Public draft | Gezondheidsraad

1 # Styrene

2 Evaluation of the carcinogenicity and mutagenicity

3 Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert
4 Committee on Occupational Safety, a committee of the Health Council of the
5 Netherlands
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The Health Council would like to give you the opportunity to comment on the draft advisory report. The draft has been presented to the Working Conditions Committee of the Social Economic Council of the Netherlands, and to experts of employer's organisations and trade unions. Other interested parties or persons are also invited to comment. The comments will be taken into account when drafting the final version of the advisory report.

Please follow the instructions for review, see www.healthcouncil.nl.

Please note that this is a draft report that will be finalised after comments received during public consultation have been considered. When citing from this report, please indicate that you are citing from a draft version.

Comments may be submitted until **December 2, 2024**
By e-mail: draftOSH@gr.nl

Attn: Ms. L. Souhoka
        Subcommittee on the Classification of Carcinogenic Substances
        The Health Council of The Netherlands

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1 **Contents**

1 Samenvatting

2 Op verzoek van de minister van Sociale Zaken en Werkgelegenheid (SZW) heeft de
3 Gezondheidsraad beoordeeld of beroepsmatige blootstelling aan styreen een
4 mutageen effect heeft en/of kanker kan veroorzaken. Op basis daarvan heeft de
5 commissie een classificatievoorstel opgesteld. Dit advies is tot stand gekomen in de
6 Subcommissie Classificatie van carcinogene stoffen, van de Commissie Gezondheid
7 en beroepsmatige blootstelling (GBBS). Op www.gezondheidsraad.nl staat informatie
8 over de taken van deze vaste commissie van de Gezondheidsraad. De samenstelling
9 van de commissie staat achterin dit advies.
10

11 Over styreen
12 De stof styreen wordt gebruikt om polystyreen en synthetisch rubber te maken. Styreen
13 zit in verpakkingsmateriaal en in isolatiemateriaal voor gebouwen, en in met glasvezel
14 versterkte kunststofproducten zoals boten, containers en windmolenwieken.
15 Beroepsmatige blootstelling aan styreen vindt plaats bij de productie van styreen en
16 van op styreen gebaseerde materialen. Blootstelling vindt voornamelijk plaats via de
17 luchtwegen. In de beoordeling is ook de stof styreen-7,8-oxide meegenomen. Dit is een
18 stof die in het lichaam ontstaat na blootstelling aan styreen.
19

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

29 Evaluatie van de gegevens
30 Naar mogelijke mutagene effecten van styreen in geslachtscellen is slechts één
31 epidemiologisch onderzoek gedaan in de mens. Hieruit bleek dat er onvoldoende
32 bewijs was voor een mogelijk effect. Uit andere epidemiologische onderzoeken is het
33 bewijs voor chromosomale afwijkingen en genomische instabiliteit beperkt. Proefdier-
34 gegevens over de mogelijke mutageniteit van styreen in kiemcellen zijn niet

1 beschikbaar. De onderzoeken in knaagdieren die werden blootgesteld aan styreen of
2 styreen-7,8-oxide toonden óf geen bewijs voor chromosomale afwijkingen aan, óf
3 leverden tegenstrijdige resultaten, waardoor er geen eenduidige conclusie getrokken
4 kon worden. In zoogdiercellen was het effect van styreen wisselend. Wel werd bewijs
5 gevonden voor DNA-schade. Meerdere in vitro onderzoeken in menselijke cellen
6 toonden aan dat styreen en styreen-7,8-oxide mutagene effecten veroorzaakten. De
7 commissie concludeert daarom dat er voldoende bewijs is om styreen te classificeren
8 als stof die ervan verdacht wordt mutageen te zijn in geslachtscellen.
9
10 Het verband tussen blootstelling aan styreen en de ontwikkeling van kanker bij mensen
11 is onderzocht in meerdere grote epidemiologische onderzoeken. Deze onderzoeken
12 toonden beperkt bewijs voor carcinogene eigenschappen. Onderzoeken in
13 knaagdieren na chronische blootstelling met styreen of styreen-7,8-oxide toonden ook
14 beperkt bewijs voor carcinogeniteit. In muizen werd een toename gevonden in
15 tumoren, maar deze tumoren waren óf goedaardig, óf worden door de commissie als
16 niet-relevant voor de mens geacht. Bij ratten werd geen significante toename in de
17 ontwikkeling van tumoren gevonden na blootstelling aan styreen. Met name het
18 onderzoek bij mensen wijst op mogelijk kankerverwekkende eigenschappen van
19 styreen. Dit onderzoek geeft echter geen uitsluitsel. Daarom adviseert de commissie
20 styreen te classificeren als stof die verondersteld wordt kankerverwekkend te zijn.

21 **Advies aan de staatssecretaris**
22
23 De commissie adviseert om styreen:
24
25 • te classificeren als stof die ervan verdacht wordt mutageen te zijn in
26 geslachtscellen (overeenkomend met een classificatie in categorie 2) en aan te
27 duiden als H341 (verdacht van het veroorzaken van genetische effecten);
28 • te classificeren als stof die verondersteld wordt kankerverwekkend te zijn
29 (overeenkomend met een classificatie in categorie 1B) en aan te duiden als H350
30 (kan mogelijk kanker veroorzaken).
31
32
33
34

Classificatie mutagene en kankerverwekkende stoffen

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

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

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

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

1

2

1 # Executive summary

2 At the request of the Minister of Social Affairs and Employment, the Health Council of
3 the Netherlands assessed whether occupational exposure to styrene may induce
4 mutagenic effects and/or may cause cancer. Based on the assessment, they
5 formulated a recommendation for classification for mutagenicity and carcinogenicity.
6 The assessment was performed by the Subcommittee on Classifying carcinogenic
7 substances of the Dutch Expert Committee on Occupational Safety (DECOS) of the
8 Health Council. More information on the tasks of this committee can be found on the
9 website www.gezondheidsraad.nl. The members of the committee are listed on the last
10 page of the assessment.
11
12 **About styrene**
13 Styrene is used in the production of polystyrene and synthetic rubbers. It can be found
14 in packaging and building insulation, and in fibreglass-reinforced plastic products such
15 as boats, industrial containers and wind turbine blades. Occupational exposure to
16 styrene occurs during the manufacturing of these products, and during the production
17 of styrene-based materials. The primary route of exposure is inhalation. Once
18 absorbed into the body, styrene is extensively metabolized to styrene-7,8-oxide.
19 This metabolite has therefore been included in this evaluation as well.
20
21 **Assessment of mutagenicity and carcinogenicity**
22 Based on the available scientific literature, the committee assesses the potential
23 mutagenetic and carcinogenic properties of the substance in question. If there are
24 indications for such properties, it recommends classifying the substance in two hazard
25 categories, which represent the weight of evidence that the substance is mutagenic in
26 germ cells, and that the substance is carcinogenic. The categories are based on the
27 globally harmonized system criteria for assessing hazard categories, which are also
28 used by the European Commission (EU-guideline (EG) 1272/2008).
29
30 **Evaluation of the data**
31 Only one epidemiological study focused on the effect of styrene on mutagenicity in
32 germ cells. This study did not show sufficient evidence for a significant effect. No
33 animal data was available on mutagenicity of styrene in germ cells. Studies in rodents
34 exposed to styrene or styrene-7,8-oxide, its metabolite, gave either negative or
35 inconclusive results regarding cytogenetic effects. In mammalian cells, effects of

1 exposure to styrene varied. However, evidence was found for DNA damage, and
2 epidemiological studies showed limited evidence for chromosomal aberrations and
3 genome instability. Additionally, in vitro studies in human cells consistently showed that
4 both styrene and its metabolite, styrene-7,8-oxide, caused genotoxic effects. Based on
5 these findings, the committee recommends classifying styrene as a substance
6 suspected to induce heritable mutations in the germ cells of humans.
7
8 Several large epidemiological studies focused on the relationship between human
9 exposure to styrene and the development of cancer. These studies showed limited
10 evidence of carcinogenicity. Animal studies also showed limited evidence of
11 carcinogenicity. In mouse studies, exposure to styrene caused an increased incidence
12 in tumours, but these tumours were either benign or considered not relevant to
13 humans. Studies in rats did not consistently show a statistically significant increase in
14 tumour incidence after exposure to styrene. Overall, although no definite conclusion
15 could be drawn and evidence is limited, particularly the human data do suggest a
16 carcinogenic effect. Therefore, the committee recommends classifying styrene as a
17 substance presumed to be carcinogenic to humans.

18 **Recommendation**

19 The committee recommends classifying styrene:

20 • as a substance suspected to induce heritable mutations in the germ cells of
21   humans (which corresponds with classification in category 2), and to label
22   styrene with H341 (suspected of causing genetic effects).
23
24 • as a substance presumed to be carcinogenic to humans (which corresponds
25   with classification in category 1B), and to label styrene with H350 (may cause
26   cancer).

Classification for mutagenicity and carcinogenicity

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

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

EU hazard categories for carcinogenicity
- Category 1A   Known to be carcinogenic to humans (H350)
- Category 1B   Presumed to be carcinogenic to humans (H350)
- Category 2   Suspected to be carcinogenic to humans (H351)

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

1

2

1 # 1 Scope

2 ## 1.1 Background

3 As a result of the Dutch regulation on registration of carcinogenic compounds that
4 came into force on 11 October 1993, the Minister of Social Affairs and Employment
5 requested the Health Council of the Netherlands to classify compounds for their
6 carcinogenicity. This classification is performed by the Health Council's Subcommittee
7 on the Classification of Carcinogenic Substances of the Dutch Expert committee on
8 Occupational Safety (DECOS). In addition to this classification, the Health Council
9 assesses the mutagenic properties of the substance in question, and proposes a
10 classification on germ cell mutagenicity. The request letter can be found on the website
11 of the Health Council.
12
13 The classification is performed according to European Union Regulation (EC)
14 1272/2008 on classification, labelling and packaging (CLP) of substances and
15 mixtures. The CLP regulation is based on the Globally Harmonised System of
16 Classification and Labelling of Chemicals (GHS). The subcommittee's advice on the
17 classification will be applied by the Ministry of Social Affairs and Employment to extend
18 the existing list of compounds classified as mutagens (category 1A,1B and 2) or
19 carcinogens (category 1A, 1B and 2).

20 ## 1.2 Committee and procedure

21 This document comprises the recommendations for classification of styrene by the
22 Health Council's Subcommittee on the Classification of Carcinogenic Substances,
23 hereafter called the committee. The members of the committee are listed on the last
24 page of this report. The classification is based on the evaluation of published
25 epidemiological and animal studies concerning adverse effects with respect to
26 mutagenicity and carcinogenicity.

27 The criteria for the classification categories are based on the Globally Harmonized
28 System, which has been incorporated into the system and guideline used by the
29 European Union (Regulation (EC) No 1272/2008) for the classification, labelling, and
30 packaging of substances and mixtures (the CLP regulation).
31 In 2023, the Health Council published a Guideline for the classification of carcinogenic

1 substances.[1] This is a guideline for recommendations on classification of mutagenic
2 and carcinogenic substances, and the assessment of the carcinogenic mode of action.
3 The classification systems on mutagenicity and carcinogenicity are based on a weight
4 of evidence assessment, in which more weight is given to evidence obtained from
5 human data than to evidence obtained from animal studies or laboratory data.
6 Furthermore, the weight of evidence depends on the number of reliable studies that
7 show clear associations between exposure and the occurrence of mutagenicity or
8 carcinogenicity. This implies that studies with significant shortcomings contribute to a
9 lesser extent to the overall weight of evidence.
10

Classification for mutagenicity and carcinogenicity

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

EU Hazard statement codes
- H340   May cause genetic effects
- H341   Suspected of causing genetic effects

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

EU Hazard statement codes
- H350   May cause cancer
- H351   Suspected of causing cancer

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11 **1.3 Data**

12 The evaluation and recommendation of the committee are based on scientific data that
13 are publicly available. A literature summary published by the National Institute for
14 Public Health and the Environment (RIVM), which was prepared at the request of the
15 Health Council, was used as a starting point for the evaluation.[2] Another important
16 source of information was the evaluation by the International Agency for Research on

1   Cancer (IARC). The original sources of the studies, which are mentioned in the IARC-

2   monograph, were only evaluated by the committee when these were considered most

3   relevant in assessing the carcinogenicity and mutagenicity of the substance in

4   question. In the case of styrene, such an IARC-monograph is available.[3]

5   Data published after the last IARC evaluation was retrieved from the online databases

6   Medline, Toxline, Chemical Abstracts, and RTECS. The last online search was

7   performed in September 2023. The literature search was based on the following key

8   words: Styrene; CAS No.100-42-5; Styrene-7,8-Oxide; CAS No 96-09-3; toxicity;

9   occupational exposure; adverse health effects; dose-response relationship; hazard

10   assessment; risk assessment; acute toxicity; chronic toxicity; genotoxicity;

11   mutagenicity; carcinogenicity; tumourigenesis; cancer mortality. All data retrieved (i.e.,

12   data from the IARC Monograph and new data) is summarized in tables in the annexes

13   of the present advisory report. Furthermore, available data with styrene-7,8-oxide, the

14   most important metabolite of styrene (see paragraph 2.2), is considered as supporting

15   evidence for mutagenicity and carcinogenicity of styrene.

16   **1.4   Quality assessment**

17   For the assessment of the mutagenic and carcinogenic properties of styrene, the

18   committee retrieved the individual studies summarised in the RIVM document and the

19   IARC Monograph.[2,3] As mentioned above, the committee only evaluated the original

20   sources of the studies when these were considered most relevant in assessing the

21   mutagenicity and carcinogenicity of styrene.

22   For mutagenicity, the committee only evaluated the quality of the original studies with

23   clastogenic and aneugenic outcome measures, as these are considered most

24   important for the assessment of mutagenicity. For the studies with miscellaneous

25   outcome measures, the committee followed the quality assessment of the IARC.

