen et al. (2018),[184] Nissen et al. (2018),[185] Coggon et al. (2015),[187] Boffetta et al. (1998),[178] Kolstad et al. (1995),[180] (1 of 6 countries) Kogevinas et al. (1994),[177] Kolstad et al. (1994),[181](1 of 6 cohorts countries) Kogevinas et al. (1993),[176] Coggon et al. (1987),[210] (1 of 6 countries) Study population: 37,021-40,688 (all cohorts combined)) workers at reinforced plastics production plants in the 6 countries, organised into 8 subcohorts. Inclusion criteria: Ever employed at one of the plants included in the eight subcohorts	Exposure estimation based on job histories and environmental and biological monitoring data Production records and payroll records of all workers were abstracted	Health outcomes: Cancer mortality, based on cause-specific national death registries	See results of the selected key publication Loomis et al. (2019)	• Risk of bias: no information on potential confounders • HWSB not addressed, 40% of workers <1 year employed • Risk of exposure misclassification; relation exposure measurements to actual individual exposures not very clear; in particular, no quantitative data provided on exposure; moreover, 19,404 workers classified as having 'unspecified tasks'; finally, exposure generally declined over time • Findings in the subgroup analyses are likely the result of (uncontrolled) multiple testing and small numbers • Mortality rates based on individual cohorts and on small numbers and not consistent between countries	• Styrene levels decreased considerably during study period in 5 of the 6 countries • More short-term workers in highly exposed groups (43% versus 34%). Proportions varied considerably between countries • Large study, but follow-up period of mean 13 years probably not enough to detect differences in mortality from lymphatic and haematopoietic cancers • Lack of quantitative data on exposure reduces the value of this study
Loomis et al. (2019), [179] Retrospective cohort study See Kogevinas et al. (1994). Here only differences mentioned.	See Kogevinas et al. (1994). Here only differences mentioned. Exposure categories Exposed (laminators, production workers with mixed tasks or in small plants, and workers who regularly entered areas where	Health outcomes: Mortality from specific cancers. Health assessment ICD 8 and 9 codes of previous study Kogevinas et al. (1994) were regrouped into WHO classification.	Total number of person-years 506,459, of which 407,459 in exposed jobs, and 61,514 of those with exposure duration ≥5 years.	See Kogevinas et al. (1994).	See Kogevinas et al. (1994). Here only differences mentioned. Mean duration of employment was 3.1 years, and workers spent mean 2.2 years in exposed jobs.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Study population: 37,021 reinforced plastics workers at reinforced plastics production plants in the 5 countries. The cohort from Norway (had contributed 9% of person-time) was excluded due to new privacy protection legislation. Furthermore, no new mortality data were added for the English and Danish cohorts Reference population: Unexposed jobs in the cohort Follow-up: Varied per country. Overall: 1945-1991. Mean follow-up 12.8 years. Lost to follow-up approximately 3% Left censoring: First data for which complete payroll records were available for those already employed at start follow-up	styrene was handled) versus unexposed Measurements: • In addition to first study, here mentioned around 18,000 measurements of styrene metabolites mandelic and phenoglyoxylic acid in urine. Exposures before 1965 set equal to Denmark data at 200 ppm and then linearly declining to arithmetic mean of earliest measurement • Mean exposure estimated at 63.1 ppm (in exposed jobs) and mean cumulative exposure at 158.0 ppm-years using the job exposure matrix. Statistical analysis: Poisson regression, ungrouped form (equal to discrete time hazard model), to calculate (hazard) rate ratios (RRs) with likelihood-based 95% CIs. • Follow-up time as time axis (person-year). • Adjustment for age, calendar time, sex, country (all categorically) length of follow-up and time since first exposure (both continuous), with retainment in model of those that changed RR 'appreciably' (not specified). Various exposure indicators were used: exposed versus	Of special note: since previous report classification of leukemias and lymphomas changed, with multiple myeloma and chronic lymphoid leukemia now classified as subtypes of non-Hodgkin's lymphoma. Thus, codes for lymphosarcoma and reticulosarcoma (200), other malignant neoplasms of lymphoid and histiocytic tissue (202) and chronic lymphoid leukemia (201.1) and multiple myeloma (203) were aggregated under non-Hodgkins' lymphoma. Acute and chronic myeloid leukemia (ICD 8/9 205.0 and 205.1) were combined.	*Exposed versus unexposed workers*: • All-cause mortality RR 1.01 (95% CI 0.89-1.14) • All cancer mortality RR 1.01 (0.81-1.17) • Oesophageal cancer mortality RR 3.50 (0.46-26.82) • Prostate cancer mortality RR 1.85 (0.81-6.15) Other cancers RR round 1 *Most highly exposed workers (laminators) versus unexposed* • Oesophageal cancer mortality RR 2.71 (1.00-7.37) • Pancreas cancer mortality RR 1.18 (0.53-2.61) • Prostate cancer mortality RR 1.85 (0.64-5.36) *Exposed workers employed 2-<5 years or >5 years versus those employed, <2 years* • Non-Hodgkin's lymphoma (NHL) mortality RR 1.40 (0.51-3.79) • Pancreas cancer mortality RR 2.12 (0.93-4.38) No increase in mortality >5 years, except for prostate cancer mortality RR 1.35 (0.57 to 3.16) and lung cancer Lung cancer: • Exposure 5-<10 years RR 1.02 (0.65-1.60) • 10-<20 years RR 1.29 (0.77-2.15) • ≥20 years RR 1.56 (0.49-4.97)		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	unexposed, employment as laminator (highest exposure), exposure duration, cumulative exposure (ppm-years) Evaluation of latency: lag times for mean and cumulative exposures of 0,5,10 and 20 years for lymphohaematopoietic cancers and 0, 10 and 20 years for other cancers. Additional analyses for lung cancer, using penalized splines to model exposure-response *Sensitivity analyses*: exclusion of Denmark (in order to assess potential exposure misclassification and bias due to lack of exposure data for years before 1970		No significant trends with duration for any of the cancers *Exposure-response* (only significant results shown): • NHL RR per 100 ppm, 2.31 (1.29-4.12) (only 0-year lag shown) • Oesophageal cancer mortality, cumulative, RR per 100 ppm-year, 20-year lag, 1.16 (1.03-1.31) • Oesophageal cancer mortality, mean, RR per 100 ppm, 20-year lag, 3.36 (1.74-6.49) (also 0 and 10 year lag significant) • Pancreas cancer mortality, mean exposure, no lag, RR 1.89 (1.17-3.06) per 100 ppm. Sensitivity analysis: Exclusion of workers exposed before 1970 resulted in lung cancer mortality RR, cumulative exposure, 1.11 (1.02-120)		

