



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

An overview of the **available data** on the mutagenicity and carcinogenicity of styrene

Colophon

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Synopsis

Overview of available data on the mutagenicity and carcinogenicity of styrene

Styrene is a substance that is being used to produce polystyrene. Polystyrene is often used as packaging material. Additionally, there are a number of styrene-based plastics and rubbers.

At the request of the Health Council of the Netherlands, RIVM has conducted literature research on the potential mutagenicity and carcinogenicity of styrene. The Health Council is preparing a recommendation for the hazard classification of styrene. As a starting point, the Health Council will use this literature research for an independent evaluation of the mutagenic and carcinogenic properties of styrene, as requested by the Minister for Social Affairs and Employment.

With respect to mutagenicity and carcinogenicity endpoints, RIVM summarised a total of 73 studies on styrene toxicity in either laboratory animals or humans.

Keywords: styrene, mutagenicity, carcinogenicity, harmful properties, hazard classification

Publiekssamenvatting

Overzicht van de beschikbare informatie over mutageniteit en carcinogeniteit van de stof styreen

De stof styreen wordt gebruikt om polystyreen te maken. Polystyreen wordt bijvoorbeeld gebruikt als verpakkingsmateriaal (piepschuim). Daarnaast worden een aantal soorten rubbers en plastics op basis van styreen gemaakt.

Het RIVM heeft in de wetenschappelijke literatuur onderzocht wat er bekend is over twee schadelijke eigenschappen van de stof. De vraag is of styreen kankerverwekkend is en erfelijke veranderingen kan veroorzaken door schade aan het DNA (mutageen).

Het RIVM deed dat in opdracht van de Gezondheidsraad. De Gezondheidsraad gaat een voorstel doen om de stof in een bepaalde 'gevarenklasse' in te delen. Als voorbereiding daarop gebruikt de Gezondheidsraad het overzicht van het RIVM om te beoordelen of styreen een mutagene of kankerverwekkende stof is. De minister van Sociale Zaken en Werkgelegenheid (SZW) heeft om dit advies gevraagd.

Het RIVM heeft de bevindingen van in totaal 73 studies in proefdieren en mensen samengevat.

Kernwoorden: styreen, mutageniteit, carcinogeniteit, schadelijke eigenschappen, gevarenklasse

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1 Summary

RIVM has summarised the available literature on the potential carcinogenicity and mutagenicity of styrene and one of its metabolites styrene-7,8-oxide. Styrene is primarily used as a monomer in the production of polystyrene polymers and styrene-based plastics and rubbers. 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.

In the current report, a total of 73 studies were summarised. Of these, 15 were mutagenicity studies in humans and 26 were carcinogenicity studies in humans. There were 24 animal studies on styrene and 8 on styrene-7,8-oxide. The studies that are summarised here can be used to assess the potential carcinogenicity and mutagenicity of styrene. Such an assessment was beyond the scope of the current report.

2 Introduction

The aim of current research is to summarize the available data from studies with laboratory models, test animals and humans on the substance styrene. The focus of the current literature overview will be on the mutagenic and carcinogenic properties of this substance. At the request of the Dutch Minister of Social Affairs and Employment, the Health Council of the Netherlands will use the summaries to assess the mutagenic and carcinogenic properties and to provide a recommendation for its classification.

The current RIVM-report does not include an assessment of the reported mutagenic and carcinogenic effects of styrene, nor does it provide a recommendation for classification of the substance based on the CLP-criteria (Regulation EC No 1272/2008¹).

The literature search strategy as applied by the Health Council of the Netherlands which forms the basis of current literature overview is presented in chapter 2. In chapter 3 the substance identity of styrene is provided. Chapter 4 presents information on international classifications of styrene. Available information on monitoring (i.e. environmental and biological exposure monitoring) and manufacture and use is presented in chapters 5 and 6, respectively. A summary of the (toxico)kinetics of styrene is described in chapter 7. Chapter 8 describes an overview of the data on mutagenicity. Finally, the data on carcinogenicity are presented in chapter 9.

¹ <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32008R1272>

3 Literature search strategy

The Health Council of the Netherlands has performed a literature search using PubMed and Scopus. The literature search retrieved 771 results. Of these results, only cohort studies, case-control studies, animal carcinogenicity studies or meta-analyses were selected for an extensive summary by the Health Council. This resulted in 56 studies. Additionally, a brief summary was requested for three other studies which were published after 2018. For the endpoint mutagenicity, the literature for which a summary was requested was limited to the time period > 2018. For the endpoint carcinogenicity, no restriction of the time period was performed.

For the current report, RIVM summarized the data of the selected studies. RIVM also consulted the REACH registration dossier of styrene and styrene-7,8-oxide (publicly available on ECHA website)^{2 3} and secondary sources, which included the IARC Monograph vol. 121 (2019) and ATSDR Toxicological profile for styrene (2010). These were used to retrieve information on substance identification, classification, manufacture, monitoring and toxicokinetics.

² <https://echa.europa.eu/registration-dossier/-/registered-dossier/15565>

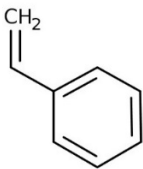
³ <https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/14585/>

4 Substance identification

4.1 Name and other identifiers of the substance

The identity of styrene is presented in Table 1 below. Table 2 presents the identity of styrene-7,8-oxide, a degradation product of styrene.

Table 1 Substance identity and information related to the molecular and structural formula of styrene

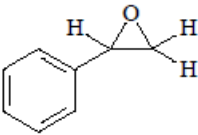
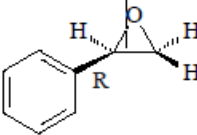
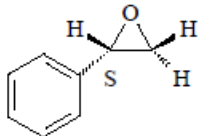
Name(s) in the IUPAC nomenclature or other international chemical name(s)	Styrene
Other names (usual name, trade name, abbreviation)	Ethenylbenzene; Vinylbenzene; Phenylethene; Phenylethylene; Cinnamene
ISO common name (if available and appropriate)	N/A
EC/EINECS number (if available and appropriate)	202-851-5
EC name (if available and appropriate)	Styrene
CAS number	100-42-5
Other identity code (if available)	N/A
Molecular formula	C ₈ H ₈
Structural formula	
SMILES notation (if available)	C=CC1=CC=CC=C1
Molecular weight or molecular weight range	104.15 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVBC substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	Almost all compositions have a purity of >98%. One composition has a purity of >80%. Some registered compositions are noted to have impurities or additives. ⁴

⁴ Ethylbenzene CAS 100-41-4 is the most reported impurity (Flam. Liq. 2. (H225), Acute Tox. 4* (H332), Asp. Tox. 1 (H304), STOT RE 2 (H373; hearing organ). There is one noted additive: 4-tert-butylpyrocatechol CAS 98-29-3 (no harmonized classification).

Other impurities are: m-xylene CAS 103-38-3; Cumene CAS 98-82-8, p-xylene CAS 106-42-3; isopropylbenzene CAS 98-82-8; propylbenzene CAS 103-65-1; 2-ethyltoluene CAS 611-14-3; 2-phenylpropene CAS 98-83-9; p-isopropylstyrene CAS 2055-40-5; benzaldehyde CAS 100-52-7; 2-phenoxyethanol CAS 122-99-6; phenylacetylene CAS 536-74-3; divinylbenzene CAS 1321-74-0; oxygen CAS 7782-44-7.

N/A: Not applicable

Table 2 Substance identity and information related to the molecular and structural formula of styrene-7,8-oxide

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(Epoxyethyl)benzene
Other names (usual name, trade name, abbreviation)	2-Phenyloxirane; 1,2-(epoxyethyl)benzene; 1,2-epoxy-1-phenylethane; α,β -epoxystyrene; phenethylene oxide; 1-phenyl-1,2-epoxyethane; phenylethylene oxide; phenyloxirane; styrene epoxide; styrene oxide; styryl oxide
ISO common name (if available and appropriate)	N/A
EC/EINECS number (if available and appropriate)	202-476-7
EC name (if available and appropriate)	Styrene-7,8-oxide
CAS number	Styrene-7,8-oxide: CAS 96-09-3; (R)-(+)-styrene-7,8-oxide: CAS 20780-53-4; (S)-(-)-styrene-7,8-oxide: CAS 20780-54-5; (\pm)-styrene-7,8-oxide: CAS 67253-49-0
Other identity code (if available)	N/A
Molecular formula	C ₈ H ₈ O
Structural formula	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>(\pm)-styrene-7,8-oxide</p> </div> <div style="text-align: center;">  <p>(R)-(+)-styrene-7,8-oxide</p> </div> </div> <div style="text-align: center; margin-top: 20px;">  <p>(S)-(-)-styrene-7,8-oxide</p> </div>
SMILES notation (if available)	C1OC1C1=CC=CC=C2
Molecular weight or molecular weight range	120.15 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Styrene-7,8-oxide exists as two optical isomers and the commercial product is a racemic mixture (1)

Description of the manufacturing process and identity of the source (for UVBC substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	N/A

4.2 Physico-chemical properties of styrene

The physico-chemical properties of styrene were obtained from the REACH registration dossier of styrene (2) and styrene-7,8-oxide (3) are presented in Table 3 and 4.

Table 3 Summary of physico-chemical properties of styrene

Properties	Value
State of the substance at normal temperature and pressure	Liquid
Melting/freezing point	-31 °C at 101.3 kPa
Boiling point	145 °C at 101.3 kPa
Relative density	0.9 at 20 °C
Vapour pressure	6.67 hPa at 20 °C
Surface tension	Not applicable
Water solubility	160.01 - 343.7 mg/L at 25 °C
Partition coefficient n-octanol/water	0.35 - 2.96 at 25 °C and pH 7
Flash point	31 - 34.4 °C at 101.3 kPa
Flammability	Flammable liquid
Explosive properties	Non-explosive
Self-ignition temperature	490 °C at 101.3 kPa
Oxidising properties	No oxidising properties
Granulometry	Not applicable
Stability in organic solvents and identity of relevant degradation products	Not applicable
Dissociation constant (pKa)	Not applicable
Viscosity	0.696 mPa/s (dynamic) at 25°C 0.77 mm ² /s (kinematic) at 25 °C

Table 4 Summary of physico-chemical properties of styrene-7,8-oxide

Properties	Value
State of the substance at normal temperature and pressure	Liquid
Melting/freezing point	-35.6 °C at 101.3 kPa
Boiling point	194.1 °C at 101.3 kPa
Relative density	1.049 g/cm ³ at 25 °C
Vapour pressure	34.9 Pa at 20 °C
Surface tension	Not applicable
Water solubility	1.49 g/L at 20 °C

Properties	Value
Partition coefficient n-octanol/water	1.61
Flash point	74 °C at 101.3 kPa
Flammability	Non-flammable
Explosive properties	Non-explosive
Self-ignition temperature	498 °C at 101.3 kPa
Oxidising properties	No oxidising properties
Granulometry	Not applicable
Stability in organic solvents and identity of relevant degradation products	Not applicable
Dissociation constant (pKa)	Not applicable
Viscosity	Not applicable

5 International classifications

5.1 European Commission

Styrene has currently a harmonized classification in Annex VI of the CLP-Regulation (EC) 1272/2008 (entry nr: 601-026-00-0) as:

- Flam. Liq. 3 (H226: Flammable liquid and vapour)
- Skin Irrit. 2 (H315: Causes skin irritation)
- Eye Irrit. 2 (H319: causes serious eye irritation)
- Acute Tox. 4* (H332: Harmful if inhaled)
- STOT RE 1 (H372: Causes damage to organs (hearing organs) through prolonged or repeated exposure)
- Repr. 2 (H361d: suspected of damaging the unborn child)

Styrene-7,8-oxide currently has a harmonized classification in Annex VI of the CLP-Regulation (EC) 1272/2008 (entry nr: 603-084-00-2) as:

- Acute Tox. 4* (H312: Harmful in contact with skin)
- Eye Irrit. 2 (H319: Causes serious eye irritation)
- Carc. 1B (H350: May cause cancer)

5.2 The Health Council

Styrene and styrene-7,8-oxide have not previously been evaluated by the Health Council of the Netherlands for its mutagenic and carcinogenic properties. Styrene has been evaluated by the Health Council of the Netherlands in 2001 for effects on reproduction (4). They recommended no classification of styrene for effects on fertility, for developmental toxicity and for effects during lactation due to a lack of appropriate data.

5.3 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 (1). IARC considered that there is limited evidence in humans for the carcinogenicity of styrene. IARC further considered that there is now sufficient evidence in experimental animals for the carcinogenicity of styrene. Overall, IARC concluded in 2019 that styrene is probably carcinogenic to humans (Group 2A).

Styrene-7,8-oxide (CAS 96-09-3) is considered a metabolite of styrene. This chemical is considered by IARC a group 2A carcinogen, based on sufficient evidence in experimental animals (1).

5.4 Other countries

Styrene:

- In the state of California, styrene is considered a substance causing cancer.⁵
- Styrene is also included in the Report on Carcinogens (15th edition) as reasonably anticipated to be a human carcinogen.⁶

⁵ <https://oehha.ca.gov/media/downloads/proposition-65//p65list091319.pdf>

⁶ <https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc/index.html#toc>

- In Germany, styrene is not included as a carcinogenic substance in the national list of CMR substances in the context of worker protection.⁷
- Styrene is currently not included in the list of substances NIOSH considers to be potential occupational carcinogens.⁸
- Styrene has the following classification in Australia⁹:
 - Flam. Liq. 3 (H226: Flammable liquid and vapour)
 - Repr. 2 (H361d: Suspected of damaging the unborn child)
 - Acute tox. 4 (H332: Harmful if inhaled)
 - STOT RE 1 (H372: Causes damage to the hearing organs through prolonged or repeated exposure)
 - Skin Irrit. 2 (H315: Causes skin irritation)
 - Eye Irrit. 2 (H319: Causes serious eye irritation)
 - Muta. 2 (H341: Suspected of causing genetic defects)
 - STOT SE 3 (H335: May cause respiratory irritation)
 - STOT SE 3 (H336: May cause drowsiness or dizziness)
- Styrene has the following classification in Japan¹⁰:
 - Flam. Liq. 3 (H226: Flammable liquid and vapour)
 - Acute tox. 4 (H332: Harmful if inhaled)
 - Skin Irrit. 2 (H315: Causes skin irritation)
 - Eye Irrit. 2A (H319: Causes serious eye irritation)
 - Muta. 2 (H341: Suspected of causing genetic defects)
 - Carc. 1B (H350: May cause cancer)
 - Repr. 2 (H360: May damage fertility or the unborn child)
 - STOT SE 1 (H370: Causes damage to central nervous system)
 - STOT SE 3 (H335: May cause respiratory irritation)
 - STOT SE 3 (H336: May cause drowsiness or dizziness)
 - STOR RE 1 (H372: 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)
 - Asp. Tox. 1 (H304: May be fatal if swallowed and enters airways)

Styrene-7,8-oxide:

- In the state of California, styrene-7,8-oxide is considered a substance causing cancer¹¹.
- Styrene-7,8-oxide is also included in the Report on Carcinogens (15th edition) as reasonably anticipated to be a human carcinogen¹².
- In Germany, styrene-7,8-oxide is currently not included as a carcinogenic substance in the national list of CMR substances in the context of worker protection¹³.

⁷ https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf?__blob=publicationFile

⁸ <https://www.cdc.gov/niosh/topics/cancer/npotocca.html>

⁹ <http://hcis.safeworkaustralia.gov.au/HazardousChemical/Details?chemicalID=4232>

¹⁰ <https://www.nite.go.jp/chem/english/ghs/20-mhlw-2111e.html>

¹¹ <https://oehha.ca.gov/media/downloads/proposition-65/p65list091319.pdf>

¹² <https://ntp.niehs.nih.gov/ntp/roc/content/profiles/styreneoxide.pdf>

¹³ https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf?__blob=publicationFile

- Styrene-7,8-oxide is currently not included in the list of substances NIOSH considers to be potential occupational carcinogens.¹⁴
- Styrene-7,8-oxide has the following classification in Australia¹⁵:
 - Acute tox. 4 (H312: Harmful in contact with skin)
 - Eye irrit. 2A (H319: Causes serious eye irritation)
 - Skin sens. 1 (H317: May cause an allergic skin reaction)
 - Carc. 1B (H350: May cause cancer)
- Styrene-7,8-oxide has the following classification in Japan¹⁶:
 - Flam. Liq. 4 (H227: Combustible liquid)
 - Acute tox. 4 (H302: Harmful if swallowed)
 - Acute tox. 3 (H311: Toxic in contact with skin)
 - Acute tox. 3 (H332: Harmful if inhaled)
 - Skin Irrit. 2 (H315: Causes skin irritation)
 - Eye Irrit. 2A (H319: Causes serious eye irritation)
 - Skin sens. 1 (H317: May cause an allergic skin reaction)
 - Carc. 1B (H350: May cause cancer)
 - Repr. 2 (H361: Suspected of damaging fertility or the unborn child)
 - STOT SE 1 (H370: Causes damage to respiratory organs)
 - STOT SE 3 (H336: May cause drowsiness or dizziness)

¹⁴ <https://www.cdc.gov/niosh/topics/cancer/npotocca.html>

¹⁵ <http://hcis.safeworkaustralia.gov.au/HazardousChemical/Details?chemicalID=4231>

¹⁶ <https://www.nite.go.jp/chem/english/ghs/20-mhlw-2047e.html>

6 Monitoring

6.1 Environmental exposure monitoring

An overview of analysis methods in different matrices was provided by IARC, 2019 and is summarized in the table below (1). Of note is that NIOSH has a standard method for styrene measurements in workplace air (Method 1501; NIOSH, 1994) and an EPA method (Method 8260B; EPA 1996) can be used to determine the concentration of styrene in various matrices, such as groundwater, aqueous sludges, waste solvents, oily wastes, tars, soils and sediments.

Table 5 Overview of methods for the analysis of styrene and styrene-7,8-oxide in the environment

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference (as cited by IARC)
Styrene Air (workplace)	Adsorbed (charcoal); desorbed (carbon disulfide)	GC/FID	0.4 µg/sample	NIOSH, 1994
Styrene Air (workplace)	Adsorbed (solid); desorb(ethyl acetate)	GC/FID	Not further specified	Tornero-Velez et al., 200
Styrene Air (workplace)	Thermal desorption	GC/MS	Not further specified	Fernandéz-Villarrenaga Martín et al., 2000
Styrene Air (ambient/indoor)	Extraction with acetone and/or carbon disulfide	GC/MS	0.6 µg/m ³ or 0.01-0.05 µg/m ³	Adgate et al., 2004 or Rehwagen et al., 2003
Styrene-7,8-oxide Air	Solid sorbent; thermal desorption (ethyl acetate or carbon disulfide)	GC/MS or GC/FID	2 ng/m ³	Krost et al., 1982
Styrene Environmental samples	Direct injection; purge-and-trap (PT); closed-system vacuum distillation; static headspace (solids); desorption from trapping media (air)	GC/MS	5 µg/L (groundwater); 5 µg/kg/wet weight (low-level soil and sediment); 250 µg/L (water-miscible liquid waste); 625 µg/kg (high-level soil and sludge); 2500 µg/L (non-water-miscible waste)	EPA, 1996
Styrene-7,8-oxide Environmental samples	Reaction in aqueous solution with 4-nitrothiophenol to form thioethers	Detection of thioethers by HPLC	<1 ppb	Cheh & Carlson, 19981

GC: Gas Chromatography, FID: Flame Ionisation Detection, MS: Mass Spectrometry, HPLC: High-Performance Liquid Chromatography

6.2 Biological exposure monitoring

An overview of analysis methods in different matrices was provided by IARC, 2019 and is summarized in the table below (1).

Table 6 Overview of methods for the analysis of styrene, styrene-7,8-oxide and styrene metabolites in blood and urine

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference (as cited by IARC)
Styrene Urine and blood		PT-GC		Prieto et al., 2000, 2002
Styrene and styrene-7,8-oxide Blood		isotope-dilution GC-MS	2.5 µg/L (styrene) 0.05 µg/L (styrene-7,8-oxide)	Tornero-Velez et al., 2001
Styrene-7,8-oxide Blood	reaction with valine, derivatization with pentafluorophenyl isothiocyanate	GC-MS/MS	0.025 µg/L	Tornero-Velez et al., 2001
Styrene Urine	headspace solid-phase microextraction	GC/MS	0.2 µg/L	Fustinoni et al., 2008
Mandelic acid (MA) and phenylglyoxylic acid (PGA) (metabolites) Urine		HPLC or LC-MS/MS	15 mg/L (MA) and 2 mg/L (PGA) or 0.1 mg/L (both MA+PGA)	Ghittori et al., 1997; Marhuenda et al., 1997 or Manini et al., 2002

PT: Purge and trap, GC: Gas Chromatography, MS: Mass Spectrometry, HPLC: High-Performance Liquid Chromatography, LC: Liquid Chromatography

The concentration of styrene measured in air and the concentrations of styrene and its biomarkers in urine and blood are strongly correlated (IARC, 2019). A log-linear correlation ($r=0.746$) was found between blood and salivary levels of styrene in exposed subjects (5). 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.

7 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 $\geq 1\,000\,000$ to $< 10\,000\,000$ tonnes (2). The majority of styrene (90%) is produced by the dehydrogenation of ethylbenzene (6). Styrene is used by consumers, in articles, 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.

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 (1). 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 the polyester resin using a roller (hand laminating) or by spraying. The evaporation of styrene from unsaturated polyester resin into the work environment during processing in the glass fibre-reinforced plastics can result in significant exposures to styrene.

Between 1981 and 1997, workers from 14 reinforced plastic industry companies in The Netherlands using hand or spray lamination were invited to participate in a cohort study (7). In a study performed by TNO in 1991, exposure measurements were performed in 4 large plants and exposure was assessed qualitatively in 12 small plants. The techniques used by the workers were filament winding, spraying and hand laminating (8).

In 2002, the total amount of polyester resin used in the Benelux was 22.200 tons (4% of the total European amount) (9). Results of model calculations, based on data of personal styrene exposure measurements retrieved from reports, databases and peer-reviewed papers, implied a significant decline of styrene concentrations in the breathing zone of European glass reinforced plastics open mould workers of 5.3% per year during the period 1966–1990 ($P < 0.0001$; $n = 213$), but by only 0.4% annually in the period after 1990 (7).

8 (Toxico)kinetics

The summary of the toxicokinetics of styrene is based on IARC, 2019 and can be found below (1). The quoted references below are the studies cited in the IARC report. Styrene is extensively metabolized to styrene-7,8-oxide in humans and animals. Hence, external exposures to styrene encompasses internal exposures to both styrene and styrene-7,8-oxide. An extensive overview of the kinetics of styrene-7,8-oxide, mainly in animal studies, can be found in IARC, 2019.

Briefly,

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

Detailed information on toxicokinetics can be found in 8.1 (human) and 8.2 (animal).

8.1 Human data

8.1.1 Absorption

Styrene is absorbed by inhalation, dermal contact, or ingestion through consumption of food.

Dermal absorption of styrene was reported to be very low in occupational exposures (Limasset et al., 1999), and occurred up to 4% using urinary styrene metabolites and exhaled styrene as markers in experimental studies (Berode et al., 1985). Inhalation is the predominant exposure route in the occupational setting (Berode et al., 1985). Under experimental conditions, the pulmonary uptake of styrene ranged from 63%-68% (Wigaeus et al., 1984; Löf et al., 1986). In reinforced plastics workers, an average blood concentration of 15.3 µM has been reported (Brugnone et al., 1993) and in another study average styrene blood concentrations were 5.4 µM versus 0.67 µM in controls (Vodicka et al., 2004). Additionally, blood concentrations of the metabolites styrene-7,8-oxide and styrene glycol were in the nanomolar

or low micromolar range, respectively in workers (Wigaeus et al., 1983). In volunteers, the clearance of styrene from blood was biphasic (Ramsey et al., 1980).

8.1.2 *Distribution*

After exposure by inhalation, styrene is rapidly absorbed into the blood and is distributed throughout the body. Concentrations of styrene in subcutaneous adipose tissue exceeded blood concentrations in industrial workers and volunteers (Wigaeus et al., 1983). Approximately 8% of the styrene was retained in adipose tissues and the adipose tissue half-life was 2.8-5.2 days (Engstrom et al., 1978ab). No constant increase in the mean values of urinary styrene metabolites was observed in workers exposed over a 4-day period, suggesting that styrene does not continuously accumulate in the body (Pekari et al., 1993).

8.1.3 *Metabolism*

Much is known about the metabolism of styrene in humans. An extensive overview can be found in IARC, 2019. Briefly, styrene is initially oxidized by cytochrome P450s (CYPs) through three distinct pathways:

1. Epoxidation of the vinyl double bond (Figure 1), the major metabolic pathway;
2. Oxidation on the vinyl group (Figure 1);
3. Oxidation on the phenyl ring (Figure 2).

Metabolites from all pathways have been detected in humans exposed to styrene and in experimental studies.

1. Epoxidation of the vinyl double bond (Figure 1)
Based on in vitro studies, styrene is metabolised on the vinyl double bond to styrene-7,8-oxide by a group of human CYPs: CYP1A2, CYP2B6, CYP2C8, CYP2E1, CYP2F1, CYP3A3/3A4/3A5, CYP4B1 and CYP2A13 (Nakajima et al., 1994, Carlson et al., 2008, Fukami et al., 2008). CYP2E1 was found to play a primary role in styrene metabolism in human liver samples (Kim et al., 1997, Wenker et al., 2001). Styrene-7,8-oxide can be metabolized into styrene glycol by human liver microsomal epoxide hydrolases (Oesch et al., 1974) and in lung microsomes (Nakajima et al., 1994a). Also, styrene-7,8-oxide can be conjugated by GSTs (Pachecka et al., 1979) which are catabolized to isomeric phenylhydroxyethylmercapturic acids (PHEMAs: M1 and M2 in figure 1). Epoxidation of the vinyl group of styrene results in formation of S- and R-styrene-7,8-oxides which are in turn hydrated to R-styrene and S-styrene glycol (no specific reference in IARC). Three mercapturic acids, degradation products of styrene-7,8-oxide-glutathion conjugates, were detected in human urine (Ghittori et al., 1997).

From styrene glycol, also glucuronide and sulfate conjugates can be formed (Korn et al., 1985). Additionally, styrene glycol is metabolized to mandelic acid (MA) probably via alcohol dehydrogenase and aldehyde dehydrogenase (Weng 2016). MA is metabolized to phenylglyoxylic acid (PGA) by alcohol dehydrogenase (Nagwekar and Kostenbauder, 1970; Gao et al., 2009). MA and PGA are metabolized into the end products benzoic acid (Nagwekar and

Kostenbauder, 1970), hippuric acid (Johanson et al., 2000), phenylglycine (Manini et al., 2002; Fustinoni et al., 2008) and hydroxymandelic acids (Pekari et al., 1993) which have all been detected in human urine. This is the predominant route within this main metabolic pathway.

2. Oxidation on the vinyl group (Figure 1)

Alternatively, styrene can be oxidized on the vinyl group by CYPs and then further metabolised into phenylethanols (Cosnier et al., 2012; Korn et al., 1985) or phenylacetaldehyde (Wang et al., 2009). Phenylacetic acid is the oxidation product of phenylacetaldehyde. The precise mechanisms are unclear.

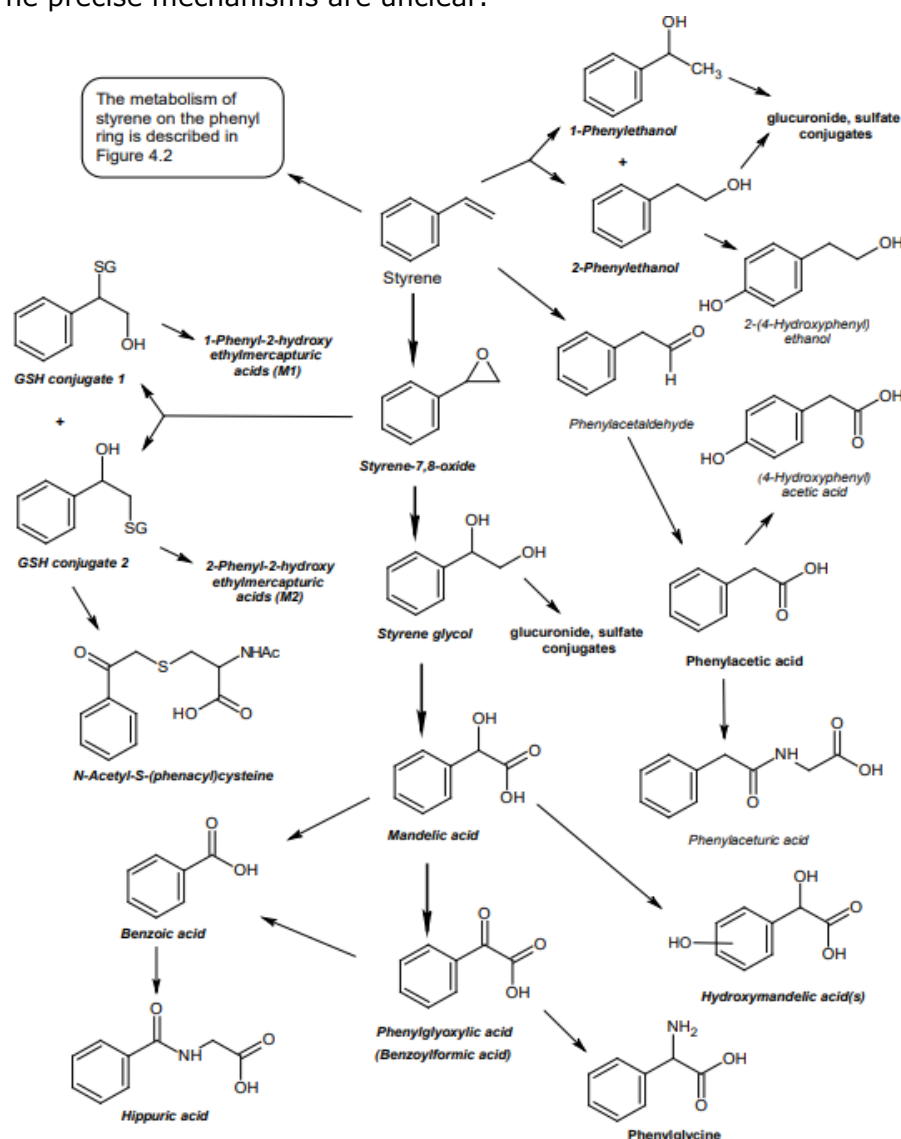


Figure 1 Metabolism of styrene based on human and experimental studies (IARC, 2019).

Metabolites in bold were found in human studies, metabolites in italics were found in experimental studies, and metabolites in both bold and italics were found in both human and experimental studies. Main pathways are indicated by thick arrows.

3. Oxidation of the phenyl ring (Figure 2)

Oxidation of the phenyl ring forms styrene-1,2-oxide, styrene-2,3-oxide and styrene-3,4-oxide (no specific reference in IARC). These can rearrange into 3- (no specific reference in IARC), 2-, or 4-vinylphenol (Watabe et al., 1982). 4-Vinylphenol is conjugated to glucuronic acid and sulfate in humans which make up 0.5-1% of the metabolite excretion (Linhart et al., 2012).

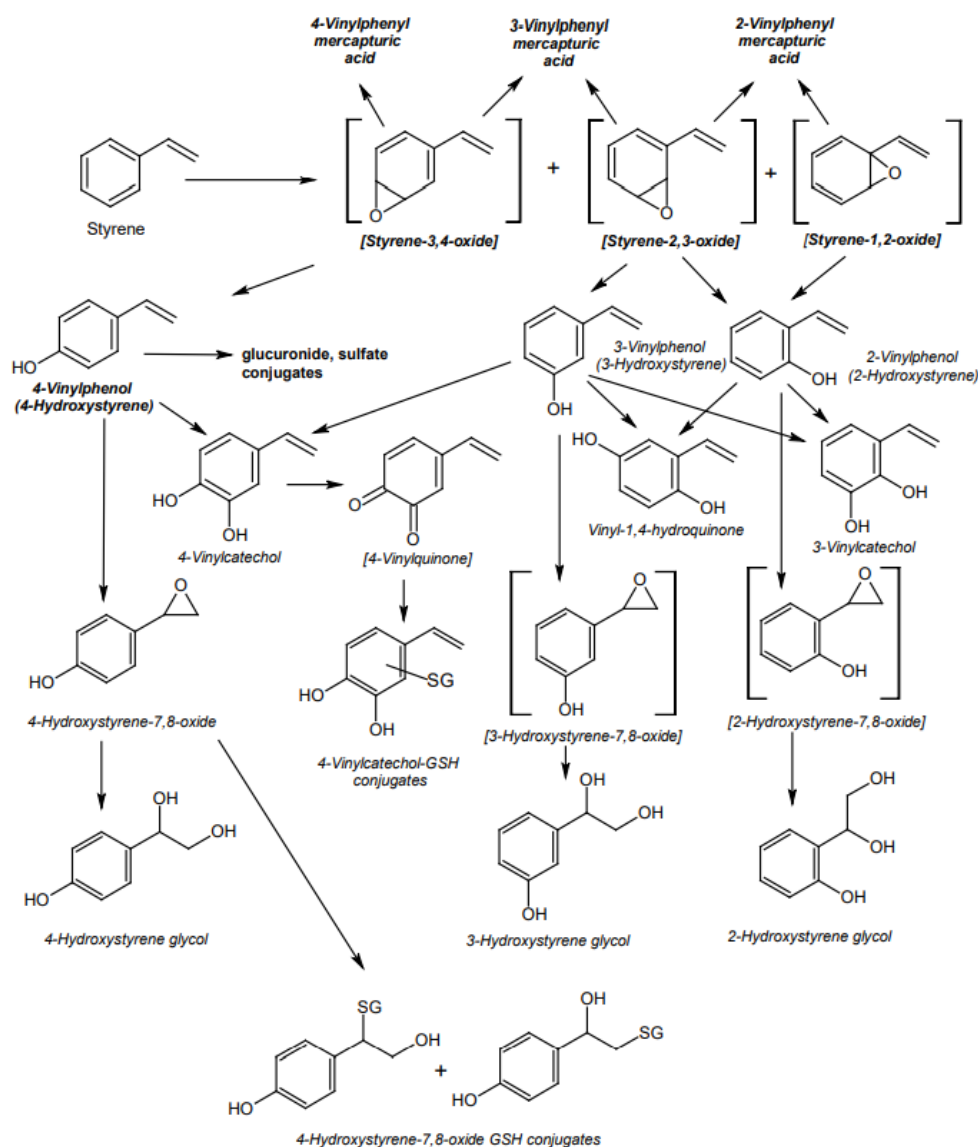


Figure 2 Metabolism of styrene on the phenyl ring based on human and experimental studies (IARC, 2019).

Metabolites in bold were found in human studies, metabolites in italics were found in experimental studies, and metabolites in both bold and italics were found in both human and experimental studies. Metabolites in brackets are putative.

Genetic polymorphisms in CYP2E1, GST's, epoxide hydrolase and aldehyde dehydrogenase can play a role in the metabolism of styrene in humans, and changes in urinary concentrations of the corresponding metabolites have been associated with these polymorphisms (IARC, 2019).

Co-exposure to ethanol, a CYP2E1 inducer, lowered urinary MA and PGA levels (Cérny et al., 1990). Contradicting results exist as to whether co-exposure to acetone affects styrene metabolism (IARC, 2019).

8.1.4 *Excretion*

Micromolar levels of unmetabolized styrene were found in the urine of occupational (Ghittori et al., 1997) and experimental subjects (Johanson et al., 2000). About 92% of the absorbed dose is metabolized and 37% was eliminated in the urine as MA and 54% as PGA after 8 hours (Caperos et al., 1979). In another chamber exposure study, the cumulative percentage of MA and PGA excreted was 58% after 28 hours of exposure (Wigaeus et al., 1983). Presence of a variety of other metabolites in human urine was confirmed as well (paragraph 7.1.3). Urinary PHEMA's account for 1% (De Palma et al., 2001) and phenylacetic acid and/or phenylaceturic acid for less than 5% (Johanson et al., 2000) of the excreted metabolites.

8.2 **Animal data**

8.2.1 *Absorption*

Rodents exposed to styrene by inhalation experienced pulmonary absorption resulting in rapid blood uptake (IARC 1994, 2002). The uptake efficiencies of styrene in the upper respiratory tract of male CD-1 mice and male Sprague-Dawley rats were inversely related to the exposure concentration (Morris, 2000). Dermal contact with gaseous styrene (3000 ppm, 4h) resulted in a maximum blood concentration of 10 µg/mL (96 µM) in male Fischer 344 rats (McDougal et al., 1990). In male B6C3F1 mice, the concentrations of styrene and styrene-7,8-oxide in blood were 21.8 µg/mL (200 µM) and 2.25 µg/mL (20 µM), respectively, after exposure by inhalation to 500 ppm styrene for 6 hours per day, for 14 days (Mahler et al., 1999). In an isolated lung perfusion system, mean styrene-7,8-oxide concentrations in mouse lungs were about twice as high as those in rat lungs at equal styrene exposure (Hofmann et al., 2006).