26   For carcinogenicity, the committee evaluated all the selected carcinogenicity studies on

27   their quality. Study quality may vary, and therefore, the committee assessed the quality

28   of the study based on reliability (quality of methodology and reporting), on the

29   relevance for the purpose of the assessment, and on adequacy (usefulness), according

30   to the current views in the scientific community. The quality evaluation was performed

31   to assess the weight of evidence for an association between substance exposure and

32   mutagenicity and/or risk of cancer development. The committee's considerations for

33   determining the quality of a study can be found in the Guideline for the classification of

34   carcinogenic substances.[1]

2 General information

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

Styrene (C_8H_8; CAS number 100-42-5; EC/EINECS number 202-851-5) is a colorless, viscous liquid with a pungent odour. It is one of the most important monomers for polymers and copolymers that are used in a wide range of applications. Styrene polymerizes readily at room temperature in the presence of oxygen and oxidizes on exposure to light and air.

Styrene-7,8-oxide (C_8H_8O; CAS number 96-09-3; EC/EINECS number 202-476-7) is the major metabolite of styrene. It is primarily used to produce epoxy resins. Human exposure during the manufacture of styrene-7,8-oxide, or during the production or use of epoxy resins, is not well understood. Occupational exposure has been documented in the reinforced plastics industry, where styrene 7,8-oxide co-occurs with styrene, at concentrations that are typically 3 orders of magnitude lower than those of styrene.

2.1 Manufacture and uses

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

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

1 plastic products, and in the production of styrene, polystyrene and styrene-based

2 plastics and rubbers. The primary route of exposure is inhalation.

3 In the Netherlands, occupational studies have mostly been performed in the fibre-

4 reinforced plastics industry. Styrene can be a component of the polyester resin used in

5 reinforced plastics. Fibres can be impregnated with polyester resin using a roller (hand

6 laminating) or by spraying. The evaporation of styrene from unsaturated polyester resin

7 into the work environment during processing in the glass fibre-reinforced plastics can

8 result in significant exposures to styrene.

9 **2.2 (Toxico)kinetics**

10 The summary of the (toxico)kinetics of styrene is based on IARC Monograph (2019)

11 and can be found below. For more detailed information, the committee refers to the

12 IARC Monograph (2019).[3]

13 *Absorption*

14 In humans, styrene is absorbed after inhalation (the major route), skin contact, or

15 ingestion, after which styrene is rapidly absorbed into the blood and has been shown to

16 distribute to adipose tissue.

17 *Distribution*

18 Styrene, styrene-7,8-oxide, and styrene glycol have been measured in the blood of

19 exposed humans. In experimental animals, styrene is widely distributed to tissues.

20 *Metabolism*

21 Styrene is extensively metabolized to styrene-7,8-oxide in humans and animals.

22 Hence, external exposures to styrene encompasses internal exposures to both styrene

23 and styrene-7,8-oxide. In both humans and experimental systems, styrene is

24 metabolized mainly by CYP2E1, CYP2F, CYP2A13, and CYP2B to enantiomers of

25 styrene-7,8-oxide, which are further metabolized by epoxide hydrolase to styrene

26 glycol. The rates of metabolism of styrene to styrene-7,8-oxide were higher in

27 microsomes from mouse lung compared with rat lung, and much higher compared with

28 human lung.

29 While some biological similarity was recognized between key events in mice and

30 humans, the mode of action in mice appears to occur less likely in humans due to

1   quantitative differences in the metabolic capacity and qualitative differences in the type

2   of pre-neoplastic and neoplastic lesions that occur. It is noted that the critical role of

3   mouse lung-specific Cyp2 F2 metabolism in mouse lung cancer caused by styrene

4   suggests that this response is not directly comparable to humans, both in terms of

5   quality and quantity.[6,7]

6   *Excretion*

7   Approximately 60% of the excretion products formed from inhaled styrene come from

8   styrene-7,8-oxide, the majority eliminated via urine as mandelic acid and

9   phenylglyoxylic acid.

10   ## 2.3   Monitoring

11   The concentration of styrene measured in air and the concentrations of styrene and its

12   biomarkers in urine and blood are strongly correlated.[3] Measurements of the main

13   metabolites mandelic acid (MA) and phenylglyoxylic acid (PGA) in urine are the most

14   commonly used biological exposure markers of exposure to styrene. Styrene itself can

15   be measured in alveolar air, blood, and urine, and styrene-7,8-oxide and the

16   haemoglobin adducts of styrene-7,8-oxide can be measured in blood.

17   ## 2.4   International classifications

18   ### 2.4.1   European commission

19   The European commission has classified styrene as a flammable liquid and vapour

20   (H226) that is causes skin irritation (H315), causes serious eye irritation (H319), is

21   harmful if inhaled (H332), causes damage to organs (hearing organs) through

22   prolonged or repeated exposure (H372) and is suspected of damaging the unborn child

23   (H361d).

24   Styrene-7,8-oxide is classified as harmful in contact with skin (H312), causes serious

25   eye irritation (H319) and may cause cancer (H350; 1B).

26   ### 2.4.2   IARC

27   IARC has re-evaluated styrene multiple times in 1994, 2002 and 2019 as new data

28   became available over the years. The most recent re-evaluation of styrene has been

1   conducted by IARC in 2019.[3] IARC concluded that there is limited evidence in humans

2   for the carcinogenicity of styrene. However, they found the evidence in experimental

3   animals to be sufficient.

4   Overall, IARC concluded in 2019 that styrene is probably carcinogenic to humans

5   (Group 2A). They considered styrene-7,8-oxide to be a group 2A carcinogen, based on

6   sufficient evidence in experimental animals.[3]

7   It should be noted that although the assessment of human and experimental animal

8   carcinogenicity data by the committee is similar to the IARC procedures, the IARC uses

9   a different classification scheme, with different groups[a].

10 **2.4.3 Other countries**

11 The United States of America has included styrene in the Report on Carcinogens (15th

12 edition) as reasonably anticipated to be a human carcinogen.[8]

13 The state of California considers styrene a substance causing cancer.[9] However,

14 styrene is currently not included in the list of substances NIOSH considers to be

15 potential occupational carcinogens.[10]

16

17 In Germany, styrene is not included as a carcinogenic substance in the national list of

18 CMR substances in the context of worker protection.[11]

19 In Australia, styrene is classified as a flammable liquid and vapour (H226), suspected

20 of damaging the unborn child (H361d), harmful if inhaled (H332), causes damage to

21 the hearing organs through prolonged or repeated exposure (H372), causes skin

22 irritation (H315), causes serious eye irritation (H319), suspected of causing genetic

23 defects (H341), may cause respiratory irritation (H335), may cause drowsiness or

24 dizziness (H336).[12]

25

26 In Japan, styrene is classified as a flammable liquid and vapour (H226), harmful if

27 inhaled (H332), causes skin irritation (H315), causes serious eye irritation (H319),

28 suspected of causing genetic defects (H341), may cause cancer (H350), may damage

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[a] IARC classification for carcinogenic agents (not just chemicals)

Group 1. The agent is carcinogenic to humans

Group 2.

Group 2A. The agent is probably carcinogenic to humans

Group 2B. The agent is possibly carcinogenic to humans

Group 3. The agent is not classifiable as to its carcinogenicity to humans

Group 4. The agent is probably not carcinogenic to humans

1   fertility or the unborn child (H360), causes damage to central nervous system (H370),

2   may cause respiratory irritation (H335), may cause drowsiness or dizziness (H336),

3   causes damage to the hearing organs, central nervous system, peripheral nervous

4   system, auditory organs, visual organs, respiratory organs and liver through prolonged

5   or repeated exposure (H372), may be fatal if swallowed and enters airways (H304).[13]

6

3   Mutagenicity

Mutagenicity studies can have many different types of outcomes. The committee considers the outcome measures for chromosomal aberration, micronuclei, aneuploidy and gene mutation as most important for the assessment of mutagenicity, as these adverse effects are irreversible. For these outcome measures, the committee performed its own quality assessment on the individual studies found. For other outcome measures, the quality assessment of the IARC-monograph was followed.

3.1   Human data

A summary of the information on the mutagenicity of styrene in epidemiological studies is presented below. An overview of the data subtracted from the IARC Monograph considered most important is presented in Table A1 in annex A. No new studies on mutagenicity in humans were published after the IARC-monograph. Two meta-analysis by Collins et al. were published after the IARC-monograph, which also did not report new data.[14,15] As both publications did not contain new information, they were not taken into account by the committee. One study published in Chinese was not considered by the committee as they could not determine its quality.[16]

3.1.1 Clastogenic and aneugenic effects

Chromosomal aberration
A total of 28 studies investigated the association between styrene exposure and chromosomal aberrations. Eleven studies were seen as studies of adequate quality [17-27], six studies had high styrene exposure concentrations [28-33] and eleven studies were of low quality and therefore disregarded.[34-44] Eight studies of adequate quality did not find a statistically significant association [19-25,27], although some of the studies were limited in their sample size.[19,21,24,25] Although the study from Somorovská et al. (1999) was limited in size, it showed a dose-response relationship with the highest frequency of chromosomal aberration, expressed as frequency of aberrant cells, in the highest exposure group (3.75 ± 1.13; P<0.001), fewer chromosomal aberrations in the lower exposure group (3.27 ± 0.70; P<0.004) and still an association in the control exposure group but with fewer chromosomal aberrations (2.50 ± 0.85; P=0.0001).[26] Four studies with high levels of styrene exposures found associations between styrene and chromosomal aberrations.[28-31]

1 *Micronuclei*
2 Thirteen studies investigated the association between styrene exposure and
3 micronucleus induction.[20-22,27,31,32,35,44-49] Nine were seen as studies of adequate
4 quality[20-22,45-50], three of moderate quality [31,32,35] and one study was disregarded
5 because of co-exposure.[44] Four studies of adequate quality found an association.[27,45,47]
6 Migliore et al. (2006) showed a statistically significant effect (p<0.001) in a fairly large
7 study[45], as did Vodička et al. (2004; p=0.002)[27], while the study of Högstedt (1984)
8 also found a statistically significant effect (p<0.005) in a smaller setting[47]. Five studies
9 of adequate quality found no statistically significant association.[20-22,46,48] The study of
10 Yager et al. (1993) looked at the effect of styrene within the same subjects, but found
11 no statistically significant effect.[46]

12 *Aneuploidy and diploidy*
13 Only one study of adequate quality studied frequencies of sperm cells with aneuploidy
14 and diploidy in individuals occupationally exposed to styrene.[51] Cytogenetic analysis
15 conducted on semen samples did not show a statistically significant difference in the
16 incidence of aneuploidy and diploidy between the group of 18 exposed workers and the
17 13 unexposed controls. The only statistically significant finding was an excess of
18 nullisomy in the exposed non-smokers.[51]

19 *Gene mutation*
20 Five studies of adequate quality looked at the effect of styrene on gene mutations.[52-56]
21 None of these studies showed a convincing association.

22 **3.1.2 Miscellaneous**
23
24 Fifteen studies looked at DNA damage in relation to styrene exposure.[26,27,48,49,57-67]
25 About half found an association[26,27,57-64] and the other half did not find a statistically
26 significant association.[26,27,48,49,63,65-67] Twelve studies looked at sister-chromatid
27 exchange with mixed results,[16,20,22,24,29,30,33-35,38,44,46,68] but most of these studies did not
28 find an association.[16,20,22,24,30,33-35,38,68] Eight studies looked at DNA adducts in relation to
29 styrene exposure.[65,69-76] All but one found positive associations.[65] Two studies found an
30 increase in the rate of gaps.[23,24]

1 ## 3.2 Animal data

2 A summary of the information on the mutagenicity of styrene in animal studies is
3 presented below. An overview of the mutagenic data subtracted from the IARC
4 Monograph considered most important can be found in Table A2 in Annex A. Next to
5 the available data subtracted from the IARC, the committee also evaluated four
6 additional recent studies.[77-80]

7 ### 3.2.1 Clastogenic and aneugenic effects

8 *Chromosomal aberration*
9 In mice, styrene exposure did not cause chromosomal abnormalities. One inhalation
10 study found no chromosomal aberrations in the spleen and lung tissue of female
11 B6C3F1 mice, and two oral studies found no chromosomal aberrations in the bone
12 marrow of male and female CD-1 mice.[81-83] Furthermore, negative results were found
13 for chromosomal aberrations in the bone marrow of male C57BL/6 mice after styrene
14 exposure by intraperitoneal injection.[84]
15 When mice were exposed to styrene-7,8-oxide, one study reported chromosomal
16 aberrations in male and female CD-1 mouse bone marrow after oral administration,
17 and similar results were found in male CD-1 mouse bone marrow after intraperitoneal
18 injection.[82,85] In contrast, one study found either negative or inconclusive results for
19 chromosomal aberrations in the bone marrow, fetus and spermatocytes of BALB/c
20 mice exposed to styrene-7,8-oxide by intraperitoneal injection.[86]
21
22 In rats exposed to styrene by inhalation, no chromosomal aberrations were observed in
23 female Fischer 344 rat lymphocytes.[81] Additionally, male Fischer 344 rats and male
24 and female Sprague-Dawley rats showed no increase for chromosomal aberrations
25 after inhalation exposure.[87,88]
26
27 In hamsters exposed to styrene, no increases for cytogenetic changes were reported in
28 the bone marrow of male Chinese hamsters after inhalation exposure.[89]Regarding
29 hamsters exposed to styrene-7,8-oxide, male Chinese hamsters showed negative
30 results for chromosomal aberrations when exposed via inhalation. However, when
31 exposed through intraperitoneal injection, the results were equivocal for both
32 cytogenetic tests.[90]

1 *Micronuclei*

2 In mice exposed to styrene by inhalation, no increases were observed for micronucleus

3 induction in the bone marrow of male NMRI mice, as well as in the spleen and

4 peripheral blood of female B6C3F1 mice.[81,91] An equivocal outcome for micronucleus

5 induction was reported in the bone marrow of male NMRI mice after inhalation

6 exposure, while weak micronucleus induction was observed in the bone marrow of

7 male LACA Swiss mice after intraperitoneal injection, and C57BL/6 mice.[92,93]

8 In rats exposed to styrene, the micronucleus assay showed no increases in

9 micronucleus induction in the bone marrow of female Fischer 344 rats during a 3-week