Table B1.3 Workers in the reinforced plastics and composites industry, USA wide.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Collins et al. (2013),[175] Wong et al. (1994),[174] Wong et al. (1990),[173] • Retrospective cohort study • US wide study • 15,908 workers (number for first study, Wong et al. (1990)) at 30 reinforced plastics manufacturing plants, selected based on study feasibility; 24.4% women • Period of exposure 1948-1977 Inclusion criteria: Having worked in an area with potential styrene exposure at any of the 30 plants for at least six months, in the period 1948-1977 Reference population: General white US population (information on race missing for most of cohort, therefore assumed to be white) Follow-up: Latest 2008 (Collins et al.) Censoring: Left: 1 January 1948 Right: those lost to follow-up were censored at last date of contact (mostly end of employment)	Exposure assessment: based on work histories and occasional measurements Work history assessment: Based on employment records, record job title lists were generated for each cohort member. Jobs were grouped according to similar exposure potential, taking into account weighted average exposure values (ppm, categorised into 10 ppm increments) and peak range exposures (ppm). The final result was a grouping of jobs in 173 exposure categories Exposure assessment: Not clear how this was performed: 'with help of a consultancy firm', who visited individual plants around 1980 and performed measurements. Wong et al. (1994) mentions that time weighted average exposures for jobs ranged from 1-200 ppm. In addition to the consultancy firm there was routine exposure monitoring Measures of exposure: • Cumulative exposure grouped into tertiles: 5-<500 ppm; ≥500-<5,000 ppm; ≥5,000 ppm Duration of exposure • Peak exposures • Mean exposure	Health outcomes: Mortality and cancer-Deaths and cause specific mortality. Health assessment: • Deaths among active employees and annuitants identified through company records • Vital status of ex-employees through social security administration records, supplemented with inquires to plant personnel • Death certificates retrieved from state vital statistics departments; causes of death coded according to different versions of ICD (in effect at time of death)	See results of the selected key publication Collins et al. (2013)	• Risk of exposure misclassification is difficult to evaluate, as exposure measurements are not described • No information for the whole cohort on other potential toxic exposures (including smoking), during employment, outside work, during follow-up, and prior to follow-up • Also no information on socioeconomic status, which could be a confounder (would lead to more expected deaths) • No assessment of HWSB	• Most workers exposed relatively shortly: only 22.1% employed at least 5 years • Regarding exposure assessment: this was done by a consulting firm, but no information is provided on the measurements performed. • Entire cohort was assumed to be white (only 1.3% non-white)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	• Time since first exposure (employment) Statistical analyses: • Calculation of (age, sex and calendar year) standardised mortality ratios (SMR) (as percentages) • Cause-specific deaths standardised for age, race, and five-year periods (1948-1977) • Mortality in relation to exposure				
Collins et al. (2013),[175] See general information above • Number of cohort members reduced to 15,826 after removal of duplicates and revision of work histories Follow-up: End of 2008	See general information above In addition: Four measures of exposure were examined: • Cumulative exposure: Mean time-weighted average exposure for an 8-hour workday estimated at 28 ppm. • Peak exposure was set at 100 ppm and 15 minutes of the working day above that limit, and days with at least one peak counted. 100 ppm based on lowest level at which irritation occurs. Mean number of peaks across workers was 113; 6% had >5 years of cumulative peak exposures. • Mean duration of exposure was 4.3 years. • Average exposure: the arithmetic mean of average exposure was obtained by dividing total cumulative exposure by total cumulative duration.	See general information above Deaths were in addition identified from Social Security data, the National Center for Health Statistics and a commercial bureau Causes of death coded by a nosologist according ICD version in effect at time of death.	Total person years 561,530 5026 (32%) deaths identified Whole cohort • All-cause mortality SMR 1.08 (95% CI 1.05-1.11) • All cancers SMR 1.12 (1.05-1.18) • All lymphatic and haematopoietic cancers SMR 0.84 (0.69-1.02) • Respiratory system cancers (ICD10 C30-C39) SMR 1.34 (1.23-1.45) • Non-Hodgkin's lymphoma SMR 0.72 (0.50-1.00) • Leukemia SMR 0.84 (0.60-1.14) • Pancreatic cancer SMR 0.96 (0.73-1.22) • Lung cancer SMR 1.34 (1.23-1.46) • Diabetes mellitus SMR 1.29 (1.09-1.51) • Ischaemic heart disease SMR 1.08 (1.02-1.15) • Nonmalignant respiratory disease SMR 1.15 (1.05-1.27)	See general information above	See general information above • For this study also information was used that at 19 plants asbestos was used (but exposure levels or area specific usage patterns not known). Seems no effect on lung cancer • Lost to follow-up reduced to <1 % • Average exposure were lower in 1977 (25 ppm) than a decade earlier (35 ppm) • Entire cohort was assumed to be white (only 1.3% non-white)?? • No nested case-control study to examine cigarette smoking as potential causes of excess of death, but lung cancer deaths and other deaths commonly related to cigarette smoking including bladder cancer; kidney cancer; bronchitis, emphysema, and asthma; and heart disease were examined in more detail

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	Statistical analysis: Cox proportional hazards for cumulative time-weighted averages (units of 100 ppm-months), adjusted for sex, year of hire and year of birth, with age as time scale Exposure-response trend for smoking related cancers		Restricted to at least 15-year latency similar results (only minor changes in SMRs) Subgroup analysis according to asbestos use at plant showed somewhat higher SMRs for lung cancer at asbestos using versus not asbestos using plants: SMR 1.35 (1.23-1.48) versus 1.30 (1.05-1.58). Similarly for bronchitis, emphysema and asthma: 1.42 (1.21-1.65) versus 1.04 (0.69-1.51) Analysis per cumulative exposure categories (with cut-offs 150 ppm-months, 400, and 1,200 ppm-months (only P-values shown for significant trends): • Lung cancer: P trend=0.003 • Kidney cancer: P trend=0.045 • All heart diseases: P trend=0.028 Cox proportional hazards: • Pancreatic cancer HR 1.008 (1.002-1.015), but poor model fit (P=0.196) • Kidney cancer HR 1.009 (1.000-1.017) Analysis per peak exposure categories: There are no major differences among the risk estimates of the four exposure categories. No trends with peak exposures are seen.		