8.2.2 *Distribution*

Styrene distributes throughout the body in CD-1 mice and male Sprague-Dawley rats after nose-only exposure to radiolabelled styrene (160 ppm, 6h). Radioactivity levels were observed in many organs of both species. Radioactivity levels were highest in the rat and mouse nasal mucosa and mouse lung and nasal levels were significantly higher compared to the rat (Boogaard et al., 2000a). In male CD2F1 mice given a single intraperitoneal injection of styrene (200 mg/kg), styrene levels peaked in brain, heart, lungs, liver, kidneys, and spleen within 5–30 minutes and then declined rapidly. In perirenal fat, in which the highest concentration of styrene was measured, styrene levels peaked later (Pantarotto et al., 1980).

8.2.3 *Metabolism*

The metabolism of styrene in experimental systems is qualitatively similar to that described for humans with some quantitative differences.

The Cyps involved in styrene metabolism in rat liver are Cyp2c11/6, Cyp2b1/2, Cyp1a1/2 and Cyp2e1 and in rat lung only cyp2b1/2 is active

(Nakajima et al., 1994b). In the mouse liver Cyp2e1 is involved in styrene metabolism and in the mouse lung both Cyp2e1 and Cyp2f2 are involved (Carlson, 1997a; Green et al., 2001a).

The rate of styrene metabolism to styrene-7,8-oxide was greater in mouse lung club (Clara) cells compared with mouse type-II pneumocytes, and greater in mouse club cells compared with rat club cells (Hynes et al., 1999).

Microsomal metabolism of styrene to styrene-7,8-oxide was highest in mouse liver, followed by rat liver, followed by human liver (Nakajima et al., 1994a).

Male B6C3F1 mice given a single intraperitoneal dose of styrene (400 mg/kg bw), metabolized styrene to urinary styrene glycol, MA, two isomeric hydroxymandelic acids and two mercapturic acids which represent 10-15% of the given dose. PGA was a minor metabolite (Linhart et al., 2000).

8.2.4 *Excretion*

The primary route of excretion is urine in male F344 rats, male CD-1 mice, and male B63CF1 mice after nose-only exposure to radiolabelled styrene (Summer 1997). The overall quantitative excretion of styrene and metabolites was similar in male CD-1 mice and male Sprague-Dawley rats after nose-only exposure to radiolabelled styrene (160 ppm) (Boogaard 2000a).

Male Fischer 344 or Sprague-Dawley rats exposed to styrene by inhalation at 75 ppm and 250 ppm for 4 days excreted increased levels of MA, PGA, and hippuric acid in their urine compared with controls. After 1 day of exposure, the urinary MA and PGA concentrations of Sprague-Dawley rats exposed to styrene at 250 ppm were 256 ± 55 mg/g creatinine and 672 ± 258 mg/g creatinine, respectively (Cosnier et al., 2012). Multiple studies detected MA, PGA, M1, M2, PHEMA, phenyl-glycine, N-acetyl-S-(phenacyl)cysteine, glucuronide, and sulfate conjugates of 4-vinylphenol and of styrene glycol in urine of Sprague-Dawley rats exposed to styrene under different conditions (Manini et al., 2002, Truchon et al., 1990). In Male B6C3F1 mice exposed to a single intraperitoneal styrene injection, mercapturic acids were the major excreted metabolites, followed by MA and styrene glycol (Linhart et al., 2000). 2-Vinylphenol, 3-vinylphenol, and 4-vinylphenol were measured in the urine of male NMRI mice exposed to styrene (600 ppm and 1200 ppm for 6 hours). Urinary concentrations of 4-vinylphenyl mercapturic acid after exposure at 600 ppm and 1200 ppm were 0.75 ± 0.1 mg/L and 1.09 ± 0.07 mg/L, which represented 0.047% and 0.043%, respectively, of the adsorbed dose of styrene (Linhart et al., 2010).

9 Mutagenicity

Data on mutagenicity of styrene and styrene-7,8-oxide can be found in IARC, 2019.

9.1 Summary of *in vitro* mutagenicity tests

In vitro mutagenicity studies of the time period > 2018 were not identified with current literature search of the Health Council of the Netherlands.

9.2 Summary of *in vitro* cytogenetic tests

In vitro cytogenetic studies of the time period > 2018 were not identified with current literature search of the Health Council of the Netherlands.

9.3 Summary of *in vivo* animal mutagenicity tests

In vivo animal mutagenicity studies of the time period > 2018 were not identified with current literature search of the Health Council of the Netherlands.

9.4 Summary of human data on mutagenicity

Human studies included in this overview are three systematic reviews and meta-analyses, one cohort study, one case-control study and six cross-sectional studies.

Yager et al. (1993) prospectively assessed dose-response relations in a cohort of 48 workers at a reinforced plastic boat manufacturing facility. Concentrations of styrene, in ambient air and in breath, were measured for each worker on 7 randomly chosen days over a year and related to frequencies of sister chromatid exchanges (SCEs) and micronuclei in peripheral blood lymphocytes. Regression analysis showed that at a mean 8-hour time-weighted average styrene air concentration of 64.2 mg/m³, mean SCEs per cell were related to air exposure as $Y=6.094+0.022 \cdot X$ ($P=0.007$).

Kolstad et al. (1996) performed a case-control study nested within a study of workers in the Danish reinforced plastics industry. Cases were 19 myeloid leukemia patients (12 with clonal chromosomal aberrations), who were matched with 57 employees from similar industries without styrene exposure. Styrene exposure was associated with a 2.5-fold increased risk for myeloid leukemia with clonal chromosome aberrations (95% CI 0.2-25.0).

A cross-sectional study by Vodicka et al. (2001), comparing 44 reinforced plastics workers to 18 unexposed controls, in multivariate regression analysis found several associations between DNA single-strand breaks, chromosomal aberration frequencies, genotypes of xenobiotic-metabolising enzymes and styrene exposure.

In an earlier study, Vodicka et al. (1999) compared assays of genotoxicity in 11 hand-lamination workers to 7 controls. Amongst others, they found *O*⁶-guanine styrene DNA adduct levels to be higher in exposed group ($P=0.001$).

Buschini et al. (2003) cross-sectionally studied 48 workers in the production of polyester resins or glass-fiber reinforced plastics and 14 unexposed colleagues. Levels of DNA damage, as assessed by comet assay of peripheral blood leukocytes, were higher in exposed workers, while the relation was affected by glutathion S-transferase gene polymorphisms.

A cross-sectional study by Somorovská found a higher number of DNA strand breaks, assessed by comet assay, in 44 styrene exposed hand laminators compared to 19 unexposed controls ($P < 0.001$), as well as a higher frequency of chromosomal aberrations ($P < 0.0001$ for highly exposed versus unexposed).

Hallier et al. (1994) analysed sister chromatid exchanges (SCEs) in peripheral blood lymphocytes of 28 styrene-exposed workers (manual laminators and 'formers') and 20 controls. The laminators had significantly more SCEs/cell than controls (P-value not reported).

A cross-sectional study from 1980 by Andersson et al. compared 36 styrene-exposed workers in a boat-building factory to 37 unexposed colleagues and measured more chromosomal aberrations in peripheral blood lymphocytes of exposed workers ($P < 0.001$), as well as more sister-chromatid exchanges ($P < 0.05$).

The review and meta-analysis by Collins et al. (2021) included 18 cross-sectional studies, comparing altogether 505 styrene exposed workers to 532 non-exposed controls; seven of these studies were case-control studies with some form of matching. The endpoint of interest was counts of chromosome aberrations, mainly in peripheral blood lymphocytes. The analysis found a meta-mean difference (of standardised mean differences) between exposed and controls of 0.361 (95% CI -0.084 to 0.807, random effects model; 0.209 (0.073 to 0.345), fixed effects model), but heterogeneity between studies was large.

In a similar meta-analysis, but with a different endpoint, Collins et al. (2019) included 12 cross-sectional studies, comprising 15 comparisons between in total 516 styrene-exposed workers to 497 non-exposed controls. Five of these studies were case-control studies applying some form of matching. The endpoint of micronucleus frequencies, mainly in peripheral blood, was greater in the exposed: meta-mean difference 1.19 (95% CI 0.20 to 2.18), but heterogeneity between studies was large. This study was an update of Costa et al. (2016).

All selected human studies on mutagenicity of styrene are summarized briefly in Table 7.

Table 7 Summary table of human data on mutagenicity of styrene

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
REVIEWS AND META-ANALYSES					
Collins et al. (2021), (10) Critical review and meta-analysis, including 18 cross-sectional studies with controls (of which seven case-control studies with some form of matching). Inclusion criteria: studies (period 1975-2020) reporting chromosomal aberrations evaluations (but studies including 'gaps' in chromosome aberration counts were excluded, as were a few others for technical reasons), among styrene exposed workers	Occupational exposure to styrene: workers at polyester manufacturing plants, reinforced plastics plants, or boat building facilities. Studies categorized as high or low exposure, based on air monitoring values (cut-off high versus low 20 ppm (87 mg/m ³)) or mandelic acid (MA) and phenyl-glyoxylic acid (PGA) in urine (cut-off (MA+PGA) creatinine 400 mg/g). Comparison between exposed and unexposed controls	Counts of chromosome aberrations mainly in peripheral blood lymphocytes (also other cell types). Method of evaluating aberrations needed to meet certain quality criteria	18 studies, together comprising 20 comparisons of a total of 505 styrene exposed workers to 532 (workers) controls: meta-mean difference (of standardized mean differences) 0.361 (95% CI -0.084 to 0.807, random effects model, 0.209 (0.073 to 0.345), fixed effects model.; 7 studies reported statistically significant increases in exposed, 3 studies significant decreases); I ² =90.11, P<0.001, fixed effect model	Lack of consistency across studies, as evaluated by I ² , and no exposure response (lower differences at higher exposures): meta-mean difference in studies with high exposure 0.407 (-0.168 to 0.982, random effects model) versus 0.494 (-0.089 to 1.077). Effect confounding: In studies with matching meta-mean difference 0.699 (0.172 to 1.226, random effects model) compared to the unmatched cross-sectional studies of 0.218 (-0.464 to 0.146).	This review contains quite detailed discussions of individual studies.
Collins et al. (2019), (11)	Occupational exposure to styrene;	Micronucleus frequencies mainly	12 studies, comprising 15	Authors found some evidence for	Note that the outcome measure

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Update of a previous meta-analysis, Costa et al. (2016). See below. Studies published in period 1975-2018 were considered. All studies cross-sectional, 5 with matching for covariates.	all but one study concerned reinforced plastics industry. All studies reported mean Styrene concentration in the air and/or the urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) concentrations. Eight populations classified as high exposure (> 20 ppm (87 mg/m) or > 400 mg/g (MA+PGA) creatinine). Comparison exposed versus non-exposed	in peripheral blood lymphocytes (also other cell types)	comparisons, comparing a total of 516 styrene exposed workers compared to 497 non-exposed: meta-mean difference (of standardized mean differences) 1.19 (95% CI 0.20 to 2.18, random effects model), but $I^2=97.47$, $P<0.001$, fixed effect model	publication bias ($P=0.2$ for one-sided Egger's test) with small studies of negative findings not being published. They took care to eliminate double counting of study subjects appearing in more than one publication. Effect confounding: matched studies meta-mean difference 0.58 (-0.03 to 0.82) versus 1.58 (0.03 to 3.12) for unmatched studies; Exposure level: low exposure studies meta-mean difference 0.44 (-0.93 to 1.82) versus 1.79 (0.38 to 3.21) for high exposure	here considers the difference between exposed and control; in Costa et al. (2016) below, it is the ratio.
Costa et al. (2016), (12) Systematic review and meta-analysis, including <i>in</i>	Occupational exposure (mostly reinforced plastics industry) to styrene, assessed by	Micronucleus frequencies (cytokinesis-block micronucleus assay) mainly in peripheral blood	11 studies (but see column Remarks) together including 479 styrene-exposed workers and 510	Effect of confounders assessed 1) by meta-regression (gender, age, smoking): Meta-MR 1.38 (1.28 to 1.50), $P<0.001$, i.e.	From Colliens et al. (2016), above, it appears that two included studies concerned

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<i>vivo</i> human exposure studies (published in period 2004-2012) that quantified micronuclei with the cytokinesis-block micronucleus assay (reported with sufficient detail on experimental protocol); excluded were studies without control group and/or concurrent exposure to other known genotoxicants	questionnaire, measurement of air levels, or biomarkers. Type of assessment accounted for in (overall) quality score of study, which also included technical parameters and consideration of confounders. Comparison of outcome: exposed versus controls.	lymphocytes (also other cell types). Effect size expressed as Meta-MR: ratio of mean micronucleated cell frequency of exposed versus control, weighted for variances of studies	controls, and 13 comparisons (exposed versus control). All studies cross-sectional. Meta-MR (see Health Assessment) 1.34 (95% CI 1.18 to 1.52, random effects model), but $I^2=67\%$, $P<0.001$, fixed effect model	similar; 2) Stratification for tertiles of Quality Score (similar results). No evidence for publication bias (Egger test and funnel plot). No study disproportionately influential (sensitivity analysis) The heterogeneity I^2 between studies (high I^2) might be partly due to the relatively low number of individuals included	subsets of two other studies that were included as independent studies
Kirsch-Volders et al. (2018), (13) Synthesis of 14 systematic reviews and/or meta-analyses that used same selection and evaluation criteria. Methodology of individuals meta-analyses same as	Exposure to various chemicals, either occupational or environmental, amongst which styrene (The study Costa et al. (2016), see above. Together, the 14 reviews assessed occupational	Micronucleus frequencies as measured with the lymphocyte Cytokinesis-Block Micronucleus Assay	Result for styrene: reviews found consistent increases in micronuclei in exposed versus controls; micronuclei not induced under the recommended threshold limit	Purpose of this review was to evaluate the validity of this test as a biomarker for DNA damage induced by human exposure to chemical with different modes of action, and to compare the frequency fold changes between substances	This review of reviews is not very relevant. The information on styrene is derived from Costa et al. (2016).

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Cosat et al. (2016) above.	exposure to heavy metals (As, Cr, Ni, Hg, Pb, Cd), vinyl chloride, formaldehyde, "miscellaneous", pesticides, cytostatics/antineoplastic agents, anaesthetic gasses, dust/asbestos/other fibers, polycyclic aromatic hydrocarbons, ethylene oxide, butadiene, styrene and petroleum/derivatives. Exposures were higher in workers in open moulding process than in 'closed' process workers			with different modes of action.	

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
COHORT STUDIES					
Yager et al. (1993), (15) Prospective cohort study including 48 workers at a reinforced plastic boat manufacturing facility	Concentrations of styrene in breath and 8-hour time-weighted average exposures measured for everyone at 7 randomly chosen days for 1 year. Mean personal breathing zone styrene concentrations were 64.2 (S.D. 71.5) mg/m ³ , range 0.88-235.35 mg/m ³ . Mean breath styrene concentrations 1.65 (S.D. 1.82) mg/m ³ , range 0-7.16 mg/m ³	Sister chromatid exchanges (SCEs) were analysed twice in peripheral blood lymphocytes and micronuclei (MN) 4 times during this year	Mean values 6.4 SCEs per cell, S.E 0.2, range 4.7-9.5 SCEs per cell. Dose-response relation: Regression of mean SCEs per cell on styrene concentration in air $Y=6.094 + 0.022 \cdot X$, $P=0.007$, $R^2=0.150$. Regression of mean SCEs per cell on styrene concentration in breath $Y=6.035 + 0.243 \cdot X$, $P=0.0013$, $R^2=0.211$. Including smoking in regression: smoking was found to contribute 62% and styrene exposure 25% to total variability		
Yager et al. (1990), (16)					

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Presentation of preliminary results of the study above					

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
CASE-CONTROL STUDIES					
Kolstad et al. (1996), (17) Case-control study, nested within Danish reinforced plastics industry including 19 myeloid leukaemia patients as cases and 57 employees from similar industries without exposure as referents	Occupational exposure to styrene	Myeloid leukaemia with clonal chromosome aberrations (12 of the 19 cases)	Risk of myeloid leukemia with clonal chromosome aberrations 2.5 (95% CI 0.2-25.0) in workers at companies with styrene exposure	Purpose of the study was to investigate whether the increased risk of leukaemia in styrene exposed is due to an association between myeloid leukaemia with clonal chromosome aberrations	
CROSS-SECTIONAL STUDIES					
Vodicka et al. (2001), (18) Cross-sectional study comparing 44 reinforced plastics workers (hand-laminators) to 18 unexposed controls working at same	Mean values of styrene measured in workplace air, styrene in blood and in exhaled air	Comet assay and cytogenetic analysis on peripheral blood lymphocytes for DNA single-strand breaks (SBB), frequency of chromosomal aberrations (CA),	Multivariate regression found SSBs to be associated with styrene exposure and with CYP2E1 heterozygosity ($r^2=0.614$); CA correlated with years of employment ($P=0.004$) and with	Probably an extension of the study below (Vodicka et al. (1999), but not stated explicitly	

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
plant (but unexposed) (Czech Republic)		mutation frequency in hypoxanthine guanine phosphoribosyl transferase (HPRT), and polymorphisms in CYP1A1, CYP2E1, GSTM1, GSTT1 and GSTP1, EPHX.	combinations of <i>EPHX</i> genotypes (exon 3, Tyr/His and exon 4, His/Arg), ($P=0.044$, $r^2=0.614$). ANOVA showed HPRT mutant frequencies to be associated with years of employment ($F=6.9$, $P=0.0001$), styrene concentration in blood ($F=10.1$, $P=0.0001$), and heterozygosity in CYP2E1 (intron 6; $F=13.5$, $P=0.0008$) and GSTP1 (exon 5; $F=3.6$, $P=0.038$)		
Vodicka et al. (1999), (19) 'Extended' cross-sectional study (repeated measurements over approximately 3-year period, i.e., December 1992-March 1995) comparing 11 hand-lamination workers in lamination plant	Styrene concentrations measured in breathing zone, and its metabolite mandelic acid in urine	Blood analysis: O^6 -guanine styrene DNA adducts, N-terminal valine adducts of styrene in haemoglobin, comet assays for single stranded breaks (SSB), and hypoxanthine guanine phosphoribosyl transferase (HPRT)	<ul style="list-style-type: none"> O^6-guanine styrene DNA adduct levels higher in exposed group (5.9 ± 4.9 adducts/10^8 dNp versus 0.7 ± 0.8 adducts/10^8 dNp, $P=0.001$). DNA adduct levels significantly correlated with haemoglobin adducts, SSB 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
in Bohemia, Czech Republic, to 7 controls working at same plant (but unexposed)		mutant frequencies (MF) in T-lymphocytes	<p>parameters and years of employment.</p> <ul style="list-style-type: none"> • N-terminal valine adducts detected only in exposed • N-terminal valine adducts correlated strongly with external exposure indicators, DNA adducts and HPRT MF • HPRT MF higher in exposed ($22.3 \pm 10.6/10^6$ versus $14.2 \pm 6.5/10^6$) <p>1 Women showed higher SSB parameters</p>		
<p>Buschini et al. (2003), (20)</p> <p>Cross-sectional study including 48 workers exposed to styrene and 14 unexposed healthy controls at factories producing polyester resins or glass-fiber reinforced plastics.</p>	Personal inhalation exposure monitored by passive air sampling and GC/MS; urinary mandelic acid and phenylglyoxylic acid monitoring	DNA damage in peripheral blood leukocytes by comet assay and polymorphisms in glutathione S-transferase genes GSTM1, GSTT1 and GSTP1 and the epoxide hydrolase encoding gene EPHX.	<ul style="list-style-type: none"> • Exposed workers higher levels of DNA damage compared to controls. Exposed versus unexposed: Tail moment > 99th percentile (TM99) 12.4 (SD 4.9) vs 34.1 (14.0), $P < 0.001$ > 95th percentile 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
		In unexposed, in vitro assessment of styrene-7,8-oxide-(SO) induced DNA damage	<p>reference distribution (URL95) 5.1(4.9) vs 15.1(9.8), $P < 0.001$</p> <ul style="list-style-type: none"> • > 99th percentile reference distribution (URL99) 0.7(0.9) vs 4.6(3.4), $P < 0.001$ Within exposed group GSTM1 positive genotype significantly higher proportion of damaged nuclei compared to null genotype, while this was reversed for GSTT1. • A dose-response relationship observed for SO <i>in vitro</i>. Homozygous GSTP1 wildtype showed less damage compared to individuals bearing at least one GSTP1 variant allele.. 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Somorovská et al. (1999), (21) Cross-sectional study of 44 workers at a hand lamination plant with case-control component involving 19 unexposed controls	Exposure to styrene in workplace air and in exhaled air, and styrene measured in blood	DNA strand breaks (SBs), measured by modified comet assay, and oxidised bases in mononuclear leukocytes, chromosomal aberrations in lymphocytes, polymorphisms in CYP1A1, EPHX, GSTM1 and GSTP1 genes and immune parameters	Higher number of SBs in styrene-exposed versus controls ($P < 0.001$). Correlation between SBs and years of exposure $r = 0.545$, $P < 0.001$. Also increased frequency of chromosomal aberrations ($P < 0.0001$ for highly exposed group, $P < 0.004$ for medium exposed group, and $P = 0.0001$ for low-exposed group.	<ul style="list-style-type: none"> • Various effects on immune parameters mentioned: The proliferative response of T-lymphocytes to concanavalin A stimulation suppressed in exposed ($P < 0.05$). Increase in percentage monocytes in differential white blood cell counts in exposed ($P < 0.05$). Also flow cytometric increased expression of CD62L, CD18, CD11a, CD11b, CD49d and CD54 ($P < 0.05$) 	
Hallier et al. (1994), (22) Cross-sectional study followed by an intervention, on 28 workers (14 laminators (manually applying plastic polymers	Exposure to styrene inhalation. Before intervention workers were exposed to estimated time-weighted average of 40 ppm (laminators) or 10 ppm (formers), with	Sister chromatid exchanges (SCEs) in peripheral blood lymphocytes	Laminators versus controls: 9.59 ± 0.77 SCEs/cell vs 7.23 ± 1.00 SCEs/cell) in smokers, and 10.25 ± 1.08 SCEs/cell vs 5.98 ± 0.60 SCEs/	<ul style="list-style-type: none"> • The intervention consisted of a lowering of the occupational exposure limit for styrene from 100 ppm to 20 ppm and the consequent technical and 	

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
dissolved in styrene onto prepared surfaces) and 14 formers (manufacturing plastic products from unformed masses with the help of machinery), and 20 controls in Germany	estimates based on ambient air monitoring and urinary mandelic acid.		<p>cell in non-smokers. (Both statistically significant but P-values not reported)</p> <p>One year after the intervention, SCE frequency had dropped to 9.02 ± 1.19 SCEs/cell in the laminator smokers and 7.74 ± 0.59 SCEs/cell in the laminator non-smokers. (insufficient numbers for statistics)</p> <p>Formers versus controls: No difference in SCE frequency</p>	hygienic improvements. This led to an estimated reduction from 40 ppm to 20 ppm for laminators	
Andersson et al. (1980) , (23) Cross-sectional study comparing 36 exposed to styrene to 37 unexposed workers from same factory	Styrene exposure of workers in reinforced plastics boat factory in Sweden. Styrene concentration in the air measured for various production	Chromosomal aberrations and sister-chromatid exchanges (SCE) in peripheral blood lymphocytes. Blood	<ul style="list-style-type: none"> • Increase of aberrations in exposed workers, mean 7.9 aberrations/100 cells, versus unexposed, mean 3.2 aberrations/100 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	processes. Air samples taken 6 times in period 1973-1978. Total exposure expressed as 8 hour work shift averaged air concentration (mg/m ³) times employment duration (years). Categorised into high (1204 mg/m ³) versus low (137 mg/m ³)	samples taken in 1978	cells, P<0.001). No significant difference between high-exposed versus low exposed, but within low exposed group a dose response was observed (r=0.576) Slight increase of SCE in exposed workers (n=20), mean 8.4 SCE/cell, versus unexposed (n=21), mean 7.5 SCE/cell, P<0.05).		

10 Carcinogenicity

10.1 **Summary of animal experiments on styrene**

The carcinogenicity studies of styrene in experimental animal studies are summarized in Table 8 followed by a summary in text. In general, only statistically significant results are presented in the table below. In studies where statistical significance of the results was not reported, the listed tumour incidences in the table were limited to the control group and groups where actual lesions occurred.

Table 8 Summary table of in vivo animal experiments with styrene

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
<i>Oral</i>					
Maltoni et al., 1982 (31)	Rat, Sprague-Dawley 13 weeks old Males and females: 40/sex/group	Carcinogenicity study (brain tumours) All animals included until spontaneous death. Statistics not reported.	Test item: styrene (purity not stated, in olive oil) 0 (vehicle), 50, 250 mg/kg bw/day 4-5 days weekly for 52 weeks. Oral via gavage	<i>Observations</i> Examination of animals on gross changes every two weeks. Full autopsy and histopathology on each animal. Extra examination of brain. <i>Results</i> Incidence in total brain tumour bearing animals in males (control: 0/40; 50 mg/kg bw/day: 1/40; 250 mg/kg bw/day: 1/40) and in females (control: 1/40; 50 mg/kg bw/day: 4/40; 250 mg/kg bw/day: 1/40).	Non-GLP Non-guideline Limited reporting on data and methods.
Beliles et al., 1985 (32)	Charles River COBS (SD) BR rats Male: - 76 controls - 50/exposure group Female: - 106 controls - 70/exposure group	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; At 52 weeks, 10 rats/sex/group were sacrificed. Statistical analyses:	Test item: styrene (in deionised water) Purity: 98.9% Nominal dose: 0 (vehicle), 125, 250 ppm in drinking water (corresponding to 0, 8.9, 17.9 mg/kg bw/day) ^a ; Oral, drinking water; continuous exposure for 2 years	<i>Observations</i> Twice weekly observation. Body weights of 16 rats/sex/group were measured weekly until 90 days and monthly thereafter. Food consumption was measured on these animals weekly. Water consumption was measured daily for 4 weeks and twice each month thereafter. At week 19, 26, 52, 70, 86 and 102 body weight and water and food consumption were obtained on all animals. Blood and urine analysis: 5 rats/sex/dose in the high dose and control group at week 4, 26, 52 and 76 and from 14 rats/sex/dose for all dose groups at week 13 and at termination.	Non-GLP Non-guideline Only the results of the chronic toxicity segment are reported in this table and the text below.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		<ul style="list-style-type: none"> - No statistics for tumour incidences - Dunnet's t-test or Wilcoxon Rank sum test for other parameters 		<p>Ophthalmic examination at 35, 51 and 104 weeks on all rats.</p> <p>At 52 weeks (10 rats/sex/group) or at study termination (all remaining rats), brain, heart, liver, spleen, kidneys, testes, ovaries and uteri were weighed. For each rat, 36 representative tissues and additional grossly visible lesions were examined histologically and microscopically.</p> <p><i>Results</i> Weekly analytical mean concentrations were approximately 90% of nominal concentrations.</p> <p>Survival: not significantly different from controls.</p> <p>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).</p> <p>Non-neoplastic lesions: non-treatment related pathological changes across all groups, no details reported.</p> <p>Neoplastic lesions: no significant increase in treatment-related tumour incidences in rats treated for two years.</p>	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
Conti et al., 1988 (33)	Sprague-Dawley rats 13 weeks old Males and females, 40/sex/dose group	Carcinogenicity study Males and females, included until spontaneous death.	Test item: styrene (in olive oil) Purity: 99.8% 0 (vehicle), 50 and 250 mg/kg bw/day, for 4-5 days per week for 52 weeks Oral, via gavage	<i>Observations</i> Three times daily status and behavioural observations, twice weekly clinical observation. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. Full necropsies and histopathological examinations were performed on all animals. <i>Results</i> 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).	Non-GLP, Non-guideline. No details on statistical analyses reported, limited reporting on the data.
NCI, 1979a (34)	F344 rats, 6 weeks old Males and females Controls: 20/sex Exposed: 50/sex/dose group	Carcinogenicity study Animals were sacrificed 29 weeks after the treatment period. Statistical analyses: - Survival: Kaplan Meier	Test item: mixture of styrene (70%) and β -nitrostyrene (30%) (in corn oil). Males: 0 (vehicle), 150, 300 mg/g bw, 3 times per week	<i>Observations</i> Twice daily inspection for mortality. Body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals. Full necropsies and histopathological examinations were performed on all animals.	Non-GLP Non-guideline Authors report one-tailed p-values.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		<ul style="list-style-type: none"> - 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 	<p>Females: 0 (vehicle), 75, 150 mg/kg bw, 3 times per week</p> <p>Exposure for 79 weeks.</p> <p>Oral, via gavage.</p>	<p><i>Results</i></p> <p>Survival was not affected by styrene. Mean body weight was decreased in male rats (300 mg/kg bw) compared to control.</p> <p>No significant effects in tumour incidences.</p>	
NCI, 1979a (34)	<p>B6C3F1 mice, 6 weeks old</p> <p>Males and females</p> <p>Controls: 20/sex</p> <p>Exposed: 50/sex/dose group</p>	<p>Carcinogenicity study</p> <p>Animals were sacrificed 14 weeks after the treatment period.</p> <p>Statistical analyses:</p> <ul style="list-style-type: none"> - 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 	<p>Test item: mixture of styrene (70%) and β-nitrostyrene (30%) (in corn oil).</p> <p>0 (vehicle), 87.5 and 175 mg/kg bw, 3 times per week, for 78 weeks</p> <p>Exposure for 78 weeks via oral gavage.</p>	<p><i>Observations</i></p> <p>Twice daily inspection for mortality. Body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals.</p> <p>Full necropsies and histopathological examinations were performed on all animals.</p> <p><i>Results</i></p> <p>Survival and body weight:</p> <ul style="list-style-type: none"> -In males, a dose-response relation for mortality was observed ($P=0.007$). -Mean body weight was decreased in female mice (175 mg/kg bw) compared to control. <p>Non-neoplastic lesions:</p>	<p>Non-GLP</p> <p>Non-guideline</p> <p>Authors report one-tailed p-values.</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>-Increased incidence of haemorrhage and necrosis in the liver of males compared to low dose and control (175 mg/kg) (175 mg/kg: 16/50; 87.5 mg/kg: 3/50; 0 mg/kg: 1/20).</p> <p>Neoplastic lesions:</p> <p>-Increased incidence of combined lung alveolar/bronchiolar carcinoma or adenomas in male mice in low dose (87.5 mg/kg bw, P=0.016) compared to control (0 mg/kg: 0/20; 87.5 mg/kg: 11/49; 175 mg/kg: 2/36).</p>	
NCI 1979b (35)	<p>F344 rats, 6 weeks old</p> <p>Males and females</p> <p>Controls: 2 control groups of 20/sex</p> <p>Exposed: 50/sex/dose group</p>	<p>Carcinogenicity study</p> <p>Rats were sacrificed at 27 weeks (1000 and 2000 mg/kg bw) or 1 week (500 mg/kg bw) after the exposure period.</p> <p>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.</p> <p>Statistical analyses:</p> <p>- Survival: Kaplan Meier</p>	<p>Test item: styrene (in corn oil). Purity not mentioned.</p> <p>0 (two groups), 500, 1000 and 2000 mg/kg bw, 5 days per week</p> <p>Exposure for 78 weeks for 0 (first control), 1000 and 2000 mg/kg bw group, for 103 weeks for 0 (second control) and 500 mg/kg bw rats.</p> <p>Oral exposure via gavage.</p>	<p><i>Observations</i></p> <p>Twice daily inspection for mortality. Body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals.</p> <p>Full necropsies and histopathological examinations were performed on all animals.</p> <p><i>Results</i></p> <p>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.</p> <p>Neoplastic lesions:</p>	<p>Non-GLP Non-guideline.</p> <p>Authors report one-tailed p-values.</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		<ul style="list-style-type: none"> - 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 		There was no significant increase in tumour incidences.	
NCI 1979b (35)	<p>B6C3F1 mice, 6 weeks old</p> <p>Males and females</p> <p>Controls: 20/sex</p> <p>Exposed: 50/sex/dose group</p>	<p>Carcinogenicity study</p> <p>Mice were sacrificed 13 weeks after the exposure period.</p> <p>Statistical analyses:</p> <ul style="list-style-type: none"> - 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 	<p>Test item: styrene (in corn oil). Purity not mentioned.</p> <p>0, 150 and 300 mg/kg bw, 5 days per week</p> <p>Exposure for 78 weeks.</p> <p>Oral exposure via gavage.</p>	<p><i>Observations</i></p> <p>Twice daily inspection for mortality. Body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals.</p> <p>Full necropsies and histopathological examinations were performed on all animals.</p> <p><i>Results</i></p> <p>Survival and body weight: In males, mortality was increased in all dose groups. Mortality was not affected in females. Slight dose-related mean body weight depression was observed in females.</p> <p>Neoplastic lesions:</p> <ul style="list-style-type: none"> - In males (300 mg/kg bw), a significant increase in combined adenomas and carcinomas 	<p>Non-GLP Non-guideline.</p> <p>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.</p> <p>Authors report one-tailed p-values.</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>of the lung compared to control (0 mg/kg bw: 0/20; 150 mg/kg bw: 6/44; 300 mg/kg bw: 9/43, P=0.024).</p> <p>- In females, a positive association between dose and incidence of hepatocellular adenomas was observed (0 mg/kg bw: 0/20; 150 mg/kg bw: 1/44; 300 mg/kg bw: 5/43, P=0.034). However, comparison of individual groups to control was not significant.</p>	
Ponomarkov et al., 1978 (36)	BD IV rats 21 exposed and 10 control pregnant dams and their offspring.	<p>Carcinogenicity study</p> <p>All animals were sacrificed at 120 weeks.</p> <p>Statistics: No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.</p>	<p>Test item: styrene (in olive oil) Purity: 99%</p> <p>0 (vehicle), and 1350 mg/kg bw (dams) or 500 mg/kg bw (offspring)</p> <p>Single administration on day 17 of gestation (pregnant dams), weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan.</p> <p>Oral, via gavage.</p>	<p><i>Observations</i> Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.</p> <p><i>Results</i> Survival and body weights: Prewaning mortality in offspring of styrene-treated pregnant females increased (offspring, styrene: 10%; offspring, olive oil: 2.5%). No differences in survival or body weights.</p> <p>Non-neoplastic lesions: Several lesions in all animals such as congestion of lung and kidney and necrotic areas in liver, forestomach and kidney.</p> <p>Neoplastic lesions: - Increased incidence (not statistically significant) in tumour-bearing females receiving</p>	Non-GLP Non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>a single styrene administration during pregnancy (styrene: 65%; olive oil: 59%).</p> <ul style="list-style-type: none"> - Stomach tumours occurred (females pregnancy, styrene: 1/20; offspring females, styrene: 2/68; offspring females, olive oil: 1/35). - 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. 	
Ponomarkov et al., 1978 (36)	<p>O20 mice</p> <p>29 exposed and 9 control pregnant dams and their offspring.</p> <p>Extra control group of 54 males and 47 females.</p>	<p>Carcinogenicity study</p> <p>All animals were sacrificed at 120 weeks.</p> <p>Statistics: No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.</p>	<p>Test item: styrene (in olive oil) Purity: 99%</p> <p>0 (olive oil or untreated) and 1350 mg/kg bw</p> <p>Single administration on day 17 of gestation (pregnant dams), weekly administration to offspring from the time of weaning.</p>	<p><i>Observations</i> Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.</p> <p>Treatment of offspring was suspended after 16 weeks due to toxicity.</p> <p><i>Results</i> Survival: - Prewaning 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</p>	Non-GLP Non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
			<p>Offspring treated for whole lifespan.</p> <p>Oral, via gavage.</p>	<p>died. Observed lesions: liver necrosis, spleen hypoplasia, congestion of lungs.</p> <p>- 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.</p> <p>Neoplastic lesions:</p> <p>- 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%).</p> <p>- 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.</p> <p>- 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).</p>	
Ponomarko v et al., 1978 (36)	C57 BL mice 15 exposed and 5 control	Carcinogenicity study All animals were sacrificed at 120 weeks.	Test item: styrene (in olive oil) Purity: 99%	<p><i>Observations</i></p> <p>Full necropsies and histopathological examinations were performed on all animals.</p>	Non-GLP Non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
	pregnant dams and their offspring. Extra control group of 51 males and 49 females.	Statistics: No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	0 (olive oil or untreated) and 300 mg/kg bw Single administration on day 17 of gestation (pregnant dams), weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan. Oral, via gavage.	No further details on observations are mentioned. <i>Results</i> 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. - Increased incidence of lymphomas (females, styrene: 10/12; females, olive oil: 3/5 females, untreated: 20/47; not statistically significant). - Increased incidence of hepatocellular carcinomas or adenoma in males: (styrene: 3/24; olive oil: 1/12; untreated: 1/47)	
<i>Inhalation</i>					
Jersey et al., 1978 Not published, based on secondary sources Described by NTP in 2008. (28)	Rat, Sprague-Dawley 7-8 weeks old 96/97 males/group and 96 females/group	Carcinogenicity study Interim sacrifices of 5/6 animals/sex/group after 6 and 12 months. Exposure until 50% mortality. Observation until death or 24 months.	Test item: styrene Purity: 99.5% 0, 600 or 1000 ppm (first 2 months at 1200 ppm) (corresponding to: 0, 2556 or 4260 mg/m ³) ^a Inhalation, 6h/day, 5 days/week for 18.3	<i>Observations</i> No details. <i>Results</i> After 2 months, excessive toxicity in 1200 ppm group. The dose was reduced to 1000 ppm. Survival was lower in males (attributed to chronic murine pneumonia) than in females: controls (5 males, 30 females), 600 ppm (18 males, 30 females), 1000 ppm (6 males, 22 females).	Non-GLP. Non-guideline Secondary sources (McConnell and Swenberg, 1994) noted that "this study was seriously flawed by the presence of