10 inhalation study, as well as in the peripheral blood reticulocytes of male Fischer 344

11 rats during a 4-week inhalation study.[81,94] When rats were exposed to styrene by

12 intraperitoneal injection, no increases were obtained for micronucleus induction in the

13 bone marrow of male Porton rats.[93]

14

15 In hamsters exposed to styrene and styrene-7,8-oxide by intraperitoneal injection, no

16 increases for micronucleus induction in the bone marrow of male Chinese hamsters

17 were reported.[95]

18 **3.2.2 Miscellaneous**

19

20 *DNA damage*

21 In mice exposed to styrene and styrene-7,8-oxide through various routes, including

22 inhalation and intraperitoneal injection, DNA damage was detected in various organs,

23 including bone marrow, liver, kidney, lung, testis, and brain.[92,96-99]

24

25 In contrast, rats exposed to styrene through inhalation did not show DNA damage in

26 lymphocytes , although an increase in DNA damage was observed in leukocytes on the

27 3rd day of treatment, but not on day 20, in the presence of formamido pyrimidine

28 glycosylase (Fpg).[94,100] Similarly, rats exposed to styrene-7,8-oxide through inhalation

29 showed no increases for DNA damage in leukocytes during a 4-week inhalation study

30 in male Fischer 344 rats.[94]

31

32 *Sister-chromatid exchange*

33 In mice exposed to styrene by inhalation, the sister-chromatid exchange assay showed

34 positive results in bone marrow, liver, and alveolar macrophages of male BDF1 mice,

35 while equivocal results were obtained in the lung, spleen, and lymphocytes of female

B6C3F1 mice.[81,101] In mice exposed to styrene by intraperitoneal injection, the sister-chromatid exchange test in male LACA Swiss mouse splenocyte yielded equivocal results, while the sister-chromatid exchange test in male C57BL/6 mouse bone marrow showed negative results.[84,93]

In mice exposed to styrene-7,8-oxide by intraperitoneal injection, only the S-enantiomer tested in male CD-1 mouse bone marrow yielded positive results for sister-chromatid exchange without including gaps.[85]

In rats exposed to styrene by inhalation, positive results for the sister-chromatid exchange test in female Fischer 344 rat lymphocytes were obtained.[81] Additionally, lymphocytes in male Fisher 344 rats showed negative results after inhalation exposure with stryrene, while for splenocytes of male Porton rats positive results were observed for sister-chromatid exchange after styrene exposure by intraperitoneal injection.[87,93] An inhalation study with male Chinese hamsters exposed to styrene-7,8-oxide resulted in negative results for sister-chromatid exchange assays, whereas intraperitoneal injection yielded equivocal results.[90]

Unscheduled DNA synthesis
In female CD-1 mice exposed to styrene by inhalation, no induction of unscheduled DNA synthesis was observed in the liver.[102]

DNA adducts
In mice, DNA adducts were detected in the lung, liver, spleen and urine through various routes, while in one study no adducts were found in the lungs after styrene exposure by inhalation.[92,103-107] In rats exposed to styrene by inhalation, DNA adducts were detected in the lung and liver, while one study found equivocal results in the liver.[105,106,108]

3.2.3 Recent studies

The committee reviewed four recent additional studies: one peer-reviewed publication and three study reports, which are summarized below. [77-80]

The mutagenicity of styrene was investigated using the transgenic MutaMouse gene mutation assay (OECD TG488).[77] Styrene was orally administered at doses of 0 (corn oil; negative control), 75, 150, and 300 mg/kg/day for 28 days, and mutant frequencies were determined using the lacZ assay in the liver and lung (five male mice/group) (see Table 1). No deaths were observed in mice treated with styrene up to the highest dose.

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

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

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

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

In another transgenic rodent gene mutation study , the mutant frequency was determined in glandular stomach, lung, liver, and duodenum tissues obtained from Big Blue® hemizygous B6C3F1 male mice.[78] These mice were orally administered either vehicle (corn oil; group 1), or styrene at doses of 75, 150, or 300 mg/kg daily for 28 consecutive days (groups 2 to 4, respectively), or a positive control (N-ethyl-N-nitrosourea [ENU] 40 mg/kg; Group 5) on days 1, 2, and 3. Following a further fixation period of 28 days, all animals were necropsied on day 56. There were no test substance-related clinical observations or effects on body weights, body weight gains, food consumption or organ weights. No statistically significant increase was observed in mutant frequency at the cII gene in lung, glandular stomach or duodenum of the mice treated with styrene at doses of 75, 150 or 300 mg/kg/day (see Table 2). A statistically significant increase in mutant frequency was observed in the liver at dose levels of 75 and 300 mg/kg/day, but not at 150 mg/kg/day. This increase did not show a dose-response relationship and the mean mutant frequency values from all styrene treated-groups remained within the 95% control limits of the historical vehicle control data. However, it is not clear whether the historical control data were sufficiently robust to provide a reliable distribution of negative control data. Although the OECD TG 488

1   criteria for a clear positive result were not met, the committee noted that the criteria
2   were also not met for a clear negative result, and therefore no firm conclusion can be
3   drawn on mutagenity based on these increases in mutant frequency. Furthermore, One
4   animal in group 1 was identified as a jackpot mutation animal yielding a high
5   background mutant frequency in the lung, and was also considered an outlier in the
6   liver as the mutant frequency fell outside of the upper 99% historical liver control limit.
7   This animal was therefore excluded from group 1 mean calculations, and replaced by a
8   different animal. Additionally, two more animals across each treatment group for the
9   liver were examined to address an outlier result within the group and to help determine
10   the biological relevance of the result.

11
12   *Table 2 Mean Mutant Frequency ± SD (× 10-6) in Big Blue® C57BL/6 mice glandular stomach, lung, liver*
13   *and duodenum after styrene exposure for 28 days*

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

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

18   In an oral 29-day study, 40 male B6C3F1 mice (8 per group) were administered one of
19   3 dose levels of styrene (75, 150, or 300 mg/kg/day) or the vehicle control (corn oil)
20   daily for 29 consecutive days.[79] The animals in the positive control group received ethyl
21   nitrosourea (ENU) daily during the initial 3 days (days 1-3), followed by ethyl
22   methanesulfonate (EMS) for the final 3 days (days 27-29). On day 29, collected blood
23   was used to assess Pig-a mutant frequency and micronucleus frequency. Samples
24   from the duodenum, glandular stomach, kidneys, liver and lungs were collected for
25   evaluation of DNA damage using the comet assay. No adverse clinical observations
26   were associated with exposure to styrene. Additionally, there were no changes in body
27   weight or body weight gain attributed to styrene exposure. Doses of styrene at 75, 150
28   or 300 mg/kg/day did not induce increases in mutagenesis, clastogenesis, or DNA
29   damage in B6C3F1 mice liver, lung, stomach and kidney, as assessed by the
30   mammalian erythrocyte Pig-a gene mutation assay, the mammalian erythrocyte

1 micronucleus test and the in vivo mammalian alkaline comet assay, respectively. The
2 committee noted that the acceptance criteria were met for the Pig-a mutant frequency
3 and micronucleus frequency assays. However, for the comet assay, only the stomach
4 samples fell within the historical negative control range of the test facility for this strain.
5 The percent tail DNA values for the liver, kidney, and lung background were consistent
6 with those found in the literature and the frozen tissue of the laboratory. The duodenum
7 comet assay did not meet the acceptance criteria (i.e. the percentage tail DNA values
8 measured for the vehicle and styrene groups fell outside of the laboratory's historical
9 data and the positive control did not induce a statistically significant response) and thus
10 the assay in duodenum was not considered valid. See Table 3, 4 and 5 for an overview
11 of the results.
12
13

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

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

16 Abbreviations: N = number of animals; EMS = ethyl methanesulfonate; ENU = N-ethyl-N-nitrosourea,
17 PCE = polychromatic erythrocytes; RBC = red blood cells
18 Values are group mean ± standard deviation
19 a Samples from the first 6 surviving animals in each group were assayed
20 * Statistically significant at $p < 0.05$ (t-test, 1-sided)
21
22 *Table 4 Summary Micronucleus (MN) Assay Results in Male B6C3F1 PCE and RBC after styrene*
23 *exposure for 29 days*

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

24 Abbreviations: N = number of animals; EMS = ethyl methanesulfonate;
25 ENU = *N*-ethyl-*N*-nitrosourea; MN-PCE = micronucleated PCE;

1 PCE = polychromatic erythrocytes Values are group mean ± standard deviation
2 a Samples from the first 6 surviving animals in each group were assayed
3 * Statistically significant at $p < 0.05$ (t-test, 1-sided)
4
5 *Table 5 Summary Comet Assay Results in Male B6C3F1 duodenum, kidney, liver, lung and stomach after*
6 *styrene exposure for 29 days*

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

7 Abbreviations: N = number of animals; EMS = ethyl methanesulfonate; ENU = N-ethyl-N-nitrosourea Values are group
8 mean ± standard deviation
9 a Samples from the first 6 surviving animals in each group were assayed
10 * Statistically significant at $p < 0.05$ (t-test, 2-sided)

11 In another recent study, potential DNA damage was measured in glandular stomach,
12 duodenum, lung, liver, and kidney cells of 40 male Fischer 344 rats (8 per group)
13 exposed for up to 28 days by oral gavage to styrene at dose levels of 100, 250, 500
14 mg/kg/day, or the vehicle control (corn oil), using the Comet assay (OECD TG 489).[80]
15 Administration of styrene at doses up to 500 mg/kg/day did not result in any effect on
16 mortality, physical examinations, observations before and after dosing, body weight, or
17 food consumption. No significant increases in the % Tail DNA compared to the
18 respective vehicle controls were found in the styrene treated animal multi-well slides,
19 except for the glandular stomach. However, these increases were within the historical
20 control range. Overall, no significant increases in DNA damage were observed in
21 duodenum, lung, liver, and kidney in male rats after administration up to 500
22 mg/kg/day. The committee noted that the percentage Tail DNA in the vehicle control
23 group was above the historical vehicle control distribution for the duodenum and
24 kidney. See Table 6 for the complete results.
25
26

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

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

3 a Ethyl methanesulfonate (EMS), positive control for Comet assay, administered orally once daily for two consecutive
4 days (Study Days 27 and 28). The second dose was administered 20 ± 0.5 hrs after the first dose.
5 S.D. = Standard Deviation
6 *$p \leq 0.05$ (Student's t-test); Statistically significant increase relative to the vehicle control
7 §$p \leq 0.05$ (Kruskal-Wallis, Dunnett's test); Statistically significant increase relative to the vehicle Control
8 @ $p \leq 0.01$ (Jonckheere's test): Statistically significant decreasing trend relative to the vehicle control, which is not
9 considered to be biologically relevant.

10 ## 3.3   In vitro data

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

14 *Human cells*
15 Various studies examining cytogenic effects of styrene in human whole-blood
16 lymphocytes showed positive results for chromosomal aberrations and micronuclei,
17 and sister-chromatid exchange, without exogenous metabolic activation systems.[109-118]
18 Additionally, the comet assay detected DNA damage caused by styrene in isolated
19 human leukocytes treated in vitro and in human skin treated in vitro in the absence of
20 metabolic activations.[119,120]
21 For styrene-7,8-oxide, results were consistently positive for similar endpoints.

22 *Mammalian cells*
23 Two studies examining the induction of chromosomal aberrations by styrene in
24 Chinese hamster lung cells yielded negative results in the absence of exogenous
25 metabolic activation, while showing weakly positive results with activation.[121,122]
26 Styrene caused mutations at the Hprt locus in Chinese hamster lung V79 cells when an

1 exogenous metabolic activation system was present .[123,124]

2 In Chinese hamster ovary cells, sister-chromatid exchanges were induced when an

3 exogenous metabolic activation system was present.[125] In contrast, sister-chromatid

4 exchanges were induced in rat lymphocytes in the absence of exogenous metabolic

5 activation.[126]

6 Furthermore, a study in isolated male mouse hepatocytes showed increased DNA

7 damage after styrene exposure, and styrene was shown to induce DNA strand breaks

8 in rat primary hepatocytes.[127,128]

9 Styrene-7,8-oxide induced genetic alterations in various cells, including sister-

10 chromatid exchanges, micronuclei formation, and Hprt locus mutations in Chinese

11 hamster lung V79 cells, without exogenous metabolic activation.[123,124,129,130] It also

12 caused DNA damage in Chinese hamster lung V79 cells.[131] In mouse lymphoma

13 L5178Y cells, mutations at the Tk locus occurred without metabolic activation, while

14 DNA strand breaks were induced in rat primary hepatocytes and rat

15 pheochromocytoma PC12 cells without metabolic activation.[128,132,133]

16 *Non-mammalian cells*

17 In Drosophila melanogaster, styrene showed mixed results, causing sex-linked

18 recessive lethal mutations, but not leading to aneuploidy, nor induction of somatic

19 mutations.[95,134,135] In Allium cepa, styrene caused chromosomal aberrations.[109,110]

20 Yeast cells also exhibited genotoxic effects from styrene, such as gene conversion and

21 reverse mutation.[136] When tested on various bacterial strains (e.g., Salmonella

22 typhimurium, Escherichia coli), styrene's mutagenic potential varied, often showing

23 negative results without metabolic activation but some positive results with metabolic

24 activation.[137-146] One study showed that styrene exposure led to significant DNA

25 damage in non-mammalian species, such as fish and mussels, with continued

26 exposure over a week.[147]

27 Styrene-7,8-oxide was extensively tested in various bacterial strains, both with and

28 without external metabolic activation. Positive results were consistently seen in strains

29 detecting base substitution mutations, while strains detecting frameshift mutations

30 mostly yielded negative results. Additionally, styrene-7,8-oxide showed positive results

31 for sex-linked recessive lethal mutations in Drosophila melanogaster and induced

32 chromosomal aberrations and micronuclei in Allium cepa root tip cells.[109,110,134] Notably,

33 styrene-7,8-oxide showed positive outcomes in DNA damage tests without metabolic

1 activation.[138,142,146,148-156] Overall, these findings align with observations regarding the
2 mutagenicity of styrene in similar assays.