B2 Summary table of carcinogenicity tests with styrene in animals

Table B2.1 Oral studies with styrene in mice

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
NCI, 1979a[197]	Mice B6C3F1 Males and females Controls: 20/sex Exposed: 50/sex/dose group	Test item: Styrene (70%) and β-nitrostyrene (30%) in corn oil Oral gavage 3 times/week 78 weeks Dosing males/females (expressed in β-nitrostyrene) 0 (vehicle) 87.5 mg/kg bw 175 mg/kg bw Endpoint: 14 weeks after the treatment Observations: Full necropsies and histopathological examinations were performed on all animals.	Survival and body weight: • In males: a dose-response relation for mortality (P=0.007). • In females: mean body weight was decreased in 175 mg/kg bw group Non-neoplastic lesions: • Haemorrhage and necrosis in the liver of males: 0 mg/kg: 1/20 87.5 mg/kg: 3/50 175 mg/kg: 16/50 Neoplastic lesions: • Combined lung alveolar/bronchiolar carcinoma or adenomas in <u>males</u>: 0 mg/kg: 0/20 87.5 mg/kg: 11/49 (P=0.016) 175 mg/kg: 2/36	Statistical analyses: • Survival: Kaplan Meier • Dose-response relations: Cox method with Tarone's extension • Tumour incidence: Fisher exact test (with Bonferroni correction) • Cochran-Armitage test for linear trend in proportions Authors report one-tailed p-values	Non-GLP. Non-guideline. Authors conclude that there is no convincing evidence for carcinogenicity in mice as lung tumours increased at medium dose, not at highest dose. Lower tumour incidence in highest dose group is probably due to high mortality rate. For mice, the Fisher Exact was significant; the Cochran-Armitage not significant Not clear what happened to 14 males lost in high dose group. Only mice surviving at least 52 weeks included. Survival till end was respectively: 18/20, 43/50, 33/55
NCI 1979b[198]	Mice B6C3F1 Males and females Controls: 20/sex Exposed: 50/sex/dose group	Test item: Styrene (in corn oil). Purity not mentioned. Oral gavage 5 days/week 78 weeks Dosing males/females 0 (vehicle) 150 mg/kg bw 300 mg/kg bw	Survival and body weight: • In males: mortality was increased in all dose groups. • In females: slight dose-related mean body weight depression, mortality was not affected. Neoplastic lesions: • Combined lung alveolar/bronchiolar carcinoma or adenomas in <u>males</u>: 0 mg/kg bw: 0/20 150 mg/kg bw: 6/44 300 mg/kg bw: 9/43 (P=0.024)	Survival: Kaplan Meier • Dose-response relations: Cox method with Tarone's extension • Tumour incidence: Fisher exact test (with Bonferroni correction) • Cochran-Armitage test for linear trend in proportions Authors report one-tailed p-values.	Non-GLP. Non-guideline. The study authors note a large variation and higher incidence in occurrence of lung tumours in untreated historical control male mice compared to the vehicle controls in the current study.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Endpoint:13 weeks after treatment Observations: Full necropsies and histopathological examinations were performed on all animals.	• Hepatocellular adenomas in females: 0 mg/kg: 0/20 50 mg/kg: 1/44 300 mg/kg: 5/43 (P=0.034)		
Ponomarkov et al., 1978[199]	Mice O20 Pregnant dams Control: 9 Exposed: 29 Offspring males Control: 20 Exposed: 45 Offspring females Control: 22 Exposed: 39 Extra control group Males: 54 Females: 47	Test item: Styrene (in olive oil) Purity: 99% Oral gavage Single administration on day 17 of gestation (pregnant dams) Weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan. Dosing: 0 (olive oil or untreated) 1350 mg/kg bw Endpoint: 120 weeks Observations: Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned. Treatment of offspring was suspended after 16 weeks due to toxicity.	Survival: • Preweaning mortality was higher in the styrene group (43% versus 22% in olive oil controls). • High mortality in styrene progeny group: at 20 weeks, 50% of males and 20% of females died. • Observed lesions: liver necrosis, spleen hypoplasia, congestion of lungs. • Average age of death: 32 weeks (males, styrene), 49 weeks (females, styrene), 88 weeks (vehicle males), 85 weeks (vehicle females). Observed lesions (survival <45 weeks): liver inflammation around necrosis area, bronchitis and peribronchitis. Observed lesions (survival>45 weeks): abscess cavities in liver, calcium deposits. Neoplastic lesions: • Increased incidence in total tumour bearing animals in offspring of styrene-treated dams in males (styrene: 89%, vehicle: 52%) and females (styrene: 100%, vehicle: 67%). • Increase in lung tumours in treated offspring of styrene-treated dams in males (styrene: 89%, vehicle: 42%) and females (styrene: 100%, vehicle: 67%), P<0.01 for both sexes.	No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non-GLP. Non-guideline. An increase in lung tumours was observed, although the background level of lung tumours in these mice is already high. These mice are highly susceptible to developing lung tumours. Lung tumours were more likely to occur in the group treated with styrene (effective number). The average age of animals with lung tumour was lower in the progeny treated with styrene.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			• Incidence adenocarcinomas in male progeny: Untreated: 12/53 (22.6%) Olive oil: 4/19 (21.1%) Styrene: 8/23 (34.8%) • Incidence adenocarcinomas in female progeny: 14/47 (29.8%) 4/8 (50.0%) 4/21 (19.0%), 7/20 (35.0%) 18/32 (56.2%)* • Lung tumours occurred earlier in styrene-treated group compared to control. Average age of death in mice with lung tumours differed: males (styrene: 49 weeks, vehicle: 84 weeks) and females (styrene: 58 weeks, vehicle: 85 weeks).		
Ponomarkov et al., 1978[199]	Mice C57 BL Pregnant dams Control: 5 Exposed: 15 Offspring males Control: 12 Exposed: 27 Offspring females Control: 13 Exposed: 27 Extra control group Males: 51 Females: 49	Test item: Styrene (in olive oil) Purity: 99% Oral gavage Single administration on day 17 of gestation (pregnant dams), weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan. Dosing: 0 (olive oil or untreated) 300 mg/kg bw Endpoint:120 weeks Observations: Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.	Litter size, preweaning mortality, offspring mortality and body weights did not differ between the groups. Neoplastic lesions: • Increased incidence in tumour-bearing females receiving a single styrene administration during pregnancy. • Lymphomas in females styrene: 10/12 olive oil: 3/5 untreated: 20/47; not statistically significant). • Hepatocellular carcinomas or adenoma in males: styrene: 3/24; olive oil: 1/12; untreated: 1/47	No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non-GLP. Non-guideline. Increased incidence of lymphomas is not statistically significant. Dosage is much higher in the 020 mice than in the BL mice. Only one dose tested, which does not provide enough information about a dose-response relationship.