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		Cochran-Armitage exact trend test on tumour incidences, conducted by NTP.	months (males) or 20.7 months (females).	<p>Neoplastic lesions:</p> <ul style="list-style-type: none"> - Increased incidence of mammary adenocarcinoma in females at 600 ppm (8.2%) compared to control (1.2%). No increase compared to historical control (mean 5.8%, range 0-9%). Trend: P=0.002 - Combined incidence of lymphosarcoma and leukemia in females (controls: 1.2%; 600 ppm: 7.1%; 1000 ppm: 7.1%) and males (controls: 1.2%; 600 ppm: 5.8%; 1000 ppm: 1.2%). 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 	<p>chronic murine pneumonia, which caused a high rate of mortality in both control and exposed male rat."</p> <p>Not clear whether nose-only or whole body inhalation applied</p>
Maltoni et al., 1982 (31)	<p>Rat, Sprague-Dawley 13 weeks old</p> <p>Males and females (styrene): 30/sex/group</p> <p>Controls: 60/sex/group</p>	<p>Carcinogenicity study (brain tumours)</p> <p>All animals included until spontaneous death.</p> <p>Statistics not reported.</p>	<p>Test item: styrene (purity not stated)</p> <p>0 (control), 25, 50, 100, 200 and 300 ppm (corresponding to: 0, 106, 213, 426, 852, 1278 mg/m³)^a.</p> <p>Inhalation, styrene in air, 4 hours/day, 5 days/week for 52 weeks.</p>	<p><i>Observations</i> Examination of animals on gross changes every two weeks. Full autopsy and histopathology on each animal. Extra examination of brain.</p> <p><i>Results</i> Incidence in total brain tumour bearing animals in males (control: 0/60; 25 ppm: 1/30; 100 ppm: 1/30) and in females (control: 0/60; 25 ppm: 1/30; 100 ppm: 3/30).</p>	<p>Non-GLP. Non-guideline</p> <p>Limited reporting on data and methods.</p> <p>Not clear whether nose-only or whole body inhalation applied</p>
Cruzan et al., 1997	Rat, CD	Subchronic inhalation study (13 weeks)	Test item: styrene Purity: >99.4%	<i>Results</i>	Non-GLP. Non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
(37)	6-7 weeks of age Males and females: 10/sex/group Extra satellite groups	Statistics: - Parametric: One-way ANOVA, Williams' test for dose response - Non-parametric: Kruskal-Wallis Satellite group: - 15 males/group, cell proliferation (BdrU assay) examined at 2, 5 or 13 weeks of exposure.	0 (control), 200, 500, 1000, 1500 ppm (Corresponding to: 0, 852, 2130, 4260, 6390 mg/m ³) ^a Inhalation, styrene vapour, whole body, 6h/day 5 days/week for 13 weeks (65 exposures)	Analytical concentrations were within 2% of target concentrations. Survival and clinical observations: - No effects on survival. All styrene-exposed rats showed signs indicative of irritating properties during exposure. - Males (1500 ppm) weighed 10% less and consumed 7% less food compared to controls at week 13. - Dose-related increase in water consumption (males and females, 1000 and 1500 ppm). Females (1500 ppm) drank twice as much as controls. - Urine pH was decreased in a dose-related manner. Non-neoplastic lesions in olfactory epithelium of nasal passage at 500-1500 ppm: - Focal disorganization with rosette formation was seen in males (0 ppm: 0/10; 500 ppm: 1/10; 1000 ppm: 5/10; 1500 ppm: 10/10) and females (0 ppm: 0/10; 500 ppm: 1/10; 1000 ppm: 4/10; 1500 ppm: 5/10). - Focal hyperplasia was seen in males (0 ppm: 1/10; 500 ppm: 1/10; 1000 ppm: 7/10; 1500 ppm: 10/10) and females (0 ppm: 0/10; 1000 ppm: 1/10; 1500 ppm: 5/10). - Single cell necrosis was seen in males (0 ppm: 0/10; 1000 ppm: 1/10; 1500 ppm: 10/10) and females (0 ppm: 0/10; 1500 ppm: 1/10).	Statistical significance was not consistently reported in the results section.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>- Apparent cell loss was seen in males (0 ppm: 0/10; 1500 ppm: 7/10) and females (0 ppm: 0/10; 1500 ppm: 1/10).</p> <p>No effects observed in the BrdU assay at 2, 5 or 12 weeks of exposure.</p>	
Cruzan et al., 1997 (37)	<p>CD-1 Mice 4 weeks of age</p> <p>Males and females: 20/sex/group</p> <p>B6C3F1 Mice 4-5 weeks of age</p> <p>Males and females: 20/sex/group</p>	<p>2-week inhalation study</p> <p>Statistics: - Parametric: One-way ANOVA, Williams' test for dose response - Non-parametric: Kruskal-Wallis</p>	<p>Test item: styrene Purity: >99.4%</p> <p>0 (control), 15, 60, 250, 500 ppm (Corresponding to: 0, 64, 256, 1065, 2130 mg/m³)^a</p> <p>Inhalation, styrene vapour, whole body, 6h/day 5 days/week for 2 weeks (10 exposures)</p>	<p><i>Observations</i> Clinical observations: daily individual observation before and after exposure, group observation during exposure. Body weight was determined weekly.</p> <p>Full necropsy and histopathological examinations were performed on 10 animals/sex/group.</p> <p><i>Results</i> Survival and clinical observations: - Styrene-exposed mice showed signs indicative of irritating properties during (all groups) or between (250 and 500 ppm groups) exposure. - Mortality was increased in 250 and 500 ppm groups. In females mortality at 250 ppm was more severe than at 500 ppm in both strains.</p> <p>Non-neoplastic lesions: - Liver toxicity at 250 and 500 ppm with increased liver weights, macroscopic changes, and microscopically centrilobular hepatocyte</p>	Non-GLP, non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				necrosis. In females microscopic lesions were more severe at 250 ppm than at 500 ppm. - Microscopic changes seen in B6C3F1 mice were more severe compared to CD-1 mice.	
Cruzan et al., 1997 (37)	CD-1 Mice 4 weeks of age Males and females: 10/sex/group Extra satellite groups	Subchronic inhalation study (13 weeks) Satellite groups: - 5/sex/group (liver effects), 1 week of exposure -30 males/group, cell proliferation examined at 2, 5 or 13 weeks of exposure Statistics: - Parametric: One-way ANOVA, Williams' test for dose response -Non-parametric: Kruskal-Wallis	Test item: styrene Purity: >99.4% 0, 50, 100, 150, 200 ppm (Corresponding to: 0, 213, 426, 639, 852 mg/m ³) ^a Inhalation, styrene vapour, whole body, 6h/day 5 days/week for 13 weeks (65 exposures)	<i>Observations</i> - Clinical observations: daily individual observation before and after exposure, group observation during exposure. - Body weight was determined weekly. Food and water consumption were monitored throughout study. - Blood and urine analysis of all animals at 13 weeks. - First satellite group: examined after 1 week for serum SDH, ALT, total bile acids and histopathological changes in liver. - Second satellite group: changes in cell proliferation with in vivo BrdU assay, staining of lung and liver. Full necropsies and full histopathological examinations were performed on all control and 200 ppm animals. Necropsies and histopathological examination of nasal passages, lungs and liver was performed on all animals. <i>Results</i> Analytical concentrations were within 1% of target concentrations.	Non-GLP. Non-guideline Statistics were not fully reported in the results section. Only incidences in the control group and groups where lesions occurred are mentioned here.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>Survival (and histopathology), and body weights:</p> <ul style="list-style-type: none"> - 2 females (200 ppm) died during the first week of exposure due to liver toxicity (multiple macroscopic lesions and centrilobular hepatocyte necrosis) and with severe nasal lesions. No clinical signs or mortality in the other groups. - Males (200 ppm) had reduced body weights and food consumption during the study. <p>Non-neoplastic lesions (1 week exposure):</p> <ul style="list-style-type: none"> - Macroscopic and microscopic liver lesions (hepatocyte loss, inflammation and necrosis within areas of histiocytosis) in 5/5 females (200 ppm). <p>Non-neoplastic lesions in liver (13 weeks exposure):</p> <ul style="list-style-type: none"> - Focal loss of hepatocytes with siderophages in males (0 ppm: 0/10; 200 ppm: 1/10) and females (0 ppm: 0/10; 200 ppm: 3/8). - Centrilobular aggregates of siderophages in males (0 ppm: 0/10; 200 ppm: 2/10) and females (0 ppm: 1/10; 150 ppm: 3/10; 200 ppm: 5/8). - Centrilobular hepatocyte necrosis in females (0 ppm: 0/10; 150 ppm: 1/10). - Areas of fibrosis and mineralization with siderophages in females (0 ppm: 0/10; 150 ppm: 1/10). 	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>- Interlobular adhesions in females (0 ppm: 0/10; 200 ppm: 2/8).</p> <p>Non-neoplastic lesions in nasal passages (13 weeks exposure): Many lesions occurred, the principal lesions are:</p> <ul style="list-style-type: none"> - Atrophy in olfactory epithelium in males (0 ppm: 0/10; 50 ppm: 5/10; 100 ppm: 10/10; 150 ppm: 10/10; 200 ppm: 9/10) and females (0 ppm: 0/10; 50 ppm: 4/10; 100 ppm: 10/10; 150 ppm: 10/10; 200 ppm: 8/10). - Metaplasia in olfactory epithelium in males (0 ppm: 0/10; 100 ppm: 1/10; 150 ppm: 3/10; 200 ppm: 5/10) and females (0 ppm: 0/10; 100 ppm: 3/10; 150 ppm: 2/10). - Atrophy of olfactory nerve fibers in males (0 ppm: 0/10; 50 ppm: 1/10; 100 ppm: 6/10; 150 ppm: 9/10; 200 ppm: 10/10) and females (0 ppm: 0/10; 50 ppm: 3/10; 100 ppm: 8/10; 150 ppm: 7/10; 200 ppm: 8/10). - Dilatation, hypertrophy and hyperplasia of Bowman's glands in males (0 ppm: 0/10; 50 ppm: 6/10; 100 ppm: 9/10; 150 ppm: 10/10; 200 ppm: 10/10) and females (0 ppm: 3/10; 50 ppm: 6/10; 100 ppm: 10/10; 150 ppm: 10/10; 200 ppm: 8/10). <p>Non-neoplastic lesions in lungs (13 weeks exposure):</p> <ul style="list-style-type: none"> - Decreased eosinophilia of bronchiolar epithelial cells in males (0 ppm: 0/10; 50 ppm: 	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>8/10; 100 ppm: 10/10; 150 ppm: 10/10; 200 ppm: 8/10) and females (0 ppm: 0/10; 50 ppm: 9/10; 100 ppm: 10/10; 150 ppm: 10/10, 200 ppm: 7/10).</p> <p>- Focal crowding of nonciliated epithelial cells in bronchioles in males (0 ppm: 0/10; 100 ppm: 3/10; 150 ppm: 4/10; 200 ppm: 5/10) and females (0 ppm: 0/10; 100 ppm: 2/10; 150 ppm 2/10; 200 ppm: 2/10).</p> <p>BrdU assay:</p> <p>- Large variation in labelling index was noted.</p> <p>- After 5 weeks, BrdU-labeled hepatocytes decreased at 100, 150 or 200 ppm ($P < 0.05$).</p> <p>- After 2 or 5 weeks, BrdU-labelled Clara cells increased at 150 (3-fold) or 200 ppm (4-fold) styrene ($P < 0.05$), but not in type II pneumocytes.</p>	
Cruzan et al., 1998 (27)	Rat, CD 4 weeks of age 70/sex/group	<p>Chronic toxicity/oncogenicity study</p> <p>intermittent kills: 9-10 rats/sex/group sacrificed after 52 weeks</p> <p><i>Statistics:</i> Tumour incidence was analysed using methodology described</p>	<p>Test item: styrene (purity: 99.5-99.7%)</p> <p>Concentrations: 0, 50, 200, 500, or 1000 ppm (corresponding to 0, 213, 852, 2130 or 4260 mg/m³)^a</p> <p>Inhalation, styrene vapour, whole body, 6h/day 5 days/week</p>	<p><i>Observations:</i></p> <p>- Body weight weekly for the first 13 weeks, thereafter every 4 weeks.</p> <p>- Overnight water consumption daily at week 1, 4, 12, 25, 51, 77, and 103.</p> <p>- Ophthalmic examination before exposure, at week 52 and 104.</p> <p>- Haematology and clinical chemistry after overnight fasting: 10 males and 10 females at week 13, 26, 52, 78, and 104.</p> <p>- Urine collected overnight after week 13, 26, 52, 78, and 104.</p>	GLP-study

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		by IARC (1980). Other pathologic data were analysed using Fisher's exact test.	for 104 weeks (520 exposures)	<p>- Blood sample taken during the 6h at week 95 exposure to measure concentration of styrene and styrene-7,8-oxide (5/sex/group).</p> <p>Full necropsies and full histopathological examinations were performed on all control and 1000 ppm animals. Histopathologic examination of the nasal passages, lungs, liver, kidneys, testes/epididymides, and macroscopic abnormalities was performed on the animals of all lower exposure levels.</p> <p><i>Results:</i> 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).</p> <p><i>Survival:</i>¹⁷ - No effect on survival of male rats. Dose-related increase in survival of female rats (500 or 1000 ppm).</p> <p>Body weights, food and water consumption: -Males (50 ppm): increased weight gain (15%) compared to control.</p>	

¹⁷ 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.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>- 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).</p> <p>- 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 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.</p> <p>- Males and females (200 ppm): increased water consumption in the first month (112% of control).</p> <p>Clinical observations, clinical pathology and necropsy:</p> <p>- Clinical signs only observed during exposure: salivation with restlessness, hunched posture.</p> <p>- No adverse effects on clinical pathology</p> <p>- No adverse effects on organ weights</p> <p>- No effects at interim necropsy</p> <p>- Terminal necropsy: increased incidences of testis masses (500 ppm and 1000 ppm males),</p>	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>decreased incidences of enlarged pituitary (500 and 1000 ppm females), increased incidence of pale foci in lung (1000 ppm females).</p> <p>Non-neoplastic lesions:</p> <ul style="list-style-type: none"> - 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. <p>Neoplastic lesions:¹⁸</p> <ul style="list-style-type: none"> - 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. - 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 	

¹⁸ 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.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				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).	
Cruzan et al., 2001 (26)	CD-1 mice 70/sex/group Males 104 weeks Females 98 weeks Follow-up study: 55 males	Chronic/oncogenicity study and a follow-up study Interim kills: 10 animals/sex/group terminated at week 52 and 78. Follow up study: - 13 weeks exposure, 13 weeks recovery to examine the course of lung and olfactory effects. - 5 males/group were terminated after 1, 2, 4, 7, 10, 20, 40 and 65 exposures and 4, 8 or 13 weeks recovery time.	Test item: styrene (Purity: >99.5%) 0, 20, 40, 80, and 160 ppm (equivalent of 0, 85, 170, 341, and 682 mg/m ³) ^a Follow up study: 0, 40, and 80 ppm (equivalent of 0, 170, and 341 mg/m ³) ^a Inhalation, styrene vapour, whole body, 6h/day 5 days/week for 104 weeks (males), 98 (females) weeks or	<i>Observations:</i> - Individual observation: daily. Body weight: weekly first 13 weeks and every 4 weeks thereafter. Food consumption: weekly. Overnight water consumption: daily on weeks 1, 4, 12, 25, 51, 77, and 96 (females) or 103 (males) - Ophthalmic examination prior at initial exposure and at 96 (females) or 103 (males) weeks. - Haematology and clinical chemistry after overnight fasting: 10/sex/group at week 13, 26, 52, 78, 96 (females) and 104 (males) Urine collected overnight after week 13, 26, 52, 78, 96 (females) or 104 (males). - Blood sample taken during the 6h exposure at week 74 to measure concentration of styrene and styrene-7,8-oxide (10/sex/group). Full necropsies and full histopathological examinations were performed on all control and	GLP-study

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
		<p>Statistics: Tumour incidence was analysed using methodology described by IARC (1980). Other pathologic data were analysed using Fisher's exact test.</p>	<p>13 weeks (males, follow-up).</p>	<p>160 ppm animals. Histopathologic examination of the nasal passages, nasal passages, lungs, liver, kidneys and macroscopic abnormalities, including all masses was performed on the animals of all lower exposure levels. In the follow-up study all tissues examined in necropsy and histopathology was performed on the nasal tissues and lungs.</p> <p><i>Results:</i> Blood levels of styrene and styrene-7,8-oxide were proportional to the exposure concentration.</p> <p>Survival, observations and body weight:</p> <ul style="list-style-type: none"> - 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. <p>Neoplastic lesions:</p> <ul style="list-style-type: none"> - No effects at week 52 and 78 interim necropsies. 	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>Terminal necropsy:</p> <ul style="list-style-type: none"> - Increase of 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 ($P<0.05$)). - Increased incidence of bronchioloalveolar adenomas in males (control: 15/50; 20 ppm: 21/50; 40 ppm: 35/50 ($P<0.05$); 80 ppm: 30/50 ($P<0.05$); 160 ppm: 33/50 ($P<0.05$)). - Increased 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). - Increased 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 ($P<0.05$)). <p>Non-neoplastic lesions:</p> <ul style="list-style-type: none"> - Styrene exposure induced changes in the lungs and nasal cavity. <p>Lung:</p> <ul style="list-style-type: none"> - Increase of incidence in areas of bronchioloalveolar hyperplasia in males (40, 80 and 160 ppm) ppm and in females (all exposures) after 24 months. - 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. 	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>- At 40 ppm, bronchiolar epithelial hyperplasia and greater at 12 months and at 20 ppm and greater at 18 and 24 months.</p> <p>- 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.</p> <p>Nasal passage: Respiratory metaplasia of the olfactory 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.</p> <p><i>Follow-up study:</i> - No effects in lungs at all exposures.</p> <p>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</p>	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				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 (25)	CD-1 mice C57BL/6 wild-type (WT) mice CYP2F2(-/-) (KO) mice CYP2F2(-/-) 2F1,2A13, mice 2B6-transgenic (TG) 6-7 weeks old 75 animals per group males only	Chronic/oncogenicity study (focussing on lung) <i>Statistics:</i> Body weight: one-way ANOVA Survival: Kaplan and Meier procedure Lung neoplasms and nonneoplastic lesions: Fisher's Exact test	Test item: Styrene monomer PO-11 Bulk Grade (CAS No. 100-42-5, 99.95% pure) 0, 120 ppm (equivalent to 0, 511 mg/m ³) ^a styrene vapor 6h/day, 5 days/week, except holidays	<i>Clinical observations:</i> - Mortality: twice a day (week) and once a day (weekend). - Body weight: weekly for 13 weeks, monthly for 72 weeks, and weekly thereafter. - Histopathology and cell proliferation: 5 mice/group euthanised after 1, 26, 52, and 78 weeks. <i>Results:</i> - No signs of styrene-induced toxicity in any of the 4 strains of mice. - 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. - 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).	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

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>- Cell proliferation in terminal bronchioles was 4- to 5-fold increased at week 1 in exposed CD-1 and WT mice ($P < 0.05$).</p> <p><i>Non-neoplastic lesions:</i></p> <p>- 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 was 10/53 (CD-1 mice) and 34/50 (WT mice) up to 104 weeks of exposure.</p> <p>- 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 was 50/67 versus 0/67 in controls.</p> <p>- 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 was 55/70 versus 0/69 in controls.</p> <p><i>Neoplastic lesions:</i> No statistical significant increase in lung adenomas or adenocarcinomas.</p>	
Conti et al., 1988 (33)	<p>Sprague-Dawley rats</p> <p>Males and females: - 60/sex in control group</p>	<p>Carcinogenicity study</p> <p>Males and females, included until spontaneous death.</p>	<p>Test item: styrene Purity: 99.8%</p> <p>0, 25, 50, 100, 200 and 300 ppm (corresponding to 0,</p>	<p><i>Observations</i></p> <p>Three times daily status and behavioural observations, twice weekly clinical observation. Body weights were recorded every 2 weeks during treatment and then every 8 weeks.</p>	<p>Non-GLP, Non-guideline.</p> <p>No detailed report on statistical analyses,</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
	- 30/sex/group		106, 213, 426, 852, 1278 mg/m ³) ^a Inhalation, whole body, 4h daily, 5 days per week for 52 weeks	Full necropsies and histopathological examinations were performed on all animals. <i>Results</i> Survival and clinical observations: survival was not affected by styrene exposure. No relevant body weight differences were observed. Neoplastic lesions: - Higher incidence of total number of malignant tumours per 100 animals (100 ppm: male 26.7, female: 50.0; control: male: 18.3, female: 28.3) which is not due to the increase in any specific type of tumours. - Higher percentage of animals with mammary tumours in females (control: 56.7%; 25 ppm: 80.0%; 50 ppm: 70.0%; 100 ppm: 76.7%; 200 ppm: 80.0%; 300 ppm: 83.3%). - Higher percentage of animals with malignant mammary tumours in females compared to control (control: 10.0%; 25 ppm: 20.0%; 50 ppm: 13.3%; 100 ppm: 30.0%; 200 ppm: 40.0%; 300 ppm: 30.0%). Increased incidence of malignant tumours was statistically significant (no details).	limited reporting on the data.
<i>Intraperitoneal</i>					
Conti et al., 1988 (33)	Sprague-Dawley rats	Carcinogenicity study	Test item: styrene Purity: 99.8%	<i>Observations</i> Three times daily status and behavioural observations, twice weekly clinical observation.	Non-GLP, Non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
	Males and females: 40/sex/dose group	Males and females, included until spontaneous death.	0 (control) and 50 mg, 4 times at 2 month interval. (total duration not reported) Intraperitoneal injections	Body weights were recorded every 2 weeks during treatment and then every 8 weeks. Full necropsies and histopathological examinations were performed on all animals. <i>Results</i> Survival and clinical observations: survival was not affected by styrene exposure. No relevant body weight differences were observed. No significant increase in the incidence of any tumour types.	No detailed report on statistical analyses, limited reporting on the data.
Brunneman et al., 1992 (38)	A/J mice, 6-8 weeks old Females: 25/group	Carcinogenicity study Females sacrificed at 20 weeks after the final administration. Statistics: student's t-test	Test item: styrene (in olive oil) Purity: 99% 0 (vehicle), 200 µmol/mouse (20 injections of 10 µmol), corresponds to 1042 mg/kg bw ^a Intraperitoneal injection, 3 times weekly, total of 20 injections.	<i>Observations</i> Full necropsies and histopathological examinations were performed on all animals. No further details on observations. <i>Results</i> Data on survival and body weights not reported. Styrene did not significantly increase lung adenomas, or other tumours.	Non-GLP; Non-guideline. Limited reporting.
Cruzan et al., 2013 (39)	C57BL/6 (wild-type) 7-12 weeks old	Non guideline 5-day study to evaluate human relevance for mouse lung tumours.	Test item: styrene Purity: 99.9% 200 mg/kg bw/day (divided into 3 doses)	<i>Observations</i> Daily visual observation. Body weight was recorded before the first dose and the day after the last dose.	Non-GLP; Non-guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
	5 males and females CYP2F2(-/-) /CYP2F1/2A13/2B6 (transgenic, from C57BL/6 mice) 5 males and females	In vivo BrdU assay (cell proliferation) Statistics: - BrdU assay: Two-way analysis of variance with post-hoc tests.	at 2h interval) for 5 days Control: corn oil (vehicle) Intraperitoneal injections	Necropsy and histopathology of lung. BrdU staining in lung. <i>Results</i> - 5-10 fold increase in BrdU labelling in terminal bronchioles in styrene-treated wild-type mice (P<0.05). No effect in transgenic mice.	Number of controls not reported.
<i>Subcutaneous</i>					
Conti et al., 1988 (33)	Sprague-Dawley rats Males and females: 40/sex/dose group	Carcinogenicity study Males and females, included until spontaneous death.	Test item: styrene Purity: 99.8% 0 (control), 50 mg Subcutaneous injection (single injection)	<i>Observations</i> Three times daily status and behavioural observations, twice weekly clinical observation. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. Full necropsies and histopathological examinations were performed on all animals. <i>Results</i> Survival and clinical observations: survival was not affected by styrene exposure. No relevant body weight differences were observed. No significant increase in the incidence of any tumour types.	Non-GLP, Non-guideline. No detailed report on statistical analyses, limited reporting on the data.

^a Converted conform the CLP-Guidance (https://echa.europa.eu/documents/10162/2324906/clp_en.pdf/58b5dc6d-ac2a-4910-9702-e9e1f5051cc5)

10.1.1 *Overview of animal studies with styrene*

Oral studies

Carcinogenicity study in rats exposed to styrene orally (Maltoni et al., 1982).

A carcinogenicity study in Sprague-Dawley rats was performed by Maltoni et al. (31). Rats, 40/sex/group were exposed to styrene in olive oil via oral gavage at dose levels of 0 (vehicle), 50, 250 mg/kg bw/day, 4-5 days weekly during 52 weeks. Animals were included until spontaneous death. Animals were examined for gross changes every two weeks. Full autopsy and histopathology was performed on each animal with a more detailed examination of the brain. Statistics were not performed.

No information on general toxicity was reported. The incidence of total brain tumour bearing animals in males was 0/40 (controls), 1/40 (50 mg/kg bw) and 1/40 (250 mg/kg bw). The incidence of total brain tumour bearing animals in females was 1/40 (controls), 4/40 (50 mg/kg bw) and 1/40 (250 mg/kg bw). The reporting on data and methods was limited.

Chronic toxicity and reproduction study (Beliles et al., 1985)

A chronic toxicity and reproduction study was performed by Beliles et al. (32). 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 (corresponding to 0, 8.9, 17.9 mg/kg bw/day as converted by the CLP guidance). It is noted that the weekly analytical mean concentrations in drinking water were approximately 90% of nominal concentrations.

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 male 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.

Carcinogenicity study in rats (Conti et al., 1988)

A carcinogenicity study in Sprague-Dawley rats was performed by Conti et al. (33). Rats were exposed to styrene (purity: 99.8%) orally via gavage. Details of the exposure are given below. Rats were observed three times daily and clinical observations were done twice weekly. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. All rats were included until spontaneous death. Full necropsies and histopathological examinations were performed on all animals. This was a non-guideline study and the reporting on the data is limited. Details on statistical analysis were not reported.

Male and female rats (40/sex/dose group) were exposed orally to styrene at dose levels of 0 (olive oil), 50 and 250 mg/kg bw/day via gavage 4-5 days per week, for 52 weeks. There was an increased mortality rate in females of the highest dose group (250 mg/kg bw/day). No significant increase in the incidence of any tumour type was reported. However, a lower incidence of total benign and malignant

tumours and of total mammary tumours in females of the highest dose group (250 mg/kg) was observed. This was attributed to the lower survival in the females according to the authors.

Carcinogenicity study of a mixture of styrene and β -nitrostyrene in rats (NCI, 1979a)

A carcinogenicity study in Fischer 344 rats was performed by the NCI (34). 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. Animals were sacrificed 29 weeks after the end of the treatment period. Tumour incidences were statistically analysed with a Fisher exact test (one-tailed). The animals were inspected twice daily for mortality and body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals. 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. There were no significant effects in tumour incidences.

Carcinogenicity study of a mixture of styrene and β -nitrostyrene in mice (NCI, 1979a)

A carcinogenicity study in B6C3F1 mice was performed by the NCI (34). 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 78 weeks. Mice were exposed at dose levels of 0, 87.5 and 175 mg/kg bw/day. Animals were sacrificed 14 weeks after the end of the treatment period. Tumour incidences were statistically analysed with a Fisher exact test (one-tailed). The animals were inspected twice daily for mortality and body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals. Full necropsies and histopathological examinations were performed on all animals. 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. An increased incidence of haemorrhage and necrosis in the liver of males (175 mg/kg bw) compared to low dose and control was observed. Also, a statistically significant increased incidence of combined lung alveolar/bronchiolar carcinoma and adenomas in low dose male mice compared to control was noticed ($P=0.016$). The high dose Fisher exact test and the Cochran- Armitage test, however, were not significant for these neoplastic lesions.

Carcinogenicity study of styrene in rats (NCI, 1979b)

A carcinogenicity study in Fischer 344 rats was performed by the NCI (35). 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, 1000 and 2000 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. Animals were sacrificed at 27 weeks (1000 and 2000 mg/kg bw and control) or 1 week (500 mg/kg bw and control) after the end of the exposure period. Tumour incidences were statistically analysed with a Fisher exact test (one-tailed). The animals were inspected twice daily for mortality and body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals. Full necropsies and histopathological examinations were performed on all animals. 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.

Carcinogenicity study of styrene in mice (NCI, 1979b)

A carcinogenicity study in B6C3F1 mice was performed by the NCI (35). 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. Animals were sacrificed 13 weeks after the end of the exposure period. Tumour incidences were statistically analysed with a Fisher exact test (one-tailed). The animals were inspected twice daily for mortality and body weights were recorded once per week for the first 6 weeks, every 2 weeks for the next 12 weeks and monthly for the rest of the study. Food consumption data were collected monthly from 20% of the animals. Full necropsies and histopathological examinations were performed on all animals. Mortality was increased in all dose groups in males. Combined alveolar/bronchiolar adenomas and carcinomas of the lung compared to control were significantly increased in males (300 mg/kg bw, $P = 0.024$). The study authors noted that a large variation in occurrence of lung tumours exists in historical untreated control male mice and that incidence in vehicle controls was lower than expected based on this data. In females, a slight dose-related mean body weight depression was observed. Also, a positive association between dose and incidence of hepatocellular adenomas was observed ($P = 0.034$). However, comparison of individual groups to control was not significant.

Carcinogenicity study of styrene in rats (Ponomarev et al., 1978)

A carcinogenicity study in BD IV rats was performed by Ponomarev et al. (36). 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. Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned and details of statistical analysis were not reported.

Prewaning 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. Several non-neoplastic lesions

were reported in all animals such as congestion of lungs and the kidneys as well as necrotic areas in the liver, forestomach and kidney. An increased incidence in tumour-bearing females receiving a single styrene administration during pregnancy was observed (not statistically significant).

Carcinogenicity study of styrene in mice (Ponomarkov et al., 1978)

A carcinogenicity study in O20 mice and C57 BL mice was performed by Ponomarkov et al. (36).

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. Full necropsies and histopathological examinations were performed on all animals. No further details on observations were mentioned. Details on statistical analyses were not mentioned.

Treatment of offspring had to be suspended after 16 weeks due to severe toxicity. Prewaning 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 increase in lung tumours was observed in treated offspring of styrene-treated dams in males and females ($P < 0.01$ for both sexes). Lung tumours occurred earlier in the styrene-treated group compared to control and the average age of death in mice with lung tumours was also lower.

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. Full necropsies and histopathological examinations were performed on all animals. No further details on observations were mentioned. Details on statistical analyses were not mentioned.

Litter size, preweaning mortality, offspring mortality and body weights 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 occurred in treated males (no details on statistics).

Inhalation studies

Carcinogenicity study in rats exposed to styrene via inhalation (Jersey et al. 1978, as described in NTP 2008)

Jersey et al. performed a carcinogenicity study in 1978. This study is not published and data were summarized by the NTP based on information retrieved from secondary sources where the study of Jersey et al. was reviewed (28). 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). 96/97 males/group and 96 females/group were included and 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. No details on observations are given.

Survival was lower in males than in females. It is 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 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.

Carcinogenicity study in rats exposed to styrene via inhalation (Maltoni et al., 1982).

A carcinogenicity study in Sprague-Dawley rats was performed by Maltoni et al. (31). Rats, 30/sex/exposure group were exposed to styrene via inhalation at concentrations of 25, 50, 100, 200 and 300 ppm (corresponding to: 106, 213, 426, 852, 1278 mg/m³ conform the CLP-guidance) for 4 hours per day and 5 days per week during 52 weeks. Animals were included until spontaneous death. Control groups of 60 animals per sex were included as well. Animals were examined for gross changes every two weeks. Full autopsy and histopathology was performed on each animal with a more detailed examination of the brain. Statistics were not reported.

No information on general toxicity was reported. The incidence of total brain tumour bearing animals in males and females was higher compared to controls (no details on statistics). The reporting on data and methods was limited.

Subchronic 13 week inhalation study in rats (Cruzan et al., 1997)

A subchronic inhalation study in CD rats was performed by Cruzan et al. (37). Rats (10/sex/group) were exposed to styrene vapour (purity styrene: >99.4%) via whole body inhalation at concentrations of 0 (control), 200, 500, 1000 and 1500 ppm (corresponding to: 0, 825, 2130, 4260, 6390 mg/m³ conform the CLP-guidance) for 6h/day and 5 days per week for a total of 13 weeks amounting to 65 exposures. There was one satellite group included, with 15 male rats/exposure group

where cell proliferation with an in vivo BrdU assay was assessed after 2, 5 or 13 weeks of exposure (staining of lung only). Animals were observed individually before and after exposure and as a group during exposure. Body weight was determined weekly. Food and water consumption were monitored throughout study. Blood and urine of all animals was analysed at 13 weeks. Full necropsies and full histopathological examinations were performed on all control and 1500 ppm animals. Full necropsies and histopathological examination of nasal passages, lungs and liver was performed on all animals.

There were no effects on survival. All styrene-exposed rats showed signs indicative of styrene irritating properties during exposure. Males (1500 ppm) weighed 10% less and consumed 7% less food compared to controls at week 13. There was a dose-related increase in water consumption (males and females, 1000 and 1500 ppm). Females (1500 ppm) drank twice as much as controls. Urine pH was decreased in a dose-related manner.

Non-neoplastic lesions were observed in the olfactory epithelium of nasal passage in males and females at doses of 500-1500 ppm. No effects were observed in the BrdU assay.

Two week inhalation study in mice (Cruzan et al., 1997)

A 2-week inhalation study in CD-1 mice and B6C3F1 mice was performed by Cruzan et al. (37). Mice (20/sex/group for each strain) were exposed to styrene vapour (purity styrene: >99.4%) via whole body inhalation at concentrations of 0 (control), 15, 60, 250 and 500 ppm (corresponding to: 0, 64, 256, 1065, 2130 mg/m³ conform the CLP-guidance) for 6h/day and 5 days per week for a total of 2 weeks amounting to 10 exposures. Animals were observed individually before and after exposure and as a group during exposure. Body weight was determined weekly. Full necropsies and full histopathological examinations were performed on 10 animals/sex/group for each strain. Styrene-exposed mice showed signs indicative of irritating properties during exposure (all groups) or between exposures (250 and 500 ppm groups). Mortality was increased in the 250 and 500 ppm groups. In females, mortality at 250 ppm was more severe than at 500 ppm. Liver toxicity was observed at 250 and 500 ppm with increased liver weights, macroscopic changes, and microscopically centrilobular hepatocyte necrosis. In females microscopic lesions were more severe at 250 ppm than at 500 ppm. Generally, microscopic changes seen in B6C3F1 mice were more severe compared to CD-1 mice.