3 ## 3.4 Evaluation of mutagenicity

4 Classification in category 1A for germ cell mutagens requires positive evidence from
5 human epidemiological studies. Since there is only one epidemiological study with
6 styrene on mutagenicity in germ cells, which did not show sufficient evidence of an
7 effect, the committee concludes that the available data does not indicate that styrene
8 should be classified in category 1A for germ cell mutagenicity.

9 A substance can be classified in category 1B if mutagenicity is observed in germ cells
10 in mammals in vivo or in somatic cells in mammals in vivo combined with evidence
11 indicating the potential to cause mutations in germ cells. Since there is no in vivo data
12 on mutagenicity in germ cells for styrene, and the one germ cell mutagenicity test in
13 styrene-7,8-oxide was negative, classification in category 1B for mutagenicity is not
14 applicable.

15 A substance can be classified in category 2 if there is evidence for mutagenicity from
16 experiments in somatic cells in mammals in vivo or other in vivo somatic cell
17 genotoxicity tests supported by in vitro data. In general, studies on rodents exposed to
18 styrene or styrene-7,8-oxide yielded either negative or inconclusive outcomes
19 regarding cytogenetic effects. Effects in mammalian cells varied depending on the
20 presence of metabolic activation, and specific responses varied across different
21 organisms and test systems. However, evidence was found for DNA damage and DNA
22 adducts, and epidemiological studies showed limited evidence for chromosomal
23 aberrations and micronuclei. Additionally, in vitro studies in human cells have
24 consistently shown that both styrene and its metabolite, styrene-7,8-oxide, cause
25 genotoxic effects. Based on these findings, the committee considers classification in
26 Category 2 warranted.

27 ## 3.5 Recommendation on the classification for mutagenicity

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

31

4 Carcinogenicity

Data on carcinogenicity is summarized in the RIVM report [2] and the IARC Monograph.[3] The committee did not find relevant additional or new data in the literature.

4.1 Human data

There are three main cohort studies on the effects of styrene exposure with results published in multiple articles. Two of these studies are American: one among boatbuilders in the Washington state, and one nationwide study among workers in the reinforced plastics and composites industry. The third cohort is a combined cohort from different countries in Europe among workers at reinforced plastics production plants. The committee selected key publications within these three cohorts presenting the most recent and complete study results. An overview of the carcinogenicity data in humans can be found in Table B1 in Annex B.

The boatbuilder study in Washington State, USA is a retrospective cohort study that has resulted in several publications.[157-162] 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.

IARC concluded that the strengths of this study were the high concentrations of styrene exposure in general, the few competing risk factors, and the long follow-up. Limitations were the lack of individual quantitative styrene exposure and information on smoking.[3] The committee agrees with the conclusions of IARC on the quality of the studies within this large cohort. The committee wants to emphasize the results of the internal analyses among exposed employees, because the chance of bias is lower in these studies, the committee selected two key publications.[160,162] Bertke et al. (2018) has the

1   most recent data, up to 2016, on mortality figures.[160] They found no excess deaths
2   from lymphohematopoietic cancers, but internal analyses indicated that the relative risk
3   increased with duration of employment. Cancer mortality due to trachea, bronchus and
4   lung tumours combined was significantly elevated (SMR 1.37, 1.19-1.57), without
5   evidence of a dose-response relationship.[2,160] Daniels et al. (2020) did not update
6   mortality any further.[162] However, they extended analyses by making fuller use of
7   available employment information and exposure measurement data. They estimated
8   mean, respectively median, cumulative exposures to have been 31, respectively 5.7
9   ppm-years. Furthermore, they concluded that there was a monotonic relation elation
10   between styrene exposure and risk of leukemia (hazard ratio HR per 50 ppm-years
11   1.46, 1.04-1.97) and risk of bladder cancer (1.64, 1.14-2.33). Similar results were found
12   among workers with longer exposure time, although statistically non-significant due to
13   small numbers.[2,162]

14   There are three publications within the cohort study among workers in the reinforced
15   plastics and composites industry in the United States of America.[163-165] For these
16   studies, a cohort of almost 16,000 workers working at one of 30 reinforced plastics
17   manufacturing plants in various US states in the period 1948-1977 was formed to
18   analyse the health effects of styrene exposure. IARC concluded that the strengths of
19   this study were the long follow-up, the high number of cases, the high concentrations of
20   styrene exposure, and the lack of known carcinogenic occupational co-exposures
21   within the industry. Quantitative styrene exposure metrics were applied but information
22   on the exposure assessment was sparse; no styrene intensity information was
23   apparently available for a substantial part of the exposure period, namely between
24   1948 and 1976, and for 27% of the cohort exposure data after 1977 was missing.[3] The
25   committee noticed that the cohort was formed with aid of the industry. The key
26   publication within this cohort, Collins et al (2013), provided the latest update with
27   follow-up until the end of 2008. At this point, they only found significant differences with
28   the general population for lung cancer (SMR 1.34, 1.23–1.46), but with an inverse trend
29   with cumulative exposure.[2,165]

30   The six-country study on workers at reinforced plastics production plants is a study of
31   workers in the reinforced plastics industry in Denmark, Finland, Italy, Norway, Sweden
32   and the UK, conducted at the initiative of the IARC. Altogether, the cohort includes over
33   40,000 workers at one of more than 600 reinforced plastics production plants. Four
34   publications report on the full cohort,[166-169] and six publications only on the Danish
35   cohort [170-175] and another two publications on the UK cohort alone.[176,177] Loomis et al.

1 (2019) is the latest study on this six-country cohort, in which the data was re-analysed

2 (excluding the Norwegian cohort), finding the mean level of styrene exposure to be

3 associated with an increased risk of dying from non-Hodgkin's lymphoma (RR 2.31,

4 1.29-4.12 per 100 ppm), from cancer of the oesophagus (2.44, 1.11-5.36 per 100 ppm),

5 or of the pancreas (RR 1.89, 1.17-3.09). Oesophageal cancer mortality was also

6 associated with cumulative styrene exposure 20 years after the start of exposure (RR

7 1.16, 1.03-1.31).[2,169] IARC noted that the strengths of this study were the large study

8 population of workers of small- and medium-sized companies, with expected

9 homogeneous and high-concentration exposure to styrene, and a long and almost

10 complete follow-up. The limitations were the lack of quantitative estimates of exposure

11 to styrene or any information on the prevalence of smoking.[3]

12 In addition, there are smaller cohort studies on workers in the synthetic rubber industry,

13 all based on North American workers in the styrene–butadiene rubber (SBR) industry.

14 These workers were exposed to styrene at lower concentrations, but for longer times.

15 Within these studies elevated risk of mortality from leukemia was found which is in line

16 with the results from the boat builders cohorts.[3,178-180]

17 Several case–control studies have investigated the association between workplace

18 exposure to styrene and the risk of various cancers. Cancers of the lymphoid and

19 haematopoietic tissues, as well as renal cell carcinoma and cancer of the lung, have

20 received particular attention and elevated risks were observed.[3]

21

1 **4.2 Animal data**

2 A summary of the provided information on the carcinogenicity of styrene and styrene-
3 7,8-oxide is presented below. An overview of the carcinogenicity data in animals can
4 be found in Table B2 and B3 in Annex B. Several studies have been excluded from the
5 evaluation of carcinogenicity, including dose-range finding studies [181]. Studies by
6 intraperitoneal- and subcutaneous injection have been excluded as this route of
7 exposure is not considered relevant for styrene in humans [182-184]. Additionally, the
8 studies of Maltoni and Conti et al. have been excluded from the evaluation due to poor
9 study quality [183,185,186].

10 **4.2.1 Studies with styrene in mice**

11 Six studies with styrene in mice were considered for the evaluation of carcinogenicity,
12 of which two studies by oral gavage, two studies involving transplacental exposure
13 followed by oral exposure by gavage in male and female pups, and two studies by
14 inhalation.

15 *Oral studies*

16 A carcinogenicity study in B6C3F1 mice was performed by the National Cancer
17 Institute (NCI) [187]. Male and female mice (20 controls/sex and 50/sex/dose group) were
18 exposed to a mixture of 70% styrene and 30% β-nitrostyrene 3 times per week for 78
19 weeks via oral gavage. Mice were exposed at dose levels of 0, 87.5 and 175 mg/kg
20 bw/day. These dosages are defined in terms of the β-nitrostyrene present in the
21 styrene solution. In males, a dose-response relation for increased mortality upon
22 treatment was observed (P=0.007). In females, mean body weight was decreased (175
23 mg/kg bw) compared to control. A statistically significant increased incidence of
24 combined lung alveolar/bronchiolar carcinoma and adenomas in low dose male mice
25 was noticed compared to control (P=0.016), although there was no significant increase
26 in malignant tumours. However, the high dose Fisher exact test and the Cochran-
27 Armitage test were not significant for these neoplastic lesions. The committee observed
28 a lack of clarity regarding the number of mice that dropped out during the study.
29 Additionally, they noted a higher attrition rate among male mice in the high-dose group.
30 Since the analysis only included mice that survived for a minimum of 52 weeks, the
31 exclusion of those mice lost in the high-dose group could have potentially affected the
32 outcome of the study.

A carcinogenicity study in B6C3F1 mice was performed by the NCI [188]. Male and female mice (20 controls/sex and 50/sex/dose group) were exposed to styrene 5 days per week for 78 weeks via oral gavage. Mice were exposed at 0, 150 and 300 mg/kg bw/day. Mortality was increased in all dose groups in males. In females, a slight dose-related mean body weight depression was observed, but mortality was not affected. Combined alveolar/bronchiolar adenomas and carcinomas of the lung compared to control were significantly increased in males (300 mg/kg bw, P=0.024). However, no difference was found in carcinomas. The study authors noted that a large variation in occurrence of lung tumours exists in historical control data of untreated male mice and that incidence in vehicle controls was lower than expected based on this data. Hepatocellular adenomas were observed in female mice, but a statistically significant increase was only found at the highest dose (300 mg/kg bw, P=0.034). Although a noticeable trend was indicated by the Cochran-Amirage test, the comparison of individual groups to the control group was not significant.

A carcinogenicity study in O20 mice and C57 BL mice was performed by Ponomarkov et al [189]. For O20 mice, pregnant dams (29 exposed, 9 control) were given a single oral gavage administration of styrene (1350 mg/kg bw, purity: 99%) or olive oil at gestation day 17. Their offspring was treated weekly from the time of weaning for the whole lifespan with the same dose of styrene or olive oil via oral gavage. An extra control group of 54 untreated males and 47 untreated females was included. Treatment of offspring had to be suspended after 16 weeks due to severe toxicity. Preweaning mortality was higher in the styrene group compared to control. Overall mortality was high in the styrene progeny group: at 20 weeks, 50% of males and 20% of females died. Survival rates of other groups (styrene pregnancy, vehicle pregnancy, vehicle progeny) were not affected. The average age of death was lower in exposed animals (32 weeks, males; 49 weeks females) compared to controls (88 weeks, males; 85 weeks, females). There was an increased incidence in total tumour bearing animals in offspring of styrene-treated dams in males and females (no details on statistics). An increased incidence of lung adenoma and adenocarcinoma combined was observed in treated offspring of styrene-treated dams in males and females compared to the olive oil control group (P<0.01 for both). However, no details on the statistics used were provided. Lung tumours appeared earlier in the styrene-treated progeny groups (both male and female) compared to control. Additionally, the committee noted that O20 mice are sensitive for developing lung tumours.
For C57 BL mice, pregnant dams (15 exposed, 5 control) were given a single oral gavage administration of styrene (300 mg/kg bw, purity: 99%) or olive oil at gestation

1   day 17. Their offspring was treated weekly from the time of weaning for the whole
2   lifespan with the same dose of styrene or olive oil via oral gavage. An extra control
3   group of 51 untreated males and 49 untreated females was included. Litter size,
4   preweaning mortality, offspring mortality and body weights did not differ between the
5   groups. An increased incidence in tumour-bearing females receiving a single dose of
6   styrene during pregnancy was observed. This was due to an increased incidence of
7   lymphomas which was not statistically significant. There was an increased incidence in
8   hepatocellular carcinoma or adenoma in treated males. However, no details on
9   statistics were reported.

10 *Inhalation studies*

11 In a GLP study, CD-1 mice(70/sex/group) were exposed to styrene vapour (whole
12 body) at concentrations of 0, 20, 40, 80, or 160 ppm for 6h/day during 5 days/week for
13 104 weeks (males) or 98 weeks (females) [190].
14 Styrene did not impact the survival in male mice. The remaining exposed females had
15 a slightly higher survival rate than the control group. An increase in the total number of
16 tumour-bearing mice was observed in females exposed to 40 ppm and 160 ppm
17 compared to the control group (both $P<0.05$). An increased tumour incidence was
18 predominantly seen in the lung. In males, there was an increased incidence of
19 bronchioloalveolar adenomas at 40 ppm, 80 ppm, and 160 ppm (all $P<0.05$). In
20 females, an increased incidence of bronchioloalveolar adenomas was observed at 20
21 ppm and 40 ppm (both $P<0.05$), as well as an increased incidence of
22 bronchioloalveolar carcinomas at 160 ppm ($P<0.05$). Non-neoplastic lesions in male
23 and female CD1-mice are briefly summarized in Table B2, Annex B. It should be noted
24 that no historical control data was available from inhalation studies conducted at the
25 testing laboratory for bronchioloalveolar adenoma and carcinoma in CD-1 mice.
26
27 A follow-up study was conducted in which 55 males were exposed to styrene [190]. No
28 effects in the lung were observed. In the 40 ppm, there were slight changes in the
29 olfactory epithelium. In the 80 ppm group, single-cell necrosis occurred in the olfactory
30 epithelium. After 2, 4 and 7 exposures, there was an increase in degree of lesions and
31 changes in the Bowman's glands. After 40 or 65 exposures, more pronounced atrophy
32 and disorganization leading to respiratory metaplasia was seen.