Table B2.2 Inhalation studies with styrene in mice

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Cruzan et al., 2001[200]	Mice CD-1 Chronic/oncogenicity study and a follow-up study 70/sex/group Males 104 weeks Females 98 weeks Follow-up study: 55 males	Test item: Styrene (Purity: >99.5%) Inhalation, styrene vapour, whole body, 6h/day 5 days/ week for 104 weeks (males), 98 (females) weeks or 13 weeks (males, follow-up). Dosing: 0, 20, 40, 80, and 160 ppm (equivalent of 0, 85, 170, 341, and 682 mg/m3)a Follow up study: 0, 40, and 80 ppm (equivalent of 0, 170, and 341 mg/m3)a Interim kills: 10 animals/sex/ group terminated at week 52 and 78. Full necropsies and full histopathological examinations were performed on all control and 160 ppm animals.	Blood levels of styrene and styrene-7,8-oxide were proportional to the exposure concentration. Survival, observations and body weight: • At 160 ppm, 1 female died during the first week and a second died in the second week (both with hepatocyte necrosis). Inhalation of styrene had no effect on survival of male mice. • No effects of styrene exposure on the appearance, behaviour or clinical observations. • Weight gain was decreased in males (80 ppm: -23%; 160 ppm: -31%) and females (160 ppm: -15%). Food consumption decreased in these groups. • No effect on water consumption. Neoplastic lesions: • No effects at week 52 and 78 interim necropsies. Terminal necropsy: • Total number of tumour bearing mice in females Control: 27 20 ppm: 34 40 ppm: 37 (P<0.05) 80 ppm: 28 160 ppm: 37 (P<0.05) • Incidence of bronchioloalveolar adenomas in males • Control: 15/50 20 ppm: 21/50	Tumour incidence was analysed using methodology described by IARC (1980). Other pathologic data were analysed using Fisher's exact test.	GLP-study. Lung: • Increased incidence in areas of bronchioloalveolar hyperplasia in males (40, 80 and 160 ppm) ppm and in females (all exposures) after 24 months. However, the dose-response varies between de groups.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			40 ppm: 35/50 (P<0.05) 80 ppm: 30/50 (P<0.05 160 ppm: 33/50 (P<0.05) • Incidence of bronchioloalveolar adenomas in females Control: 6/50 20 ppm: 16/50 (P<0.05) 40 ppm: 16/50 (P<0.05); 80 ppm: 11/50 160 ppm (24/50). • Incidence of bronchioloalveolar carcinomas in females Control: 0/50 20 ppm: 0/50 40 ppm: 2/50 80 ppm: 0/50 160 ppm: 7/50 (P<0.05)). Non-neoplastic lesions: • Styrene exposure induced changes in the lungs and nasal cavity. • In the terminal bronchioles of the lung, decrease in the eosinophilic staining of the Clara cells at all concentrations at 12, 18 and 24 months. • At 40 ppm, bronchiolar epithelial hyperplasia and greater at 12 months and at 20 ppm and greater at 18 and 24 months. • At 160 ppm, bronchiolar epithelial hyperplasia extending into alveolar ducts after 12 months, at >40 ppm after 18 months and at >20 ppm after 24 months. Nasal passage: Respiratory metaplasia of the olfactory		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			epithelium and changes of the underlying Bowman's glands (present at all intervals in all groups), including dilatation, respiratory metaplasia, epithelial hyperplasia, eosinophilic material/debris and cholesterol clefts. The lesions were time-dependant. Focal loss of bone from the turbinate increased with time. Cellular damage and irritation: all exposure groups at each time interval. These included degeneration, necrosis and atrophy. Follow-up study: • No effects in lungs at all exposures. 80 ppm: • After single exposure: single-cell necrosis in olfactory epithelium of mice. • After 2, 4 and 7 exposures, increase in degree of lesions and changes in the Bowman's glands. - After 40 or 65 exposures: more pronounced atrophy and disorganization leading to respiratory metaplasia. • No recovery occurred. 40 ppm: • Minimal focal changes to the olfactory epithelium; the effects became slightly more severe.		
Cruzan et al., 2017[201]	Mice CD-1 Mice C57BL/6 wild-type (WT) Mice CYP2F2(-/-) (KO)	Test item: Styrene monomer PO-11 Bulk Grade (CAS No. 100-42-5, 99.95% pure) Inhalation 6h/day, 5 days/week, except holidays	• No signs of styrene-induced toxicity in any of the 4 strains of mice based on general observations of behavior or activity. • CD-1, WT and KO mice exposed to styrene weighed less than controls (2-13%; 2-10%; up to 7% respectively). No difference with TG mice.	Body weight: one-way ANOVA Survival: Kaplan and Meier procedure Lung neoplasms and nonneoplastic lesions: Fisher's Exact test	Non-GLP. Non-guideline. An inhibitor of styrene polymer formation, t-butyl catechol, was added to the styrene by the producer at 10-15 ppm. Not clear how long exposure took place.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	Mice 2B6-transgenic (TG), CYP2F2(-/-) 2F1,2A13, 6-7 weeks old 75 animals per group males only Chronic/oncogenicity study (focussing on lung)	Dosing: 0, 120 ppm (equivalent to 0, 511 mg/m3)a styrene vapor	• Mean body weights were lower compared to control at 1, 52 and 78 weeks (CD-1 mice P<0.05), at 1, 24, 52 and 78 weeks (WT mice P<0.05) and at 24 and 78 weeks (KO mice P<0.05). • Cell proliferation in terminal bronchioles was 4- to 5-fold increased at week 1 in exposed CD-1 and WT mice (P<0.05). Non-neoplastic lesions: • Increased incidence of epithelial cell degeneration in terminal bronchioles occurred in WT and CD-1 mice at 1 and 26 weeks (3, 4 or 5 out of 5 mice) and in WT mice at 52 and 78 weeks (1 out of 5 mice). Overall, the incidence up to 104 weeks of exposure CD-1 mice:10/53 WT mice: 34/50 • Hyperplasia occurred in terminal bronchioles in exposed CD-1 mice exposed at week 1, 26, 78 or 104 (P<0.05 at this time point). Overall incidence Control: 0/67 Exposed: 50/67 • Hyperplasia occurred in the terminal bronchioles in WT mice at week 1, 26, 52, 78 and 104 (P<0.05 at this time point). Overall incidence Control: 0/69 Exposed: 55/70		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			Neoplastic lesions: The incidence of bronchioloalveolar carcinoma was significantly increased in CD-1 mice [P<0.05] exposed to styrene (17/67) compared with the CD-1 control mice (7/67) There were no statistically increased tumours in the genetically modified mice (KO, TG)		