Subchronic 13 week inhalation study in mice (Cruzan et al., 1997)

A subchronic inhalation study in CD-1 mice was performed by Cruzan et al. (37). Mice (10/sex/group) were exposed to styrene vapour (purity styrene: >99.4%) via whole body inhalation at concentrations of 0 (control), 50, 100, 150 and 200 ppm (corresponding to: 0, 213, 426, 639, 852 mg/m³ conform the CLP-guidance) for 6h/day and 5 days per week for a total of 13 weeks amounting to 65 exposures. There were two satellite groups included. One with 5 mice/sex/group and 1 week of exposure after which liver effects were assessed. The second satellite group with 30 males/group at 2, 5 or 13 weeks of exposure was incorporated to study cell proliferation with an in vivo BrdU assay (staining of lung and liver). Animals were observed individually before

and after exposure and as a group during exposure. Body weight was determined weekly. Food and water consumption were monitored throughout study. Blood and urine of all animals was analysed at 13 weeks. Full necropsies and full histopathological examinations were performed on all control and 200 ppm animals. Full necropsies and histopathological examination of nasal passages, lungs and liver was performed on all animals.

Two females (200 ppm) died during the first week of exposure due to liver toxicity and severe nasal lesions. No clinical signs or mortality occurred in the other groups. Males (200 ppm) had reduced body weights and food consumption during the study.

In the first satellite group, macroscopic and microscopic liver lesions were observed in 5/5 females (200 ppm) after 1 week exposure. After 13 weeks, multiple liver lesions were observed in both males and females. These lesions were generally less severe than those seen after 1 week of exposure. No liver effects were seen in males and females exposed to 50 or 100 ppm or in males at 150 ppm.

Additionally, non-neoplastic lesions in the nasal passages and lungs occurred at 13 weeks of exposure in both exposed males and females. In the cell proliferation assay a large variation in labelling index was noted. Still, after 5 weeks BrdU-labeled hepatocytes decreased at 100, 150 or 200 ppm. After 2 or 5 weeks, BrdU-labelled Clara cells increased at 150 (3-fold) or 200 ppm (4-fold) styrene ($P < 0.05$), but not in type II pneumocytes.

Carcinogenicity study in rats (Cruzan et al., 1998)

A chronic toxicity/oncogenicity study was performed by Cruzan et al. (27). Rats (70/sex/group) were exposed to styrene at 0, 50, 200, 500, or 1000 ppm (corresponding to 0, 213, 852, 2130 or 4260 mg/m³, as converted conform the CLP-guidance) for 104 weeks. The exposure was performed by inhalation (whole body) of styrene vapour 6h/day 5 days/week for 104 weeks (520 exposures) under GLP conditions. Analytical concentrations were within 1% of the target concentrations. Blood levels of styrene and styrene-7,8-oxide at week 95 after exposure were proportional to the exposure concentration. Animals were observed weekly for the first 13 weeks and thereafter every four weeks. Full details on observations are provided in the summary table. Necropsies and full histopathological examinations were performed on all control and 1000 ppm animals. Histopathologic examination of the nasal passages, lungs, liver, kidneys, testes/epididymides, and macroscopic abnormalities was performed on the animals of all lower exposure levels.

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.

Males (500 and 1000 ppm) and females (200, 500 and 1000 ppm) had a decreased weight gain compared to controls during the first year. Females (500 and 1000 ppm) continued to gain less weight and consume less food compared to controls. There was a dose-related increase in water consumption in males during the whole study and in females during the first 6 months. Clinical signs were only observed

during exposure. There were no adverse effects on clinical pathology, organ weights or at the interim necropsy. At the terminal necropsy an increased incidences of testis masses (control: 500 ppm and 1000 ppm males), decreased incidences of enlarged pituitary (500 and 1000 ppm females) and increased incidences of pale foci in lung (1000 ppm females).

Non-neoplastic treatment-related histopathological findings in rats were confined to the olfactory epithelium of the nasal passages. The changes included atrophic and/or degenerative changes in the olfactory epithelium and changes in the underlying Bowman's glands compared to control rats. The incidences of these lesions increased with increasing dose levels in both males and females.

No statistically significant treatment-related increase of number of animals bearing tumours was observed in males and females. There was a treatment related decrease 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.

Carcinogenicity study in mice (Cruzan et al., 2001)

A chronic toxicity/oncogenicity study was performed with CD-1 mice (26). Mice (70/sex/group) were exposed to styrene vapour (whole body) at concentrations of 0, 20, 40, 80, or 160 ppm (equivalent of 0, 85, 170, 341, 682 mg/m³ conform the CLP-guidance) for 6h/day during 5 days/week for 104 weeks (males) or 98 weeks (females). Levels of styrene and styrene-7,8-oxide in the blood at week 74 were proportional to exposure concentration, except that at 20 ppm the styrene-7,8-oxide level was below the limit of detection.

Styrene had no effect on survival in males. Two high-dose females died (acute liver toxicity) during the first 2 weeks; the remaining exposed females had a slightly higher survival than control mice. There were no changes of toxicological significance in haematology, clinical chemistry, urinalysis or organ weights. Mice exposed to 80 or 160 ppm gained slightly less weight than the controls.

Styrene-related non-neoplastic histopathological changes were found only in the nasal passages and lungs. In the nasal passages of males and females at all exposure concentrations, the changes included respiratory metaplasia of the olfactory epithelium with changes in the underlying Bowman's gland; the severity increased with styrene concentration and duration of exposure. Loss of olfactory nerve fibres was seen in mice exposed to 40, 80 or 160 ppm. In the lungs, there was decreased eosinophilia of Clara cells in the terminal bronchioles and bronchiolar epithelial hyperplasia extending into alveolar ducts.

There was an increase of total number of tumour bearing mice observed in females at 40 ppm and 160 ppm compared to control (both $P < 0.05$). Increased tumour incidence occurred only in the lung. In males, there was an increased incidence of bronchioloalveolar adenomas at 40 ppm, 80 ppm and 160 ppm compared to control (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 compared to control ($P < 0.05$). No difference in lung tumours between control and styrene-exposed mice was seen in the intensity or degree of immunostaining, the location of tumours relative to bronchioles or histological type (papillary, solid, or mixed).

A follow-up study was conducted in which 55 males were exposed to styrene where 5 males/group were terminated after 1, 2, 4, 7, 10, 20, 40 and 65 exposures and 4, 8 or 13 weeks recovery time. 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.

Carcinogenicity study in mice (Cruzan et al., 2017)

A chronic toxicity/oncogenicity study (focussing on lung) was performed in mice for a duration of 104 weeks with 75 males per groups (6-7 weeks of age) (26). The objective of this study was to examine the role of CYP2F2 metabolism on the lung toxicity and tumorigenicity for chronic (up to 24 months) exposure to styrene. The design included evaluation of the human relevance of the CYP2F mediated bioactivation with observations in CYP2F2 knockout and CYP2F1 humanized mice. Four different strains were included, i.e. CD-1 (used in Cruzan et al., 2001), C57BL/6 (wild-type for knockout mice, referred to as WT), CYP2F2-knockout (KO), and CYP2F2(KO) 2F1,2A13, 2B6-transgenic (TG). They were divided in 2 groups at target concentrations: 0, or 120 ppm (corresponding to 0, or 511 mg/m³, as converted conform the CLP-guidance). Analytical concentrations were within 1% of the target concentrations. The exposure was performed by inhalation (whole body) of styrene vapour 6h/day 5 days/week for 104 weeks.

No signs of styrene-induced toxicity were observed in any of the 4 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.

In WT and CD-1 mice, an increased incidence of epithelial cell degeneration in terminal bronchioles occurred at multiple points in time. Overall the incidence was 10/53 (CD-1 mice) and 34/50 (WT mice) up to 104 weeks of exposure. No degeneration of epithelial cells was noticed in KO or TG mice (control of styrene-treated).

Cell proliferation in the terminal bronchioles was 4- to 5-fold increased at week 1 in exposed CD-1 and WT mice ($P < 0.05$). Proliferative changes were found in styrene-treated CD-1 and WT mice. These consisted of hyperplasia in the terminal bronchioles characterized by increased numbers of unevenly sized epithelial cells "piling" up in multicellular layers, sometimes extending into the alveolar ducts and tumours.

Hyperplasia was seen in styrene-treated CD-1 mice at week 1, 26, 78 or 104 ($P < 0.05$ at this point in time). Overall 50 of 67 CD-1 mice exposed to styrene during this study had hyperplasia in the terminal bronchioles compared to 0/67 control CD-1 mice. Similarly, in WT mice (55 of 70), hyperplasia in the terminal bronchioles was found at 1, 26, 52, 78, and 104 ($P < 0.05$ at this point in time) weeks upon styrene-treatment compared to 0/69 WT controls. Terminal bronchiole hyperplasia was not observed in control or treated KO and TG mice. Six mice, including one each in the CD-1, WT, and KO controls, had epithelial hyperplasia that encompassed areas of bronchiolar and/or alveolar tissue, but did not have features of an adenoma. This epithelial hyperplasia was distinguished from the hyperplasia limited to the terminal bronchioles.

No statistical significant increase in lung adenomas or adenocarcinomas were observed in the 4 mouse strains.

Carcinogenicity study in rats (Conti et al., 1988)

Inhalation study

A carcinogenicity study in Sprague-Dawley rats was performed by Conti et al. (33). Rats were exposed to styrene (purity: 99.8%) via inhalation. Details of the exposure are given below. Rats were observed three times daily and clinical observations were done twice weekly. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. All rats were included until spontaneous death. Full necropsies and histopathological examinations were performed on all animals. This was a non-guideline study and the reporting on the data is limited. Details on statistical analysis were not reported.

Male and female rats were exposed to styrene daily for 4h via whole body inhalation at concentrations of 0, 25, 50, 100, 200 and 300 ppm (corresponding to 0, 106, 213, 426, 852, 1278 mg/m³; converted conform the CLP guidance) 4h daily, 5 days per week for 52 weeks. Survival was not affected by styrene exposure and no relevant body weight differences were observed. There was a higher incidence of the total number of malignant tumours per 100 animals at 100 ppm in both males and females which is not due to the increase in any specific type of tumours. In females, a higher percentage of animals with mammary tumours compared to control was observed in all exposure groups compared to control. Additionally, a higher percentage of malignant mammary tumours in females compared to controls was seen in all exposure groups. This increased incidence of malignant tumours was statistically significant (no details reported).

Intraperitoneal studies

Carcinogenicity study in rats (Conti et al., 1988)

Intraperitoneal study

A carcinogenicity study in Sprague-Dawley rats was performed by Conti et al. (33). Rats were exposed to styrene (purity: 99.8%) via intraperitoneal injection. Details of the exposure are given below. Rats were observed three times daily and clinical observations were done twice weekly. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. All rats were included until spontaneous death. Full necropsies and histopathological examinations

were performed on all animals. This was a non-guideline study and the reporting on the data is limited. Details on statistical analysis were not reported.

Male and female rats were exposed intraperitoneally to styrene (50 mg) four times at 2-month intervals (total treatment duration is not mentioned). Survival was not affected by styrene exposure. No relevant body weight differences were observed. There was no significant increase in the incidence of any tumour type.

Carcinogenicity study of styrene in mice (Brunnemann et al., 1992)

A carcinogenicity study in A/J mice was performed by Brunnemann et al (38). Female A/J mice (25/group) were given intraperitoneal injections of styrene (purity: 99%) or olive oil, for three times a week for a total of 20 injections (total dose of 200 µmol, corresponding to 1040 mg/kg bw). Mice were sacrificed 20 weeks after the final administration. Full necropsies and histopathological examinations were performed on all animals, but no further details on observations were provided. Differences between groups were statistically analysed with a student's t-test. Data on survival and body weights were not reported. Styrene did not significantly induce lung adenomas or any other tumours.

Five day intraperitoneal study in mice (Cruzan et al., 2013)

A study in C57BL/6 mice was performed to evaluate the human relevance for the occurrence of mouse lung tumours after styrene exposure and to explore the role of in vivo CYP2F1 (human) metabolism of styrene (39). To this end, styrene (purity 99.9%) was injected intraperitoneally in wild-type and CYP2F2(-/-) /CYP2F1/2A13/2B6 transgenic humanized mice. Five animals/sex/strain were exposed to styrene 200 mg/kg bw/day (divided into 3 doses at 2h intervals) for 5 days. Vehicle controls were also included, but the number of controls was not reported. To perform an in vivo BrdU assay, BrdU was given via osmotic pumps throughout the 5 day treatment period. Mice were observed daily and body weight was recorded before the first dose and the day after the last dose. BrdU staining in the lung was quantified and necropsy and histopathology of the lungs was performed. In styrene-treated wild-type mice, a 5-10 fold increase in BrdU labelling was observed in terminal bronchioles compared to controls ($P < 0.05$). In transgenic mice there were no differences in BrdU labelling compared to control.

Subcutaneous studies

Carcinogenicity study in rats (Conti et al., 1988)

A carcinogenicity study in Sprague-Dawley rats was performed by Conti et al. (33). Rats were exposed to styrene (purity: 99.8%) via a subcutaneous injection. Details of the exposure are given below. Rats were observed three times daily and clinical observations were done twice weekly. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. All rats were included until spontaneous death. Full necropsies and histopathological examinations were performed on all animals. This was a non-guideline study and the

reporting on the data is limited. Details on statistical analysis were not reported.

Male and female rats were given a single subcutaneous injection with styrene (50 mg). Survival was not affected by styrene exposure. No relevant body weight differences were observed. There was no significant increase in the incidence of any tumour type.

10.2 Summary of animal experiments on styrene-7,8-oxide

The carcinogenicity studies of styrene-7,8-oxide in experimental animal studies are summarized in Table 9 followed by a summary in text. In general, only statistically significant results are presented in the table below. In studies where statistical significance of the results was not reported, the listed tumour incidences in the table were limited to the control group and groups where actual lesions occurred.

Table 9 Summary table of in vivo animal experiments with styrene-7,8-oxide

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
<i>Oral</i>					
Maltoni et al., 1979 (40)	Rat, Sprague-Dawley 13 weeks old Males and females: 40/sex/group	Carcinogenicity study (stomach tumours) All animals included until spontaneous death. Study duration: 156 weeks. Statistical analysis not reported.	Test item: styrene-7,8-oxide (purity not stated, in olive oil) Oral via gavage 0 (vehicle), 50, 250 mg/kg bw/day 4-5 days weekly for 52 weeks.	<p><i>Observations</i> Examination of animals on gross changes every two weeks. Animals were weighed every two weeks, and then every eight weeks. Full autopsy and histopathology on each animal.</p> <p><i>Results</i> Survival at 51 weeks in males (control: 37/40; 50 mg/kg bw/day: 31/40; 250 mg/kg bw/day: 28/40) and in females (control: 28/40; 50 mg/kg bw/day: 31/40; 250 mg/kg bw/day: 30/40). Survival at 135 weeks in males (control: 0/40; 50 mg/kg bw/day: 3/40; 250 mg/kg bw/day: 3/40) and females (control: 3/40; 50 mg/kg bw/day: 0/40; 250 mg/kg bw/day: 4/40)</p> <p>Incidence in total forestomach epithelial tumour (both papillomas and squamocellular carcinomas) bearing animals in males (control: 0/40; 50 mg/kg bw/day: 6/40; 250 mg/kg bw/day: 14/40) and females (control: 0/40; 50 mg/kg bw/day: 6/40; 250 mg/kg bw/day: 15/40).</p> <p>Incidence in papillomas in males (control: 0/40; 50 mg/kg bw/day: 0/40; 250 mg/kg bw/day: 3/40) and females (control: 0/40; 50 mg/kg bw/day: 2/40; 250 mg/kg bw/day: 6/40)</p>	<p>Non-GLP, Non-guideline</p> <p>Limited reporting on data and methods.</p> <p>Data reported is preliminary, until week 135 (experiment was ongoing at the time of publication).</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>Incidence in total squamocellular carcinomas in males (control: 0/40; 50 mg/kg bw/day: 6/40; 250 mg/kg bw/day: 12/40) and females (control: 0/40; 50 mg/kg bw/day: 6/40; 250 mg/kg bw/day: 15/40)</p> <p>Incidence in in situ squamocellular carcinomas in males (control: 0/40; 50 mg/kg bw/day: 5/40; 250 mg/kg bw/day: 11/40) and females (control: 0/40; 50 mg/kg bw/day: 6/40; 250 mg/kg bw/day: 12/40)</p> <p>Incidence in invasive squamocellular carcinomas in males (control: 0/40; 50 mg/kg bw/day: 2/40; 250 mg/kg bw/day: 4/40) and females (control: 0/40; 50 mg/kg bw/day: 1/40; 250 mg/kg bw/day: 6/40)</p> <p>Authors note that carcinomas often metastasize to the liver and that precursor lesions in forestomach are often found (both not quantified).</p> <p>Incidence of papillomas in historical controls is below 1% (both olive oil treated and untreated rats).</p>	
Maltoni et al., 1982 (31)	Rat, Sprague-Dawley 13 weeks old	Carcinogenicity study (brain tumours)	Test item: styrene-7,8-oxide (purity not stated, in olive oil)	<i>Observations</i>	Non-GLP, Non-guideline

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
This is the same experiment as Maltoni et al., 1979	Males and females: 40/sex/group	All animals included until spontaneous death. Statistical analysis not reported.	Oral via gavage 0 (vehicle), 50, 250 mg/kg bw/day 4-5 days weekly for 52 weeks.	Examination of animals on gross changes every two weeks. Full autopsy and histopathology on each animal. Extra examination of brain. <i>Results</i> Incidence in total brain tumour bearing animals in males (control: 1/40; 50 mg/kg bw/day: 1/40; 250 mg/kg bw/day: 0/40) and in females (control: 0/40; 50 mg/kg bw/day: 1/40; 250 mg/kg bw/day: 2/40).	Limited reporting on data and methods.
Lijinsky, 1986 (41)	Rat, F344 9 weeks old Males and females: 52/sex/group	Chronic study Animals sacrificed at 107 or 108 weeks. Statistics: Fisher exact test and Cochran-Armitage test	Test item: styrene-7,8-oxide (in corn oil) Purity: 96.6% Oral gavage 0 (vehicle), 275 and 550 mg/kg bw/day, 3 times per week, 104 weeks	<i>Observations</i> - 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. <i>Results</i> 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	Non-GLP, non-guideline 3.3% of the styrene-7,8-oxide solution consisted of benzaldehyde, benzene and one other unspecified compound

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>mg/kg bw: 10/52; 550 mg/kg bw: 9/51) and females (control: 0/52; 275 mg/kg bw: 8/52; 550 mg/kg bw: 9/52).</p> <p>Neoplastic lesions:</p> <ul style="list-style-type: none"> - 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) and 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) and 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) and 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). 	
Lijinsky, 1986 (41)	Mouse, B6C3F1 7 weeks old	Chronic study Animals sacrificed at 107 or 108 weeks. Statistics:	Test item: styrene-7,8-oxide (in corn oil) Purity: 96.6%	<p><i>Observations</i></p> <ul style="list-style-type: none"> - 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). 	<p>Non-GLP, Non-guideline</p> <p>3.3% of the styrene-7,8-oxide solution</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
	Males and females: 52/sex/group	Fisher exact test and Cochran-Armitage test	0 (vehicle), 375 and 750 mg/kg bw/day, 3 times per week, 104 weeks	<p>Full necropsies and full histopathological examinations on all animals.</p> <p><i>Results</i> 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).</p> <p>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 males (control: 0/51; 375 mg/kg bw: 2/51; 750 mg/kg bw: 2/52) and females (control: 1/51; 375 mg/kg bw: 6/50; 750 mg/kg bw: 3/51).</p> <p>Neoplastic lesions: - Increased liver carcinomas + adenomas in males (control: 12/51; 375 mg/kg bw: 28/52, $P < 0.001$; 750 mg/kg bw: 15/52). - Increased forestomach carcinomas + papillomas in males (control: 2/51; 375 mg/kg bw: 37/51, $P < 0.001$; 750 mg/kg bw: 21/52, $P < 0.001$) and females (control: 0/51; 375 mg/kg bw: 24/50, $P < 0.001$; 750 mg/kg bw: 20/51, $P < 0.001$).</p>	consisted of benzaldehyde, benzene and one other unspecified compound

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<ul style="list-style-type: none"> - Incidence of carcinomas of the forestomach in males (control: 0/51; 375 mg/kg bw: 16/51; 750 mg/bw: 15/52) and females (control: 0/51; 375 mg/kg bw: 10/50; 750 mg/bw: 3/51). - Incidence of papillomas of the forestomach in males (control: 2/51; 375 mg/kg bw: 22/51; 750 mg/bw: 8/52) and females (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, $P=0.01$). 	
Ponomarkov et al., 1984 (42)	Rat, BDIV 14 exposed dams and their offspring (62 females and 42 males). 14 control dams and their offspring (55 female and 49 male).	<p>Carcinogenicity study</p> <p>All animals were sacrificed at 120 weeks of the experiment.</p> <p>Statistics: No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.</p>	<p>Test item: styrene-7,8-oxide (in olive oil) Purity: 97%</p> <p>Pregnant dams: 0 (olive oil) and 200 mg/kg bw Single administration on day 17 of gestation</p> <p>Offspring: 0 (olive oil) and 100-150 mg/kg bw, 96 weekly doses from 4 weeks of age (weaning) until</p>	<p><i>Observations</i> Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.</p> <p><i>Results</i> Litter size, preweaning mortality, offspring mortality and body weights did not differ between the groups.</p> <p>Non-neoplastic and neoplastic lesions: Incidence in tumour-bearing pregnant dams was 57% (controls) and 31% (styrene-7,8-oxide).</p> <p>Effects in offspring:</p>	Non GLP, Non guideline.

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
			<p>termination of experiment</p> <p>Oral, via gavage</p>	<p>-Incidence in tumour-bearing animals in treated rats was 77% (females) and 52% (males) and in controls 58% (females) and 20% (males).</p> <p>Increased incidence in forestomach tumours:</p> <ul style="list-style-type: none"> - 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, $P<0.02$) and 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, $P<0.0001$) and males (control: 0/49; styrene-7,8-oxide: 10/42, $P<0.0002$). <p>Early changes of squamous epithelium frequently observed in styrene-7,8-oxide groups (though not statistically significant):</p> <ul style="list-style-type: none"> - 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). 	
Conti et al., 1988 (33)	<p>Sprague-Dawley rats, 13 weeks old</p> <p>Males and females, 40/sex/dose group</p>	<p>Carcinogenicity study</p> <p>Males and females, included until spontaneous death.</p>	<p>Test item: styrene-7,8-oxide (in olive oil). Purity not stated.</p> <p>0 (olive oil), 50 and 250 mg/kg bw, for 4-5 days per week for 52 weeks</p>	<p><i>Observations</i></p> <p>Three times daily status and behavioural observations, twice weekly clinical observation. Body weights were recorded every 2 weeks during treatment and then every 8 weeks.</p> <p>Full necropsies and histopathological examinations were performed on all animals.</p>	<p>Non-GLP, Non-guideline.</p> <p>No details on statistical analyses reported, limited reporting on the data.</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
			Oral, via gavage	<p><i>Results</i></p> <p>Increased mortality in males (50 and 250 mg/kg). No body weight differences.</p> <p>Neoplastic lesions:</p> <ul style="list-style-type: none"> - Increase in total tumour bearing animals (combined benign and malignant tumours) in treated males (control: 22.5%; 50 mg/kg: 35%; 250 mg/kg: 50%) and females (control: 25%; 50 mg/kg: 40%; 250 mg/kg: 55%) and increase in total tumour bearing animals (malignant tumours) in treated males (control: 15.0%; 50 mg/kg: 27.5%; 250 mg/kg: 45.0%) and females (control: 17.5%; 50 mg/kg: 22.5%; 250 mg/kg: 50.0%) due to forestomach neoplasias. <p>Forestomach neoplasias:</p> <ul style="list-style-type: none"> - Precursor lesions in males (control: 2.5%; 50 mg/kg: 12.5%; 250 mg/kg: 35.0%) and females (control: 5.0%; 50 mg/kg: 17.5%; 250 mg/kg: 25.0%). - Papillomas and acanthomas in males (control: 0%; 50 mg/kg: 7.5%; 250 mg/kg: 22.5%) and females (control: 0%; 50 mg/kg: 7.5%; 250 mg/kg: 12.5%). - Squamous cell carcinomas in males (control: 0%; 50 mg/kg: 27.5%; 250 mg/kg: 75.0%) and females (control: 0%; 50 mg/kg: 20.0%; 250 mg/kg: 82.5%). Both in situ and invasive carcinomas increased. 	

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
				<p>Other neoplasias</p> <ul style="list-style-type: none"> - Increase in benign and malignant mammary tumours in males (control: 2.5%; 50 mg/kg: 0%; 250 mg/kg: 25.0%) and females (control: 10.0%; 50 mg/kg: 17.5%; 250 mg/kg: 22.5%). - Increase in pheochromocytomas in males (control: 5.0%; 50 mg/kg: 10.0%; 250 mg/kg: 15.0%). 	
Cruzan et al., 2013 (39)	<p>C57BL/6 (wild-type) 7-12 weeks old 5 males and females</p> <p>CYP2F2(-/-)/CYP2F1/2A13/2B6 (transgenic, from C57BL/6 mice) 5 males and females</p>	<p>Non guideline study to evaluate human relevance for mouse lung tumours.</p> <p>In vivo BrdU assay (cell proliferation)</p> <p>Statistics: - BrdU assay: Two-way analysis of variance with post-hoc tests.</p>	<p>Test item: S- and R-styrene-7,8-oxide (in corn oil) CAS nr: 20780-53-4</p> <p>Purities: 99.4% (S-isomer), 98.2 (R-isomer)</p> <p>200 mg/kg bw/day (divided into 3 doses at 2h interval) for 5 days</p> <p>Controls: vehicle</p> <p>Intraperitoneal injection</p>	<p><i>Observations</i> Daily visual observation. Body weight was recorded before the first dose and the day after the last dose.</p> <p>Necropsy and histopathology of lung. BrdU staining in lung.</p> <p><i>Results</i> - 5-10 fold increase in BrdU labelling in terminal bronchioles in R- and S-styrene-7,8-oxide treated wild-type mice ($P < 0.05$). No effect in transgenic mice.</p>	<p>Non-GLP; Non-guideline.</p> <p>Number of controls not reported.</p>

Reference	Species	Experimental period and design	Concentration and route	Observations and results	Remarks
<i>Dermal</i>					
Weil et al., 1963 (43)	Mice, CH3 90 days old 30-40 mice/group	Carcinogenicity study Whole-lifetime observation Statistics not performed.	Test item: styrene-7,8-oxide (in acetone) One brush of 10 or 5 % solution of compound. 3 applications per week for a lifetime	<p><i>Observations</i> Observations for papillomas and carconomas were made during each painting period.</p> <p><i>Results</i> Survival (at 12-24 months) in mice treated with 10% styrene-7,8-oxide was lower compared to the 5% group. After 12, 17 and 24 months, 18, 2 and 0 mice, respectively, were alive in 10% group versus 37, 33, 17 mice in 5% group.</p> <p>No mice with tumours were observed.</p>	<p>Non GLP, non-guideline.</p> <p>Limited reporting on methods and data.</p> <p>No control animals.</p>

10.2.1 *Overview of studies with styrene-7,8-oxide* **Oral studies with styrene-7,8-oxide**

Carcinogenicity study in rats exposed to styrene-7,8-oxide orally (Maltoni et al., 1979)

Preliminary data (collected until week 135 of the study) on the incidence of stomach tumours in a carcinogenicity were reported by Maltoni et al. (40). Sprague-Dawley rats 40/sex/group were exposed to styrene-7,8-oxide in olive oil via ingestion at dose levels of 0 (vehicle), 50, 250 mg/kg bw/day, 4-5 days weekly during 52 weeks. Animals were included until spontaneous death and the study lasted 156 weeks in total. Animals were examined for gross changes every two weeks. Body weight was recorded every two weeks, and then every eight weeks. Full autopsy and histopathology was performed on each animal. Reporting on data and methods is limited.

After 51 weeks survival in males was 37/40 (controls), 31/40 (50 mg/kg bw/day) and 28/40 (250 mg/kg bw/day). Survival in females at 51 weeks was 28/40 (control), 31/40 (50 mg/kg bw/day) and 30/40 (250 mg/kg bw/day). After 135 weeks survival most animals in the control and exposed groups had died.

The incidence of forestomach epithelial tumours (papillomas and in situ or invasive squamocellular carcinomas) were reported. No tumours occurred in control animals. Increased incidences of all tumour types in males and females were observed in all exposed groups compared to control. It is noted that statistics were not performed.

A carcinogenicity study in rats exposed to styrene-7,8-oxide orally (Maltoni et al., 1982)

A carcinogenicity study in Sprague-Dawley rats was performed by Maltoni et al. (31). Rats, 40/sex/group were exposed to styrene-7,8-oxide in olive oil via ingestion at concentrations of 0 (vehicle), 50, 250 mg/kg bw/day, 4-5 days weekly during 52 weeks. Animals were included until spontaneous death. Animals were examined for gross changes every two weeks. Full autopsy and histopathology was performed on each animal with a more detailed examination of the brain. Statistics were not performed.

No information on general toxicity was reported. The incidence of total brain tumour bearing animals in males was 1/40 (controls), 1/40 (50 mg/kg) and 0/40 (250 mg/kg). The incidence of total brain tumour bearing animals in females was 0/40 (controls), 1/40 (50 mg/kg) and 2/40 (250 mg/kg). The reporting on data and methods was limited.

Chronic oral study in rats exposed to styrene-7,8-oxide (Lijinsky, 1986)

A chronic toxicity/carcinogenicity study was performed in 9 weeks old F344 rats by Lijinsky (41). 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. Dose-selection was based on previous 24-week study to identify the maximum tolerable dose (MTD). 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. Animals were inspected twice daily on mortality. Body weight was recorded once a week for the first 4 months, then every two weeks for the next 4

months and finally once every 4 weeks for the rest of the study. Full necropsies and full histopathological examinations was performed on all animals. Fisher exact tests and Cochran-Armitage tests were performed, but it is not clear on what data these were applied.

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 hyperplasia in the forestomach was seen in males and females. Increased incidence of combined carcinomas and papillomas in the forestomach in treated males and females was observed, which was statistically significantly different from controls for the male 275 mg/kg group ($P < 0.001$). Increased incidence of carcinomas of the forestomach in males and females was observed. Finally, increased incidence of papillomas of the forestomach in males and females was observed. 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 leukemia 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.

Chronic oral study in mice exposed to styrene-7,8-oxide (Lijinsky, 1986)

A chronic toxicity/carcinogenicity study was performed in 7 weeks old B6C3F1 mice by Lijinsky (41). To determine the maximum tolerable dose (MTD), a 24-week subchronic study was performed. 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. 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. Animals were inspected twice daily on mortality. Body weight was recorded once a week for the first 4 months, then every two weeks for the next 4 months and finally once every 4 weeks for the rest of the study. Full necropsies and full histopathological examinations was performed on all animals. Fisher exact tests and Cochran-Armitage tests were performed, but it is not clear on 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 incidences were not reported. Lipoid degeneration, focal necrosis and haemorrhage of liver occurred in males (750 mg/kg bw). Incidence of hyperplasia in forestomach was reported in males and females (see overview table). Increased incidences in combined liver carcinomas and adenomas was observed in males which was statistically significantly different from controls in the 375 mg/kg group ($P < 0.001$). Increased incidence in combined forestomach carcinomas and papillomas was observed in males which was statistically significantly different from controls at 375

and 750 mg/kg bw ($P < 0.001$) as well as in females which was statistically significant at 375 and 750 mg/kg bw ($P < 0.001$). Incidence of carcinomas of the forestomach alone was increased in males and females. Incidence of papillomas of the forestomach alone was increased in males and females. There was a decreased incidence of malignant lymphoma and leukemia in females (750 mg/kg bw, $P = 0.01$).

Carcinogenicity study of styrene in rats (Ponomarev et al., 1984)

A carcinogenicity study in BDIV rats was performed by Ponomarev et al. (42). 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 administered via oral gavage. Full necropsies and histopathological examinations were performed on all animals. No further details on observations were mentioned. Details on statistical analyses were not mentioned.

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 57% (controls) and 31% (styrene-7,8-oxide). 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 number of papillomas in males was increased ($P < 0.003$). Carcinomas in situ in both females ($P < 0.02$) and males ($P < 0.04$) was increased. Finally, early carcinomas or carcinomas in females ($P < 0.0001$) and males ($P < 0.0002$) were increased. Early changes of squamous epithelium frequently observed in styrene-7,8-oxide groups. Nervous system tumours occurred in exposed males and lung tumours occurred in exposed females although these increases were not statistically significant.

Oral carcinogenicity study in rats (Conti et al., 1988)

A carcinogenicity study in Sprague-Dawley rats was performed by Conti et al. (33). Male and female rats (40/sex/dose group) were exposed orally to styrene-7,8-oxide at dose levels of 0 (olive oil), 50 and 250 mg/kg bw/day via a stomach tube for 52 weeks. Rats were observed three times daily and clinical observations were done twice weekly. Body weights were recorded every 2 weeks during treatment and then every 8 weeks. All rats were included until spontaneous death. Full necropsies and histopathological examinations were performed on all animals. This was a non-guideline study and the reporting on the data is limited. Details on statistical analysis were not reported.

There was an increased mortality rate in males (50 and 250 mg/kg bw/day). No body weight differences occurred.

Total benign and malignant tumours were increased in both exposed male and females and could be attributed to increase of forestomach neoplasia's. Several types of forestomach neoplasias occurred such as precursor lesions in males and. Additionally, papillomas and acanthomas were observed in males and. Finally, squamous cell carcinomas were

observed in males and females. Both in situ and invasive squamous cell carcinomas increased.

Other tumour types were also observed in styrene-7,8-oxide treated rats. There was an increase in benign and malignant mammary tumours in males and females. Finally an increase in pheochromocytomas was seen in males.

Intraperitoneal studies with styrene-7,8-oxide

5 day intraperitoneal study in mice (Cruzan et al., 2013)

A study in C57BL/6 mice was performed to evaluate the human relevance for the occurrence of mouse lung tumours after styrene-7,8-oxide exposure and to explore the role of in vivo CYP2F1 (human) metabolism of styrene (39). To this end, S- and R-styrene-7,8-oxide (purity 99.4% and 98.2% respectively) was injected intraperitoneally in wild-type and CYP2F2(-/-) /CYP2F1/2A13/2B6 transgenic humanized mice. Five animals/sex/strain were exposed to either styrene-7,8-oxide isomer at 200 mg/kg bw/day, divided into 3 doses at 2h intervals, for 5 days. Vehicle controls were also included, but the number of controls was not reported. To perform an in vivo BrdU assay, BrdU was given via osmotic pumps throughout the 5 day treatment period. Mice were observed daily and body weight was recorded before the first dose and the day after the last dose. BrdU staining in the lung was quantified and necropsy and histopathology of the lungs was performed.

In styrene-7,8-oxide treated wild-type mice, a 5-10 fold increase in BrdU labelling was observed in terminal bronchioles compared to controls ($P < 0.05$). In transgenic mice there were no differences in BrdU labelling compared to control.

Dermal studies with styrene-7,8-oxide

Dermal carcinogenicity study in mice (Weil et al., 1963)

A dermal carcinogenicity study in CH3 mice (90 days old) was performed by Weil et al. (43). Styrene-7,8-oxide in a 5 or 10% solution in acetone was applied dermally via a brush stroke on shaved skin. The mice (30-40 animals per group) received 3 applications per week for the whole lifetime, during which observations for papillomas and carcinomas were made. Control animals were not included and statistics were not performed. Overall, the reporting on methods and results is limited. Survival in mice treated with 10% styrene-7,8-oxide was lower compared to the 5% group. After 12, 17 and 24 months, respectively 18, 2 and 0 mice were alive in the 10% group versus respectively 37, 33, 17 mice in the 5% group. No tumours were reported to occur in the mice.

10.3 Summary of observations in humans

The carcinogenic effects of styrene as observed in cohort studies are summarized in Table 10. The case control studies are summarized in Table 11 (extensive summaries) and Table 12 (brief summaries). The cross-sectional studies are briefly summarized in Table 13. All tables are followed by a summary in text.