33 In another inhalation study (whole-body exposure), groups of 75 male CD-1, C57BL/6
34 wildtype (WT), *Cyp2f2*(−/−) knockout (KO), and *Cyp2f2*KO-*Cyp2f1* transgenic (TG)

1 mice were exposed to styrene at 0 ppm or 120 ppm for 6 hours per day, 5 days per

2 week, for a duration of 104 weeks [191]. Treated wildtype mice showed significantly

3 higher survival rates compared to the control group. No statistically significant increase

4 in lung adenomas or adenocarcinomas was observed in any of the four strains of mice.

5 CD-1, WT and KO mice exposed to styrene weighed less than controls (2-13%; 2-10%;

6 up to 7% respectively). Mean body weights in exposed CD-1, WT and KO mice were

7 statistically significantly lower compared to controls at multiple time points. Non-

8 neoplastic lesions observed in the four strains of mice are briefly summarized in Table

9 B2, Annex B.

10 **4.2.2 Studies with styrene in rats**

11 Six studies with styrene in male and/or female rats were considered for the evaluation

12 of carcinogenicity: three studies by oral gavage, two studies by inhalation, and one

13 study involving transplacental exposure followed by oral exposure by gavage in male

14 and female pups.

15

16 *Oral studies*

17 A carcinogenicity study in Fischer 344 rats was performed by the NCI [187]. Male and

18 female rats (20 controls/sex and 50/sex/dose group) were exposed to a mixture of 70%

19 styrene and 30% β-nitrostyrene 3 times per week via oral gavage for a duration of 79

20 weeks. Males were exposed at dose levels of 0, 150 or 300 mg/kg bw/day and females

21 at dose levels of 0, 75 and 150 mg/kg bw/day. Tumour incidences were statistically

22 analysed with a Fisher exact test (one-tailed). Survival was not affected by styrene.

23 Mean body weight was decreased in male rats (300 mg/kg bw) compared to control.

24 There were no significant effects on tumour incidences.

25 A carcinogenicity study in Fischer 344 rats was performed by the NCI [188]. Male and

26 female rats (20 controls/sex and 50/sex/dose group) were exposed to styrene 5 days

27 per week via oral gavage. Rats were exposed at dose levels of 0, 1000 and 2000

28 mg/kg bw/day for 78 weeks and 0 and 500 mg/kg bw/day for 103 weeks. The 500

29 mg/kg bw group and extra control group were added later due to excessive mortality in

30 the high dose groups. Tumour incidences were statistically analysed with a Fisher

31 exact test (one-tailed). Mortality was significantly higher in high-dose male and female

32 rats compared to control (both $P<0.001$). A slight dose-related mean body weight

depression was observed in males. There was no significant increase in tumour incidences.

A chronic toxicity and reproduction study was performed by Beliles et al [192]. In the chronic toxicity part of the study, male (76 controls and 50/exposure group) and female (106 controls and 70/exposure group) Charles River COBS (SD) BR rats were continuously exposed to styrene (purity: 98.9%) orally for two years via drinking water at concentrations of 0, 125 and 250 ppm. Survival of both male and female rats was not affected by styrene exposure. A decrease in terminal body weight and increased relative brain weight was observed in females (250 ppm). Water consumption was decreased in both males and females (125 ppm and 250 ppm) and a dose-response relationship was established. There were no reported treatment-related increased incidences of non-neoplastic lesions or neoplastic lesions.

A carcinogenicity study in BD IV rats was performed by Ponomarkov et al [189]. Pregnant dams (21 exposed, 10 control) were given a single oral administration of styrene (1350 mg/kg bw, purity: 99%) or olive oil via gavage at gestation day 17. Their offspring was treated from the time of weaning weekly for the whole lifespan with 500 mg/kg bw styrene or olive oil via oral gavage. Details of statistical analysis were not reported. Preweaning mortality of the offspring of styrene-treated females given a single administration of styrene during pregnancy was higher compared to the offspring of olive-oil treated dams. There were no other differences in survival or body weight. A non-significant increased incidence was observed in tumour-bearing females receiving a single styrene administration during pregnancy.

Inhalation studies

Jersey et al. performed a carcinogenicity study in 1978. This study is not published and data was summarized by the NTP based on information retrieved from secondary sources in which the study of Jersey et al. was reviewed [193]. The NTP also performed a Cochran-Armitage exact trend test on tumour incidences. Sprague-Dawley rats (7-8 weeks old) were exposed to styrene (purity 99.5%) via inhalation at concentrations of 0, 600 or 1000 ppm (corresponding to 0, 2556 or 4260 mg/m3 conform the CLP-guidance). Each group consisted of 96/97 males and 96 females, and they were exposed for 5 days/week until 50% mortality was reached at 18.3 (females) or 20.7 (males) months. Initially, the high-dose group was exposed to 1200 ppm styrene, but due to excessive toxicity, this was reduced to 1000 ppm after 2 months. Survival was

1   lower in males than in females. It is noted that others (McConnell and Swenberg, 1994)
2   state that the presence of chronic murine pneumonia caused excessive mortality in
3   control and exposed males.
4   In females, the incidence of mammary adenocarcinoma was increased at 600 ppm
5   compared to control, but not when compared to historical controls. The P-value for
6   trend was 0.002. A statistically significant increased incidence of combined
7   lymphosarcomas and leukemia was observed in females compared to incidences in
8   historical controls, but not when compared to the concurrent controls. The P-value for
9   trend was 0.035. However, the committee agreed with McConnell and Swenberg, 1994
10   that this study was seriously flawed by the presence of chronic murine pneumonia,
11   which caused a high rate of mortality in both controls and exposed male rats.

12   A chronic toxicity/oncogenicity study was performed by Cruzan et al [194]. Rats
13   (70/sex/group) were exposed to styrene at 0, 50, 200, 500, or 1000 ppm for 104 weeks.
14   The exposure was performed by inhalation (whole body) of styrene vapour 6h/day 5
15   days/week for 104 weeks (520 exposures). During week 61, eight males in the 1000
16   ppm group and six males in the 500 ppm group received a massive dermal exposure of
17   styrene due to a technical problem. All died or were sacrificed and were not included in
18   the analysis. There were no further effects on survival of male rats. A dose-related
19   increase in survival of female rats was noticed. No statistically significant treatment-
20   related increase of number of animals bearing tumours was observed in males and
21   females. There was a treatment related decrease noted in pituitary adenomas in
22   females. Additionally, a treatment-related decrease in mammary adenocarcinomas in
23   females was noted as well as a treatment related decrease in mammary
24   fibroadenomas in females. Summary of additional animal data with styrene-7,8-oxide

25   A summary of the provided information on the carcinogenicity of styrene-7,8-oxide is
26   presented below. An overview of the carcinogenicity data can be found in Table B3 in
27   Annex B. Dermal and intraperitoneal studies were not included in this assessment
28   because these exposure routes are not considered relevant for styrene-7,8-oxide
29   [182,195]. The studies by Maltoni et al. and Conti et al. have been excluded due to limited
30   study quality [183,185,186].

31   **4.2.3 Studies with styrene-7,8-oxide in mice**

32   One study by oral gavage with styrene-7,8-oxide in male and female mice was
33   considered for the evaluation of carcinogenicity [196].

1 *Oral studies*

2 B6C3F1 mice (52/sex/group) were treated with styrene-7,8-oxide via oral gavage at
3 concentrations of 0 (vehicle), 375 mg/kg bw and 750 mg/kg bw, 3 times per week for
4 104 weeks (Lijinsky, 1986). Styrene-7,8-oxide was dissolved in corn oil (purity 96.6%)
5 and the authors noted that 3.3% of the solution consisted of benzaldehyde, benzene
6 and one other unspecified compound. Fisher's exact tests and Cochran-Armitage tests
7 were performed, but it is not clear to what data these were applied. Survival of animals
8 (750 mg/kg bw) was lower compared to control; half of the group died by 60 weeks.
9 Weight gain was reduced in males and females (375 and 750 mg/kg bw) compared to
10 control and weight loss was observed in males (375 and 750 mg/kg bw) after 75 weeks
11 (no details). Some non-neoplastic lesions occurred, although their incidences were not
12 reported, as summarized in Table B3, Annex B. Increased incidences in combined liver
13 carcinomas and adenomas were observed in males, which were statistically
14 significantly different from the controls in the 375 mg/kg group (P<0.001).
15 Increased incidences of papillomas (in males and females), carcinomas (in males), and
16 the combination of both (in males and females) were observed in the forestomach,
17 which were statistically significantly different from controls at doses of 375 and 750
18 mg/kg bw (P<0.001). There was a decreased incidence of malignant lymphoma and
19 leukemia in females (750 mg/kg bw, P=0.01).

20 **4.2.4 Studies with styrene-7,8-oxide in rats**

21 Two studies with styrene-7,8-oxide in rats were considered for the evaluation of
22 carcinogenicity, of which one study by gavage in males and females, [196]and one study
23 involving transplacental exposure followed by oral exposure by gavage in male and
24 female pups [196,197].

25 *Oral studies*

26 F344 rats (52/sex/group) were treated with styrene-7,8-oxide via oral gavage at
27 concentrations of 0 (vehicle), 275 mg/kg bw and 550 mg/kg bw, 3 times per week for
28 104 weeks [196]. Styrene-7,8-oxide was dissolved in corn oil (purity 96.6%) and the
29 authors noted that 3.3% of the solution consisted of benzaldehyde, benzene and one
30 other unspecified compound. Survival and weight gain of animals in the 550 mg/kg bw
31 group was reduced compared to control. A small weight loss was observed in males
32 (550 mg/kg bw) after 75 weeks (no details reported). Increased incidence of combined

1 carcinomas and papillomas in the forestomach was observed in treated males and

2 females, which was statistically significantly different from controls in the males at 275

3 mg/kg (P<0.001). This styrene-related increased incidence in forestomach tumours

4 was also confirmed by an increased incidence of hyperplasia in the fore stomach.

5 Because some of the rats given the high dose died relatively early with neoplasms

6 attributable to the treatment, the incidences of some of the common spontaneous

7 neoplasms, such as islet cell adenomas and/or carcinomas of the pancreas, mammary

8 fibroadenomas, neoplastic nodules of the liver in females, and endometrial stromal

9 polyps, were lower in the treated animals than in the controls. There was a decreased

10 incidence of leukemia in males and females (both 550 mg/kg bw) compared to control,

11 which was, according to the study authors, considered less likely due to the early

12 deaths.

13 A carcinogenicity study in BDIV rats was performed by Ponomarkov et al [197]. Pregnant

14 dams (14 exposed, 14 control) were given a single oral administration of styrene-7,8-

15 oxide (200 mg/kg bw, purity: 97%) or olive oil at gestation day 17. Their offspring was

16 treated with 96 weekly doses of styrene-7,8-oxide (100-150 mg/kg bw) or olive oil from

17 week 4 of age (weaning) until termination of the experiment at 120 weeks. Styrene-7,8-

18 oxide was administrated via oral gavage. Litter size, preweaning mortality, offspring

19 mortality and body weights did not differ between the groups. No carcinogenic effects

20 were observed in the pregnant dams except that the incidence in tumour-bearing

21 pregnant dams was decreased compared to the control group (31% for styrene-7,8-

22 oxide and 57% in controls). In treated offspring, the percentage of tumour-bearing

23 animals was 77% (females) and 52% (males) versus 58% (females) and 20% (males)

24 in the control group. An increased incidence in several types of forestomach tumours

25 was observed in treated offspring. The incidence of carcinomas in situ and early

26 carcinomas or carcinomas increased significantly in both females, ranging from

27 P<0.0001 to P<0.04.The number of papillomas was increased in males only (P<0.003).

28 Early changes of squamous epithelium frequently observed in styrene-7,8-oxide

29 groups. Other increased tumour incidences were not statistically significant.

30 **4.3   Evaluation of carcinogenicity**

31 Classification of a substance in category 1A requires sufficient evidence from

32 epidemiological studies to support the existence of a causal relationship between

33 human exposure and the development of cancer. There are several epidemiological

34 studies available that are large and well performed, although none of the studies

1 performed in workers are without flaws. The committee considers the evidence from
2 the boatbuilder study in Washington State and the European cohort as most relevant
3 as they present dose-response relationships within exposed workers only as this
4 reduces the impact of bias. The study of Daniels et al (2020) showed an elevated risk
5 for leukemia and bladder cancer and the study of Loomis et al (2019) showed an
6 elevated risk for non-Hodgkin-lymphoma, oesophageal and pancreatic cancer. Overall,
7 the committee concludes that there is limited evidence of carcinogenicity from human
8 studies, and bias and confounding cannot be excluded. Therefore, category 1A is not
9 applicable.

10 Classification in category 1B (presumed to be carcinogenic to humans) requires a
11 marked increase in the number of malignant tumours, which has been obtained in at
12 least two experimental animal species, or in a single species in two or more
13 independent studies.
14 In several studies, exposure to styrene led to an increased incidence of lung tumours in
15 B6C3F1, O20, and CD-1 mice. In most of these studies, the increased incidence in
16 lung tumours consisted of adenomas (benign) or a combination of adenomas and
17 carcinomas (malignant). However, the committee carefully evaluated the evidence of
18 the increased incidence of these tumours, taking into account the crucial role of mouse
19 lung-specific Cyp2 F2 metabolism in the carcinogenicity induced by styrene. This
20 indicates that this tumour response is not relevant, either qualitatively or quantitatively,
21 to humans.[6,7]
22 In rats, the effects of styrene exposure on tumour incidence varied depending on the
23 route of exposure. Inhalation exposure was associated with an increased risk of certain
24 tumours in females, including combined lymphosarcomas/leukemia and mammary
25 adenocarcinoma.[193] However, this study was seriously flawed by the presence of
26 chronic murine pneumonia, and the incidence of lymphosarcomas/leukemia was
27 increased compared to historical control data rather than concurrent controls. The
28 mammary adenocarcinomas were statistically significant only in the low dose group.
29 Other routes of exposure, such as oral gavage or drinking water, did not show a
30 significant increase in tumour incidence.
31 Carcinogenicity observed after styrene-7,8-oxide exposure in male mice and both male
32 and female rats includes increased tumour incidences of the forestomach. The
33 committee considers these not relevant for humans based on the WOE Decision
34 Criteria for Assessing the Relevance of Forestomach Tumors in Human Cancer Risk
35 Assessment.[198,199]
36

1 Although no significant increase of relevant malignant tumours in at least two
2 experimental animal species or studies was found after styrene exposure, the
3 committee considers classification in category 1B warranted based on the limited
4 evidence of carcinogenicity in epidemiological studies and limited evidence of
5 carcinogenicity in animal studies.