Table B2.3 Oral studies with styrene in rats

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Beliles et al., 1985[202]	Rats Charles River COBS (SD) BR Male: Control: 76 Exposure: 50 Female: Control: 106 Exposure: 70	Test item: Styrene (in deionised water) Purity: 98.9% Oral, drinking water Continuous exposure for 2 years Nominal dose 0 (vehicle) 8.9 mg/kg bw/day 17.9 mg/kg bw/day Chronic toxicity (and three-generation reproduction study) Males (10-15) and females (20-30) from each group were mated after 90 days and returned to chronic toxicity study after weaning; Endpoint: At 52 weeks, 10 rats/sex/group were sacrificed.	Weekly analytical mean concentrations were approximately 90% of nominal concentrations. Survival: not significantly different from controls. Clinical findings: decreased mean terminal body weight and increased relative brain weight (250 ppm females; P<0.05), water consumption decreased (125 ppm and 250 ppm males and females; P<0.05; dose-response effect). Non-neoplastic lesions: non-treatment related pathological changes across all groups, no details reported. Neoplastic lesions: no significant increase in treatment-related tumour incidences in rats treated for two years.	• No statistics for tumour incidences • Dunnet's t-test or Wilcoxon Rank sum test for other parameters	Non-GLP. Non-guideline. Only the results of the chronic toxicity segment are reported in this table and the text below. No symptoms were reported. This study is negative but not very informative. Applied dose levels were not high enough due to lack of toxicity. There is no reduced survival due to exposure, which was the case in the oral gavage study in mice.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
NCI, 1979a[197]	Rats F344 Males and females Control: 20/sex Exposed: 50/sex/dose group	Test item: Styrene (70%) and β-nitrostyrene (30%) (in corn oil). Oral, via gavage 3 times/week 79 weeks Dosing: Males: 0 (vehicle) 150 mg/kg bw 300 mg/kg bw Females: 0 (vehicle) 75 mg/kg bw 150 mg/kg bw Endpoint: Animals were sacrificed 29 weeks after the treatment period. Full necropsies and histopathological examinations were performed on all animals.	Survival was not affected by styrene. Mean body weight was decreased in male rats (300 mg/kg bw) compared to control. No significant effects in tumour incidences	• Survival: Kaplan Meier • Dose-response relations: Cox method with Tarone's extension • Tumour incidence: Fisher exact test (with Bonferroni correction) • Cochran-Armitage test for linear trend in proportions	Non-GLP. Non-guideline. Authors report one-tailed p-values. No effects were found in rats exposed to mixture of styrene (70%) and β-nitrostyrene (30%). These results suggest that rats may be less sensitive to the effects of styrene compared to mice.
NCI 1979b[198]	Rats F344 Males and females Control: 2 control groups of 20/sex Exposed: 50/sex/dose group	Test item: Styrene (in corn oil). Purity not mentioned. Oral exposure via gavage. Dosing: 0 (two groups), 500, 1000 and 2000 mg/kg bw, 5 days per week Exposure for 78 weeks for 0 (first control), 1000 and	Mortality was significantly higher in male and female rats compared to control (both P<0.001, 2000 mg/kg bw). Slight dose-related mean body weight depression was observed in males. Neoplastic lesions: There was no significant increase in tumour incidences.	• Survival: Kaplan Meier • Dose-response relations: Cox method with Tarone's extension • Tumour incidence: Fisher exact test (with Bonferroni correction) • Cochran-Armitage test for linear trend in proportions	Non-GLP. Non-guideline. Authors report one-tailed p-values. No effects were found in rats exposed to styrene. These results suggest that rats may be less sensitive to the effects of styrene compared to mice.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		2000 mg/kg bw group, for 103 weeks for 0 (second control) and 500 mg/kg bw rats. Endpoint: Rats were sacrificed at 27 weeks (1000 and 2000 mg/kg bw) or 1 week (500 mg/kg bw) after the exposure period. Initially groups were 60/sex/ dose, this was reduced to 50 due to excessive mortality in week 8 of the study. The 500 mg/kg bw group and extra control group were added later. Full necropsies and histopathological examinations were performed on all animals.			
Ponomarkov et al., 1978[199]	Rats BD IV Pregnant dams Control:10 Exposed: 21 Offspring males Control: 36 Exposed: 73 Offspring females Control: 39 Exposed: 71	Test item: Styrene (in olive oil) Purity: 99% Oral, via gavage Dosing: 0 (vehicle), and 1350 mg/kg bw (dams) or 500 mg/kg bw (offspring) Single administration on day 17 of gestation (pregnant dams), weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan.	Survival and body weights: Preweaning mortality in offspring of styrene-treated pregnant females increased (offspring, styrene: 10%; offspring, olive oil: 2.5%). No differences in survival or body weights. Non-neoplastic lesions: Several lesions in all animals such as congestion of lung and kidney and necrotic areas in liver, forestomach and kidney. Neoplastic lesions: • Stomach tumours occurred Females pregnancy, styrene: 1/20 Offspring females, styrene: 2/68 Offspring females, olive oil: 1/35	No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non-GLP. Non-guideline. There is no increased incidence of tumourgenis observed in rats, unlike the observations in 020 mice. This indicates strain dependency.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Endpoint: All animals were sacrificed at 120 weeks Full necropsies and histopathological examinations were performed on all animals.	• Liver tumours: Offspring females, styrene: 1/68 Other groups: none • Two neurinomas (heart, n. trigeminus) were found in two styrene-treated progeny males. • One neurinoma of the intestine was found in a female treated during pregnancy. One meningioma was observed in a male progeny control.		
Maltoni et al., 1982[196]	Rat Sprague-Dawley Males and females: 40/sex/ group	Test item: Styrene (purity not stated, in olive oil) Oral gavage 4-5 days/week 52 weeks Dosing 0 (vehicle) 50 mg/kg bw/day 250 mg/kg bw/day Endpoint: All animals included until spontaneous death	Total brain tumour bearing animals in males: Control: 0/40; 50 mg/kg bw/day: 1/40 250 mg/kg bw/day: 1/40 Total brain tumour bearing animals in females: Control: 1/40 50 mg/kg bw/day: 4/40 250 mg/kg bw/day: 1/40	Statistics not reported.	Non-GLP Non-guideline Limited reporting on data and methods. No incidence of brain tumours. Results are not significant. Survival data is missing. The committee excludes this study from the evaluation of carcinogenicity due to its poor quality.
Conti et al., 1988[193]	Rats Sprague-Dawley Males and females, 40/sex/ dose group	Test item: Styrene (in olive oil) Purity: 99.8% Oral, via gavage for 4-5 days per week for 52 weeks Dosing: 0 (vehicle), 50 mg/kg bw/day 250 mg/kg bw/day	Survival: Increased mortality rate in females (250 mg/kg bw/day). Neoplastic lesions: • No significant increase in the incidence of any tumour types. • Lower incidence of total benign and malignant tumours and of total mammary tumours in females (250 mg/kg bw/day).	No details on statistical analyses reported.	Non-GLP. Non-guideline. Limited reporting on the data. A higher mortality rate was observed in females at 250 mg/kg bw. It is possible that these deaths may be attributed to other factors, potentially preventing them from having sufficient time to develop tumours.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Endpoint: Males and females, included until spontaneous death. Full necropsies and histopathological examinations were performed on all animals.	• Percentage total benign and malignant mammary tumours in females: Control: 60 50 mg/kg bw/day: 75 250 mg/kg bw/day: 37.5 • Percentage malignant mammary tumours in females: Control: 12.5 50 mg/kg bw/day: 15.0 250 mg/kg bw/day: 12.5		Overall, there is no increase in tumour incidence. The committee excludes this study from the evaluation of carcinogenicity due to its poor quality.