Table 10 Summary table of cohort studies

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Bertke et al. (2021), (44) Daniels et al. (2020), (45) Bertke et al. (2018), (46) Ruder et al. (2017), (47) Ruder et al. (2016), (48) Ruder et al. (2004), (49) Okun et al. (1985), (50) • Retrospective cohort study • Washington State, USA • Boat building Follow-up: Job information 1959-1978 Health outcomes until end 2016 for the last study Censoring:	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	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	1 year styrene exposure at > 30 parts per million () accelerated time to lung cancer death by 2.29 years (95% CI: 1.53, 2.94) • Strong evidence for Healthy Worker Survivor Bias (HWSB)	• 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 fiberglass, solvents, wood dust, or wood finishing agents • No information on hospitalisation	• Job exposure was also possible to acetone (TWA 50.6 ppm plant 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

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<p>Left censoring: 1959 (use of styrene started in 1957) Right censoring: end 1978 for exposure and work histories</p> <p>Inclusion criteria: employed ≥ 1 day in glass fiber-reinforced plastic and composites boat manufacturing between 1959 and 1978.</p> <p>Study population: 5,163 boatbuilders working at one of two boat building facilities in Kelso (plant A) and Bellingham (plant B), Washington, USA.</p> <p>Reference population:</p>	<p>plant B. Low exposure estimated at 5 ppm/day</p> <ul style="list-style-type: none"> • 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 <p>Statistical analyses: Mostly calculation of standardised mortality ratios (SMR), both for overall mortality and cause-specific (cancer) deaths, and 95% CI's</p>			<ul style="list-style-type: none"> • 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 	<p>possible at the job</p> <ul style="list-style-type: none"> • Information on exposure of cohort members since 1978 not available. In 1978 at time of job data collection 772 workers were still employed

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general population in the state Washington • Number of exposed and non-exposed; total amount of person-years;	based on Poisson distribution				
Okun et al. (1985), (50) See general information above Study population: 5,201 boatbuilders (out of 5409, 208 of whom not included because of lacking information) working at one or two plants • Of those, 2060 classified as high exposure group (3102 to minimal exposure; 39 could not be classified due to lacking work information)	See also general information above In this study workers were divided into a high exposure group (fibrous glass or lamination departments) and a low exposure group (all other job categories). Exposure concentration: In high exposure group: composite mean concentration of airborne styrene 42.5 ppm (SE 2.6) at one and 71.7 ppm (SE 5.2) at the other facility.	Health outcomes: Mortality and cause-specific mortality, in particular lymphoma and leukemia deaths Health assessment: Based on death certificates with cause of death ICD coded by qualified nosologist, according to ICD version in effect at time of death	Whole cohort: • SMR overall: 90 (0.9), not significant (p-value and CI not reported) • SMR cause-specific, all nonsignificant (p-values and CIs not reported) • No lymphoma or leukemia deaths High exposure: • SMR overall: 113 (%), not significant (p-value and CI not reported) • SMR cause-specific deaths, including malignancies, nonsignificant (p-values and CIs not reported) • Subgroup analysis in white males only: SMR for all cause death 135 (1.35) (p = 0.05, CI not reported)	• Age distribution strongly skewed to younger ages: 81% of person-years accumulated in individuals under age 45, which may lead to bias • Healthy worker effect not assessed (confounding)	See also general information above Regarding this study: • Cohort 96% complete. Data on 208 individuals lacking. Death certificates lacking in 6% of deceased • Relatively small study group, with relatively brief exposure time and relatively young population at end of follow-up, so not much power to detect excess lymphoma or leukemia deaths and to take into account latency

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<p>Reference population: Age and calendar specific death rates of US general population</p> <p>Follow-up: Vital status and causes of death as at 31 December 1978</p> <p>Censoring: Right censoring in 1978: work history for still active workers have incomplete work history records</p>	<p>All other jobs minimal (figures not reported)</p> <p>Statistical analyses: Comparison of observed versus expected number of cause specific deaths, for whole cohort and separately for high and low exposure group</p> <ul style="list-style-type: none"> • Excess cause specific mortality tested with two-tailed Poisson distribution • Covariates included the analysis: age, race, sex, calendar year; effects of time since first employment and duration of employment in five-year intervals 		<p>Minimal exposure:</p> <ul style="list-style-type: none"> • SMR overall: 85, nonsignificant (p-values and CI not reported) • For the 15 lung cancer deaths found, versus 8 expected, a descriptive breakdown according to duration of employment was done, but without statistical analysis 		<ul style="list-style-type: none"> • Relatively few workers with long observation: in high exposure group only 39% > 10 years • Even though healthy worker effect not assessed, authors observe that lack of death deficit in overall and cardiovascular mortality was unexpected • 39 workers not included in analysis

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Ruder et al. (2004), (49) See general information above Study population: 5,204 workers • Of those, 2060 classified as high exposure group Reference population: Age and calendar specific death rates of Washington State and US general population. Follow-up: Vital status through end 1998	See general information above for exposure assessment • Cumulative exposure grouped into tertiles: 5- <500 ppm; ≥500-<5,000 ppm; ≥5,000 ppm Statistical analyses: See general information above. In addition: • Race- and gender-specific person years at risk accumulated across 5-year age and calendar intervals, beginning with qualified data of first exposure until date of death, last known alive, or December 31,	Health outcomes: Mortality and cause-specific mortality, in particular lymphoma and leukemia deaths Health assessment: See general information above for health assessment	Whole cohort (135,707 or 135.588 (inconsistency text versus table) person-years at risk): • SMR all-cause mortality 1.09 (95% CI 1.02-1.17) • SMR malignant neoplasms overall 1.17 (1.02-1.33) • SMR esophageal cancer 2.30 (1.19-4.02) • SMR prostate cancer 1.71 (1.09-2.54) • SMR lymphatic and haematopoietic cancer 0.74 (0.42-1.20) • SMR accidents 1.26 (1.02-1.53) • SMR 'other and unspecified sites 1.68 (1.01-2.62)' • SMR other diseases of the heart 0.59 (0.40-0.85) • SMR cirrhosis of the liver 1.67 (1.15-2.34) High exposure: (54,122 person-years at risk) • SMR all-cause mortality 1.26 (1.10-1.43) • SMR malignant neoplasms overall 1.26 (0.96-1.63) • SMR urinary tract cancer	See also general information above -Healthy worker effect not assessed -As above, no information on lifestyle-related factors, but excess mortality from mental disorders and alcoholism. In short term workers excess deaths from oesophageal cancer and liver cirrhosis	See also general information above Regarding this study: • 3 workers were excluded, because of missing information • Relatively small study group, with relatively brief exposure time: only 1,678 individuals worked in the plants for more than 1 year. • Even though healthy worker effect not assessed, authors observe that lack of death deficit in overall and cardiovascular mortality was unexpected • For SMRs the study used both Washington State mortality data and US general population. Quoted

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	<p>1998.</p> <ul style="list-style-type: none"> Multiple-cause-of death analysis to investigate possible excesses in nonmalignant chronic disease (using U.S. rates as reference) 		<p>3.44 (1.26-7.50)</p> <ul style="list-style-type: none"> SMR lymphatic and haematopoietic cancer 0.72 (0.20-1.84) Pneumoconiosis and other respiratory diseases SMR 2.54 (1.31-4.44) SMR accidents 1.55 (1.14-2.07) SMR tuberculosis 15.79 (1.91-57.0) <p>Low exposure: (81,466 person-years at risk)</p> <ul style="list-style-type: none"> SMR all-cause mortality 1.04 (0.96-1.13) SMR malignant neoplasms overall 1.14 (0.98-1.32) SMR prostate cancer 1.67 (1.03-2.55) SMR lymphatic and haematopoietic cancer 0.71 (0.19-1.81) SMR oesophagus cancer 2.42 (1.16-4.44) SMR pneumoconiosis and other respiratory diseases 1.13 (0.74-1.64) SMR other & unspecified sites 1.84 (1.05-2.99) SMR other diseases of the heart 0.57 (0.36-0.86) 		<p>are the Washington State references SMRs. For MCOD's US mortality data were used as reference</p> <ul style="list-style-type: none"> The tables with SMRs for all types of ICD codes are too extensive to reproduce here. Only significant ones, or those pertaining to study hypotheses shown 39 workers mentioned as unclassified in Okun et al. (1985) included here in low exposure group

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			<ul style="list-style-type: none"> • SMR cirrhosis of liver 1.90 (1.25-2.77) <p>Multiple causes of death analysis (MCOB):</p> <ul style="list-style-type: none"> • In multiple cause of death analysis, SMR cancer 1.15 (1.03-1.27) • Prostate cancer SMR 1.50 (0.99-2.19) • Diabetes deaths in high-exposure group SMR 1.73 (1.06-2.67) • Alcoholism deaths in whole cohort SMR 2.76 (1.94-3.80); in high exposure 2.16 (0.99-4.10); in low exposure 3.03 (2.01-4.38) • Deaths from 'other mental disorders' in whole cohort SMR 1.55 (1.13-2.07); in low exposure 1.56 (1.08-2.17) <p>Subgroup analyses:</p> <ul style="list-style-type: none"> • SMR for overall mortality for white males and females were 1.11 (1.03-1.19) and 0.98 (0.72-1.31), respectively. • In analysis using different subcategories of haematopoietic 		

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			<p>cancers no significant results (details not reported)</p> <ul style="list-style-type: none"> • Lung cancer in white females SMR 1.82 (0.78-3.59) <p>Analysis of latency: All-cancer deaths: latency < 15 years SMR 1.16 (0.89-1.49) versus latency ≥ 15 years (n=170) SMR 1.18 (1.01-1.37) Oesophageal cancer: latency < 15 years (n=3) SMR 2.61 (0.52-8.54) versus latency ≥ 15 years SMR 2.23 (1.02-4.35) Prostate cancer: latency < 15 years (n=5) SMR 2.33 (0.75-5.78) versus latency ≥ 15 years SMR 1.60 (0.96-2.52)</p> <p>Duration of exposure: Workers < 1 years employment SMR all cancers 1.35 (1.14-1.59), with inverse relation with duration (but data not shown); SMR overall mortality 1.24 (1.14-1.35) Workers ≥ 1 year; employment (n=1678) SMR all cancers 0.97 (0.78-1.20);</p>		

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			<p>SMR overall mortality 0.91 (0.81-1.01)</p> <p>Association with cumulative exposure: Overall death rates negatively associated with estimated cumulative styrene exposure;</p> <ul style="list-style-type: none"> – Lowest exposure tertile (5-500 ppm) SMR 1.28 (1.13-1.45) – Middle tertile (500-5,000 ppm) SMR 1.13 (1.01-1.26) – Highest tertile (>5,000 ppm) SMR 0.93 (0.82-1.05) <p>Also inverse relationship for overall cancer and oesophageal cancer (but results not shown)</p> <p>Positive trend for urinary tract cancer:</p> <ul style="list-style-type: none"> – Lowest tertile SMR 0.85 (0.10-3.70) – Middle tertile 1.26 (0.34-3.49) – Highest tertile 1.96 (0.79-4.21) 		
Ruder et al. (2016), (48)	See also general information above	See also general information above	58,594 person-years at risk (21,007 with potentially high	See also general information above	See also general information above

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>See general information above</p> <p>Study population: 5,203 (instead of the 5,204 mentioned in Ruder et al. (2004))</p> <p>Follow-up: Vital status through end 2011 (update of Ruder et al. (2004))</p>	<p>for exposure assessment</p> <p>Exposure Based on industrial hygiene measurements, exposure for styrene-exposed estimated 42.5 ppm/day (plant A) and 71.7 ppm/day (plant B); all other departments, set at 5 ppm/day. Tertiles of estimated cumulative potential styrene exposure for those ever employed at department with styrene exposure: 0 to <3500, 3500 to <1 582 000, ≥82 000 ppm) with roughly equal numbers of deaths in each tertile.</p>		<p>styrene exposure. Overall, 598 deaths occurred:</p> <ul style="list-style-type: none"> • All-cause mortality SMR 0.96 (95% CI 0.8-1.04) • Lung cancer (SMR 1.23 (0.95-1.56)) • Ovarian cancer SMR 3.08 (1.00-7.19) • COPD SMR 1.15 (0.81-1.58). <p>Potentially highly exposed (n=580):</p> <ul style="list-style-type: none"> • COPD SMR 2.02 (1.08-3.46). <p>Internal comparisons per tertiles of cumulative styrene exposure not shown in article, but described by authors as follows: no positive association with all-cause mortality; deaths from ovarian cancer, pancreatic cancer and cardiomyopathy concentrated in highest tertile; significant increases with increasing exposure for pancreatic cancer and cardiomyopathy, but small numbers</p>		<ul style="list-style-type: none"> • A priori hypothesis for this study: excess leukemia and lymphoma mortality would be found

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	Statistical analysis: <ul style="list-style-type: none"> In addition to SMR analyses, internal comparisons were done according to tertiles of estimated cumulative styrene exposure 				
Ruder et al. (2017), (47) See general information above Study population: 3,704 out of 5,203 workers; <ul style="list-style-type: none"> Workers still living in Washington State between 1991 (the year at which cancer registration started) and end 2007. A residence history of each worker, derived from 	See general information above for exposure assessment <ul style="list-style-type: none"> Race- and gender-specific person-years at risk (PYAR) accumulated for each worker across 5-year age and calendar year intervals Tertiles of cumulative exposure: 0- <3,500 ppm; ≥3500- < 82,000 ppm; ≥ 82,000 ppm 	Health outcomes: Cancer incidence evaluated as standardised incidence ratios (SIRs) and standardised rate ratios (SRRs). Health assessment Cancer diagnosis according to ICD Oncology Third Edition (ICD-O-3). Incident cases defined as all primary invasive cancers and in situ	<ul style="list-style-type: none"> Overall cancer incidence 516 cases in 63,117 person-years at risk, SIR 0.83 (95% CI 0.76-0.90) (in text, but in table 0.89 (0.81-0.97)) Mean time after start employment to diagnosis is 33.7 years (range 14.6-52.0 years) Individual cancer incidences SIR > 1 for: <ul style="list-style-type: none"> Cancer trachea, bronchus, lung SIR 1.11 (0.89-1.37). In high exposure SIR 1.42 (1.00-1.95) Lymphatic and haematopoietic cancers SIR overall 1.03 (0.77-1.35); SIR high exposure 0.99 (0.59-1.57); low exposure 1.05 	See also general information above -Healthy worker effect not assessed -As above, no information on lifestyle-related factors, in particular smoking and alcohol -Selective migration or competing causes of death might have led to bias	See also general information above Regarding this study: <ul style="list-style-type: none"> Loss to follow-up: 39 workers were lost to follow-up prior to 1991, 510 had died before 1991, and 950 believed to have moved out of Washington State were excluded Cancer registry only started in 1991, hence prior cancers not detected (loss of

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<p>various sources, was created in order to ascertain residence in Washington State during 1991-2007.</p> <ul style="list-style-type: none"> • 580 classified as potentially high exposure group <p>Censoring: Workers who left Washington State or died before end 2007 censored at date of migration or death.</p> <p>Reference population: Age and calendar specific cancer incidence rates of Washington State from the Washington State Cancer Registry. Follow-up: Vital status through end 2011</p>	<p>Statistical analyses: See also general information above.</p> <ul style="list-style-type: none"> • Calculation of total cancer and specific cancers Standardised incidence ratios (SIRs) • Standardised rate ratios (SRRs plus 95% CIs) comparing incidence across high versus low exposure. Also analyses restricted to workers > 1 year employment 	<p>bladder cancers. Diagnosis dates assigned to June 30 if only year known (only two cases).</p>	<p>(0.73-1.46)</p> <ul style="list-style-type: none"> • Ovarian cancer in high exposure SIR 2.26 (0.62-5.78) <p>High exposure versus low exposure: All cancers together increased, but none of specific cancers, except buccal and pharyngeal cancer:</p> <ul style="list-style-type: none"> • All cancers SRR 1.28 (1.05-155) • Trachea, bronchus and lung cancer SRR 1.41 (0.87-2.29) <p>Workers > 1 year employment:</p> <ul style="list-style-type: none"> • Trachea, bronchus and lung cancer SRR 0.66 (0.33-1.34) 		<p>power)</p> <ul style="list-style-type: none"> • State of residence had to be assumed for 14% of person-years at risk 1991-2007. • 39 diagnoses in workers who first left catchment area and later returned were excluded • At time of data collection on work history 772 workers still employed, so exposure after 1978 not known (of those 152 excluded due to migration criterion • Cohort relatively young: median age 44 at beginning of follow-up in 1991 and 65 at end of follow-up.

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					<p>Together with relatively small sample size, this implies power to detect excess cancer incidence low</p> <ul style="list-style-type: none"> Analyses were performed using the NIOSH LTAS.NET life table analysis system
<p>Bertke et al. (2018), (46) See general information above</p> <p>Study population: 5,201 workers (after 2 removed for missing birth date resp. duplicate entry), of whom 1960 in high exposure group</p> <p>Reference population:</p>	<p>See further general information above for exposure assessment. Further: Jobs divided into 5 groups with respect to exposure level, but in analyses dichotomised into high versus low</p> <p>Two exposure metrics used:</p> <ul style="list-style-type: none"> Ever/never worked in high exposure job 	<p>Health outcomes: See information above, with follow-up extended to 2016.</p> <p>Health assessment All causes of death evaluated based on NDI Plus, coded following ICD version in effect at time of death. 28 workers lost to follow-up before</p>	<p>Mortality: Total person-years at risk 203,404 with 2111 deaths (41% of cohort)</p> <ul style="list-style-type: none"> All-cause mortality whole cohort SMR 1.19 (95%CI 1.14-1.24); employment ≥ 1 year SMR 0.99 (0.92-1.06) All cancers SMR whole cohort 1.23 (1.13-1.33); employment ≥ 1 year SMR 1.07 (0.93-1.23) Lymphohaematopoietic cancers whole cohort SMR 0.99 (0.74-1.30); employment ≥ 1 year SMR 0.85 (0.51-1.35) 	<p>See also general information above</p> <ul style="list-style-type: none"> Healthy worker effect not assessed, but observed that mortality much lower in administrative jobs (e.g. SMR 0.73 versus 1.21 in fiberglass or plastic workers) As above, no information on lifestyle-related factors, in particular smoking 	<p>See also general information above</p> <p>Regarding this study:</p> <ul style="list-style-type: none"> More details on employment duration relatively short and strongly skewed: nearly two-thirds employed < 1 year; median years employed 0.4 (0.1-1.5), for whole cohort Cohort relatively

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<p>General population of Washington State, 1960-2014.</p> <p>Censoring: Exposure person-time for workers still active in 1978 truncated at October 1, 1988</p> <p>Follow-up: Additional follow-up since 2011 through 2016 using the (US) National Death Index (NDI)</p>	<ul style="list-style-type: none"> • Employment duration (administrative jobs excluded). Exposure duration lagged 10 years and for workers still employed in 1978 exposure truncated at 1988. To account for skewed distribution of employment duration in high-exposed employment group, duration was further modelled with two-piece linear spline with a knot at 10 years (approximately 99th percentile) <p>Statistical analyses: See also general information above.</p> <ul style="list-style-type: none"> • Calculation of Standardised mortality ratios (SMRs) as ratio of expected versus observed 	<p>1979 (start NDI) and 19 emigrants classified as 'vital status unknown' and censored at date last observed</p>	<ul style="list-style-type: none"> • Lung cancer SMR 1.37 (1.19-1.57) whole cohort; employment ≥ 1 year SMR 1.20 (0.95-1.51) <p>Cox regression <i>Exposed versus not exposed:</i></p> <ul style="list-style-type: none"> • All cancers RR 1.2 (1.0-1.4) • Lung cancer RR 1.0 (0.8-1.4) • Lymphohaematopoietic cancers RR 1.2 (0.6-2.2) • Leukemia RR 1.6 (0.5-4.5) <p><i>Duration employed in high exposure group (log-linear):</i></p> <ul style="list-style-type: none"> • All cancers RR 1.0 (1.0-1.1) • Lung cancer RR 0.9 (0.7-1.1) • Lymphohaematopoietic cancers RR 1.2 (1.0-1.4) • Leukemia RR 1.3 (1.0-1.5) <p><i>Duration employed in high exposure group (2 piece spline, RRs for slope of first piece of spline):</i></p> <ul style="list-style-type: none"> • All cancers RR 1.1 (1.0-1.2) • Lung cancer RR 0.9 (0.8-1.1) • Lymphohaematopoietic cancers RR 1.4 (1.1-1.7) • Leukemia RR 1.6 (1.2-2.2) 	<p>and alcohol. For internal analyses, persons employed in the administrative group removed because potentially confounding lifestyle and socioeconomic factors</p> <p>-Exposure misclassification due to lack of information on specific job titles and variation in exposure within the high exposure group. One aspect of the risk of exposure misclassification addressed by truncation of exposure accumulation for workers still employed in 1978 at 1988, and by modelling with</p>	<p>young: median age 44 at beginning of follow-up in 1991 and 65 at end of follow-up. Together with relatively small sample size, this implies power to detect excess cancer incidence low</p> <ul style="list-style-type: none"> • Analyses were performed using the NIOSH LTAS.NET life table analysis system • As seen previously among those employed less than a year, there were excess deaths from diseases associated with generally adverse lifestyle factors such as diabetes mellitus (45

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	<p>numbers of death (by indirect standardisation); Person-time at risk ended at date of death, date last observed, or December 31, 2016; Person-time at risk stratified by age and calendar period (in 5-year intervals) and multiplied with general population se, race, age and calendar-specific rates to derive expected numbers of death</p> <ul style="list-style-type: none"> • (Hazard) Rate Ratios (reported as RRs) per year employed using Cox regression (after exclusion of administrative workers); risk-sets consisting of those persons at risk at the 			spline	<p>deaths, SMR: 1.42 (1.03, 1.89]), alcoholism (15 deaths, SMR: 2.13 (1.19, 3.52)), and accidents (124 deaths, SMR: 1.43 (1.19, 1.70)).</p> <ul style="list-style-type: none"> • References rates for 2010-2014 were used to calculate expected numbers of deaths during 2015-2016. (follow up is through 2016, while data reference population is through 2014

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	attained age of the case, and matched on race, gender, birth data (2.5 years margin), and employment duration (< 1 year versus ≥ 1 years)				
Daniels et al. (2020), (45) 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	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: <ul style="list-style-type: none"> • Exposure scientists blinded to case status • Work history 	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,	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, <ul style="list-style-type: none"> • 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) 	See also general information above <ul style="list-style-type: none"> -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 	<ul style="list-style-type: none"> • 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

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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	<p>based on job titles and department assignments and linked to exposure levels</p> <ul style="list-style-type: none"> • 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 airborne concentrations) 	<p>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</p>	<ul style="list-style-type: none"> • 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) • 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) <p>Same as above with SES adjustment Only minor differences</p> <p><i>Analyses restricted to exposure < 500 ppm-years</i> Of note (without SES adjustment):</p> <ul style="list-style-type: none"> • Urinary tract overall 1.43 (1.11-1.79) • Bladder and other urinary 1.64 (1.14-2.33) • Lymphatic and haematopoietic cancers overall 1.37 (1.09-1.69) 	<p>effect of smoking was explored by considering smoking-associated cancers: no association observed</p> <p>-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</p> <p>-In general: this study strongly depended on modelling and underlying assumptions</p> <p>-To account for mortality from competing sources life table analysis was used, under</p>	<p>based on the NIOSH Recommended Exposure Limit</p> <ul style="list-style-type: none"> • 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
	<p>and duration spent in each group</p> <p>Statistical analysis: <i>Cox proportional hazards regression</i></p> <ul style="list-style-type: none"> • Hazard ratios (HRs) 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 		<ul style="list-style-type: none"> • Leukemia 1.46 (1.04-1.97) (no cases in persons with cumulative exposure ≥ 500 ppm-years) <p>Restricted cubic spline models at 50 ppm-years (95% CI):</p> <ul style="list-style-type: none"> • 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) • Leukemia 4.10 (2.88-7.29) • Myeloid leukemia 11.67 (6.31-30.76) <p>Furthermore, these models showed much higher risks at low exposures than did loglinear models</p> <p>Sensitivity analyses:</p> <ul style="list-style-type: none"> • Model estimates without lag similar to those with 10-year lag 	<p>assumption that relative risk is independent of age. Assumption might be incorrect</p> <ul style="list-style-type: none"> -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
	<p>modelled</p> <ul style="list-style-type: none"> 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 <p><i>Working lifetime leukemia risks estimation</i></p> <ul style="list-style-type: none"> 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 over a working lifetime. <p>Subgroup analyses:</p>		<ul style="list-style-type: none"> Leukemia findings not appreciably different when person-time for active workers after 1978 included <p>Latency analysis</p> <ul style="list-style-type: none"> 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) <p>Risk projection</p> <p>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)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<ul style="list-style-type: none"> • 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 <p>Latency analysis:</p> <ul style="list-style-type: none"> • Models without exposure lag • Grid search over a range of lags (2-40 years) • Time since last exposure among cases, using restricted cubic splines <p>Sensitivity analysis:</p> <ul style="list-style-type: none"> • Leukemia models without person-time truncation 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Bertke et al. (2020), (45) See general information above Study population: In this study, the whole cohort of 5,163 workers was used Reference population: General US population. Censoring: The 772 workers still employed in 1978 at the end of records collection in were censored at October 1, 1978 Follow-up: Through December 31, 2016, including Health Worker Survivor Bias	See further general information above for exposure assessment. For this study the constructed job-exposure matrix was used (see above Daniels et.al. (2020)) Statistical analysis: The main purpose was to assess Health Workers Survivor Bias (HWSB). Method: • Analysis of 3 components: – (c1) the effect of prior (t_{j-1}) exposure on work status (employed/not employed) at t_j (age), assessed with Cox regression of time to end of employment stratified for age, race, sex, birth date (± 5 years from	Health outcomes: Lung cancer deaths coded according to ICD version in effect at time of death	Total person-years at risk 175,930, 176 lung cancer deaths (out of 2,095 deaths total) Exposure • Effect of average exposure (lifetime cumulative/employment duration) on employment duration, ≥ 30 ppm versus < 30 ppm: median 3.7 versus median 6.0 months • Average Exposure Intensity median 15.0 Association past exposure (component c1) with time to ending employment: Using cumulative exposure: – 0- < 5 ppm HR 1.00 (ref) – 5- < 25 HR 0.44 (95% CI 0.41-0.47); HR adjusted 0.44 (0.41-0.48) – 25- < 150 HR 0.27 (0.25-0.29); HR adj 0.26 (0.24-0.29) – ≥ 150 HR 0.14 (0.12-0.17); HR adj 0.12 (0.10-0.15) Average exposure intensity:	See also general information above – Differences in results using alternative exposure and work status variables show the sensitivity to assumptions and the risk of model misspecification – No adjustments for lifestyle factors, in particular smoking. – Exposure was dichotomised, implying loss of precision	See also general information above Regarding this study: • The explicit aim of this study was to assess the HWSB • The cut-off of 30 ppm used to dichotomise exposure in the statistical analyses was roughly the mean of individual average exposure intensity of exposed worker • Again, the power for this study was low due to the relatively brief exposure, young age, and small number of 'early' lung cancer deaths • The authors explain that the seemingly contradictory finding of opposite effects of exposure

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>index case) and SES (as a spline). Three alternative exposure variables used: cumulative, average and current exposure</p> <ul style="list-style-type: none"> – (c2) the effect of work status at t_j on subsequent exposure – (c3) the association between work status at t_j and time to lung cancer mortality (accelerated failure time), assessed with Cox regression stratified for age, race, sex, birth date (± 5 years from index case) and SES (as a spline). Three alternative variables for working status used: 0-year lag, 5-year lag (employed in previous 5 years), and duration of employment in 4 		<ul style="list-style-type: none"> – 0-<5 ppm HR 1.00 (ref) – 5-<45 HR 0.88 (95% CI 0.81-0.95); HR adjusted 0.94 (0.86-1.02) – 45-<70 HR 0.96 (0.86-1.06); HR adj 1.02 (0.91-1.13) – ≥ 70 HR 1.54 (1.41-1.67); HR adj 1.28 (1.17-1.40) <p>Current exposure: Average exposure intensity:</p> <ul style="list-style-type: none"> – 0-<5 ppm HR 1.00 (ref) – 5-<45 HR 1.18 (95% CI 1.09-1.28); HR adjusted 1.27 (1.17-1.38) – 45-<70 HR 3.07 (2.73-3.44); HR adj 3.35 (2.95-3.79) – ≥ 70 HR 3.31 (3.00-3.64); HR adj 2.87 (2.59-3.17) <p>Association time-dependent employment status and lung cancer mortality (component c3): (Only analysis with duration of employment shown):</p> <ul style="list-style-type: none"> – 0-<3 months HR 1.00 (ref) – 3 mths<1 year HR 0.86 (95% CI 0.59-1.23); HR 		<p>on work status (component c1) does not detract from the analysis method, as what is relevant is the existence of any association</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>categories</p> <ul style="list-style-type: none"> - An unknown unmeasured factor (U) representing a common cause of work status and lung cancer mortality • Exposure at time t was dichotomised with 30 ppm as cut-off • G-estimation of a structural nested model including these 3 components • Loss to follow-up and competing risks accounted for by inverse probability of censoring weights <p>SES was adjusted by using a job prestige score as described above, Daniels et al. (2020)</p> <p>Sensitivity analysis: Replication analysis without censoring</p>		<p>adjusted 0.89 (0.61-1.27)</p> <ul style="list-style-type: none"> - 1 yr < 5 yrs HR 0.89 (0.60-1.30); HR adj 0.93 (0.62-1.37) - ≥ 5 yrs HR 0.69 (0.35-1.22); HR adj 0.64 (0.33-1.17) <p>Relation exposure lung cancer mortality in nested model:</p> <ul style="list-style-type: none"> • Value of exposure-response term ψ (log of ratio survival time exposure > 30 ppm versus no exposure): 1.19 (95% CI 0.93-1.37) • 1 year of styrene exposure at > 30 ppm accelerated time to lung cancer death by 2.29 years (95% CI 1.53-2.94) <p><i>Sensitivity analysis</i> led to ψ 1.30 (1.23-1.35)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	active employees (assuming workers still employed in 1978 were unemployed and unexposed thereafter)				
General information cohort study in Loomis et al. (2019), (51) Christensen et al. (2018), (52) Nissen et al. (2018), (53) Coggon et al. (2015), (54) Boffetta et al. (1998), (55) Kolstad et al. (1995), (56) (1 of 6 countries) Kogevinas et al. (1994), (57) Kolstad et al. (1994), (58) (1 of 6 cohorts countries) Kogevinas et al. (1993), (59)	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 also general information above -Differences in results using alternative exposure and work status variables show the sensitivity to assumptions and the risk of model misspecification -No adjustments for lifestyle factors, in particular smoking -Risk of misclassification of exposure great. -Exposure was dichotomised, implying loss of precision	See also general information above Regarding this study: • The explicit aim of this study was to assess the HWSB • The cut-off of 30 ppm used to dichotomise exposure in the statistical analyses was roughly the mean of

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Coggon et al. (1987), (60) (1 of 6 countries)</p> <p>Study population: 37,021-40,688 (all cohorts combined)) workers at reinforced plastics production plants in the 6 countries, organised into 8 subcohorts.</p> <p>Inclusion criteria: Ever employed at one of the plants included in the eight subcohorts</p>					
<p>Coggon et al. (1987)</p> <ul style="list-style-type: none"> • Retrospective cohort study • UK • 1947-1984 	<p>Exposure estimation based on job histories and styrene measurements</p>	<p>Health outcomes: Vital status at 31 December 1984, cause-specific mortality and cancers.</p>	<p>(Results shown for 7 out of 8 factories)</p> <ul style="list-style-type: none"> • All-cause mortality SMR 83 (77-89) • All neoplasms SMR 80 (69-93) • Ischaemic heart disease SMR 92 (80-105) 	<ul style="list-style-type: none"> • No information on and adjustment for other risk factors, in particular smoking -Missing data many more for one factory. 	<p>See also general information above Regarding this study:</p> <ul style="list-style-type: none"> • 2,458 persons worked in exposed occupations for at

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Study population: 7,949 workers at 1 of 8 British companies manufacturing glass-reinforced plastics. Of these 3,494 were considered highly exposed (hand laminators). There were 6,638 men and 1,311 women. 405 out of 8,354 study candidates (4.8%) were excluded due to missing data.</p> <p>Inclusion criteria: Ever employed at one of the eight companies, identified from personnel and wage records</p> <p>Follow-up:</p>	<p>Job histories were obtained from personnel and wage records. Jobs were classified into 4 exposure categories from high (hand laminators), moderate, low and background, based on information from management and staff</p> <p>Styrene measurement had been measured since 1975 in 5 of the 8 factories. Based thereon, exposure levels were estimated at 40-100 ppm for the high exposure group. The most important other potential carcinogens were glass fiber,</p>	<p>Cohort members were traced through the National Health Service (Central Register) and National Insurance Index. Death certificates were obtained from the Office of Population Censuses and Surveys, with underlying cause of death coded according to ICD revision 9. Also, cancer cases occurring during follow-up were obtained from this office</p>	<ul style="list-style-type: none"> • Cerebrovascular disease SMR 82 (61-107) • Respiratory disease SMR 70 (53-90) • Digestive disease SMR 82 (48-132) • Injury and poisoning SMR 81 (58-110) • Specific cancers presented according to exposure level and exposure duration in tables 4 and 5 of article, but no statistically significant results; the authors mention a deficit of deaths from lymphoid and hemopoietic cancer (6 observed, 14.9 expected), but only provide confidence intervals for individual cancers, with numbers too small; highest rates were for lung cancer, but rates did not increase with time since first exposure (dose-response not consistent) <p>Results for the factory presented separately were similar</p>	<p>Therefore, the results for that factory are presented separately</p> <ul style="list-style-type: none"> -Risk of misclassification of exposure is great. -HWSB not accounted for 	<p>least one year.</p> <ul style="list-style-type: none"> • Small study with not much power to detect signals • Also cancer incidence was not studied due to incompleteness of cancer registration

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Until end 1984 (171 emigrated, followed-up until emigration)</p> <p>Reference population: General population of England and Wales.</p> <p>Censoring: 31 December 1984. 210 subjects lost to follow-up were censored at the date last employed</p>	<p>acetone, methyl ethyl ketone, organic peroxides and asbestos</p> <p>Statistical analysis Calculation of SMRs, with 95% CIs based on Poisson distribution</p>				
<p>Kogevinas et al. (1993), (59)</p> <ul style="list-style-type: none"> • Retrospective cohort study • 6 European countries: Denmark, Finland, Italy, Norway, Sweden, United Kingdom) • 40,683 workers 	<p>Based on job histories, environmental and biological monitoring data, and production records</p> <p>Job histories: obtained from payroll records and</p>	<p>All cause and cause-specific mortality coded nationally and converted to IARC codes over different revisions of the International Classification of Diseases (ICD) 8th revision</p>	<p>Whole cohort (544,005 patient-years)</p> <ul style="list-style-type: none"> • All-cause mortality SMR 91 (95% CI 88-95) • All cancers SMR 87 (80-94) • Lymphatic and haematopoietic cancer SMR 96 (71-126) 	<ul style="list-style-type: none"> • Risk of bias: no information on potential confounders • HWSB not addressed, 40% of workers < 1 year employed • Risk of exposure misclassification; relation exposure 	<ul style="list-style-type: none"> • Styrene levels decreased considerably during study period in 5 of the 6 countries • More short-term workers in highly exposed groups (43% versus 34%).