6 **4.4   Recommendation on the classification for carcinogenicity**

7 The committee recommends classifying styrene as *Presumed to be carcinogenic to*
8 *humans*, which corresponds with classification in category 1B with H350 (may cause
9 cancer).
10
11

References

1   The Health Council. *Guideline for the classifcation of carinogenic compounds*. https://www.healthcouncil.nl/documents/other/2023/06/19/guideline-to-the-classification-of-carcinogenic-substances. Geraadpleegd: 16-6-2024.

2   Eliesen GAM, Proquin HAA, Engelfriet PM. RIVM. *An overview of the available data on the mutagenicity and carcinogenicity of styrene*. 2023; RIVM letter report 2022-0129.

3   IARC. *Styrene, Styrene-7,8-oxide, and Quinoline*. Lyon, France 2019; IARC-Monographs Volume 121.

4   European Chemicals Agency (ECHA). *REACH registration dossier of styrene*. https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/15565/1/1. Geraadpleegd: last updated: 14-2-2022.

5   Behr A. *Styrene Production from Ethylbenzene* Faculty of Biochemical and Chemical Engineering, Dortmund University, 2017.

6   Frank EA, Meek M. *Procedural application of mode-of-action and human relevance analysis: styrene-induced lung tumors in mice*. Crit Rev Toxicol 2024; 54(2): 134-151.

7   Cohen SM, Zhongyu Y, Bus JS. *Relevance of mouse lung tumors to human risk assessment*. Journal of Toxicology and Environmental Health, Part B 2020; 23(5): 214-241.

8   U.S. Department of Health and Human Services. *Report on Carcinogens, Fifteenth Edition*. 2021. https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc#toc.

9   State of California. Environmental protection agency. *Chemicals known to the State to cause cancer or reproductive toxicity*. https://oehha.ca.gov/media/downloads/proposition-65//p65list091319.pdf. Geraadpleegd: mei 2024.

10   The National Institute for Occupational Safety and Health (NIOSH). *Occupational Cancer – Carcinogen List*. https://www.cdc.gov/niosh/topics/cancer/npotocca.html. Geraadpleegd: mei 2024.

	11	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA). *Technischen Regeln für Gefahrstoffe (TRGS)*. https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf?__blob=publicationFile. Geraadpleegd: mei 2024.
	12	Safe Work Australia. *Hazardous Chemical Information System (HCIS)*. https://hcis.safeworkaustralia.gov.au/HazardousChemical/Details?chemicalID=423. Geraadpleegd: mei 2024.
	13	National Institute of Technology and Evaluation. *GHS Classification Results by the Japanese Government*. https://www.nite.go.jp/chem/english/ghs/20-mhlw-2111e.html. Geraadpleegd: mei 2024.
	14	Collins JJ, Moore M. *A meta-analysis of epidemiologic studies of occupationally exposed styrene workers and micronuclei levels*. Mutat Res Genet Toxicol Environ Mutagen 2019; 837: 15-28.
	15	Collins JJ, Moore M. *A critical review and meta-analysis of epidemiology studies of occupationally exposed styrene workers evaluated for chromosomal aberration incidence*. Mutat Res 2021; 861-862: 503275.
	16	Huang M. *[Study of cytogenetic damages in peripheral blood of styrene exposed workers]*. Zhonghua Yu Fang Yi Xue Za Zhi 1992; 26(5): 272-274.
	17	Forni A, Goggi E, Ortisi E, Cecchetti R, Cortona G, Sesana G, et al. *Cytogenetic findings in styrene workers in relation to exposure.* Editor: Seemayer NH HWe. Environmental hygiene.: pp. 159–162. Berlin, Germany: 1988. doi:10.1007/978-3-642-73766-4_34.
	18	Oberheitmann B, Frentzel-Beyme R, Hoffmann W. *An application of the challenge assay in boat builders exposed to low levels of styrene--a feasibility study of a possible biomarker for acquired susceptibility*. Int J Hyg Environ Health 2001; 204(1): 23-29.
	19	Jablonická A, Karelová J, Poláková H, Vargová M. *Analysis of chromosomes in peripheral blood lymphocytes of styrene-exposed workers*. Mutat Res 1988; 206(2): 167-169.
	20	Sorsa M, Anttila A, Jarventaus H, Kubiak R, Norppa H, Nylander L, et al. *Styrene revisited--exposure assessment and risk estimation in reinforced plastics industry*. Prog Clin Biol Res 1991; 372: 187-195.
	21	Hagmar L, Högstedt B, Welinder H, Karlsson A, Rassner F. *Cytogenetic and hematological effects in plastics workers exposed to styrene*. Scand J Work Environ Health 1989; 15(2): 136-141.

22   Mäki-Paakkanen J. *Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene*. Mutat Res 1987; 189(4): 399-406.

23   Pohlová H, Srám RJ. *Cytogenetic analysis of peripheral blood lymphocytes of workers occupationally exposed to styrene*. J Hyg Epidemiol Microbiol Immunol 1985; 29(2): 155-161.

24   Hansteen IL, Jelmert O, Torgrimsen T, Førsund B. *Low human exposure to styrene in relation to chromosome breaks, gaps and sister chromatid exchanges*. Hereditas 1984; 100(1): 87-91.

25   Thiess AM, Fleig I. *Chromosome investigations on workers exposed to styrene/polystyrene*. J Occup Med 1978; 20(11): 747-749.

26   Somorovská M, Jahnová E, Tulinská J, Zámecníková M, Sarmanová J, Terenová A, et al. *Biomonitoring of occupational exposure to styrene in a plastics lamination plant*. Mutat Res 1999; 428(1-2): 255-269.

27   Vodicka P, Tuimala J, Stetina R, Kumar R, Manini P, Naccarati A, et al. *Cytogenetic markers, DNA single-strand breaks, urinary metabolites, and DNA repair rates in styrene-exposed lamination workers*. Environ Health Perspect 2004; 112(8): 867-871.

28   Helal SF, Elshafy WS. *Health hazards among workers in plastic industry*. Toxicol Ind Health 2013; 29(9): 812-819.

29   Camurri L, Codeluppi S, Pedroni C, Scarduelli L. *Chromosomal aberrations and sister-chromatid exchanges in workers exposed to styrene*. Mutat Res 1983; 119(3): 361-369.

30   Andersson HC, Tranberg EA, Uggla AH, Zetterberg G. *Chromosomal aberrations and sister-chromatid exchanges in lymphocytes of men occupationally exposed to styrene in a plastic-boat factory*. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 1980; 73(2): 387-401.

31   Tomanin R, Ballarin C, Bartolucci GB, De Rosa E, Sessa G, Iannini G, et al. *Chromosome aberrations and micronuclei in lymphocytes of workers exposed to low and medium levels of styrene*. Int Arch Occup Environ Health 1992; 64(3): 209-215.

32   Nordenson I, Beckman L. *Chromosomal aberrations in lymphocytes of workers exposed to low levels of styrene*. Hum Hered 1984; 34(3): 178-182.

33   Watanabe T, Endo A, Kumai M, Ikeda M. *Chromosome aberrations and sister chromatid exchanges in styrene-exposed workers with reference to their smoking habits*. Environ Mutagen 1983; 5(3): 299-309.

1   34   Artuso M, Angotzi G, Bonassi S, Bonatti S, De Ferrari M, Gargano D, et al. *Cytogenetic*
2   *biomonitoring of styrene-exposed plastic boat builders*. Arch Environ Contam Toxicol
3   1995; 29(2): 270-274.

4   35   Mäki-Paakkanen J, Walles S, Osterman-Golkar S, Norppa H. *Single-strand breaks,*
5   *chromosome aberrations, sister-chromatid exchanges, and micronuclei in blood*
6   *lymphocytes of workers exposed to styrene during the production of reinforced plastics*.
7   Environ Mol Mutagen 1991; 17(1): 27-31.

8   36   Meretoja T, Vainio H, Sorsa M, Härkönen H. *Occupational styrene exposure and*
9   *chromosomal aberrations*. Mutation Research/Fundamental and Molecular Mechanisms
10   of Mutagenesis 1977; 56(2): 193-197.

11   37   Dolmierski R, Szczepanik M, Danielewicz-Garbalińska G, Kunikowska D, Mickiewicz W,
12   Chomicz M, et al. *Mutagenic action of styrene and its metabolites. 1. Chromosome*
13   *aberration in persons exposed to the action of styrene. Introductory investigations*. Bull
14   Inst Marit Trop Med Gdynia 1983; 34(1-2): 89-93.

15   38   Meretoja T, Järventaus H, Sorsa M, Vainio H. *Chromosome aberrations in lymphocytes*
16   *of workers exposed to styrene*. Scand J Work Environ Health 1978; 4 Suppl 2: 259-264.

17   39   Fleig I, Thiess AM. *Mutagenicity study of workers employed in the styrene and*
18   *polystyrene processing and manufacturing industry*. Scand J Work Environ Health
19   1978; 4 Suppl 2: 254-258.

20   40   Smejkalova J, Hassmanova V, Emminger S, Malir F. *[Chromosome aberrations in*
21   *peripheral blood lymphocytes in workers occupationally exposed to styrene]*. Sb Ved Pr
22   Lek Fak Karlovy Univerzity Hradci Kralove Suppl 1989; 32(4): 471-480.

23   41   Högstedt B, Hedner K, Mark-Vendel E, Mitelman F, Schütz A, Skerfving S. *Increased*
24   *frequency of chromosome aberrations in workers exposed to styrene*. Scand J Work
25   Environ Health 1979; 5(4): 333-335.

26   42   Mierauskiene J, Lekevicius R, Lazutka JR. *Anticlastogenic effects of Aevitum intake in a*
27   *group of chemical industry workers*. Hereditas 1993; 118(3): 201-204.

28   43   Lazutka JR, Lekevicius R, Dedonyte V, Maciuleviciute-Gervers L, Mierauskiene J,
29   Rudaitiene S, et al. *Chromosomal aberrations and sister-chromatid exchanges in*
30   *Lithuanian populations: effects of occupational and environmental exposures*. Mutat
31   Res 1999; 445(2): 225-239.

32   44   Tates AD, Grummt T, van Dam FJ, de Zwart F, Kasper FJ, Rothe R, et al.
33   *Measurement of frequencies of HPRT mutants, chromosomal aberrations, micronuclei,*
34   *sister-chromatid exchanges and cells with high frequencies of SCEs in*
35   *styrene/dichloromethane-exposed workers*. Mutat Res 1994; 313(2-3): 249-262.

45   Migliore L, Naccarati A, Coppedè F, Bergamaschi E, De Palma G, Voho A, et al. *Cytogenetic biomarkers, urinary metabolites and metabolic gene polymorphisms in workers exposed to styrene*. Pharmacogenet Genomics 2006; 16(2): 87-99.

46   Yager JW, Paradisin WM, Rappaport SM. *Sister-chromatid exchanges in lymphocytes are increased in relation to longitudinally measured occupational exposure to low concentrations of styrene*. Mutat Res 1993; 319(3): 155-165.

47   Hogstedt B. *Micronuclei in lymphocytes with preserved cytoplasm. A method for assessment of cytogenetic damage in man*. Mutat Res 1984; 130(1): 63-72.

48   Hanova M, Stetina R, Vodickova L, Vaclavikova R, Hlavac P, Smerhovsky Z, et al. *Modulation of DNA repair capacity and mRNA expression levels of XRCC1, hOGG1 and XPC genes in styrene-exposed workers*. Toxicol Appl Pharmacol 2010; 248(3): 194-200.

49   Godderis L, De Boeck M, Haufroid V, Emmery M, Mateuca R, Gardinal S, et al. *Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers*. Environ Mol Mutagen 2004; 44(4): 293-303.

50   Vodicka P, Kumar R, Stetina R, Musak L, Soucek P, Haufroid V, et al. *Markers of individual susceptibility and DNA repair rate in workers exposed to xenobiotics in a tire plant*. Environ Mol Mutagen 2004; 44(4): 283-292.

51   Naccarati A, Zanello A, Landi S, Consigli R, Migliore L. *Sperm-FISH analysis and human monitoring: a study on workers occupationally exposed to styrene*. Mutat Res 2003; 537(2): 131-140.

52   Compton-Quintana PJ, Jensen RH, Bigbee WL, Grant SG, Langlois RG, Smith MT, et al. *Use of the glycophorin A human mutation assay to study workers exposed to styrene*. Environ Health Perspect 1993; 99: 297-301.

53   Bigbee WL, Grant SG, Langlois RG, Jensen RH, Anttila A, Pfaffli P, et al. *Glycophorin A somatic cell mutation frequencies in Finnish reinforced plastics workers exposed to styrene*. Cancer Epidemiol Biomarkers Prev 1996; 5(10): 801-810.

54   Vodicka P, Bastlová T, Vodicková L, Peterková K, Lambert B, Hemminki K. *Biomarkers of styrene exposure in lamination workers: levels of O6-guanine DNA adducts, DNA strand breaks and mutant frequencies in the hypoxanthine guanine phosphoribosyltransferase gene in T-lymphocytes*. Carcinogenesis 1995; 16(7): 1473-1481.

55   Vodicka P, Soucek P, Tates AD, Dusinska M, Sarmanova J, Zamecnikova M, et al. *Association between genetic polymorphisms and biomarkers in styrene-exposed workers*. Mutat Res 2001; 482(1-2): 89-103.

1   56   Vodicka P, Tvrdik T, Osterman-Golkar S, Vodicková L, Peterková K, Soucek P, et al. *An
2   evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study
3   of hand-lamination workers*. Mutat Res 1999; 445(2): 205-224.