Table B2.4 Inhalation studies with styrene in rats

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Jersey et al., 1978 Not published, based on secondary sources Described by NTP in 2008.[203]	Rat, Sprague-Dawley 7-8 weeks old 96/97 males/group and 96 females/group Carcinogenicity study	Test item: Styrene Purity: 99.5% Inhalation, 6h/day, 5 days/week for 18.3 months (males) or 20.7 months (females). Dosing: 0, 600 or 1000 ppm (first 2 months at 1200 ppm) (corresponding to: 0, 2556 or 4260 mg/m3)a Endpoint: Interim sacrifices of 5/6 animals/sex/group after 6 and 12 months. Exposure until 50% mortality. Observation until death or 24 months.	After 2 months, excessive toxicity in 1200 ppm group. The dose was reduced to 1000 ppm. Survival males Control: 5 600 ppm: 18 1000 ppm: 6 Survival females Control: 30 600 ppm: 30 1000 ppm: 22 Neoplastic lesions: • Incidence of mammary adenocarcinoma in females Control: 1.2% 600 ppm: 8.2% No increase compared to historical control (mean 5.8%, range 0-9%). Trend: P=0.002	Cochran-Armitage exact trend test on tumour incidences, conducted by NTP.	Non-GLP. Non-guideline. Secondary sources (McConnell and Swenberg, 1994) noted that “this study was seriously flawed by the presence of chronic murine pneumonia, which caused a high rate of mortality in both control and exposed male rat.” Survival was lower in males (attributed to chronic murine pneumonia) than in females. Not clear whether nose-only or whole body inhalation was applied. In females the incidence of mammary adenocarcinoma was increased at 600 ppm compared to control, but not when compared to historical controls. The P-value for trend was 0.002.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		No further details about observation.	• Combined incidence of lymphosarcoma and leukemia in females Controls: 1.2% 600 ppm: 7.1% 1000 ppm: 7.1%) Statistically significant increase in females compared to incidence in historical controls (no details in original paper, 1.36% (range 0-2.64%) according to NTP) but not with concurrent controls. Trend: P=0.035 • Combined incidence of lymphosarcoma and leukemia in males Controls: 1.2% 600 ppm: 5.8% 1000 ppm: 1.2%		A statistically significant increased incidence of combined lymphosarcomas and leukemia was observed in females compared to incidences in historical controls, but not when compared to the concurrent controls. The P-value for trend was 0.035.
Cruzan et al., 1998[204]	Rat, CD 4 weeks of age 70/sex/group Chronic toxicity/oncogenicity study	Test item: Styrene (purity: 99.5-99.7%) Inhalation, styrene vapour, whole body, 6h/day 5 days/ week for 104 weeks (520 exposures) Dosing: 0, 50, 200, 500, or 1000 ppm (corresponding to 0, 213, 852, 2130 or 4260 mg/m3) Endpoint: intermittent kills: 9-10 rats/sex/ group sacrificed after 52 weeks Full necropsies and full histopathological examinations were performed on all control and 1000 ppm animals. Histopathologic examination of the nasal passages, lungs,	Analytical concentrations were within 1% of the target concentrations. Levels of styrene and styrene-7,8-oxide in blood at week 95 after exposure were proportional to exposure concentration (with smaller increase for the oxide). Survival:[a] • No effect on survival of male rats. Dose-related increase in survival of female rats (500 or 1000 ppm). Body weights, food and water consumption: • Males (50 ppm): increased weight gain (15%) compared to control. • Males (500 and 1000 ppm): decreased weight gain in males (500 and 1000 ppm) compared to controls (10% and 17% respectively after 1 year) and less food consumption during the first 26 weeks. The weight differences were less at study termination. In the last 6 months the exposed males lost less weight than controls. There was a dose related increase in water consumption compared to controls (121 and 127% during whole study). • Females (200, 500 and 1000 ppm): decreased weight gain compared to controls during the first year (10, 29 and 34% less, respectively). The 500 and 1000 ppm group	Tumour incidence was analysed using methodology described by IARC (1980). Other pathologic data were analysed using Fisher's exact test.	GLP-study No tumours were found. Tumour reduction seen in three tumour types compared with control.