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>(34,556 males) in the reinforced plastics industry</p> <ul style="list-style-type: none"> • About 40% employed < 1 years • 16,253 follow-up > 10 years • Period: approximately 1945-1991, but start year ranged per country from 1945 to 1970 <p>Inclusion criteria: Having worked in the reinforced plastics industry at one of the plants included in the study at some time during the study period</p> <p>Reference population: General population</p>	<p>production records of participating plants</p> <p>Measurements: 16,367 personal environmental measurements and 18,695 urine measurements of styrene metabolites.</p> <p>Exposure categories: based on job titles and exposure measurements five exposure groups were defined: laminators (most exposed), workers with 'unspecified tasks', workers in 'other exposed jobs, workers 'not exposed', workers with unknown job titles with laminators (n=10,628) as</p>		<p>Risk with time since first exposure (in 10 year intervals): Among workers exposed > 1 year:</p> <ul style="list-style-type: none"> • Lymphatic and haematopoietic cancer at 20 years SMR 140 (70-251), Chi squared for linear trend 3.91, P<0.05 <p>Subgroup analyses: Results per exposure group (5) not reproduced here. Not for any of the exposure groups was found.</p> <ul style="list-style-type: none"> • National cohorts: increased mortality rates for pancreatic cancer in Denmark and UK, prostate and testis in Sweden, oesophagus, larynx and ovary in UK, bladder in Denmark, stomach in Finland, liver and gallbladder in Italy (Emilia Romagna) 	<p>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</p> <ul style="list-style-type: none"> • 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 	<p>Proportions varied considerably between countries</p> <ul style="list-style-type: none"> • 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

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>mortality rates from WHO mortality databank</p> <p>Follow-up; Average 13 years</p> <p>Censoring: Right: workers lost to follow-up or who emigrated (3%) Left: not reported</p> <p>Related study: Boffeta et al. (1998) and Loomis et al. (2019) etc.</p>	<p>most exposed. For some analyses categories were merged into high versus low exposure versus unexposed.</p> <p>Statistical analysis National Standardised mortality ratios (SMRs) calculated from person-years and 95% CI based on Poisson distribution (two-tailed $P < 0.05$ as significant). Standardised for sex, 5-year age group and calendar period. • Subgroup analyses per site, country, and exposure group</p>				
Kogevinas et al. (1994), (57)	See information above	Health outcomes:	Total number of person-years 539,479, of which 214,965 at	See also general information above -No information and	See also general information above

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Retrospective cohort study</p> <ul style="list-style-type: none"> • Denmark, Finland, Italy, Norway, Sweden, UK • Reinforced plastics industry <p>Study population: 40,688 reinforced plastics workers at reinforced plastics production plants in the 6 countries, organised into 8 subcohorts (2 subcohorts each in Italy and UK); 34,560 men and 6,128 women</p> <p>Inclusion criteria: Ever employed at one of the eight plants</p>	<p>Exposure estimation based on job histories and environmental and biological monitoring data</p> <p>Job histories Production records and payroll records of workers were abstracted. Finnish cohort incomplete job history data (see below). For Danish cohort (175,640 person-years) the proportion of work force employed at each plants in production of reinforced plastic was estimated. Data for Sweden also incomplete.</p> <p>Exposure assessment Exposure assessed based on about</p>	<p>All-cause mortality, non-cancer and cancer mortality, based on cause-specific national death registries. National codes were converted to ICD codes using an IARC conversion table</p>	<p>≥ 10 years since start exposure</p> <p>SMRs whole cohort See tables in article for full enumeration</p> <ul style="list-style-type: none"> • All-cause mortality SMR 92 (95% CI 81-94) • All cancers SMR 87 (81-94) • Lymphatic and hematopoietic cancers SMR 93 (71-120) • Circulatory diseases SMR 92 (87-97) • Respiratory diseases SMR 79 (67-92) <p>Specific cancers (only statistically significant)</p> <ul style="list-style-type: none"> • Buccal cavity and pharynx SMR 33 (11-77) • Rectum SMR 62 (38-95) • Breast SMR 52 (28-89) • Brain SMR 62 (37-98) <p>SMRs compared according to years of exposure (in exposed workers) All cancers, time since first exposure</p> <p>< 10 yrs - < 2 yrs exposure SMR 78</p>	<p>adjustments for lifestyle factors, in particular smoking</p> <ul style="list-style-type: none"> - No information on exposure to other potential carcinogens at work, but the authors state that in reinforced plastic there is at most minimal exposure to known carcinogens - Also no information on potential exposure outside work - Risk of misclassification of exposure great. Especially for Denmark information on exposure was not detailed, and in Finland a lower percentage of short-term workers were included - Decreasing levels of exposure over time, differing per country, might have led to 	<p>Regarding this study:</p> <ul style="list-style-type: none"> • 479 out of 41,167 potential subjects were excluded due to missing data • For one of the two UK cohorts only records of laminators were obtained • Finnish cohort comprised all workers at 157 plants identified by cross-sectional survey in 1978, but complete dates of employment only available for 598 out of 2085 workers. • The Swedish cohort comprised 30 companies identified in 1976; 16 of these were still active 1987 when the investigators

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Reference population: General population in the respective countries. Country-specific death rates based on WHO international mortality data bank</p> <p>Follow-up: Varied per country. Overall: 1945-1991. Mean follow-up 13 years. Lost to follow-up 1.4% and 1.6% emigrated</p> <p>Left censoring: First data for which complete payroll records were available for those already employed at start follow-up</p>	<p>16,500 personal environmental measurements of styrene in air conducted in 1955-1990, and 18,500 measurements of styrene metabolites in urine conducted in late 1980s.</p> <ul style="list-style-type: none"> Extensive exposure information prior to 1970 only available for Denmark. For other countries these were modelled in two different ways (model A (=level from Denmark) versus model B (extrapolated level)) Exposure levels decreased strongly over time from about 200 ppm before 		<p>(63-94) - ≥ 2 yrs SMR 94 (74-118) - Total exposure SMR 84 (72-97)</p> <p>10-19 yrs - < 2 yrs exposure SMR 105 (88-123) - ≥ 2 yrs SMR 87 (72-105) - Total exposure SMR 96 (85-109)</p> <p>≥ 20 yrs - < 2 yrs exposure SMR 86 (63-116) - ≥ 2 yrs SMR 100 (76-129) - Total exposure SMR 93 (76-113)</p> <p>Lymphatic and haematopoietic cancers overall (subtypes not reproduced here; see table in article), time since first exposure:</p> <p>< 10 yrs - < 2 yrs exposure SMR 43 (16-93) - ≥ 2 yrs SMR 92 (37-190) - Total exposure SMR 60 (32-103)</p>	<p>bias, especially with respect to cumulative exposure. For earliest periods, exposure was extrapolated. But use of alternative exposure models (A and B) led to similar results</p> <p>-HWSB not accounted for</p>	<p>located them again; only for the workers at these companies were job histories complete</p> <ul style="list-style-type: none"> Approximately 60% of workers employed < 2 years, but this varies per country from 9% to 81% Mortality from lymphatic and haematopoietic cancers greater in Denmark: 24 out of 50 identified cases

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>1970 to approximately 40 ppm in 1990.</p> <ul style="list-style-type: none"> • Duration of exposure calculated from individual payroll records combined with plant records showing dates of production of reinforced plastics (in Denmark differently and more accurately) • Cumulative exposure in ppm-years and average exposure in ppm (cumulative/total exposure time), categorised into 4 exposure categories: laminators, unspecified task, other exposed jobs, unexposed • Cumulative exposure both for 		<p>10-19 yrs - < 2 yrs exposure SMR 183 (112-283) - ≥ 2 yrs SMR 61 (22-132) - Total exposure SMR125 (82-183)</p> <p>≥ 20 yrs - < 2 yrs exposure SMR 85 (18-248) - ≥ 2 yrs SMR 173 (70-357) - Total exposure SMR 132 (64-244)</p> <p>Test for linear trend over time since first exposure (employment): chi-square 3.91, P<0.05. In unexposed SMR at ≥ 20 yrs 44 (6-176)</p> <p>Poisson regression lymphatic and haematopoietic neoplasms (adjusted for age, gender, country, calendar period, and time since first exposure; results for model without time lag reproduced here) On cumulative exposure (ppm-years)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>total exposure and ignoring 5 year period before death, loss, or end of follow-up</p> <p>Exposure categories Five mutually exclusive groups defined on basis of exposure measurements and individual job titles, of diminishing exposure levels (groups 1 through 4) and a group with unknown job titles. Workers classified according to longest held job.</p> <p>Statistical analysis:</p> <ul style="list-style-type: none"> • Calculation of Standardised Mortality Ratios (SMRs), with 95% CI based on 		<p>- < 75 Reference - 75-199 RR 0.98 (0.43-2.26) - 200-499 RR 1.24 (0.57-2.72) - ≥ 500 RR 0.84 (0.35-2.02) - Test linear trend P=0.65</p> <p>On time since first exposure (years) - < 10 Reference - 10-19 RR 2.90 (1.29-6.48) - ≥ 20 RR 3.97 (1.30-12.13) Test linear trend P=0.012</p> <p>On average exposure (ppm) - < 60 Reference - 60-99 RR 1.68 (0.59-4.79) - 100-119 RR 3.11 (1.07-9.06) - 120-199 RR 3.08 (1.04-9.08) - ≥ 200 RR 3.59 (0.98-13.14) - Test linear trend P=0.019</p> <p>Poisson regression other cancers No statistically significant results, but of note pancreas cancer at ≥500 ppm-years RR 2.56 (0.90-731) (see table 5 in article for results on all cancers, esophagus, pancreas, lung and kidney)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>Poisson distribution, with $P < 0.05$ (two-sided); national mortality reference data by gender and five-year age groups</p> <ul style="list-style-type: none"> • Test for trend in SMRs according to Breslow and Day • Poisson regression for internal comparisons (limited to exposed workers), adjusting for country, age (5 groups), gender, calendar period (4 periods) and time since first exposure (3 levels), for estimation of rate ratios (RRs). Tests for linear trend 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>according to Breslow and Day</p> <p>For Finnish cohort only 598 out of 2085 workers included in analyses involving cumulative exposure</p> <p>For some analyses exposure dichotomised into exposed versus unexposed</p>				
<p>Kolstad et al. (1994), (58)</p> <ul style="list-style-type: none"> • Retrospective cohort study • Denmark • 1964-1988 <p>Study population: 36,525 male workers in the reinforced plastics industry and 14,254 workers at similar industries</p>	<p>Exposure estimation based on overall characteristics of company, and styrene measurements at a few companies</p> <p>Each worker was classified, based on classification of the companies by expert opinion (two dealers in reinforced plastics)</p>	<p>Health outcomes: Overall cancer incidence and Incidence of lymphohaematopoietic cancers, expressed as standardized incidence ratios (SIRs). Cancer cases were identified from the Danish Cancer Registry, and classified according</p>	<p>584,556 person-years observed with mean follow-up 10.9 years</p> <p>Overall cancer incidence SIR 1.02 (95% CI 0.97-1.07)</p> <p>All workers:</p> <ul style="list-style-type: none"> • Hodgkin's disease SIR 1.09 (0.70-1.63) • Multiple myeloma SIR 0.81 (0.46-1.34) • Non-Hodgkin's lymphoma SIR 1.29 (0.99-1.66) • Leukemia SIR 1.17 (95% CI 0.90-1.51) 	<ul style="list-style-type: none"> • No information on other risk factors, in particular smoking -No adjustments for lifestyle factors, in particular smoking -Risk of misclassification of exposure great. -HWSB not accounted for -Misclassification of exposure very likely, as classification was done very roughly, 	<p>See also general information above Regarding this study:</p> <ul style="list-style-type: none"> • At an initial screening of relevant companies 552 were identified. These were further evaluated by independent reviews by two plastic dealers, and by postal questionnaires

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>not exposed to styrene.</p> <p>Inclusion criteria: Ever employed between 1964 and 1988 at one of 386 companies producing reinforced plastics for first group, and 166 companies not producing reinforced plastics or status unknown, for the second, and resident in Denmark after January 1970.</p> <p>Exclusion criteria: Being female (N=10,799)</p> <p>Follow-up: Until end 1989</p>	<p>as working in a company with 50% or more of the workforce working in the production, with less than 50% working in production, or company not producing reinforced plastic. 36,525 workers classified as exposed. For exposed workers, first and last year of exposed employment was recorded based on pension fund payments.</p> <p>Styrene measurement: Between 1964 and 1988 2,473 personal air samples from work sites were collected as part of</p>	<p>to ICD revision 7 by codes 200-205 (lymphohaematopoietic neoplasms)</p>	<p>All exposed workers:</p> <ul style="list-style-type: none"> • Hodgkin's disease SIR 1.08 (0.62-1.76) • Multiple myeloma SIR 0.99 (0.51-1.73) • Non-Hodgkin's lymphoma SIR 1.33 (0.96-1.80) • Leukemia SIR 1.22 (95% CI 0.88-1.65) <p>Subgroup analyses (only statistically significant results shown):</p> <ul style="list-style-type: none"> • Non-Hodgkin's lymphoma at companies with 1-49% of employees in reinforced plastics production SIR 2.35 (1.42-3.67) <p>Workers in the 1964-1970s (period of highest exposure)</p> <ul style="list-style-type: none"> • Leukemia SIR 1.54 (1.04-2.19); In workers employed > 10 years leukemia SIR 1.69 (1.09-2.49) <p>Exposed workers according to time since first employment</p> <ul style="list-style-type: none"> • Non-Hodgkin lymphoma in < 10 years SIR 1.68 (1.03-2.53) • Leukemia in ≥10 years SIR 1.07-2.22, with SIR 2.34 (1.43-3.61) in those < 1 year 	<p>and number of actual styrene measurements very low</p> <ul style="list-style-type: none"> - Many subgroup analyses performed, but no accounting for multiple testing - Mean follow-up of 10.9 years likely insufficient to capture all incident cases 	<p>sent to employees, with fairly strong agreements (kappa's from 0.72 to 0.94)</p> <ul style="list-style-type: none"> • Most companies were boat yards or producers of containers by hand lamination; the non-producers of reinforced plastics produced wooden boats or thermoplastics, or were within the metal industry or were dealers. 82 companies were classified as unknown. • Around 60% of workers employed < 1 year. • Loss to follow-up < 2% • In sheer numbers, the study was large, capturing almost all Danish workers in

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Reference population: General population of Denmark. Censoring: Left censoring 1 January 1970 or 1 January year following first year of employment thereafter Right censoring 31 December 1989 or date of death, emigration or disappearance	surveillance by the Danish Work Inspection Service. Mean styrene levels per period: – 1964-1970 180 ppm – 1971-1975 88 ppm – 1976-1988 43 ppm Statistical analysis Calculation of standardized incidence ratios (SIRs), standardized for gender, age and year of diagnosis, with 95% CIs based on Poisson distribution		employment • All lymphohaematopoietic malignancies in < years SIR 1.65 (1.18-2.26) in those < 1 year employment		reinforced plastics production in the time period, but due to very coarse estimations of exposure, power to detect excess cancer incidence is low. • In fact, the experts consulted estimated that on average only 40% of workers classified as exposed had actually worked in reinforced plastics production
Kolstad et al. (1995), (56) See information above, Kolstad et al. (1994)	See information above, Kolstad et al. (1994)	Health outcomes: Mortality, solid cancer incidence, specific cancer incidence, cause	Extensive tables in article. Here only results highlighted in abstract • In high probability exposed companies MMR for	Same as above, Kolstad et al. (1994)	Same as above, Kolstad et al. (1994)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Follow-up: 31 December 1990. 54 lost to follow-up (0.1%) and 1072 emigrated (2%)</p>	<p>For this study, workers at companies with 50% or more of the workforce working in production were classified as (probably) high exposure, those at companies with less than 50% working in production, as (probably) low exposure</p> <p>Statistical analysis: Calculation of standardised mortality ratios (SMRs), and standardised incidence ratios (SIRs), standardized for gender, age and calendar period, with 95% CIs</p>	<p>specific mortality, all coded according to ICD revision 8. Multiple sclerosis, Parkinson's disease and motor neuron disease grouped as degenerative disorders of the nervous system</p>	<p>degenerative nervous system disease 1.8 (95% CI 0.9-3.8), and pancreatic cancer IRR 2.2 (1.1-4.5)</p> <ul style="list-style-type: none"> • For these outcomes, there was increased occurrence in long term workers and those employed in the 1960s 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>based on Poisson distribution.</p> <p>Internal comparisons, exposed versus unexposed; Poisson regression to estimate mortality rate ratios (MRRs) and incidence rate ratios (IRRs), with as variables exposure probability (high, low, not), age (4 groups), year of first employment (< 1970 or after), duration of employment (< 1 year or longer), and time since first employment (< 10 years or longer)</p>				
Boffetta et al. (1998), (55) • Retrospective cohort study	See also above general information • The study does	All cause and cause-specific mortality assessment not described, but	380,521 patient-years, of which 196,257 contributed by workers employed < 1 year (52%)	• No information on potential confounding factors such as	• The study only indirectly considers exposure by way

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<ul style="list-style-type: none"> • 7 European countries (Denmark, Finland, Germany, Italy, Norway, Sweden, United Kingdom; 8 countries according to authors but this seems a mistake) • 29,525 male workers in the reinforced plastics and man-made vitreous fiber industries; 15,318 employed < 1 year <p>Inclusion criteria: Having worked at one of the study locations some time during the</p>	<p>not report on exposure assessment apart from employment duration</p> <ul style="list-style-type: none"> • Workers divided according to length of employment: <1 month, 1-5 months, 6-11 months (short term), ≥ 1 year <p>Statistical analysis</p> <ul style="list-style-type: none"> • National Standardised mortality ratios (SMRs) calculated from person-years and 95% CI based on Poisson distribution (two-tailed $P < 0.05$ as significant). Standardised for sex, 5-year age group and calendar period • Multivariate 	<p>authors refer to Kogevinas et al. (1994). In tables ICD-9 are used, but this is not described in the text</p>	<p>Exposure < 1 year:</p> <ul style="list-style-type: none"> • All-cause mortality 1.16 (95% CI 1.09-1.23) • All cancer mortality 0.96 (0.83-1.10) <p>Exposure ≥ 1 year:</p> <ul style="list-style-type: none"> • All-cause mortality 0.83 (0.79-0.99) • All cancer mortality 0.88 (0.79-0.99) <p>Internal comparisons:</p> <ul style="list-style-type: none"> • Poisson regression: All-cause mortality RR 1.11 (1.01-1.23) for employment < 1 year versus reference. This increased to 1.21 (1.11-1.33) when employment status was excluded. • Analyses per country showed higher all-cause mortality in short term workers in Denmark and Finland, but not in the other countries. After exclusion of Denmark RR was reduced to 0.97 (0.85-1.11) • Analysis with further subdivision of employment < 	<p>smoking or other exposures</p> <ul style="list-style-type: none"> • Only duration of employment considered, so weak relation with true exposure • Results suggest a HWSB, but a binary categorisation with cut-off at 1 year employment is rough may lead to an underestimation • Incomplete enumeration of short-term workers may have biased the results of this study if short-term workers may have biased the results of this study if short-term workers not included in the cohorts had experienced a 	<p>of length of employment. However, as it concerns the same cohort as Loomis et al. (2019) etc. it might be assumed that this is still a useful study. Therefore it is included in this summary</p> <ul style="list-style-type: none"> • This study also describes a second cohort of workers in the man-made vitreous fiber industries. That part of the study is not summarised here • The main focus was a comparison between short-term and long-term workers (and between

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>period under consideration</p> <p>Reference population: General population mortality rates from WHO mortality databank</p> <p>Follow-up 1990-1992 (depending on different studies and countries)</p>	<p>Poisson regression of mortality risk on exposure, resulting in relative risks (RRs), with employment > 1 year as reference. Adjustment for age, calendar period, country, time since first employment, and employment status; all variables categorical.</p> <ul style="list-style-type: none"> Analyses separately for each country, and stratified analyses for age at time of employment, calendar period of first employment, birth cohort, years since last employment 		<p>1 year showed increasing risk with shorter duration, with all-cause mortality RR 1.24 (1.09-1.42) for < 1 month, P trend < 0.01.</p> <p>This phenomenon was especially strong in the Nordic countries, and in workers first employed between ages 25 and 34.</p> <p>Analyses by calendar period of first employment and cohort of birth did not show any pattern in either cohort</p>	<p>mortality different from the short-term workers included</p>	<p>the two cohorts, not further mentioned here)</p>
Coggon et al. (2015), (54)	See information above Coggon et al. (1987) (only	See information above Coggon et al. (1987) (only	<p>Whole cohort</p> <ul style="list-style-type: none"> All-cause mortality SMR 0.97 (95% CI 0.93-1.00) 	See information above Coggon et al. (1987) (only	See information above Coggon et al. (1987) (only

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>See above Coggon et al. (1987) (only differences mentioned here)</p> <p>Study population: 7,970 workers (instead of 7949), of whom 3121 had died (2022 since 1990); 6650 men, 1320 women</p> <p>Follow-up: Until end 2012</p> <p>Reference population: For deaths in 2010-2012 population rates for 2005-2012 were applied</p> <p>Censoring: 31 December 2012. In addition (to previous</p>	<p>differences mentioned here)</p> <p>Exposure categories: – high, at an estimated 8 hour time-weighted average 40-100 ppm for ≥ 1 year (3488 workers, of whom 1402 at least one year) – moderate – low – background</p> <p>Statistical analysis Supplementary case-control analysis of lymphohaematopoietic cancer incidence (122 cases) in relation to exposure, lagged by 5 years, with 10 controls per case (1 excluded for lack</p>	<p>differences mentioned here)</p> <p>Health outcomes: Overall mortality and death due to lymphohaematopoietic and other cancer. For a nested case-control study also lymphohaematopoietic cancer incidence</p> <p>Causes of death coded according to ICD revision 9 (death up to end 2000) or revision 10 (thereafter)</p> <p>For the nested case-control study additional information was also obtained from cancer registrations</p>	<ul style="list-style-type: none"> • All cancers SMR 1.01 (0.95-1.08) • Lymphohaematopoietic cancer SMR 0.89 (0.68-1.14) • Lung cancer SMR 1.20 (1.08-1.34) <p>Exposed (above background) (for specific cancers from table 3 only noteworthy results reproduced here)</p> <ul style="list-style-type: none"> • All-cause mortality SMR 0.99 (0.95-1.04) • All cancers SMR 1.05 (0.97-1.13) • Lymphohaematopoietic cancer SMR 0.82 (95% CI 0.58-1.14) • Lung cancer SMR 1.27 (1.11-1.45); • Brain and nervous system cancer SMR 1.55 (1.02-2.28) <p>Highly exposed ≥ 1 year</p> <ul style="list-style-type: none"> • All cancers SMR 1.05 (0.97-1.13) • Lymphohaematopoietic cancer SMR 0.82 (95% CI 0.58-1.14) • Lung cancer SMR 1.44 (1.10-1.86); in highly exposed only SMR • Brain and nervous system 	<p>differences mentioned here)</p> <ul style="list-style-type: none"> • Lack of information on smoking especially relevant given the increased lung cancer mortality – 	<p>differences mentioned here)</p> <ul style="list-style-type: none"> • Missing data: of 8354 participants 383 were excluded because of missing data • Although still a relatively small study, the long follow-up of this study is a strong point, especially with respect to the evaluation of mortality due to specific cancers • Also, according to the authors, the number of subjects with relatively high exposure is a strong point • But no more detail was added to work histories with respect to previous study

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>study) moving to a higher exposure category (in exposure analyses); 914 lost to follow-up</p>	<p>of suitable controls) matched on sex, factory, and age (within 2 years); calculation of odds ratios (ORs) by conditional logistic regression</p> <p>Subgroup analyses: for lung cancer analysis per factory</p> <p>Sensitivity analyses: using different lag times, of 10 respectively 20 years</p>		<p>cancer SMR 2.20 (1.01-4.19) In other exposure categories no significant results</p> <p>Internal comparison, highly exposed versus background exposed</p> <ul style="list-style-type: none"> • Non-Hodgkin lymphoma/chronic lymphocytic leukemia OR 0.54 (0.23-1.27) <p>Subgroup analysis for lung cancer showed excesses of deaths at two factories but not at two others (data not shown)</p> <p>Sensitivity analyses did not lead to different insights except for less clear results for lung, oesophagus and large intestine (only shown in online supplementary tables)</p> <p>Case-control (123 cases):</p> <ul style="list-style-type: none"> • No indication for association of exposure with lymphohaematopoietic cancer; OR for Non-Hodgkin lymphoma/chronic lymphocytic leukaemia in highly exposed workers (≥ 1 year) versus 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			background exposed 0.54 (0.23-1.27)		
<p>Christensen et al. (2018), (52) See information above, Kolstad et al. (1995). • Retrospective cohort study • Denmark</p> <p>Study population: Substantial increase in study size population compared to Kolstad et al. (1995) to 73,036 workers at 456 small- and medium-sized producers of reinforced plastic in Denmark</p> <p>Inclusion criteria Being mentioned in a national pension register</p>	<p>See information above, Kolstad et al. (1994)</p> <p>Job information From Nationwide individual Survey, Population, and Housing Census in 1970 and Statistics Denmark for 1981 and onwards, with jobs categorised (according to International Standard Classification of Occupations versions 1968 and 1988) as white collar, skilled blue collar, unskilled blue collar or other. Occupational changes over time were considered. Information on work processes</p>	<p>Health outcomes: Incidence of lymphohaematopoietic malignancies (LHMs), based on Danish Cancer Registry, coded according to ICD-7 (up to 1977) or ICD-10 (1978-2011), and for myeloid malignancies also ICD-O-3 (1978-2011), and for myelodysplastic syndrome, polycythemia vera, and essential thrombocythemia also the National Patient Register (1977-2011)</p>	<p>Total of 1,581,976 person-years follow-up with 665 LHM incident case of 21 different LHM with ≥ 20 cases (Below only results with significant trends shown)</p> <ul style="list-style-type: none"> • RRs (crude, and adjusted plus 95% CI) per cumulative exposure - 1-17 mg/m³(reference) - 18-70 mg/m³ T-cell lymphoma RR crude 1.3; RR adj 1.1 (0.3-5.2); All lymphoid lymphomas RR crude 1.4, RR adj 0.9 (0.6-1.5); All chronic lymphoid lymphomas RR crude 1.3, RR adj 0.8 (0.5-1.4) - ≥ 71 mg/m³ T-cell lymphoma RR crude 4.3; RR adj 3.2 (0.9-11.8), P_{trend} 0.04; All lymphoid lymphomas RR crude 1.2, RR adj 0.6 (0.4-1.0), P_{trend} 0.04; All chronic lymphoid lymphomas RR crude 1.1, RR adj 0.6 (0.3-1.9), P_{trend} 0.04. <p>Authors also mention Acute Myeloid Leukemia and Hodgkin's</p>	<p>Same as above, Kolstad et al. (1995) In addition: • Many comparisons were made, but no correction for multiple testing • Exposure assessments cannot be considered very precise (rather probabilistic). Berkson type error ('random') not believed to lead to bias, but misclassification here might not be random</p>	<p>Same as above, Kolstad et al. (1995) • More details on exposure measurement than in previous study, but difficult to know which of the details also applied to that earlier study • Correction factor to account for increasing use of respirators since early 1990s based on urine mandelic acid measurements • A large study, comprehensively capturing almost all workers in one country in a relevant branch of industry, with long follow-up • Numbers and follow-up sufficient to detect lymphohaematopoie</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>as worker at one of the companies at any time in the period 1964 and 2007, but not only in 1964 (wash-out)</p> <p>Censoring Left censoring at April 1, 1968, or January 1 of year following year first employment. Right censoring December 31, 2011, or date of death, emigration, or diagnosis</p> <p>Follow-up: From 1968 until end 2011</p>	<p>and production was obtained from employers and from two dealers of plastic raw materials</p> <p>Exposure intensity assessment Based on 1122 personal measurements of work room styrene concentration at least 1 hour sampling time, performed at 133 reinforced plastic companies during 1970-2011. Increasing use of respirators since 1990 accounted for with correction factor of 0.2 for all measurements obtained since 1990. These measurements</p>		<p>lymphoma, with P_{trend} 0.28, respectively 0.15. (Results for AML for cumulative exposure score, highest versus reference, RR adj 1.4 (0.7-2.8))</p> <ul style="list-style-type: none"> Adjusted RRs for alternative exposure metrics and exposure times (only results with significant trend shown) <p>T-cell lymphoma, cumulative exposure score, complete work history</p> <ul style="list-style-type: none"> -1-17 mg/m³-year (reference) -18-70 mg/m³-year RR 1.1 (0.3-5.1) -≥71 mg/m³-year RR 3.2 (0.9-11.8); P_{trend} 0.04 <p>Acute Myeloid Leukemia, cumulative exposure score, 15-29 years since first exposure</p> <ul style="list-style-type: none"> -0 mg/m³-year (reference) -1-45 mg/m³-year RR 1.3 (0.6-3.0) -≥46 mg/m³-year RR 2.4 (1.2-4.6); P_{trend} 0.01 <ul style="list-style-type: none"> Post hoc analysis: 		<p>tic malignancy incidence, but imprecise exposure information reduces power</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>were combined with estimation of exposure probability per job category, to derive mean and cumulative styrene exposure scores (in mg/m³-year).</p> <p>Exposure probability assessment based on a questionnaire survey among a stratified sample of 15,107 workers from different periods, conducted in 2013-2014.</p> <p>Statistical analysis:</p> <ul style="list-style-type: none"> • Exposure intensity Styrene exposure intensity modelled by mixed-effects linear regression on company 		<p>combining exposure time windows 15-29 years and ≥ 30 years resulted in RR adj for T-cell lymphoma of 16.34 (1.74-153.01)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>characteristics (production process, product, and decade) as fixed effects and company as random effect.</p> <ul style="list-style-type: none"> • Exposure probability estimated with mixed effects logistic regression (exposure yes/no) on calendar period (decade), main production process, main product, gender, occupation, company size (fixed effects) with company as random effect • Cumulative exposure scores in mg/m³-year (product of yearly exposure intensity and probability) 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>summed of exposure time), and mean exposure intensity by dividing cumulative exposure by duration of employment</p> <ul style="list-style-type: none"> • Exposure lymphohematopoietic malignancy incidence association: discrete time hazards model relating incidence rate ratios (RRs) to tertiles of halves of styrene exposures (cut-points based on person-year exposure distributions. Duration categorised in 1, 2-4, ≥ 5 years. Only malignancies with at least 20 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>cases (after grouping) were analysed. Various exposure metrics were analysed: mean exposure, mean exposure probability, duration of employment and different time windows: < 15, 15-29, ≥ 30 years cumulative scores (in addition modelled as restricted cubic splines)</p> <ul style="list-style-type: none"> • Adjusted analyses including age, gender, and calendar year (<2000, ≥ 2000) • Tests for linear trends by including variables for different exposure metrics • Sensitivity analyses by 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	ending follow-up in 2008, since exposure information for 2008-2011 is lacking.				
<p>Nissen et al. (2018), (53) See information above, Christensen et al. (2018). In addition:</p> <p>Study population: With 73,092 workers 56 fewer than in Christensen et al. (2018)</p>	<p>See information above, Christensen et al. (2018).</p> <p>Statistical analysis</p> <ul style="list-style-type: none"> • Calculation of incidence rates (IRs) and crude incidence rate ratios (IRRs) with a discrete survival function • Estimation of odds ratios (ORs) for incidence in relation to cumulative styrene exposure, adjusted for age, sex and company. This was done as a nested case-control study with 	<p>Health outcomes: Incidence of sinonasal adenocarcinoma, based on Danish Cancer Registry, as follows:</p> <ul style="list-style-type: none"> - ICD-7 (up to 1977) code 160 - ICD-10 (1978-2011) codes C30 and C31 - ICD-O-3 (1978-2011) codes 8070/3, 8071/3 squamous cell, codes 8140/3, 8440/3, 8260/3 adenocarcinoma, and 8002/3, 8020/3, 8200/3, 8430/3, 8720/3, 9680/3, 9999/3 other histologic subtypes 	<p>A total of 1,585,772 person-years follow-up with identification of 9 cases of adenosarcoma, 15 of squamous cell carcinoma and 13 of other histological type and 370 controls</p> <p>IRs and IRRs</p> <ul style="list-style-type: none"> • Sinonasal adenocarcinoma IR 1 case per 100,000 person-years; IRR 8.00 (1.00-63.97) <p>Odds ratios (case-control)</p> <ul style="list-style-type: none"> • Adenocarcinoma high versus low cumulative exposure score adj OR 5.11 (95% CI 0.58-45.12); per 100 mg/m³ OR 1.08 (0.96-1.21) • ORs for other exposure metrics and other sinonasal cancers were lower and not significant 	See information above, Christensen et al. (2018).	See information above, Christensen et al. (2018).

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	10 controls per case, matched for age, sex, and employment in reinforced plastics company or employment in wood industry. Exposure scores were categorised into two categories (high ≥ 37 mg/m ³ -years versus low), and also continuously (per 100 mg/m ³ -years). Various exposure metrics and time windows were used.				
Loomis et al. (2019), (51) 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	Health outcomes: Mortality from specific cancers. Health assessment ICD 8 and 9 codes of previous study Kogevinas et al. (1994) were regrouped into WHO	Total number of person-years 506,459, of which 407,459 in exposed jobs, and 61,514 of those with exposure duration ≥ 5 years. <i>Exposed versus unexposed workers:</i> • All-cause mortality RR 1.01 (95% CI 0.89-1.14)	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
<p>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</p> <p>Reference population: Unexposed jobs in the cohort</p> <p>Follow-up: Varied per country. Overall:</p>	<p>with mixed tasks or in small plants, and workers who regularly entered areas where styrene was handled) versus unexposed</p> <p>Measurements: -In addition to first study, here mentioned around 18,000 measurements of styrene metabolites mandelic and phenoglyoxylic acid in urine.</p> <p>Exposures before 1965 set equal to Denmark data at 200 ppm and then linearly declining to arithmetic mean of earliest measurement</p>	<p>classification. 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</p>	<ul style="list-style-type: none"> • 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) <p>Other cancers RR round 1</p> <p><i>Most highly exposed workers (laminators) versus unexposed</i></p> <ul style="list-style-type: none"> • 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) <p><i>Exposed workers employed 2- < 5 years or > 5 years versus those employed , <2 years</i></p> <ul style="list-style-type: none"> • Non-Hodgkin's lymphoma (NHL) mortality RR 1.40 (0.51-3.79) • Pancreas cancer mortality RR 2.12 (0.93-4.38) <p>No increase in mortality > 5 years, except for prostate cancer mortality RR 1.35 (0.57 to 3.16) and lung cancer</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>1945-1991. Mean follow-up 12.8 years. Lost to follow-up approximately 3%</p> <p>Left censoring: First data for which complete payroll records were available for those already employed at start follow-up</p>	<p>-Mean exposure estimated at 63.1 ppm (in exposed jobs) and mean cumulative exposure at 158.0 ppm-years using the job exposure matrix.</p> <p>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</p>	<p>205.0 and 205.1) were combined.</p>	<p>Lung cancer:</p> <ul style="list-style-type: none"> - 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) <p>No significant trends with duration for any of the cancers</p> <p><i>Exposure-response</i> (only significant results shown):</p> <ul style="list-style-type: none"> • 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. <p>Sensitivity analysis:</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>continuous), with retainment in model of those that changed RR 'appreciably' (not specified). Various exposure indicators were used: exposed versus unexposed, employment as laminator (highest exposure), exposure duration, cumulative exposure (ppm-years)</p> <p>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.</p>		<p>Exclusion of workers exposed before 1970 resulted in lung cancer mortality RR, cumulative exposure, 1.11 (1.02-120)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>Additional analyses for lung cancer, using penalized splines to model exposure-response</p> <p><i>Sensitivity analyses:</i> exclusion of Denmark (in order to assess potential exposure misclassification and bias due to lack of exposure data for years before 1970)</p>				
<p>Niehoff et al. (2019), (61) Prospective cohort study</p> <ul style="list-style-type: none"> Population-based study (Sister Study, NIH), United States Study period: from recruitment in 2003-2009 until 	<ul style="list-style-type: none"> 29 non-metallic air toxics classified as mammary gland carcinogens in animal studies, (including styrene); exposure was assessed as a complex mixture of these 29 toxicants Exposure measurement; Air 	<ul style="list-style-type: none"> Breast cancer incidence. Also, tumor characteristics were taken into account: stage, histology and estrogen receptor (ER) status. By computer-assisted telephone interview information was obtained on demographics, lifestyle factors, medical and family history, and residential history 	<ul style="list-style-type: none"> 2,975 women newly diagnosed with breast cancer For styrene no positive association found with all breast cancer for all other styrene exposure groups versus exposure in 0-0.01 µg/m³. Same for ER+ invasive breast cancer. Associated with increased risk in combination with four other toxics (propylene dichloride, ethylene dibromide, ethylidene dichloride). 	<p>Misclassification from NATA (Concentrations at the census tract level do not fully account for variation in an individual's daily activities/sources, such as cigarette smoke, occupation, indoor air, and drinking water)</p>	<p>Study is not about occupational exposure</p> <p>The authors were particularly interested in the modifying effects of BMI and physical activity. The first because of the known increased breast cancer risk of obesity in</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>end of followed up in September 15th, 2016</p> <ul style="list-style-type: none"> • 49,718 Women who took part in the Sister Study • Inclusion: having a sister with breast cancer and self not having breast cancer at study entry <p>Follow-up: mean 8.4 years</p>	<p>concentrations measured and modelled in 2005 as part of a (US) National Air Toxics Assessment (NATA); this results in estimates of ambient concentrations of each toxicant for each census tract (administrative district); note that 94% of women enrolled in 2005 or later;</p> <ul style="list-style-type: none"> • Measurements were linked to residential addresses. Based on quintiles 5 exposure groups were formed: 0-0.01 µg/m³; 0.01-0.03 µg/m³; 0.03-0.04 µg/m³; 0.04-0.07 µg/m³; and >0.07 µg/m³; • Statistical analysis: Cox 	<ul style="list-style-type: none"> • Changes in health and risk factors were obtained by annual health updates and every three years follow-up questionnaires were administered • Response rates > 91% over follow-up 			<p>postmenopausal women, the second because physical activity could lead to greater exposure as a result of higher respiratory rate and depth. As this study considered exposure to toxicants in environmental air, it is about mixtures of toxicants</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>regression of breast cancer risk (overall and invasive ER+ cancer) on exposure, expressed as adjusted hazard ratios (HRs) plus 95% CIs for single toxicant analysis;</p> <ul style="list-style-type: none"> • Classification and regression trees to identify relevant combinations of toxicants <p>Covariates included in the analysis: BMI and physical activity as potential modifiers. Other covariates identified by directed acyclic graphs analysis were age, race, education, cigarette smoking, marital status, menopausal</p>				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	status, parity, hormone replacement therapy use, residence type, education levels. Also stratified analysis for pre-/postmenopausal status. Effect modification by BMI and physical activity assessed both on additive and multiplicative scales				
General information cohort study in Collins et al. (2013), (62) Wong et al. (1994), (63) Wong et al. (1990), (64) • Retrospective cohort study • US wide study • 15,908 workers (number for first study, Wong et	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	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	See individual studies	<ul style="list-style-type: none"> • 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, 	<ul style="list-style-type: none"> • 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