4   57   Brenner DD, Jeffrey AM, Latriano L, Wazneh L, Warburton D, Toor M, et al. *Biomarkers
5   in styrene-exposed boatbuilders*. Mutat Res 1991; 261(3): 225-236.

6   58   Shamy MY, Osman HH, Kandeel KM, Abdel-Moneim NM, El SK. *DNA single strand
7   breaks induced by low levels of occupational exposure to styrene: the gap between
8   standards and reality*. J Environ Pathol Toxicol Oncol 2002; 21(1): 57-61.

9   59   Laffon B, Pásaro E, Méndez J. *Evaluation of genotoxic effects in a group of workers
10   exposed to low levels of styrene*. Toxicology 2002; 171(2-3): 175-186.

11   60   Wongvijitsuk S, Navasumrit P, Vattanasit U, Parnlob V, Ruchirawat M. *Low level
12   occupational exposure to styrene: its effects on DNA damage and DNA repair*. Int J Hyg
13   Environ Health 2011; 214(2): 127-137.

14   61   Walles SA, Edling C, Anundi H, Johanson G. *Exposure dependent increase in DNA
15   single strand breaks in leucocytes from workers exposed to low concentrations of
16   styrene*. Br J Ind Med 1993; 50(6): 570-574.

17   62   Migliore L, Naccarati A, Zanello A, Scarpato R, Bramanti L, Mariani M. *Assessment of
18   sperm DNA integrity in workers exposed to styrene*. Hum Reprod 2002; 17(11): 2912-
19   2918.

20   63   Fracasso ME, Doria D, Carrieri M, Bartolucci GB, Quintavalle S, De Rosa E. *DNA
21   single- and double-strand breaks by alkaline- and immuno-comet assay in lymphocytes
22   of workers exposed to styrene*. Toxicol Lett 2009; 185(1): 9-15.

23   64   Marczynski B, Rozynek P, Elliehausen HJ, Korn M, Baur X. *Detection of 8-
24   hydroxydeoxyguanosine, a marker of oxidative DNA damage, in white blood cells of
25   workers occupationally exposed to styrene*. Arch Toxicol 1997; 71(8): 496-500.

26   65   Holz O, Scherer G, Brodtmeier S, Koops F, Warncke K, Krause T, et al. *Determination
27   of low level exposure to volatile aromatic hydrocarbons and genotoxic effects in workers
28   at a styrene plant*. Occup Environ Med 1995; 52(6): 420-428.

29   66   Costa C, Costa S, Silva S, Coelho P, Botelho M, Gaspar J, et al. *DNA damage and
30   susceptibility assessment in industrial workers exposed to styrene*. J Toxicol Environ
31   Health A 2012; 75(13-15): 735-746.

32   67   Manini P, De Palma G, Andreoli R, Marczynski B, Hanova M, Mozzoni P, et al.
33   *Biomarkers of nucleic acid oxidation, polymorphism in, and expression of, hOGG1 gene
34   in styrene-exposed workers*. Toxicol Lett 2009; 190(1): 41-47.

1 68 Kelsey KT, Smith TJ, Hammond SK, Letz R, Little JB. *Sister-chromatid exchanges in*
2 *lymphocytes from styrene-exposed boat builders*. Mutat Res 1990; 241(2): 215-221.

3 69 Liu SF, Rappaport SM, Pongracz K, Bodell WJ. *Detection of styrene oxide-DNA*
4 *adducts in lymphocytes of a worker exposed to styrene*. IARC Sci Publ 1988; (89): 217-
5 222.

6 70 Vodicka P, Vodickova L, Hemminki K. *32P-postlabeling of DNA adducts of styrene-*
7 *exposed lamination workers*. Carcinogenesis 1993; 14(10): 2059-2061.

8 71 Hemminki K, Vodicka P. *Styrene: from characterisation of DNA adducts to application in*
9 *styrene-exposed lamination workers*. Toxicol Lett 1995; 77(1-3): 153-161.

10 72 Horvath E, Pongracz K, Rappaport S, Bodell WJ. *32P-post-labeling detection of DNA*
11 *adducts in mononuclear cells of workers occupationally exposed to styrene*.
12 Carcinogenesis 1994; 15(7): 1309-1315.

13 73 Vodicka P, Vodickova L, Trejbalova K, Sram RJ, Hemminki K. *Persistence of O6-*
14 *guanine DNA adducts in styrene-exposed lamination workers determined by 32P-*
15 *postlabelling*. Carcinogenesis 1994; 15(9): 1949-1953.

16 74 Mikes P, Korinek M, Linhart I, Krouzelka J, Dabrowska L, Stransky V, et al. *Urinary N3*
17 *adenine DNA adducts in humans occupationally exposed to styrene*. Toxicol Lett 2010;
18 197(3): 183-187.

19 75 Buschini A, De Palma G, Poli P, Martino A, Rossi C, Mozzoni P, et al. *Genetic*
20 *polymorphism of drug-metabolizing enzymes and styrene-induced DNA damage*.
21 Environ Mol Mutagen 2003; 41(4): 243-252.

22 76 Koskinen M, Vodicka P, Hemminki K. *Identification of 1-adenine DNA adducts in*
23 *workers occupationally exposed to styrene*. J Occup Environ Med 2001; 43(8): 694-700.

24 77 Murata Y, Natsume M, Iso T, Shigeta Y, Hirose N, Umano T, et al. *In vivo mutagenicity*
25 *assessment of styrene in MutaMouse liver and lung*. Genes and Environment 2023;
26 45(1): 12.

27 78 Plastics Europe (owner). *An Oral Gavage In Vivo Mutation Assay of Styrene at the cII*
28 *Locus in Transgenic Big Blue® Hemizygous B6C3F1 Mice (Unpublished report;*
29 *Available for consultation at The Health Council of The Netherlands)*. 2024.

30 79 Plastics Europe (owner). *Combined Pig-a, Micronucleus and Comet Study in B6C3F1*
31 *Mice after Oral Administration of Styrene (Unpublished report; Available for consultation*
32 *at The Health Council of The Netherlands)*. 2023.

80 Plastics Europe (owner). *Styrene: Mammalian Alkaline Comet Study in Male Fischer 344 Rats via Oral Gavage Administration for 28 days (Unpublished report; Available for consultation at The Health Council of The Netherlands)*. 2023.

81 Kligerman A, Allen J, Erexson G, Morgan D. *Cytogenetic studies of rodents exposed to styrene by inhalation*. IARC Sci Publ 1993; (127): 217-224.

82 Loprieno N, Presciuttini S, Sbrana I, Stretti G, Zaccaro L, Abbondandolo A, et al. *Mutagenicity of industrial compounds: VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyses*. Scand J Work Environ Health 1978: 169-178.

83 Sbrana I, Lascialfari D, Rossi AM, Loprieno N, Bianchi M, Tortoreto M, et al. *Bone marrow cell chromosomal aberrations and styrene biotransformation in mice given styrene on a repeated oral schedule*. Chem Biol Interact 1983; 45(3): 349-357.

84 Sharief Y, Brown AM, Backer LC, Campbell JA, Westbrook-Collins B, Stead AG, et al. *Sister chromatid exchange and chromosome aberration analyses in mice after in vivo exposure to acrylonitrile, styrene, or butadiene monoxide*. Environ Mutagen 1986; 8(3): 439-448.

85 Sinsheimer JE, Chen R, Das SK, Hooberman BH, Osorio S, You Z. *The genotoxicity of enantiomeric aliphatic epoxides*. Mutat Res 1993; 298(3): 197-206.

86 Fabry L, Leonard A, Roberfroid M. *Mutagenicity tests with styrene oxide in mammals*. Mutat Res 1978; 51(3): 377-381.

87 Preston R, Abernethy D. *Studies of the induction of chromosomal aberration and sister chromatid exchange in rats exposed to styrene by inhalation*. IARC Sci Publ 1993; (127): 225-233.

88 Sinha AK, Jersey GC, Linscombe VA, Adams RL, Mueller AM, McClintock ML. *Cytogenetic evaluation of bone marrow cells from rats exposed to styrene vapor for one year*. Fundam Appl Toxicol 1983; 3(2): 95-98.

89 Norppa H, Sorsa M, Vainio H. *Chromosomal aberrations in bone marrow of Chinese hamsters exposed to styrene and ethanol*. Toxicol Lett 1980; 5(3-4): 241-244.

90 Norppa H, Elovaara E, Husgafvel-Pursiainen K, Sorsa M, Vainio H. *Effects of styrene oxide on chromosome aberrations, sister chromatid exchange and hepatic drug biotrans-formation in chinese hamsters in vivo*. Chem Biol Interact 1979; 26(3): 305-315.

91 Engelhardt G, Gamer A, Vodicka P, Bárta I, Hoffmann HD, Veenstra G. *A re-assessment of styrene-induced clastogenicity in mice in a subacute inhalation study*. Archives of Toxicology 2003; 77(1): 56-61.

92   Vodicka P, Koskinen M, Vodicková L, Stetina R, Smerák P, Bárta I, et al. *DNA adducts, strand breaks and micronuclei in mice exposed to styrene by inhalation*. Chem Biol Interact 2001; 137(3): 213-227.

93   Simula AP, Priestly BG. *Species differences in the genotoxicity of cyclophosphamide and styrene in three in vivo assays*. Mutat Res 1992; 271(1): 49-58.

94   Gaté L, Micillino JC, Sébillaud S, Langlais C, Cosnier F, Nunge H, et al. *Genotoxicity of styrene-7,8-oxide and styrene in Fisher 344 rats: a 4-week inhalation study*. Toxicol Lett 2012; 211(3): 211-219.

95   Penttilä M, Sorsa M, Vainio H. *Inability of styrene to induce nondisjunction in Drosophila or a positive micronucleus test in the Chinese hamster*. Toxicol Lett 1980; 6(2): 119-123.

96   Vaghef H, Hellman B. *Detection of styrene and styrene oxide-induced DNA damage in various organs of mice using the comet assay*. Pharmacol Toxicol 1998; 83(2): 69-74.

97   Solveig Walles SA, Orsen I. *Single-strand breaks in DNA of various organs of mice induced by styrene and styrene oxide*. Cancer Lett 1983; 21(1): 9-15.

98   Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S. *Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow)*. Mutat Res 1997; 391(3): 201-214.

99   Tsuda S, Matsusaka N, Madarame H, Miyamae Y, Ishida K, Satoh M, et al. *The alkaline single cell electrophoresis assay with eight mouse organs: Results with 22 mono-functional alkylating agents (including 9 dialkyl N-nitrosoamines) and 10 DNA crosslinkers*. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 2000; 467(1): 83-98.

100   Kligerman AD, Allen JW, Erexson GL, Morgan DL. *Cytogenetic studies of rodents exposed to styrene by inhalation*. IARC Sci Publ 1993; (127): 217-224.

101   Conner MK, Alarie Y, Dombroske RL. *Sister chromatid exchange in murine alveolar macrophages, bone marrow, and regenerating liver cells induced by styrene inhalation*. Toxicol Appl Pharmacol 1980; 55(1): 37-42.

102   Clay P. *Styrene monomer does not induce unscheduled DNA synthesis in the mouse liver following inhalation exposure*. Mutagenesis 2004; 19(6): 489-492.

103   Mikeš P, Kořínek M, Linhart I, Krouželka J, Frantík E, Vodičková Ľ, et al. *Excretion of urinary N7 guanine and N3 adenine DNA adducts in mice after inhalation of styrene*. Toxicol Lett 2009; 184(1): 33-37.

1   104   Pauwels W, VodiˇĉČèka P, Severi M, Plná K, Veulemans H, Hemminki K. *Adduct*
2   *formation on DNA and haemoglobin in mice intraperitoneally administered with styrene*.
3   Carcinogenesis 1996; 17(12): 2673-2680.

4   105   Boogaard P, De Kloe K, Sumner S, Van Elburg P, Wong B. *Disposition of [ring-U-14C]*
5   *styrene in rats and mice exposed by recirculating nose-only inhalation*. Toxicological
6   Sciences 2000; 58(1): 161-172.

7   106   Boogaard PJ, de Kloe KP, Wong BA, Sumner SC, Watson WP, van Sittert NJ.
8   *Quantification of DNA adducts formed in liver, lungs, and isolated lung cells of rats and*
9   *mice exposed to 14C-styrene by nose-only inhalation*. Toxicological Sciences 2000;
10   57(2): 203-216.

11 107   Otteneder M, Lutz U, Lutz W. *DNA adducts of styrene-7, 8-oxide in target and non-*
12   *target organs for tumor induction in rat and mouse after repeated inhalation exposure to*
13   *styrene*. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis
14   2002; 500(1-2): 111-116.

15 108   Otteneder M, Eder E, Lutz WK. *32P-Postlabeling analysis of DNA adducts of styrene 7,*
16   *8-oxide at the O6-position of guanine*. Chemical Research in Toxicology 1999; 12(1):
17   93-99.

18 109   Linnainmaa K, Meretoja T, Sorsa M, Vainio H. *Cytogenetic effects of styrene and*
19   *styrene oxide on human lymphocytes and Allium cepa*. Scand J Work Environ Health
20   1978; 4 Suppl 2: 156-162.

21 110   Linnainmaa K, Meretoja T, Sorsa M, Vainio H. *Cytogenetic effects of styrene and*
22   *styrene oxide*. Mutat Res 1978; 58(2-3): 277-286.

23 111   Pohlová H, Rössner P, Srám RJ. *Cytogenetic analysis of human peripheral blood*
24   *lymphocytes in culture exposed in vitro to styrene and styrene oxide*. J Hyg Epidemiol
25   Microbiol Immunol 1984; 29(3): 269-274.

26 112   Jantunen K, Mäki-Paakkanen J, Norppa H. *Induction of chromosome aberrations by*
27   *styrene and vinylacetate in cultured human lymphocytes: dependence on erythrocytes*.
28   Mutat Res 1986; 159(1-2): 109-116.

29 113   Norppa H, Vainio H. *Induction of sister-chromatid exchanges by styrene analogues in*
30   *cultured human lymphocytes*. Mutation Research/Genetic Toxicology 1983; 116(3-4):
31   379-387.