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		liver, kidneys, testes/ epididymides, and macro-scopic abnormalities was performed on the animals of all lower exposure levels.	continued to gain less weight throughout the study and consumed 10% less food than controls. Also the 500 and 1000ppm group consumed more water compared to controls in the first 6 months. • Males and females (200 ppm): increased water consumption in the first month (112% of control). Clinical observations, clinical pathology and necropsy: • Clinical signs only observed during exposure: salivation with restlessness, hunched posture. • No adverse effects on clinical pathology • No adverse effects on organ weights • No effects at interim necropsy • Terminal necropsy: increased incidences of testis masses (500 ppm and 1000 ppm males), decreased incidences of enlarged pituitary (500 and 1000 ppm females), increased incidence of pale foci in lung (1000 ppm females). Non-neoplastic lesions[b]: • Treatment-related effects on olfactory epithelium of the nasal passages: • Increased incidence in atrophic and/or degenerative changes in epithelium, number of affected animals increases with increasing dose. • Increased incidence of changes in the Bowman's glands, number of affected animals increases with increasing dose. Neoplastic lesions: • No statistically significant increase in the number of tumours. • Incidence of testes interstitial cell tumours Control: 2/60 50 ppm: 2/60 200 ppm: 2/60 500 ppm: 4/54 1000 ppm: 6/52, but incidences were within historical range.		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			• Treatment-related decreases in pituitary adenomas in females Control: 45/60 50 ppm: 42/49 200 ppm: 35/42 500 ppm: 29/37 1000 ppm: 31/60). Of the female rats that survived 2 years the incidence was 21/28 (control) and 24/49 (1000 ppm). • Treatment-related decrease in mammary adenocarcinomas in females Control: 20/60 50 ppm: 13/44 200 ppm: 9/43 500 ppm: 2/38 1000 ppm: 2/59 • Treatment-related decrease in mammary fibroadenomas in females Control: 21/60 50 ppm: 16/44 200 ppm: 13/43 500 ppm: 18/38 1000 ppm: 17/59 Of the female rats that survived 2 years the incidence was 38% (control), 64% (50 ppm), 58% (200 ppm), 61% (500 ppm), and 33% (1000 ppm).		
Maltoni et al., 1982[196]	Rat, Sprague-Dawley 13 weeks old Males and females (styrene): 30/sex/group Control: 60/sex/group Carcinogenicity study (brain tumours)	Test item: Styrene (purity not stated) Inhalation, styrene in air, 4 hours/day, 5 days/week for 52 weeks. Dosing: 0 (control), 25, 50, 100, 200 and 300 ppm (corresponding to: 0, 106, 213, 426, 852, 1278 mg/m3)[a].	Incidence in total brain tumour bearing animals in males Controls: 0/60 25 ppm: 1/30 100 ppm: 1/30 Incidence in total brain tumour bearing animals in females Controls: 0/60 25 ppm: 1/30 100 ppm: 3/30	Statistics not reported.	Non-GLP. Non-guideline Limited reporting on data and methods. Not clear whether nose-only or whole body inhalation applied There was no significant increase in brain tumours. However, little information is given on how the study was conducted.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Endpoint: All animals included until spontaneous death. Observations: Examination of animals on gross changes every two weeks. Full autopsy and histo-pathology on each animal. Extra examination of brain.			The committee excludes this study from the evaluation of carcinogenicity due to its poor quality.

[a] During week 61, eight males in the 1000 ppm group and six males in the 500 ppm group received a massive dermal exposure of styrene due to a technical problem which resulted in liquid styrene dripping into the exposure chambers in a discrete location at the start of exposure. All died or were sacrificed within the next 2 weeks and were not included in the mortality or tumour analysis.

[b] It is noted that, for the mid-dose levels (50, 200 and 500 ppm), histopathology of some tumour types is only assessed in animals with macroscopic lesions. Hence, the denominator of the incidences is the number of animals for which the histopathological effects were assessed and not the total number of animals in the group.