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>al. (1990)) at 30 reinforced plastics manufacturing plants, selected based on study feasibility; 24.4% women • Period of exposure 1948-1977</p> <p>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</p> <p>Reference population: General white US population (information on race missing for most of cohort, therefore</p>	<p>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</p> <p>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</p>	<p>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)</p>		<p>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</p>	<p>performed. • Entire cohort was assumed to be white (only 1.3% non-white)</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>assumed to be white)</p> <p>Follow-up: Latest 2008 (Collins et al.)</p> <p>Censoring: Left: 1 January 1948 Right: those lost to follow-up were censored at last date of contact (mostly end of employment)</p>	<p>ppm. In addition to the consultancy firm there was routine exposure monitoring</p> <p>Measures of exposure:</p> <ul style="list-style-type: none"> • Cumulative exposure grouped into tertiles: 5- <500 ppm; ≥500-<5,000 ppm; ≥5,000 ppm • Duration of exposure • Peak exposures • Mean exposure • Time since first exposure (employment) <p>Statistical analyses:</p> <ul style="list-style-type: none"> • Calculation of (age, sex and calendar year) standardised mortality ratios (SMR) (as 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	percentages) • Cause-specific deaths standardised for age, race, and five-year periods (1948-1977) • Mortality in relation to exposure				
Wong et al. (1990), (64) See general information above. Study also included a nested case-control study Follow-up: End of follow-up not explicitly mentioned, but seems to be end of 1977	See general information above Statistical analyses: Calculation of Standardised mortality ratios (SMR), In addition to above: Internal comparisons • Mortality in relation to exposure • Calculation of standardised mortality ratios (SMR) (as percentages), also separately per sex	See also general information above Causes of death coded according to ICD revision 7	Total person-years 122,078. 499 deaths were identified, with causes of death obtained for 452 (90.6%) of those (dates for all) Whole cohort (95% CI not reported), not all causes reproduced here • All cause mortality SMR 100.0 • Cancer overall SMR 88.1 ($P > 0.05$) • Lymphatic and haematopoietic cancers SMR 73.3 ($P > 0.05$) • Respiratory system cancer SMR 116.1 ($P > 0.05$) • Non-malignant respiratory disease SMR 51.8 ($P < 0.05$ and > 0.01 , but exact value	See also general information above • Vital status unknown for 16.1% (24.1% in women and 13.6% in men). Might have led to bias in exposure-risk estimation • No information on smoking or other toxic risk factors (apart from the nested case-control study where information on smoking and other chemicals such as resins and	See also general information above • Regarding distinction hot versus cold production processes, the authors state that hot processes are associated with lower styrene exposure • The nested case-control study is described here and not in the table for case-control studies • Average follow-

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<ul style="list-style-type: none"> • Cause-specific deaths standardised for age, race, and five-year periods (1948-1977) <p>Subgroup analyses:</p> <ul style="list-style-type: none"> • Sex • Duration of employment categories • Latency categories • High versus low exposure <p>Nested case-control study 40 cases of respiratory cancer deaths, with maximum 3 controls per case, matched on plant, age at death (five years interval), sex, and race. Additional information was collected for cases and controls on</p>		<p>not reported); specifically, pneumonia SMR 23.8 ($P < 0.05$)</p> <ul style="list-style-type: none"> • Liver cirrhosis SMR 52.5 ($P < 0.05$) <p>Employment > 5 years (shorter periods not reproduced here, except: for workers employed 6 months to 1 year all-cause mortality SMR 128.6 ($P < 0.05$))</p> <ul style="list-style-type: none"> • All-cause mortality SMR 74.9 ($P < 0.01$) • Cancer overall SMR 56.6 ($P < 0.01$) • Diseases of circulatory system SMR 69.2 ($P < 0.01$) • Non-malignant respiratory disease SMR 23.0 ($P < 0.05$) <p>According to latency:</p> <ul style="list-style-type: none"> • Only significant result Non-malignant respiratory disease at latency < 10 years SMR 35.9 ($P < 0.05$) • SMRs for lung cancer increased with latency, but none was significant (but linear trend not tested). • A separate analysis for lung 	acetone was available)	<p>up was 7.7 years. Thus weak power for detection of cancers associated with exposure</p> <ul style="list-style-type: none"> • Entire cohort was assumed to be white (only 1.3% non-white) • No assessment of HWSBS, but authors state healthy worker effect not present in cohort

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>work circumstances and potential other chemical exposures from employment outside of the plastics industry and smoking history. ORs (interpreted as RRs) computed for each of the factors included for analysis.</p>		<p>cancer amongst those employed > 20 years latency, for men employed 2-5 years SMR 716.5 ($P < 0.05$), and for women (overall) SMR 1382 ($P < 0.01$)</p> <p>High exposure versus low exposure (based on type of department): No significant results, but lower SMRs for non-malignant and digestive system disease in low exposure group</p> <p>According to mean time-weighted average exposure (TWA), high versus low among ever-exposed, cut-off 12 ppm, 6545 versus 8694 workers: <ul style="list-style-type: none"> • Larynx cancer 941.1 ($P < 0.01$) • Diseases of circulatory system SMR 63.1 ($P < 0.01$) Results per maximum TWA similar but less significant</p> <p>According to the production process, hot versus cold: In hot process, SMR respiratory system cancer 177.9 ($P < 0.05$); non-malignant respiratory disease SMR 30.1 ($P < 0.05$)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			<p>In cold process, SMR circulatory system diseases 74.3 ($P < 0.05$)</p> <p>Case-control study: respiratory cancer found no significant relations between various types of occupational exposures, but only with smoking</p>		
<p>Wong et al. (1994), (63) See general information above</p> <ul style="list-style-type: none"> • Number of cohort members reduced to 15,826 after removal of duplicates and revision of work histories <p>Follow-up: End of 1989</p>	<p>See general information above</p> <p>Exposure: Based on survey by consultancy firm, jobs were categorised into 6 exposure classes For each workers, cumulative exposures in ppm-years were calculated.</p> <p>Statistical analysis: In addition, for internal comparisons for specific causes of death Cox</p>	<p>See general information above</p> <p>Deaths were in addition identified from Social Security data, the National Center for Health Statistics and an commercial bureau</p> <p>Causes of death coded according ICD version in effect at time of death.</p>	<p>Total person-years 307,932. 1628 (10.3 %) deaths identified</p> <p>Whole cohort (only significant results reproduced):</p> <ul style="list-style-type: none"> • All-cause mortality SMR 107.9 (95% CI 102.7-133.2, $P < 0.01$) • All cancers mortality SMR 115.5 (104.8-127.1, $P < 0.01$) • Oesophagus cancer SMR 191.7 (104.8-321.7, $P < 0.05$) • Cancer of bronchus, trachea or lung SMR 140.6 (119.8-162.0, $P < 0.01$) • Cancer of cervix uteri SMR 283.5 (135.9-521.3, $P < 0.01$) • Cancer from other female genital organs SMR 201.6 (107.4-344.8, $P < 0.05$) • Hypertension with heart disease SMR 185.9 (110.2-293.8, $P < 0.05$) 	<p>See general information above</p> <ul style="list-style-type: none"> • Misclassification: exposure of 0 ppm was assigned to unknown jobs, although they were presumably exposed 	<p>See general information above</p> <ul style="list-style-type: none"> • Cases with missing vital status reduced to 3.5% • Entire cohort was assumed to be white (only 1.3% non-white)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>proportional hazards analysis, adjusted for age and sex. Mortality related to duration of exposure and cumulative exposure (as fixed variables)</p> <p>Also subgroup analyses per exposure category</p>		<ul style="list-style-type: none"> • Other non-malignant respiratory disease SMR 140.6 (104.7-184.8, $P < 0.05$) • Accidents SMR 123.9 (102.4-148.7, $P < 0.05$) <p>Analysis according to latency period (only latency ≥ 20 years and significant shown, no CI reported):</p> <ul style="list-style-type: none"> • All-cause mortality SMR 115.4 ($P < 0.01$) • All cancers mortality SMR 124.1 ($P < 0.01$) • Cancer of bronchus, trachea or lung SMR 150.5 ($P < 0.01$) <p>Analysis by duration of employment only significant results for shorter employment durations, in particular mortality for all causes, all cancers, cancer of bronchus, trachea, lung, all uterine cancers: all no longer significant at > 5 years duration</p> <p>Similarly for duration of styrene exposure</p> <p>Analysis based on cumulative exposure, in 4 categories with</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			<p>cut-off at 10 ppm-years, 30 and 100 ppm-years, i.e. approximately quartiles. No CI reported. Noteworthy in category 30-99.9 ppm-years:</p> <ul style="list-style-type: none"> - oesophagus cancer SMR 294.8 ($P < 0.05$) - cervix cancer SMR 372.4 ($P < 0.05$). <p>Noteworthy in category ≥ 100 ppm-years:</p> <ul style="list-style-type: none"> -hypertension with heart disease SMR 271.7 ($P < 0.05$) <p>Combining cumulative exposure and latency did not lead to significant results</p> <p>Analysis per exposure category and > 2 years employment showed increased SMR in:</p> <ul style="list-style-type: none"> - exposure category 4 (plant office and support) for cancer of biliary passages and liver SMR 456.4 ($P < 0.05$) (maintenance and preparation) for- bronchus, trachea or lung cancer in SMR 149.4 ($P < 0.05$) -- in exposure category 6 for all external causes of deaths SMR 31.1 ($P < 0.05$) 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			Cox proportional hazards analysis showed the following significant result (apart from age): <ul style="list-style-type: none"> • Cancer of lung: adj β for exposure duration -0.046059 (SD 0.01656), $P=0.0054$ (=inverse relationship) 		
Collins et al. (2013), (62) See general information above <ul style="list-style-type: none"> • 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: <ul style="list-style-type: none"> • 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 	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, 5,026 (32%) deaths identified Whole cohort <ul style="list-style-type: none"> • 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) 	See general information above	See general information above <ul style="list-style-type: none"> • 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

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>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.</p> <ul style="list-style-type: none"> • 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. • Statistical analysis: Cox proportional hazards for cumulative time- 		<ul style="list-style-type: none"> • 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) <p>Restricted to at least 15-year latency similar results (only minor changes in SMRs)</p> <p>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)</p> <p>Analysis per cumulative exposure categories (with cut-offs 150 ppm-months, 400, and 1,200 ppm-months (only P-values shown for significant trends):</p> <ul style="list-style-type: none"> - Lung cancer: P trend = 		<p>(35 ppm)</p> <ul style="list-style-type: none"> • 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
	<p>weighted averages (units of 100 ppm-months), adjusted for sex, year of hire and year of birth, with age as time scale</p> <p>• Exposure-response trend for smoking related cancers</p>		<p>0.003</p> <p>- Kidney cancer: P trend = 0.045</p> <p>- All heart diseases: P trend = 0.028</p> <p>Cox proportional hazards:</p> <p>- Pancreatic cancer HR 1.008 (1.002-1.015), but poor model fit (P=0.196)</p> <p>Kidney cancer HR 1.009 (1.000-1.017)</p> <p>Analysis per peak exposure categories</p> <p>There are no major differences among the risk estimates of the four exposure categories. No trends with peak exposures are seen.</p>		
<p>Bond et al. (1992), (65)</p> <ul style="list-style-type: none"> • Retrospective cohort study • United States (Michigan, Connecticut, Texas, California) • 2904 male 	<p>Jobs</p> <p>Individual job histories obtained from annual department census lists for each production or research unit.</p> <p>Exposure</p>	<p>All cause and cause specific mortality, coded according to ICD revision 8</p> <p>Vital status at 1 January 1987 and causes and dates of death retrieved from the company's central mortality</p>	<p>89,825 person-years observed, with a total of 687 deaths.</p> <ul style="list-style-type: none"> • All-cause mortality SMR 76 (70-82) • All cancer death SMR 81 (95% CI 69-95) • Lung cancer SMR 81 (61-105) 	<ul style="list-style-type: none"> • Assessment of styrene exposure was complex in this study and confounded by exposure to other toxicants • No information on smoking • Confounding by 	<p>This study is an update of a study by Ott et al. (1980)</p> <ul style="list-style-type: none"> • The study is relatively small, so power is not very high • On the other hand, follow-up

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>workers in production of styrene-based products (at The Dow Chemical Company)</p> <p>Inclusion: Males employed in period 1937-1971, potentially exposed for at least a year</p> <p>Follow-up: Through 1986. Average follow-up 30.9 years. Loss to follow-up 0.4%.</p> <p>Reference population:</p> <ul style="list-style-type: none"> • US white male population • Workers in chemical industries unexposed to styrene (from Michigan-based 	<p>Exposure levels were apparently based on measurements, but details on measurements not reported in this study (reference to: Ott et al. A mortality survey of employees engaged in the development or manufacture of styrene-based products. J Occup Med 1980;22:445-60)</p> <p>Exposures were categorised according to 5 primary agent groupings:</p> <ul style="list-style-type: none"> - Vapors 1 (Styrene and ethylbenzene), level A (1-4 ppm 8 hour time-weighted average (TWA) and level B (≥ 5 ppm TWA) 	<p>surveillance data base</p>	<ul style="list-style-type: none"> • Lymphatic and haematopoietic cancers SMR 144 (95-208) <p>Comparison exposed versus unexposed to styrene-based products:</p> <ul style="list-style-type: none"> • All-cause mortality RR 0.88 (95% CI 0.81-0.95) • All cancer mortality RR 0.87 (0.74-1.03) • Lymphatic and haematopoietic cancers RR 139 (0.92-2.08) • Multiple myeloma RR 2.45 (1.07-5.65) • Category of symptoms, senility and ill-defined conditions (ICD8 790-799) SMR 3.80 (1.68-8.56) <p>Analysis per work area showed excess mortality for category of symptoms, senility and ill-defined conditions in styrene monomer and finishing work area (SMR 299, $P < 0.05$, CI not reported). In product research and development mortality of most causes was lower than expected.</p> <p>Analysis per agent grouping showed increased mortality from</p>	<p>region (geography) might be relevant: about 80% of the styrene-based cohort were from Michigan.</p>	<p>is relatively long, so most cancer mortality would have been captured</p> <ul style="list-style-type: none"> • According to the authors the elevated rate in the category symptoms, senility and ill-defined conditions might have been due to a regional phenomenon, as 6 out of 7 deaths were from Texas • The discussion in this paper also contains a review of 7 other studies. • Multiple comparisons were not accounted for • Health workers effect

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Dow employees)</p> <p>Censoring: Left: 1 January 1940 Right: 1 January 1987</p>	<ul style="list-style-type: none"> - Vapors 2 (Benzene, alkylbenzene compounds - Vapors 3 (Styrene, ethylbenzene and acrylonitrile in approximately equal concentrations, level A (1-4 ppm TWA) and level B (≥ 5 ppm TWA) - Extrusion fumes and polymer dust, level A (predominantly dust) and level B (predominantly fume) - Colorants (wide variety pigments and dyes), level A (indirect exposure) and level B (direct exposure) <p>These groupings were then used to define 57 job</p>		<p>lymphatic and haematopoietic cancers (SMR 236 (122-411) among workers in category vapors 1 (styrene and ethylbenzene) level A 1-4 ppmTWA and from arteriosclerotic heart disease in category vapors 1 (styrene and ethylbenzene) level B ≥ 5 ppm (SMR 134 (104-171)).</p> <p>Analysis of mortality from lymphatic and haematopoietic cancers per exposure duration only showed a statistically significant mortality risk for a minimum 15-year latency period (SMR 160 (102-238)). But no trend across categories <15 years, 15-34 years, ≥ 34 years (P-trend=0.36)</p> <p>Analysis of mortality from lymphatic and haematopoietic cancers per mutually exclusive exposure and socioeconomic groupings showed SMR 177 (71-365) among professionals and SMR 263 (120-500) in production</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>categories of similar exposure. Research jobs were analysed separately.</p> <p>Statistical analysis:</p> <ul style="list-style-type: none"> • Calculation of standardised mortality rates (SMRs). • SMR trends across different exposure levels evaluated with chi-square test (1 degree of freedom) • Calculation of relative risks (RR) of exposed versus unexposed workers, adjusted for age, time since study entry, and pay status, by Mantel-Haenszel method <p>Subgroup analyses by major work area, by agent</p>		workers exposed to extrusion fumes and colorants		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	groupings, by duration of exposure (<1 yr, 1-4 yrs, ≥ 5 yrs), and by mutually exclusive exposure and socioeconomic groupings				
Lemen et al. (1990), (66) <ul style="list-style-type: none"> • (an update of) a retrospective cohort study • US, Texas • 1943-1981 (Plant A) and 1950-1982 (Plant B) • Workers at two styrene-butadiene rubber plants: 1,662 out of 3,494 workers at Plant A and 1,094 out of 2,015 at plant B Inclusion:	<p>No information on job histories and job types provided, apart from employment duration and employment start and end</p> <p>Measurements Air samples were taken from all areas (Mean (SD), range): Plant A - Styrene (55 samples) 0.94 (1.23) ppm, 0.03-6.46 ppm - 1,3-Butadiene (41 samples) 1.24 (1.20), 0.11-4.17 - Benzene (3</p>	<p>All cause and cause-specific mortality, coded by a nosologist in accordance with the ICD at the time of death and then recoded to ICD7</p>	<p>Person-years at risk accumulated, 43,341 at plant A (through 1981), and 26,314 at plant B (through 1982), and 19,582 (subgroup analysis plant A)</p> <p>No statistically significant mortality differences were found for plant A and B, in the analysis up to 1976 (no statistics reported for the updated mortality figures at 1982). Mentioned by authors (for endpoint 1976, plant A): SMR for lymphatic and hematopoietic tissue related deaths (ICD7 200-205) 155, with leukemia (ICD7 201) 203.</p> <p>Subgroup analysis (conducted because leukemia cases were</p>	<ul style="list-style-type: none"> • No adjustments other than age and calendar period • Risk of misclassification of exposure great, as only overall exposure at each of the two plants was considered with no specification per job type 	<ul style="list-style-type: none"> • The authors explain that during the study period rubber production methods changed • Besides 1,3-butadiene and styrene, also benzene exposure was studied • Small study lacking statistical power • Healthy worker effect

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>White males with at least 6 months employment</p> <p>Follow-up</p> <ul style="list-style-type: none"> • 1943-1982 (Plant A) and 1950-1981 (Plant B) • Average employment 9.48 years (A) and 10.78 years (B) <p>Reference population: General US population</p>	<p>samples) 0.10 (0.035), 0.08-0.14</p> <p>Plant B</p> <ul style="list-style-type: none"> - Styrene (35 samples) 1.99 (3.00) ppm, 0.05-12.3 ppm - 1,3-Butadiene (47 samples) 13.50 (29.90), 0.34-174.0 - Benzene (0 samples) <p>Statistical analysis</p> <p>Standardised mortality rates (SMRs, in percentages) per 5-year age group, 5-year calendar time period, male sex, and race (white). Person-years at risk were further calculated by employment duration and by time from start of</p>		<p>found to be concentrated among those who worked at plant A in the years 1943-1945) showed a deficit for total mortality, and mortality for all other cancers and accidents. The SMR for malignant neoplasms of the lymphatic and hematopoietic tissues was 212% and SMR 278 for leukemia and aleukemia (both $P < 0.05$, one-sided test)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>employment. Statistical significance testing ($P < 0.05$ two-sided) based on Poisson distribution.</p> <ul style="list-style-type: none"> • Post-hoc analysis: subgroup of 600 workers at plant A who had worked there at least 6 months in years 1943-1945 (after which production processes had changed) 				
<p>Frentzel-Beyme et al. (1978), (67)</p> <ul style="list-style-type: none"> • Retrospective cohort study • Germany • 1931-1978 • 1,960 workers in styrene and polystyrene manufacture at BASF Ludwigshafen 	<p>Jobs Information on jobs and job histories restricted to start of exposure, date of and reason for leaving the plant, and the remark that employees carried out various tasks within plants.</p> <p>Measurements Information on measurements</p>	Mortality and cause-specific mortality	<p>20,138 person-years for styrene exposure, total of 74 deaths (12 cancers)</p> <p>In the article results presented also per 10-year age groups. Reporting is mainly descriptive, mentioning numbers of (cause-specific) deaths and expected deaths. No increase of death due to cancer or other causes. No increase in mortality with exposure time</p>	<ul style="list-style-type: none"> • No information on confounding • Exposure assessment performed but not reported on 	<ul style="list-style-type: none"> • No information provided on styrene measurements. • Study too small, and subdivision into subgroups resulting in small 'cells', for statistical power • Follow-up of ex-employees was successful in 93% of German workers, but only

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Inclusion criteria All workers at least 1 month engaged in manufacture of styrene or polystyrene.</p> <p>Reference population</p> <ul style="list-style-type: none"> • General German (Federal Republic) population, and population in Ludwigshafen and Rhinehessia Palatinate • Cohort exposed to vinyl chloride (n=1,681) 	<p>referred to a previous paper presented at the American Chemical Society Meeting (Thiess and Friedheim; 1976)</p> <p>Statistical analysis</p> <ul style="list-style-type: none"> • Observed versus expected deaths. Tested for significance only when more deaths were observed than expected, assuming Poisson distribution. • Proportional mortality ratio (PMR): percentages of deaths attributable to specific causes, and then compared to the reference population • Comparison of 				<p>in 29% of non-German workers. The non-German workers were employed after 1960 and approximately half were employed < 6 months. Proportion of German versus non-German not mentioned</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>relative risks with vinyl chloride cohort (but no statistical analysis)</p> <p>Subgroup analyses:</p> <ul style="list-style-type: none"> • Groups employed before 1960 (higher exposure) versus after 1960. (Due to improvement of equipment and safety procedures through the years) • Further distinction according to exposure duration: < 5 years, 5-10 years, 10-15 years, ≥15 years (altogether 8 combinations) <p>In addition, analysis per 10-year age group</p>				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Nicholson et al. (1978), (68)</p> <ul style="list-style-type: none"> • Retrospective cohort study • United States • 1960-1975 • 560 male workers at plant manufacturing styrene and polystyrene with exposure also to benzene and ethylbenzene <p>Inclusion Being mentioned on a Workers Union's list from 1960 as having been employed at the plant for at least 5 years and having continued to work at least till reaching their 10th anniversary of employment</p> <p>Follow-up</p>	<p>Jobs Classified into high exposure work (styrene production, polystyrene polymerisation, development of special products, maintenance) and low exposure (services and utilities)</p> <p>Measurements air concentrations measured in 1974 as part of a health hazard evaluation by NIOSH and body burden of styrene metabolites measured as part of that evaluation (no details provided) - Styrene air concentrations found to generally range 5-20 ppm in high exposure</p>	<p>All cause and cause-specific mortality, based on certificates of death supplemented with clinical information, radiographs, pathological specimens and autopsy protocols where appropriate</p>	<p>83 deaths No statistical results reported.</p>	<ul style="list-style-type: none"> • No information on confounders • Possibility of misclassification mentioned by authors 	<ul style="list-style-type: none"> • Study of limited value due to the small number of workers included • Healthy worker effect

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Complete (none lost to follow-up)</p> <p>Reference population General US population</p>	<p>areas and < 1 ppm in low exposure areas</p> <p>Statistical analysis Calculation of expected numbers of deaths. Further only descriptive (observed numbers of deaths) Subgroup analyses according to 5-year calendar periods, time since onset of employment, in 10 years intervals, starting at 10 years, and high versus low exposure</p>				

10.3.1 Cohort studies: overview

Boat builders study in Washington State, USA. This concerns a retrospective cohort study that has resulted in several publications involving updates on mortality and highlighting different aspects. 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. Glass-fiber-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.

Okun et al. (1985), (50), followed mortality up until the end of 1978 and found a somewhat lower, but not significantly, all cause standardised mortality ratio (SMR) in the cohort compared to the general population. Also cause-specific SMRs did not differ significantly. A subgroup analysis found an increased SMR in white males only (SMR 1.35, no CI reported, $P=0.05$). No lymphohaematopoietic cancer deaths were observed.

Ruder et al. (2004), (49), extended follow-up through end 1998, finding somewhat increased all-cause SMRs (1.09, 95% CI 1.02-1.17) and all cancer mortality (1.17, 1.09-2.54). In particular, SMRs for oesophageal cancer and prostate cancer were increased (2.30, 1.19-4.02, respectively 1.71, 1.09-2.54). Looking at 2,062 highly exposed workers only, SMR for urinary tract cancer was increased (3.44, 1.26-7.50), with a positive trend with cumulative exposure.

Ruder et al. (2016), (48), updating the follow-up to end 2011, found excess mortality from lung cancer (SMR=1.23, 0.95-1.56) and from ovarian cancer (SMR 3.08, 1.00-7.19). Also mortality due to COPD was increased, in particular among 580 workers with potentially high styrene exposure (SMR=2.02, 1.08-3.46).

Ruder et al. (2017), (47), analysed cancer incidence rather than mortality from cancer. They observed that cancer incidence was reduced compared to the general population (standardised incidence ratio SIR: 0.83 (0.76-0.90), but internal comparison showed that potentially highly exposed had a greater risk of cancer incidence than lowly exposed workers (standardised rate ratio SRR: 1.28 (1.05-1.55).

Bertke et al. (2018), (46), further update mortality figures to end 2016. They found no excess deaths from lymphohematopoietic cancers, but internal analyses indicated that the relative risk increased with duration of employment. Lung cancer mortality was significantly elevated (SMR 1.24, 1.08-1.41), without evidence of a dose-response relationship.

Daniels et al. (2020), (45), did not update mortality any further. However, they extended analyses by making fuller use of available employment information and exposure measurement data. They estimated mean, respectively median, cumulative exposures to have been 31, respectively 5.7 ppm-years. Furthermore, they concluded that there was a monotonic relation between styrene exposure and risk of

leukemia (hazard ratio HR per 50 ppm-years 1.46, 1.04-1.97) and risk of bladder cancer (1.64, 1.14-2.33).

Bertke et al. (2021), (44), is, so far, the last study on this cohort. This study focussed on lung cancer and aimed to assess the Health Worker Survivor Bias (HWSB). This analysis, using a structural nested model, indicated that HWSB was potentially large. They estimated styrene exposure during 1 year at more than 30 ppm accelerated time to lung cancer death by 2.29 years (1.53-2.94).

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

This was 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. It was set up in 1988, after one (of two) cohorts in the UK had already been studied by Coggon et al. (1987). Altogether, the cohort includes over 40,000 workers at one of more than 600 reinforced plastics production plants. These plants had been identified in order to investigate a possible relation between the risk of lymphohaematopoietic cancer and occupational exposure to styrene. Assessments were based on job descriptions and histories. To some extent styrene concentrations were measured as part of industrial monitoring, but estimates depended strongly on expert evaluation.

Coggon et al. (1987), (60) was restricted to one of the two UK cohorts, and included almost 8,000 men and women employed between 1947 and 1984 at one of eight companies. Exposure assessment was based on type of job, job history, and styrene concentration measurements at a subset of the factories. The high exposure category was estimated to range between an 8-hour time-weighted average (TWA) of 40-100 ppm. SMRs for all-cause and all cancers mortality were lower than expected (83%, 77-89%, respectively 80%, 69-93%).

Kogevinas et al. (1993), (59) and **Kogevinas et al. (1994), (57)**, presented the analyses of the whole six countries cohort. They found no significant excess mortality, but mortality rate from lymphohaematopoietic cancers increased with time since first exposure, with a risk increasing to two-fold (SMR 197%, 85-387%) at 20 years after first exposure in workers exposed for at least one year (test for trend, $P < 0.05$). But this was not consistently associated with cumulative exposure.

Kolstad et al. (1994), (58), was a separate publication on the Danish cohort, and analysed the incidence of lymphohaematopoietic cancers. Although the authors did not find a significant increase in incidence (SIR 1.22, 0.88-1.65), incidence was increased among workers in the early phase (the 1960s) when recorded styrene concentrations were the highest (1.54, 1.04-2.19).

Also Kolstad et al. (1995), (56), was devoted to the Danish cohort only. It considered cancer and several chronic health effects. It found increased mortality ratios for degenerative nervous system disorders in companies where more than half of the workers were engaged in production. In these companies, also an increased incidence rate for cancer of the pancreas was noted (IRR 2.2, 1.1-4.5). Increased occurrence was also found in long term workers, workers of the 1960s (highest exposure, see above), and workers with observations at least 10 years since the start of employment.

Boffetta et al. (1998), (55) included workers from the six countries mentioned, to which also a German cohort was added. This latter cohort consisted of workers in the man-made vitreous fiber industry. The main purpose of this study was to compare short-term workers employed less than one year, with those employed at least one year, as 'proxy' for the effect of exposure. Mortality was higher among short-term workers, but this study was not very informative, as it concerns the same cohort as Loomis et al. (2019).

Coggon et al. (2015), (54), is a follow-up of the UK cohort only, extending the health outcomes data to the end of 2012. It also includes a nested case-control study of 122 cases of lymphohaematopoietic cancer. At this time, over 3,000 out of 7,970 workers had died without significant differences in mortality compared to the general population of England and Wales, but mortality from lung cancer was increased (SMR 1.44, 1.10-1.86).

Christensen et al. (2018), (52), extended the number of workers for the large Danish cohort (now totalling $n=73,036$), with follow-up to the end of 2012, and reported on the incidence of lymphohaematopoietic cancers among the Danish workers. Internal comparison according to levels of styrene exposure, showed that increasing cumulative styrene exposure was associated with a greater risk of acute myeloid leukemia ($P_{\text{trend}}=0.01$). Risk at high exposure during the prior 15-29 years was more than twice that of low exposure ($RR = 2.4, 1.2-4.6$).

Nissen et al. (2018), (53), was a study in parallel with Christensen et al. (2018) mentioned above, but focussing on sinonasal adenocarcinoma. The study identified nine cases of this cancer type, which corresponded to an OR of 5.11 (0.58-45.12) for those with potentially high cumulative exposure to styrene versus those with low cumulative exposure.

Loomis et al. (2019), (51), is the latest study on this six countries cohort, but did not extend the follow-up. Instead, they re-analysed the data (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 ($RR\ 1.16, 1.03-1.31$).

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

Wong et al. (1990), (64), compared mortality with that in the general population, finding no significant differences for overall and cancer-specific mortality for the cohort as a whole. However, mortality from cancer of the respiratory system amongst workers at plants where 'hot' processes were used, was more than twice that for those at plants where 'cold' processes, with higher potential exposures to styrene, were used (SMR 177.9% versus 78.3%, $P<0.05$). The study also included a nested case-control study of 40 cases of death from respiratory cancer which did not confirm this association.

Wong et al. (1994), (63), updated mortality data for this study extending follow-up until the end of 1989 and counting a total number of 1628 deaths. They found significantly higher SMRs for all-cause mortality and several cancers (all-cancer, oesophagus, respiratory, cervix uteri. However, as these deaths predominantly occurred in short-term workers and those with low cumulative exposure to styrene, the authors concluded that a causal relation was not likely.

Collins et al. (2013), (62), provided a further update of this study, 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.

Other studies

Bond et al. (1992), (65), studied mortality among 2904 workers who were employed in the chemical industry (the Dow Chemical Company) manufacturing styrene-based products in the period 1937-1971. This study reported on deaths that occurred up to the end of 1986. Overall, standardised mortality was lower in the chemical workers exposed to styrene than in the general population and in other chemical workers unexposed to styrene. An exception was mortality from multiple myeloma (RR 2.45, 1.07-5.65), but there was no indication of a relation with intensity or duration of exposure.

Lemen et al. (1990), (66), reported on mortality among workers at two plants producing styrene—butadiene rubber in Texas, USA, in the period 1943-1982. The authors found no excess mortality compared to the general US population, with the exception of lymphohaematopoietic cancer (SMR 212%, $P < 0.05$, one-sided test), and specifically leukemia (SMR 278%, $P < 0.05$), in subgroup analysis (one of the two plants)

Frentzel-Beyme et al. (1978), (67), conducted a retrospective cohort study in Germany including 1,960 workers in styrene and polystyrene manufacture at BASF, in the period 1931-1978. They observed no significant differences in overall and cancer mortality in comparison to the general population.

Nicholson et al. (1978), (68) studied 560 male workers at a facility in the US manufacturing styrene and polystyrene in the period 1960-1975, who were also potentially exposed to benzene and ethylbenzene. The 88 identified deaths that occurred during that period were lower than expected from the mortality in the general population.

Table 11 Overview table of case control studies (extensive summaries)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>Cocco et al. (2010), (69)</p> <ul style="list-style-type: none"> • case-control study (the Epilymph study) • Multicentre study, conducted in six countries: Czech Republic, France, Germany, Ireland, Italy and Spain • Period: 1998-2004 • Aim: investigate the relation between environmental exposures and lymphoid neoplasms • Hospital-based (cases) and population based (part of the controls). Cases were 	<ul style="list-style-type: none"> • Exposure to 43 potential toxicants, among which styrene, was assessed by industrial hygienists • Assessment was based on structured interviews including an inventory of all full-time jobs held for at least one years, besides questions on socio-demographics, lifestyle, and health history • Jobs were coded according to standard classification, and on the basis of job histories industrial hygienist determined 	<p>Occurrence of B-cell non-Hodgkin's lymphoma (NHL) and its major subtypes diffuse large B-cell lymphoma (DLBCL), chronic lymphatic leukemia (CLL), follicular lymphoma (FL) and multiple myeloma (MM), as well as Hodgkin's lymphoma (HL), and T-cell lymphoma (TCL). Diagnosis classified according to WHO Classification of Lymphomas (2001); about 20% of pathology slides reviewed by panel of pathologists.</p>	<p>(Only results for styrene reproduced here)</p> <ul style="list-style-type: none"> • Ever exposed OR (95% CI) for B-NHL 1.6 (1.1-2.3). $P_{trend}=0.000096$ (threshold for rejection of null $P=0,000125$) • Ever exposed OR for FL 2.6 (1.3-5.2). $P_{trend}=0.000097$ (threshold for rejection of null $P=0,000125$) 	<ul style="list-style-type: none"> • Classification semi-quantitative, with lack of precision and risk of misclassification • Effect of styrene exposure confounded by exposure to other toxicants.. Also exposure outside work not clear 	<ul style="list-style-type: none"> • Although carefully conducted, this study is of limited use: not based on actual measurements of exposure, and the great number of toxicants considered would blur any signal of styrene.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
all consecutive adult patients first diagnosed with lymphoma during the study period and residing in the referral area of participating centres (n=2348); controls (n=2462) were randomly drawn from the general population (Germany and Italy), matched by sex, 5-year age group, and residence area; in the	<p>exposure based on likelihood of exposure (possible, probable, certain), intensity (low, medium, high) and frequency (percentage of work time)</p> <p>Statistical analysis</p> <ul style="list-style-type: none"> • Unconditional logistic regression to calculate ORs, adjusted for age, sex, education and centre, with unexposed individuals as reference. Trends in ORs for the three exposure metrics (likelihood, intensity, frequency) tested with Wald test for trends. Multiple 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
other countries matched hospital controls were used with diagnoses other than cancer, infectious or immunodeficiency disease (response rates 88% in cases, 81% in hospital controls, and 52% in population controls)	testing taken in to account with Bonferroni correction				
Blanc-Lapierre et al. (2018), (70) <ul style="list-style-type: none"> • Case-control study; • Population-based study; • Canada, Montreal area 	<ul style="list-style-type: none"> • Occupational exposure to styrene, benzene, toluene and xylene (BTX for last three); in addition, there was a category of any monocyclic aromatic 	Health outcome <ul style="list-style-type: none"> • prostate cancer and tumor (Gleason's) grade • (Hospital) histologically confirmed primary prostate cancer (approximately 	<ul style="list-style-type: none"> • 538 of 1929 cases had high-grade prostate cancer • Participation response 56% for controls • Percentage of population per job type with exposure to MAHs, benzene, toluene, xylene and styrene displayed in table 2. For styrene relatively high prevalence of exposure for 	<ul style="list-style-type: none"> • Risk of misclassification: exposure estimated based on self-report and exposure classification of jobs by experts. But risk of recall bias may be small as exposure assessment is based 	<ul style="list-style-type: none"> • Strong point: most cases of prostate cancer within a metropolitan area captured • Exposure assessment was very 'qualitative' and based on self-report and expert

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>(Prostate Cancer & Environmental Study);</p> <ul style="list-style-type: none"> • Study population: 1929 cases ≤75 years diagnosed with prostate cancer in 2005-2009 versus 1989 population controls (frequency matched, ±5 years) randomly selected (from electoral list of French-speaking men); • Related: Gérin et al. (1998) 	<p>hydrocarbon (MAH)</p> <p>Exposure assessment based on expert opinion:</p> <ul style="list-style-type: none"> • Coding of occupations and industries (Canadian classifications) experts assigned exposures to 345 chemical agents, including styrene and BTX, for the jobs held by the participants. Semi-qualitative assignment low (background), medium and high and percentage of time exposed (<5%, 5-30%, 30-90%, 90-100%). Assignments also based on job-exposure profiles derived from 	<p>80% of cases in the region compared to registry data); Gleason's grade extracted from pathology reports</p>	<p>firefighters and auto mechanics, moulding occupations in the rubber and plastic industries.</p> <ul style="list-style-type: none"> • Mean exposure duration for styrene was 22.3 years • Proportion ever exposed to styrene 2.0% (n=78). For B, T, and X percentages were 11.2, 11.8 respectively 9.7, with strong correlation between BTX exposure durations and cumulative exposures. Correlations between individual BTX compounds and styrene ranged from 0.28 to 0.43. <p>Relation prostate cancer exposure</p> <ul style="list-style-type: none"> • Prostate cancer cases versus controls, for exposure ever: styrene adj. OR 1.19 (95% CI 0.74-1.91); BTX adj. OR 1.27 (95% CI 1.05-1.53) <p>Sub-analyses</p> <ul style="list-style-type: none"> • Low-grade tumours styrene adj. OR 1.41 (0.85-2.31); BTX adj. OR 1.33 (95% CI 1.08-1.64) • Low-grade, and exposure 'at substantial level' duration: ≥ 25 years styrene adj. OR 2.44 (95% 	<p>on job history</p> <ul style="list-style-type: none"> • Confounding by exposure to unassessed toxicants possible • Participation response in controls lower than in cases, which might have resulted in selection bias • Matching was 'frequency matching', but not described explicitly on which variables. There were some differences between cases and controls in age and education levels, and in having recently been screened (but no statistics on differences reported). For the latter a sensitivity analysis was performed. 	<p>opinion</p> <ul style="list-style-type: none"> • Collinearity was strong for BTX, but relatively low for styrene with BTX. Yet, the percentage of persons exposed to styrene seems very low, hence not much power to detect signals

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>exposure data of some 20,000 jobs as derived in previous studies</p> <ul style="list-style-type: none"> • Self-reported job history data elicited by trained interviewers, with work history including all jobs held ≥ 1 year and for jobs ≥ 2 years detailed information was asked on workplace characteristics, tasks, products, and equipment used and protective measures. Only exposures occurring > 5 years before date of diagnosis or interview included • In addition, Information on sociodemographic s, medical history, 		<p>CI 1.16 to 5.13).</p> <p>Sensitivity analyses</p> <p>Similar results (data not shown)</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>lifestyle factors and anthropometrics collected by trained interviewers</p> <p>Statistical analysis</p> <ul style="list-style-type: none"> • Logistic regression resulting in odds ratios (ORs) for BTX and for styrene; also, polytomous analyses for low-grade (Gleason < 7 and high-grade (Gleason > 7); various exposure metrics used: yes/no, combinations of durations and levels, and cumulative exposure • Covariates included in the analysis: age, 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>ancestry, first degree family history of prostate cancer, household income, education, BMI, type 2 diabetes, alcohol intake, smoking, occupational physical activity (missing values < 3% for each covariate): Tests for trend were conducted by treating ordinal exposure categories as continuous variables in a logistic model (Wald test)</p> <ul style="list-style-type: none"> • Sensitivity analyses: <ol style="list-style-type: none"> 1) setting alternative weights of 1,4 or 9 to concentration levels in 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	calculating cumulative exposures; 2) Restricted to subjects screened for prostate cancer within two years of index date; 3) applying 10-years or 15-year lagging times; 4) using a minimum Gleason score of 8 in defining high-grade prostate cancer; 5) stratifying ≤ 65 years versus > 65 year at index date				
Gérin et al. (1998), (71) • Study design: case-control, with different categories of controls • Study	• Occupational exposure to styrene, benzene, toluene, and xylene (BTX) estimated based on detailed job histories (including	• Type of health effect(s): diagnosed cancer of following types: oesophagus, stomach, colon, rectum, pancreas, lung (three types), prostate, bladder, kidney, skin	• Exposure to styrene limited to at most 2% of population (cases plus controls); for BTX this ranged from 12.4 to 18.8% • Correlation between exposure to BTX high Styrene exposure, ever versus	• This study was a composite of many sub-studies (per 15 cancer types and exposure to 4 substances). Multiple testing not taken in to account • Exposure estimation	• Due to subdivision into several sub-studies (for different types of cancer) and small proportion of population exposed to styrene, the power of this study was weak

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>setting: population-based; Canada, Montreal area; period 1979-1986</p> <ul style="list-style-type: none"> 3,730 cancer patients (various types of cancer) and 533 controls; controls recruited from general population of men (drawn from electoral lists), stratified for age; in addition, cancer patients (cancers at different sites from among cases) were used as controls, and mixtures of population and cancer controls. In each separate analysis (case series) a control group was formed from 533 population 	<p>detailed information on specific tasks and circumstances) elicited by semi-structured interviews; in addition, structured interviews administered with questions on potential confounders (age, smoking, socioeconomic status)</p> <ul style="list-style-type: none"> Exposure per type of job estimated by team of (blinded) experts and scored into three dimensions: levels of confidence (possible, probable, definite), frequency of exposure during normal workweek (< 5%, 5-30%, > 30%) and levels of concentration (low, 	<p>melanoma, Hodgkin's and non-Hodgkin's lymphoma. These were the cancer types with at least 50 cases.</p> <ul style="list-style-type: none"> from medical record and coded according to ICD revision 9; Lung cancer categorised as oat cell carcinoma, squameous cell carcinoma and adenocarcinoma; primary cancers at two different sites were included in analyses for both sites 	<p>unexposed adj OR (95% CI):</p> <ul style="list-style-type: none"> Oesophagus cancer adj. OR 1.0 (0.3-0.5) Stomach cancer adj. OR 0.3 (0.1-1.5) Colon cancer adj. OR 1.2 (0.6-2.5) Pancreas cancer adj. OR 0.3 (0.0-2.6) Kidney cancer adj. OR 0.3 (0.0-2.0) Melanoma adj. OR 1.0 (0.2-4.4) Non-Hodgkin's lymphoma adj. OR 2.0 (0.8-4.8) Hodgkin's lymphoma adj. OR 2.4 (0.5-11.6) <p>Styrene exposure, low respectively medium/high versus unexposed adj OR (95% CI):</p> <ul style="list-style-type: none"> Rectum cancer, low exposure adj. OR 1.0 (0.3-2.9), medium/high 5.1 (1.4-19.4) Lung cancer, low exposure adj. OR 0.3 (0.1-0.9), medium/high 0.9 (0.2-3.3) Prostate cancer, low, adj. OR 1.0 (0.4-2.9), medium/high adj. OR 5.5 (1.4-21.8) Bladder cancer, low, adj. OR 	<p>is based on expert knowledge and not directly on exposure measurements (although expert knowledge was probably partly based on actual measurements performed in the context of other studies. Thus, (non-differential) misclassification is likely.</p> <ul style="list-style-type: none"> Results on styrene likely to be confounded by simultaneous exposure to BTX Controls consisted of males only (not explained why) 	<ul style="list-style-type: none"> Styrene was the least reliable of the exposure assignments with only 45% of exposed being in the 'certain' category of reliability.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>controls and 533 cancer controls</p> <ul style="list-style-type: none"> • Cases: 82% of identified cases agreed to participate. For controls, the response rate was 71% • Inclusion criteria: men between 35 and 70 years old and resident of metropolitan area of Montreal. • Study related to Blanc-Lapierre et al. (2018). See above 	<p>medium, high, evaluated versus reference occupations).</p> <ul style="list-style-type: none"> • Exposures and job histories were combined into an 'exposure index', with concentrations and frequency levels coded as 1,4 and 9; cumulative exposures calculated from duration, frequency and concentration, summed over each job, per separate substance, and categorised as low, medium, high (cut points 70th and 99th percentiles), and high versus low when numbers were too small. Also, ever versus never exposed was used <p>Statistical analysis</p> <ul style="list-style-type: none"> • Unconditional 		<p>1.0 (0.4-2.4), medium/high adj. OR 0.7 (0.2-2.6)</p> <ul style="list-style-type: none"> • Findings for BTX were noteworthy for associations toluene with oesophagus, colon and rectum; xylene with colon, rectum, lung and prostate; and benzene with colon. • Extra analyses: combined exposure model rectum cancer (including BTX): styrene, low versus unexposed, adj. OR 0.8 (0.3-2.6), medium/high versus unexposed adj. OR 4.4 (1.1-17.3) 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>logistic regression; separate per type of cancer and kind of exposure, each time with the appropriate control group (see description study first column); for cancers with elevated ORs, also combinations of exposures were analysed (single versus multiple exposure)</p> <ul style="list-style-type: none"> • Covariates included in the analyses: age, family income, cumulative smoking index, ethnicity, smoking status, respondent status (proxy or self); for lung cancer 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>and bladder cancer also occupational exposures to number of other toxicants included (arsenic, asbestos, chromium VI, nickel, crystalline silica, beryllium, cadmium, and polycyclic aromatic hydrocarbons for lung cancer and for bladder cancer aromatic hydrocarbons)</p> <ul style="list-style-type: none"> • Cancers with fewer than 50 cases (small intestine, gallbladder, testis, penis, liver, myeloma, sarcoma, pleura, and peritoneum) not analysed as cases, but included into 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	'cancer controls' for other analyses • Diagnoses abstracted				
Seidler et al. (2007), (72) • Study design: case-control study • Study setting: population-based, six regions in Germany; • Study population: 710 cases, aged 18-80 years, with malignant lymphoma recruited prospectively, and an equal number of controls from the general population (drawn from register) matched on gender, region and age (± 1 year of birth); response	• Complete occupational history and lifestyle factors (including smoking, alcohol consumption, and leisure time activities retrieved by trained interviewer via structured personal interviews; Workers in specific jobs (higher exposure risk) were asked supplementary job task-specific questions • Exposure per job (intensity and frequency) to chlorinated hydrocarbons and	Type of health effect(s) • Malignant lymphoma, both Hodgkin's (HL) and non-Hodgkin's (NHL), as prospectively identified by hospital and ambulatory physicians in the study regions • NHLs were subdivided into B cell (B-NHL), T cell (T-NHL), combined B and T, and other; B-NHL further subdivided • Details on medical histories and medication retrieved by trained	• Participation rate in cases was 87.4% versus 44.3% in controls. 55% males • Exposure prevalence to styrene in control group estimated at 23.8% • Diagnoses were HL (n=116), B-NHL (n=554), T-NHL (n=35), combined B and T (n=1) and other (n=5); B-NHL subtypes diffuse large B-cell lymphoma DLBCL (n=158), follicular lymphoma FL (n=92), chronic lymphocytic lymphoma CLL (n=104), multiple myeloma MM (n=76), and marginal zone lymphoma MZL (n=38) Logistic regression On levels of styrene exposure (OR and (95% CI): - 0 ppm*years OR 1.0 (ref.) - $>0 \leq 1.5$ ppm*years adj. OR 0.7 (0.5-1.0) - $>1.5 \leq 67.1$ ppm*years adj. OR 1.2 (0.8-1.7) - >67.1 ppm*years adj. OR 0.6	• Although effects were estimated separately for each toxicant, no attempt was made to correct for co-exposure to multiple toxicants. Only an estimated correlation between exposure with BTX reported (0.25), but not with chlorinated hydrocarbons • Job histories were based on self-report, and exposure estimation on expert opinion. Non-differential misclassification likely. • Non-occupational exposure could be a confounder	• This was a population-based study, with unmeasured styrene exposures outside of work. Moreover, styrene exposure at work was limited. Hence, a comparison per different levels of exposure did not have much power. • The main findings were for chlorinated hydrocarbons, which might have 'overwhelmed' any signal from styrene, although also the ORs for these other hydrocarbons were not very high, except for marginal zone lymphoma • Only results for exposure categories

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>rate for controls was 44.3%</p> <ul style="list-style-type: none"> Exclusion criteria: not familiar with German language 	<p>aromatic hydrocarbons (styrene, benzene toluene and xylene) estimated by a (blinded) trained occupational physician; expert assessment coordinated within IARC</p> <ul style="list-style-type: none"> Categories of exposure: styrene exposure was categorised as low (0.5-5 ppm, set at 2.5 ppm), medium (> 5 to 50 ppm, set at 25 ppm), or high (> 50 ppm, set at 100 ppm); Frequency of exposure defined as percentage of working time, categorised as low (1-5%, set at 3%), medium (> 5 to 30%, set at 	<p>interviewer</p> <ul style="list-style-type: none"> Information on cause of death and or registry (e.g., local or nationwide registry, completeness of registry); Type of medical examinations (e.g., lung function tests, x-rays, questionnaires); standardized method; qualified investigator 	<p>(0.3-1.4) P-trend=0.43</p> <ul style="list-style-type: none"> For other hydrocarbons only significant: high cumulative exposure (>47.3 ppm*years) to chlorinated hydrocarbons (OR 2.1 (1.1-4.3) and (borderline) high exposure to trichloroethylene (>35 ppm*years) OR 2.1 (1.0-4.8) <p>Sub-analyses (OR and (95% CI) Per lymphoma type:</p> <ul style="list-style-type: none"> HL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - >0 ≤1.5 ppm*years adj. OR 0.4 (0.2-0.8) - >1.5 ≤67.1 ppm*years adj. OR 1.5 (0.7-3.1) - >67.1 no data P-trend=0.26 (neg.) B-NHL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - >0 ≤1.5 ppm*years adj. OR 0.8 (0.6-1.2) - >1.5 ≤ 67.1 ppm*years adj. OR 1.2 (0.8-1.7) - >67.1 ppm*years adj. OR 0.8 (0.4-1.8) P-trend=0.18 		<p>with at least 5 probands (cases and control subjects combined) were reported.</p>

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>17,5%), or high (> 30%, set at 65%); also, degree of confidence in exposure estimate scored as possible, probable, or certain.</p> <ul style="list-style-type: none"> • Cumulative exposures expressed in [ppm*years], calculated as product of frequency, intensity and job duration, for each job held, and summed up. For some analyses categorised at 50th and 90th percentiles of exposed controls • Statistical analyses: Conditional logistic regression 		<ul style="list-style-type: none"> • T-NHL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - >0 ≤1.5 ppm*years adj. OR 1.3 (0.5-3.6) - >1.5 ≤67.1 ppm*years adj. OR 1.6 (0.5-4.8) - >67.1 ppm*years no data <p>P-trend=0.41 (neg.)</p> <p>Per B-NHL subtype:</p> <ul style="list-style-type: none"> • DLBCL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - 0-1 ≤5 ppm*years adj. OR 0.8 (0.4-1.5) - 1.5 ≤67.1 ppm*years adj. OR 1.3 (0.7-2.3) - >67.1 ppm*years adj. OR 1.5 (0.5-4.4) <p>P-trend=0.03</p> <ul style="list-style-type: none"> • FL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - <0 ≤1.5 ppm*years adj. OR 1.1 (0.5-2.1) - >1.5 ≤67.1 ppm*years adj. OR 2.2 (1.2-4.0) - >67.1 ppm*years adj. OR 1.6 (0.5-6.0) <p>P-trend=0.20</p> <ul style="list-style-type: none"> • CLL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - <0 ≤1.5 ppm*years adj. OR 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>resulting in odds ratios (ORs) and 95% CIs;</p> <ul style="list-style-type: none"> • Covariates included in the analysis: pack years of smoking and alcohol consumption • Sub-analyses with unconditional logistic regression analysis: per type of lymphoma (n > 30 cases) and per subtype B-NHL, compared with entire control group for power, with covariates age, sex, region, smoking and alcohol 		<p>1.0 (0.5-2.2)</p> <ul style="list-style-type: none"> - >1.5 ≤67.1 ppm*years adj. OR 1.1 (0.5-2.2) - >67.1 ppm*years adj. OR 0.5 (0.2-2.3) <p>P-trend=0.37</p> <ul style="list-style-type: none"> • MM <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - >0 ≤1.5 ppm*years adj. OR 0.8 (0.3-1.9) - >1.5 ≤67.1 ppm*years adj. OR 1.0 (0.5-2.4) - >67.1 ppm*years adj. OR 0.5 (0.1-3.8) <p>P-trend=0.85</p> <ul style="list-style-type: none"> • MZL <ul style="list-style-type: none"> - 0 ppm*years OR 1.0 (ref.) - <0≤1.5 ppm*years adj. OR 1.0 (0.3-3.0) - >1.5≤67.1 ppm*years adj. OR 0.8 (0.2-2.6) - >67.1 ppm*years no data <p>P-trend=0.28</p> <p>For high exposure to chlorinated hydrocarbons significant risks were seen for FL (adj. OR 3.9 (1.3-12.1)) and MZL (adj. OR 7.0 (1.8-26.3))</p> <p>(for medium exposure to toluene and xylene significant risks were</p>		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			seen for FL, adj. OR 2.6 (1.4-5.1), respectively 3.0 (1.6-5.8))		
Scélo et al. (2004), (73) <ul style="list-style-type: none"> Case-control study conducted at 15 centers in seven European countries (Czech Republic, Hungary, Poland, Romania, Russia, Slovakia, UK) Newly diagnosed hospitalised lung cancer patients in the period 1998-2002 were the case, and controls were chosen matched on age and sex, hospital controls in most countries (excluding cancer and smoking-related diseases), population controls at two centres. After	<ul style="list-style-type: none"> Exposure to styrene, vinyl chloride, and acrylonitrile (and several other potential toxicants) were estimated for each job in the job histories by local experts in industrial hygiene, on the basis of detailed occupational questionnaires. Exposures were categorised both according to the level of confidence the evaluators had in their judgment and using cut points for estimated exposures (for styrene 5 and 50 ppm) 	Lung cancer, histologically or cytologically confirmed	<ul style="list-style-type: none"> For styrene, no association between exposure and lung cancer was observed. For example, OR of ever exposed versus never exposed was 0.70 (0.42-1.18) (ever exposure to acrylonitrile was associated with an OR of 2.20 (1.11-4.36) and a positive dose-response relation) 	<ul style="list-style-type: none"> Risk of misclassification of exposure: not based on measurements but expert opinion There was a disbalance in controls, which included both hospital-based ones (some severely ill) and population-based ones. This might have biased the results. A sensitivity analysis was performed separating these two types of controls, leading to similar results, but at the cost of power. 	<ul style="list-style-type: none"> Age and sex distribution between cases and controls were not identical because controls were chosen to also serve in other studies. Regarding exclusion: initially 3,403 cases were eligible, but 27 had already been discharged at the time of the interview, 53 were to ill, 13 had died, and 449 refused to participate. Of 3,670 potential controls, 16 had been discharged, 21 were to ill, two had died, and 511 refused to participate.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
exclusion, 2861 cases and 3118 controls remained.	<ul style="list-style-type: none"> • Also information on smoking collected. • Statistical analysis: various exposure metrics were used, including duration, frequency, intensity, and cumulative exposure. These were categorised into tertiles in unconditional logistic regression to calculate ORs, adjusted for age, sex, center, tobacco use, and exposure to other occupational agents. Also models were explored with a 20-year lag, and sensitivity analyses were conducted on the level of 				

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	confidence of the experts, and stratified analyses by sex and age at diagnosis.				
<p>Matanoski et al. (1997), (74)</p> <ul style="list-style-type: none"> • Study design: (retrospective) nested case-control study • Study setting: industrial-based, 7 US and 1 Canadian synthetic rubber plants; • From a cohort of 12,110 workers, 59 cases of lymphohaematopoietic cancer were identified based on death certificates and included in the study; 1242 controls were selected from 	<ul style="list-style-type: none"> • Exposure to styrene and butadiene; • 3,649 styrene exposure measurements were taken from 7 of the 8 plants and from NIOSH, and 3,952 butadiene measurements. Most measurements were personal monitoring data. • In addition, a rank score was used from a previous study in which industry representatives had scored each job for styrene and butadiene exposure on a scale from 1 to 10. • Estimation of 	<ul style="list-style-type: none"> • Type of health effect: death from lymphohaematopoietic, as attested by death certificate; further verification from hospital records (55/59 cases); based on that, subdivision into subtypes, according to ICD revision 9: all lymphohaematopoietic cancers (LHC) ICD 200-209; lymphomas ICD 200, 202, lymphosarcoma ICD 200; lymphoma ICD 202; Hodgkin's lymphoma ICD 201; myeloma ICD 203; leukemia ICD 204-207. 	<p>Lymphohaematopoietic cancer (LHC)</p> <ul style="list-style-type: none"> • Risk per average time-weighted styrene and butadiene exposures (in backwards regression model), ORs for butadiene not shown; also, other variables retained in final model not reproduced here: OR (95% CI) • All LHC OR per ppm styrene, final model, 2.20 (1.46-3.33) • Lymphomas OR per ppm styrene 2.67 (1.22-5.84) • Lymphosarcoma OR per ppm styrene 3.88 (1.57-9.59) • Lymphoma OR per ppm styrene 2.62 (0.40-17.15) • Myeloma OR per ppm 3.04 (1.33-6.96) • Risk per cumulative exposure • All LHC Beta per ppm styrene, final model, 0.4 (P<0.0001) 	<ul style="list-style-type: none"> • As remarked by authors, employment duration of controls slightly longer than cases, with therefore possibly higher cumulative exposure. This might have been biased towards the null. • No information on potential confounders such as smoking and alcohol and education • Misclassification of exposures not to be excluded 	<ul style="list-style-type: none"> • Disentangling the effects from styrene and butadiene difficult • Use of actual measurements, even though scarce, is a strong point of the study

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
<p>workers at the same plants (representing 1% of plant population), chosen to represent a similar age distribution and to reflect population sizes across plants</p> <ul style="list-style-type: none"> • References of earlier publications; Santos-Burgoa et al. Lymphohematopoietic cancer in styrene-butadiene polymerization workers. Am J Epidemiol 1992;136: 843-854 and Matanoski et al. (1993) (same cases, but fewer controls) 	<p>exposure for jobs without measurements for a particular plant by using z-score transformation and assuming equal relative exposures across jobs</p> <ul style="list-style-type: none"> • Each job was assigned an exposure level; • Job histories obtained from company files • Cumulative exposures calculated in ppm-months, and time-weighted average exposures in ppm, from job durations and exposure levels; • Type of statistical analyses: unconditional logistic regression with ln-transformed 		<ul style="list-style-type: none"> • Myeloma Beta per ppm styrene, final model, 0.023 (P=0.013) • Leukemia Beta per ppm styrene, final model, 0.006 (P=0.001) • Sub-analyses: per lymphoid leukemia with job variable • All leukemia's OR per ppm styrene, final model, 2.64 (1.21-5.30) • Myeloid leukemia OR per ppm styrene, final model, 3.70 (1.33-10.29) 		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	exposure measures; • Covariates included in (stepdown) multivariate models: birth year, year of first employment, age at first employment, race, and duration of employment				
Matanoski et al. (1993), (75) See above, Matanoski et al. (1997) In addition: • Controls were matched on plant worked, age at first employment, year of first employment, and duration worked. 81% of cases had been employed for 10 or more years	Methods of exposure assessment not described in this publication, but reference to Santos-Burgoa et al. (1993). Methods described also in Matanoski et al. (1997). See above. In addition: In statistical analysis, conditional	• Type of health effect: death from lymphohaematopoeitic, as attested by death certificate; further verification from hospital records (55/59 cases); based on that, subdivision into subtypes, according to ICD revision 9: leukemia (ICD 204-207), lymphosarcoma (ICD 200), Hodgkin's	Lymphohaematopoeitic cancers (LHC): in the article only the results for leukemia are shown, those for the other cancer types not being significant Risks high exposure versus low exposure (but see second column), in models including butadiene, styrene and butadiene and styrene in combination): OR (95% CI) • Leukemia OR 2.9 (0.8-10.3) for styrene alone, and 1.1 (0.2-5.0) when combined with butadiene In addition, analyses were	See above Matanoski et al. (1997)	Seem to be almost the same study as Matanoski et al. (1997). See above. Only See above. In addition: • The article also goes into the cohort study within which this case-control study was nested • The authors devote considerable analysis and discussion to potential misclassification of

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	<p>logistic regression was used, instead of unconditional. Odds ratios were calculated per exposure category. The text is not clear as to what this means. Job scores were ordinals from 1 to 10, and exposures were calculated by summing the products of score and the number of months the job was held, for all jobs held by an employee. Next, per cancer type mean scores were calculated for cases and controls. Finally, due to the skewness of the data these means were then log transferred.</p>	<p>lymphoma (ICD 201), other lymphatic cancers (ICD 202, 203, 209), all lymphohaematopoietic cancers (which included one case of polycythemia vera); however, 2 of the 55 retrieved hospital records showed these to have been misclassified (appeared to be pancreatic cancer, respectively a retroperitoneal fibrosarcoma)</p>	<p>performed per work area (irrespective of exposure). These are not reproduced here. Furthermore, re-analyses were performed for butadiene alone, with new controls and other cut points for high versus low exposure. These results are also not reproduced here.</p>		<p>exposure per job</p> <ul style="list-style-type: none"> • The authors mention that in cases the average time between first employment and death was 24 years, drawing attention to the possibly long latency for this cancer (But this could also be due to the long survival after diagnosis) • The authors describe an attempt to verify the ranking of jobs according to exposure by experts with actual measurements. NIOSH data from personal and area monitoring performed in 1986 were available for 3 plants, a total of 3,649 measurements for styrene and 3,952

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	Finally, it seems (this we infer) that exposure was categorised into high and low, with the log mean score as cut-off.				for butadiene (see Matanoski et al. (1997) above). Table 7 in the text shows these results per plant, with overall mean (SD) for styrene 3.53 (14.32) ppm. These appeared to be about one fourth of the values found in earlier reports. Due to the sparseness of the measurement data and the difficulty of applying these values to all jobs, the authors decided to stick with the ranking scores assigned by the hygienists

Table 12 Summary table of case control studies (brief summaries)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Cantor et al. (1995), (76) <ul style="list-style-type: none"> • Case-control study • US, 24 states • 1984-1989 Deaths identified from national mortality records constituted 33,509 cases, to which 117,794 controls from amongst non-cancer deaths were matched on age, sex (female) and age (ratio 1 to 4). Separate analyses were performed for blacks and whites	Occupational exposure to several substances including styrene, organic solvents such as formaldehyde, and metals and metal oxides Information on jobs was derived from death certificates (job codes), and combined with exposure estimates, expressed as an exposure probability weight running from 0 to 4, into a job-exposure matrix	Breast cancer mortality as retrieved from death certificates as underlying cause of death(ICD, 9th revision, Code 174).	<p>"Suggestive associations" were found for styrene and other molecules</p> <p>For styrene, results for white women, adjusted for age and socioeconomic status: Exposure probability (actually weight):</p> <ul style="list-style-type: none"> • 0, OR 1 (reference) • 1, OR 1.13 (95% CI 1.0-1.2) • 2, OR 1.18 (1.1-1.3) • 3, OR 1.38 (1.0-1.9) • 4, numbers too small <p>Similarly, results for black women: Exposure probability:</p> <ul style="list-style-type: none"> • 0, OR 1 (reference) • 1, OR 1.49 (95% CI 1.1-2.0) • 2, OR 1.52 (1.1-2.1) • 3, OR 1.32 (0.5-3.3) 4, numbers too small 		This study was 'hypothesis generating (In the words of the authors: "because of the methodologic limitations of this study, its primary value is in suggesting hypotheses for further evaluation"

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Santos-Burgoa et al. (1992), (77) <ul style="list-style-type: none"> •Nested case-control study •US and Canada •1943-1982 •Cases were 59 male workers at 1 of 8 styrene-butadiene rubber synthesis plants who died from a lymphohaematopoietic cancer, to which 193 controls were matched on plant, age, year of first employment, work duration and survival to the time of death of the case <p>Related to the cohort studies by Matanoski et al. (see table cohort studies)</p>	<p>Each jobs was assigned an estimated exposure rank for butadiene and styrene. Cumulative exposure ranks calculated from job history and exposure rank. Exposure was further dichotomised by a log rank score compared to the mean log scores for the total population, within subtype of cancer</p> <p>Statistical analysis by matched pair analysis, and conditional logistic regression for the combination of styrene and butadiene</p>	<p>Death by lymphohaematopoietic cancer</p> <p>Cases were workers who had died with lymphohaematopoietic cancer (ICD-8 codes 200-209) as either the underlying, contributory or other cause of death identified on the death certificate.</p>	<ul style="list-style-type: none"> • Association styrene-leukemia OR 3.13 (95% CI 0.84-11.2) • The association butadiene-leukemia was much stronger: 9.36 (2.05-22.9) <p>Logistic regression of leukemia on butadiene and styrene combined:</p> <ul style="list-style-type: none"> • Styrene OR 1.06 (0.23-4.95) <p>Butadiene OR 7.39 (1.32-41.3)</p>		<ul style="list-style-type: none"> • For this study, no use was made of measured quantitative styrene levels No information on life style factors.

10.3.2 *Case control studies: overview (extensive summaries only)*

Cocco et al. (2010), (69), studied the relation between exposure to 43 potential toxicants and the risk of several types of lymphoma's in a case-control design involving multiple centres in six European countries (the Epilymph study). 2348 cases diagnosed in the period 1998-2004 were included and matched with 2462 controls, partly hospital, partly population controls. Occupational exposure was assessed by industrial hygienist on the basis of structured interviews. Multiple statistical tests were performed. For styrene a significant association (also after correction for multiple testing) was found for having ever been exposed with the risk of B-cell non-Hodgkin's lymphoma (OR 1.6, 1.1-2.3), and with the risk of follicular lymphoma (OR 2.6, 1.3-5.2), both with significant positive trends with three metrics of degree of exposure.

Blanc-Lapierre et al. (2018), (70), performed a case-control in the Montreal area of Canada study to investigate the relation between the risk of prostate cancer and exposure to styrene, benzene, toluene or xylene. In the period 2005-2009 1929 cases with a diagnosis of prostate cancer were identified and enrolled, and matched with 1989 population controls. Assessment of exposure was based on evaluation by experts of detailed job histories. For styrene exposure (only 2% of participants were evaluated as having been exposed to styrene) only a significantly increased risk of low-grade prostate cancer was found for exposure 'at substantial level' (based on expert opinion, not specified) during 25 years or more (adj. OR 2.44 (1.16-5.13)).

Gérin et al. (1998), (71), similar to the study above, also conducted a case-control study in the Montreal area, but including 15 types of cancer. Cases consisted of 3,730 cancer patients, which were matched with 533 population controls. For styrene, a possible association was found with rectum cancer (medium/high exposure versus unexposed adjusted OR 5.1 (1.4-19.4), and for prostate-cancer (medium/high versus unexposed adjusted OR 5.5 (1.4-21.8)).

Seidler et al. (2007), (72), studied 710 patients with malignant lymphoma in six regions in Germany, and compared their exposure to solvents (chlorinated and aromatic hydrocarbons), including styrene, to an equal number of population controls matched on age, sex and region. Structured interviews were used to obtain detailed occupational histories, which were evaluated by a trained occupational physician for exposure to the substances of concern. For aromatic hydrocarbons (i.e. including styrene) no significant associations with lymphoma or its subtypes was observed.

Scélo et al. (2004), (73), conducted a case-control study at 15 centres in seven European countries to evaluate the risk of lung cancer due to exposure to styrene and several other potential toxicants. In the period 1998-2002, 2861 cases were enrolled and matched with 3118 controls. Occupational exposure was assessed by industrial hygienist using detailed occupational questionnaires. For styrene, no associations were found.

Matanoski et al. (1997), (74), was a case-control study nested in a cohort of more than 12,000 workers at one of eight plants (7 in the US and 1 in Canada) producing synthetic rubber. In this cohort 59 cases of lymphohaematopoietic cancer were identified based on death certificates and included in the study; 1242 controls were selected from workers at the same plants. Analysis revealed associations of styrene exposure (and butadiene) with several subtypes of cancer. For all

lymphohaematopoietic cancers combined, OR per ppm average time-weighted styrene was 2.20 (1.46-3.33), for lymphoma 2.67 (1.22-5.84), for lymphosarcoma 3.88 (1.57-9.59), and for myeloma 3.04 (1.33-6.96).

Matanoski et al. (1993) (75), was largely the same study as Matanoski et al. (1997). However, fewer results are shown, and risk estimates for styrene were not significant, with wide confidence intervals (OR for leukemia 2.9, 0.8-10.3)

Table 13 Summary table of cross-sectional studies (briefly summarized)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Mohammadyan et al. (2019), (78) <ul style="list-style-type: none"> • Cross-sectional study • Neyshabur city, Iran • 2017-2018 59 workers in the electronics industry (3 factories), in particular compact plastic parts production hall	<ul style="list-style-type: none"> • Occupational exposure to styrene measured in respiratory air via personal sampling pump attached to worker's collar (using NIOSH1501 method) • Information about the person's history, exposure time, and exposure frequency were collected through a questionnaire. Exposure calculations based on 8-hour exposure per day, 6 days a week, and 49 weeks per year • Average styrene levels in compact plastic parts production halls 79.61 mg m^{-3} (range 28–208.33). No statistically significant difference 	No health outcomes were measured, but theoretical risk estimates (lifetime carcinogenic risk) were made based on measured styrene and estimated exposure levels. Lifetime carcinogenic risk more than 10^{-4} is classified as definite risk, 10^{-4} to 10^{-5} is probable risk and between 10^{-5} and 10^{-6} is possible risk. According to EPA risk level for occupational workers is acceptable when lifetime carcinogenic	<ul style="list-style-type: none"> • Average lifetime carcinogenic risk estimated at The average lifetime carcinogenic risk of styrene estimated at 1.4×10^{-3} • Highest lifetime carcinogenic risk in plastic injection device users (1.9×10^{-3}) and then in shift managers (1.6×10^{-3}). 		No actual health outcome measured.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	between factories. 45.8% of subjects encountered exposure above permitted limit of 86 mg/m ³	risk is 10 ⁻³ , for general population 10 ⁻⁶			
Helal et al. (2013), (79) <ul style="list-style-type: none"> • Cross-sectional study • Egypt, El Oboor City • 40 workers in a plastics factory(exposed males, aged 18-33 years) (exposed group) and 50 healthy administrative workers from same factory (unexposed group), matched for age, sex, socioeconomic status and smoking habit 	Occupational history (interview), and measurement of blood styrene levels plus urinary mandelic acid levels Styrene levels in exposed versus unexposed, mean (SD): <ul style="list-style-type: none"> • Blood: 1117 (64.52) µg/L versus 0.24 (0.15) µg/L, P<0.001 • Urinary mandelic acid levels 246 (21.60) µmol/L versus 4.20 (1.21) µmol/L, P<0.001 	Medical history, clinical examination, spirometry (ventilatory effects), urinary β2 microglobulin and creatinine measurements (kidney effects), cytogenetic study	<ul style="list-style-type: none"> • Urinary β2 microglobulin, exposed versus unexposed, mean (SD) 145.9 (11.7) µg/L versus 52.9 (18.4) µg/L, P<0.001 • Blood creatinine, exposed versus unexposed, mean (SD) 1.02 (0.12) mg/dl versus 0.8 (6.1) mg/dl, P<0.05 • All spirometric parameters lower in exposed versus unexposed: as example FEV₁% of the predicted, mean (SD), 76.91 (6.5) versus 87.40 (3.67), P<0.001 • Correlation between duration of styrene exposure and spirometric parameters. Example FEV₁% r=-0.655, P<0.05 • More chromosomal 		This study could be described as a cross-sectional study comparing two groups of approximately equal sizes.

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			aberrations in exposed versus unexposed (see table 3 in article for details)		
Lorimer et al. (1978), (80) <ul style="list-style-type: none"> • Cross-sectional study • US 493 production workers in styrene monomer, polymerisation and extrusion facility 	Exposure categorized in low and high exposure based on exposure durations and levels obtained from job histories and measurements involving air sampling data (from company and National Institute for Occupational Safety and Health), styrene concentrations in blood and fat, and urinary concentrations of mandelic and phenylglyoxylic acids.	<ul style="list-style-type: none"> • Medical interview on a number of symptoms, and investigations including radiographs, spirometry, nerve conduction studies, sputum cytology, lab hematology and chemistry and chromosome analysis 	<ul style="list-style-type: none"> • Statistically significant differences between high and low exposure groups in prevalences of history of acute prenarctic symptoms, history of acute lower respiratory symptoms, peroneal nerve conduction velocities, relative lymphocytosis, and elevated gamma glutamyl transpeptidase (liver injury) 		Limited air sampling available. Clinically significant abnormalities were rare

11 References

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