B3 Summary table of carcinogenicity tests with styrene-7,8-oxide in animals

Table B3.1 Oral studies with styrene-7,8-oxide in mice

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Lijinsky, 1986[205]	Mouse, B6C3F1 7 weeks old Males and females: 52/sex/group Chronic study	Test item: Styrene-7,8-oxide (in corn oil) Purity: 96.6% Oral gavage 3 times per week, 104 weeks Dosing: 0 (vehicle), 375 and 750 mg/kg bw/day, Endpoint: Animals sacrificed at 107 or 108 weeks. Full necropsies and full histopathological examinations on all animals.	Survival of animals (750 mg/kg bw) was lower compared to control, half of the group died by 60 weeks. Reduced weight gain in males females (375 and 750 mg/kg bw). Weight loss in males after week 75 (375 and 750 mg/kg bw). Non-neoplastic lesions: • Lipoid degeneration, focal necrosis and haemorrhage of liver in males (750 mg/kg bw, no incidences reported). • Incidence of hyperplasia in forestomach in <u>males</u> Control: 0/51 375 mg/kg bw: 2/51 750 mg/bw: 2/52 • Incidence of hyperplasia in forestomach in <u>females</u> Control: 1/51 375 mg/kg bw: 6/50 750 mg/bw: 3/51 Neoplastic lesions: • Increased liver carcinomas+ adenomas in <u>males</u> Control: 12/51 375 mg/kg bw: 28/52 (P<0.001) 750 mg/kg bw: 15/52) • Increased forestomach carcinomas+ papillomas in <u>males</u> Control: 2/51 375 mg/kg bw: 37/51 (P<0.001) 750 mg/kg bw: 21/52 (P<0.001) • Increased forestomach carcinomas+ papillomas in <u>females</u> Control: 0/51 375 mg/kg bw: 24/50 (P<0.001) 750 mg/kg bw: 20/51 (P<0.001)	Statistics: Fisher exact test and Cochran-Armitage test	Non-GLP, Non-guideline 3.3% of the styrene-7,8-oxide solution consisted of benzaldehyde, benzene and one other unspecified compound

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			• Incidence of carcinomas of the forestomach in <u>males</u> Control: 0/51 375 mg/kg bw: 16/51 750 mg/bw: 15/52 • Incidence of carcinomas of the forestomach in <u>females</u> Control: 0/51 375 mg/kg bw: 10/50 750 mg/bw: 3/51 • Incidence of papillomas of the forestomach in <u>males</u> Control: 2/51 375 mg/kg bw: 22/51 750 mg/bw: 8/52 • Incidence of papillomas of the forestomach in <u>females</u> Control: 0/51 375 mg/kg bw: 14/50 750 mg/bw: 17/51 • Decreased incidence of malignant lymphoma and leukemia in females (750 mg/kg bw, P=0.01).		

Table B3.2 Oral studies with styrene-7,8-oxide in rats

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Lijinsky, 1986[205]	Rat, F344 9 weeks old Males and females: 52/sex/group Chronic study	Test item: styrene-7,8-oxide (in corn oil) Purity: 96.6% Oral gavage 3 times per week, 104 weeks Dosing: 0 (vehicle), 275 and 550 mg/kg bw/day Endpoint: Animals sacrificed at 107 or 108 weeks.	Survival of animals (550 mg/kg bw) was lower compared to control. Lower weight gain of animals (550 mg/kg bw). Small weight loss in males after 75 weeks (550 mg/kg bw). Non-neoplastic lesions: • Increased incidence of hyperplasia in forestomach in males Control: 2/52 275 mg/kg bw: 10/52 550 mg/kg bw: 9/51 • Non-neoplastic lesions: Increased incidence of hyperplasia in forestomach in females Control: 0/52 275 mg/kg bw: 8/52 550 mg/kg bw: 9/52	Statistics: Fisher exact test and Cochran-Armitage test	Non-GLP, non-guideline 3.3% of the styrene-7,8-oxide solution consisted of benzaldehyde, benzene and one other unspecified compound

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Observations: • Twice daily mortality checks. • Body weight was recorded once a week (first 4 months), every two weeks (next 4 months) and once every 4 weeks (rest of study). Full necropsies and full histopathological examinations on all animals.	Neoplastic lesions: • Increased incidence of combined carcinomas and papillomas in forestomach in males Control: 1/52 275 mg/kg bw: 50/52 (P<0.001) 550 mg/kg bw: 50/51 • Increased incidence of combined carcinomas and papillomas in forestomach in females Control: 0/52 275 mg/kg bw: 46/52 550 mg/kg bw: 50/52 • Increased incidence of carcinomas of the forestomach in males Control: 0/52 275 mg/kg bw: 35/52 550 mg/kg bw: 43/51 Increased incidence of carcinomas of the forestomach in females Control: 0/52 275 mg/kg bw: 32/52 550 mg/kg bw: 36/51 • Increased incidence of papillomas of the forestomach in males Control: 1/52 275 mg/kg bw: 23/52 550 mg/kg bw: 18/51 • Increased incidence of papillomas of the forestomach in females Control: 0/52 275 mg/kg bw: 21/52 550 mg/kg bw: 24/51 • Decreased incidence of leukemia in males and females (both 550 mg/kg bw).		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Ponomarkov et al., 1984[206]	Rat, BDIV 14 exposed dams and their offspring (62 females and 42 males). 14 control dams and their offspring (55 female and 49 male). Carcinogenicity study	Test item: Styrene-7,8-oxide (in olive oil) Purity: 97% Oral, via gavage Dosing: Pregnant dams: 0 (olive oil) and 200 mg/kg bw Single administration on day 17 of gestation Offspring: 0 (olive oil) and 100-150 mg/kg bw, 96 weekly doses from 4 weeks of age (weaning) until termination of experiment Endpoint: All animals were sacrificed at 120 weeks of the experiment. Observations: Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.	Litter size, preweaning mortality, offspring mortality and body weights did not differ between the groups. Non-neoplastic and neoplastic lesions: Incidence in tumour-bearing pregnant dams was 57% (controls) and 31% (styrene-7,8-oxide). Effects in offspring: • Incidence in tumour-bearing animals in treated rats was 77% (females) and 52% (males) and in controls 58% (females) and 20% (males). Increased incidence in forestomach tumours: • Papillomas in males control: 0/49; styrene-7,8-oxide: 7/42, P<0.003) • Carcinoma in situ in females control: 0/55; 200 mg/kg: 6/60, P<0.02 • Carcinoma in situ in males control: 0/49; styrene-7,8-oxide: 4/42, P<0.04 • Early carcinomas or carcinomas in females control: 1/55; styrene-7,8-oxide: 16/60, P<0.0001 • Early carcinomas or carcinomas in males control: 0/49; styrene-7,8-oxide: 10/42, P<0.0002 Early changes of squamous epithelium frequently observed in styrene-7,8-oxide groups (though not statistically significant): • Incidence in nervous system tumours in males control: 1/49; styrene-7,8-oxide: 3/42 • Incidence in lung tumours in females control: 1/55; styrene-7,8-oxide: 6/60	Statistics: No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non GLP, Non guideline.

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