32 114   Norppa H, Vainio H. *Genetic toxicity of styrene and some of its derivatives*. Scand J
33   Work Environ Health 1983: 108-114.

115 Norppa H, Vainio H, Sorsa M. *Metabolic activation of styrene by erythrocytes detected as increased sister chromatid exchanges in cultured human lymphocytes*. Cancer Res 1983; 43(8): 3579-3582.

116 Chakrabarti S, Duhr MA, Senécal-Quevillon M, Richer CL. *Dose-dependent genotoxic effects of styrene on human blood lymphocytes and the relationship to its oxidative and metabolic effects*. Environ Mol Mutagen 1993; 22(2): 85-92.

117 Lee S-H, Norppa H. *Effects of indomethacin and arachidonic acid on sister chromatid exchange induction by styrene and styrene-7, 8-oxide*. Mutation Research Letters 1995; 348(4): 175-181.

118 Bernardini S, Hirvonen A, Järventaus H, Norppa H. *Influence of GSTM1 and GSTT1 genotypes on sister chromatid exchange induction by styrene in cultured human lymphocytes*. Carcinogenesis 2002; 23(5): 893-897.

119 Laffon B, Pérez-Cadahía B, Pásaro E, Méndez J. *Individual sensitivity to DNA damage induced by styrene in vitro: influence of cytochrome P450, epoxide hydrolase and glutathione S-transferase genotypes*. Toxicology 2003; 186(1-2): 131-141.

120 Costa C, De Pasquale R, Silvari V, Barbaro M, Catania S. *In vitro evaluation of oxidative damage from organic solvent vapours on human skin*. Toxicology in Vitro 2006; 20(3): 324-331.

121 Matsuoka A, Hayashi M, Ishidates Jr M. *Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro*. Mutation Research/Genetic Toxicology 1979; 66(3): 277-290.

122 Ishidate M, Yoshikawa K. *Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation—a comparative study on mutagens and carcinogens*.Further Studies in the Assessment of Toxic Actions: Proceedings of the European Society of Toxicology Meeting, Held in Dresden, June 11–13, 1979. 1980. Springer: 1980.

123 Loprieno N, Abbondandolo A, Barale R, Baroncelli S, Bonatti S, Bronzetti G, et al. *Mutagenicity of industrial compounds: styrene and its possible metabolite styrene oxide*. Mutat Res 1976; 40(4): 317-324.

124 Beije B, Jenssen D. *Investigation of styrene in the liver perfusion/cell culture system. No indication of styrene-7, 8-oxide as the principal mutagenic metabolite produced by the intact rat liver*. Chem Biol Interact 1982; 39(1): 57-76.

125 De Raat W. *Induction of sister chromatid exchanges by styrene and its presumed metabolite styrene oxide in the presence of rat liver homogenate*. Chem Biol Interact 1978; 20(2): 163-170.

126 Norppa H, Tursi F, Einisto P. *Erythrocytes as a metabolic activation system in mutagenicity tests*. Mutagenese et toxicology genetique Editions INSERM, Paris, France 1985: 35-48.

127 Fontaine FR, DeGraaf YC, Ghaoui R, Sallustio BC, Edwards J, Burcham PC. *Optimisation of the comet genotoxicity assay in freshly isolated murine hepatocytes: detection of strong in vitro DNA damaging properties for styrene*. Toxicol In Vitro 2004; 18(3): 343-350.

128 Sina J, Bean C, Dysart G, Taylor V, Bradley M. *Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential*. Mutation Research/Environmental Mutagenesis and Related Subjects 1983; 113(5): 357-391.

129 Loprieno N, Presciuttini S, Sbrana I, Stretti G, Zaccaro L, Abbondandolo A, et al. *Mutagenicity of industrial compounds. VII. Styrene and styrene oxide: II. Point mutations, chromosome aberrations and DNA repair induction analyses*. Scandinavian Journal of Work, Environment and Health 1978; 4(2 SUPPL.): 169-178.

130 Nishi Y, Hasegawa MM, Taketomi M, Ohkawa Y, Inui N. *Comparison of 6-thioguanine-resistant mutation and sister chromatid exchanges in Chinese hamster V79 cells with forty chemical and physical agents*. Cancer Res 1984; 44(8): 3270-3279.

131 Oesch F, Herrero ME, Hengstler JG, Lohmann M, Arand M. *Metabolic detoxification: implications for thresholds*. Toxicologic pathology 2000; 28(3): 382-387.

132 Amacher DE, Turner GN. *Mutagenic evaluation of carcinogens and non-carcinogens in the L5178Y/TK assay utilizing postmitochondrial fractions (S9) from normal rat liver*. Mutation Research/Environmental Mutagenesis and Related Subjects 1982; 97(1): 49-65.

133 Dypbukt JM, Costa LG, Manzo L, Orrenius S, Nicotera P. *Cytotoxic and genotoxic effects of styrene-7, 8-oxide in neuroadrenergic Pc 12 cells*. Carcinogenesis 1992; 13(3): 417-424.

134 Donner M, Sorsa M, Vainio H. *Recessive lethals induced by styrene and styrene oxide in Drosophila melanogaster*. Mutation Research/Genetic Toxicology 1979; 67(4): 373-376.

135 Rodriguez-Arnaiz R. *Biotransformation of several structurally related 2B compounds to reactive metabolites in the somatic w/w+ assay of Drosophila melanogaster*. Environ Mol Mutagen 1998; 31(4): 390-401.

136 Del Carratore R, Giagoni P, Bauer C, Bronzetti G, Corsi C, Nieri R, et al. *Mutagenicity of styrene on metabolizing D7 strain of saccharomyces cerevisiae*. Boll Soc Ital Biol Sper 1983; 59(2): 233-238.

137 De Meester C, Poncelet F, Roberfroid M, Rondelet J, Mercier M. *Mutagenic activity of styrene and styrene oxide. A preliminary study [proceedings]*. Arch Int Physiol Biochim 1977; 85(2): 398-399.

138 de Meester C, Duverger-van Bogaert M, Lambotte-Vandepaer M, Mercier M, Poncelet F. *Mutagenicity of styrene in the Salmonella typhimurium test system*. Mutat Res 1981; 90(4): 443-450.

139 Poncelet F, De Meester C, Bogaert MD-v, Lambotte-Vandepaer M, Roberfroid M, Mercier M. *Influence of experimental factors on the mutagenicity of vinylic monomers*.Further Studies in the Assessment of Toxic Actions: Proceedings of the European Society of Toxicology Meeting, Held in Dresden, June 11–13, 1979. 1980. Springer: 1980.

140 Vainio H, Pääkkönen R, Rönnholm K, Raunio V, Pelkonen O. *A study on the mutagenic activity of styrene and styrene oxide*. Scand J Work Environ Health 1976; 2(3): 147-151.

141 Stoltz DR, Whitey RJ. *Mutagenicity testing of styrene and styrene epoxide in Salmonella typhimurium*. Bull Environ Contam Toxicol 1977; 17(6): 739-742.

142 Watabe T, Isobe M, Sawahata T, Yoshikawa K, Yamada S, Takabatake E. *Metabolism and mutagenicity of styrene*. Scand J Work Environ Health 1978; 4 Suppl 2: 142-155.

143 Busk L. *Mutagenic effects of styrene and styrene oxide*. Mutat Res 1979; 67(3): 291-298.

144 Florin I, Rutberg L, Curvall M, Enzell CR. *Screening of tabacco smoke constituents for mutagenicity using the Ames' test*. Toxicology 1980; 15(3): 219-232.

145 Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. *Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals*. Environ Mol Mutagen 1988; 11 Suppl 12: 1-157.

146 Brams A, Buchet J-P, Crutzen-Fayt M, De Meester C, Lauwerys R, Leonard A. *A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure)*. Toxicol Lett 1987; 38(1-2): 123-133.

147 Mamaca E, Bechmann RK, Torgrimsen S, Aas E, Bjørnstad A, Baussant T, et al. *The neutral red lysosomal retention assay and Comet assay on haemolymph cells from mussels (Mytilus edulis) and fish (Symphodus melops) exposed to styrene*. Aquatic toxicology 2005; 75(3): 191-201.

148 Sugiura K, Kimura T, Goto M. *Mutagenicities of styrene oxide derivatives on Salmonella typhimurium (TA 100): relationship between mutagenic potencies and chemical reactivity*. Mutat Res 1978; 58(2-3): 159-165.

149   Sugiura K, Goto M. *Mutagenicities of styrene oxide derivatives on bacterial test systems: relationship between mutagenic potencies and chemical reactivity*. Chem Biol Interact 1981; 35(1): 71-91.

150   Einistö P, Hooberman B, Sinsheimer J. *Base-pair mutations caused by six aliphatic epoxides in Salmonella typhimurium TA100, TA104, TA4001, and TA4006*. Environ Mol Mutagen 1993; 21(3): 253-257.

151   Wade D, Airy SC, Sinsheimer JE. *Mutagenicity of aliphatic epoxides*. Mutation Research/Genetic Toxicology 1978; 58(2-3): 217-223.

152   Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. *Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals*. Environ Mol Mutagen 1992; 19(S21): 2-141.

153   Głośnicka R, Dziadziuszko H. *Mutagenic action of styrene and its metabolites. II. Genotoxic activity of styrene, styrene oxide, styrene glycol and benzoic acid tested with the SOS chromotest*. Bull Inst Marit Trop Med Gdynia 1986; 37(3-4): 295-302.

154   Nakamura S-i, Oda Y, Shimada T, Oki I, Sugimoto K. *SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals*. Mutation Research Letters 1987; 192(4): 239-246.

155   von der Hude W, Seelbach A, Basler A. *Epoxides: comparison of the induction of SOS repair in Escherichia coli PQ37 and the bacterial mutagenicity in the Ames test*. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 1990; 231(2): 205-218.

156   Guyonnet D, Belloir C, Suschetet M, Siess M-H, Le Bon A-M. *Antimutagenic activity of organosulfur compounds from Allium is associated with phase II enzyme induction*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 2001; 495(1-2): 135-145.

157   Okun AH, Beaumont JJ, Meinhardt TJ, Crandall MS. *Mortality patterns among styrene-exposed boatbuilders*. Am J Ind Med 1985; 8(3): 193-205.

158   Ruder AM, Meyers AR, Bertke SJ. *Mortality among styrene-exposed workers in the reinforced plastic boatbuilding industry*. Occup Environ Med 2016; 73(2): 97-102.

159   Ruder AM, Bertke SJ. *Cancer incidence among boat-building workers exposed to styrene*. Am J Ind Med 2017; 60(7): 651-657.

160   Bertke SJ, Yiin JH, Daniels RD. *Cancer mortality update with an exposure response analysis among styrene-exposed workers in the reinforced plastics boatbuilding industry*. Am J Ind Med 2018; 61(7): 566-571.

161   Bertke SJ, Keil A, Daniels RD. *Lung Cancer Mortality and Styrene Exposure in the Reinforced Plastics Boatbuilding Industry: Evaluation of Healthy Worker Survivor Bias*. Am J Epidemiol 2021:

162   Daniels RD, Bertke SJ. *Exposure-response assessment of cancer mortality in styrene-exposed boatbuilders*. Occup Environ Med 2020; 77(10): 706-712.

163   Wong O. *A cohort mortality study and a case-control study of workers potentially exposed to styrene in the reinforced plastics and composites industry*. Br J Ind Med 1990; 47(11): 753-762.

164   Wong O, Trent LS, Whorton MD. *An updated cohort mortality study of workers exposed to styrene in the reinforced plastics and composites industry*. Occup Environ Med 1994; 51(6): 386-396.

165   Collins JJ, Bodner KM, Bus JS. *Cancer mortality of workers exposed to styrene in the U.S. Reinforced plastics and composite industry*. Epidemiology 2013; 24(2): 195-203.

166   Kogevinas M, Ferro G, Saracci R, Andersen A, Biocca M, Coggon D, et al. *Cancer mortality in an international cohort of workers exposed to styrene*. IARC Sci Publ 1993; (127): 289-300.

167   Kogevinas M, Ferro G, Andersen A, Bellander T, Biocca M, Coggon D, et al. *Cancer mortality in a historical cohort study of workers exposed to styrene*. Scand J Work Environ Health 1994; 20(4): 251-261.

168   Boffetta P, Sali D, Kolstad H, Coggon D, Olsen J, Andersen A, et al. *Mortality of short-term workers in two international cohorts*. J Occup Environ Med 1998; 40(12): 1120-1126.

169   Loomis D, Guha N, Kogevinas M, Fontana V, Gennaro V, Kolstad HA, et al. *Cancer mortality in an international cohort of reinforced plastics workers exposed to styrene: a reanalysis*. Occup Environ Med 2019; 76(3): 157-162.

170   Kolstad HA, Juel K, Olsen J, Lynge E. *Exposure to styrene and chronic health effects: mortality and incidence of solid cancers in the Danish reinforced plastics industry*. Occup Environ Med 1995; 52(5): 320-327.

171   Kolstad HA, Lynge E, Olsen J, Breum N. *Incidence of lymphohematopoietic malignancies among styrene-exposed workers of the reinforced plastics industry*. Scand J Work Environ Health 1994; 20(4): 272-278.

172   Kolstad HA, Pedersen B, Olsen J, Lynge E, Jensen G, Lisse I, et al. *Clonal chromosome aberrations in myeloid leukemia after styrene exposure*. Scand J Work Environ Health 1996; 22(1): 58-61.

173 Christensen MS, Hansen J, Ramlau-Hansen CH, Toft G, Kolstad H. *Cancer Incidence in Workers Exposed to Styrene in the Danish-reinforced Plastics Industry, 1968-2012*. Epidemiology 2017; 28(2): 300-310.

174 Christensen MS, Vestergaard JM, d'Amore F, Gørløv JS, Toft G, Ramlau-Hansen CH, et al. *Styrene Exposure and Risk of Lymphohematopoietic Malignancies in 73,036 Reinforced Plastics Workers*. Epidemiology 2018; 29(3): 342-351.

175 Nissen MS, Stokholm ZA, Christensen MS, Schlünssen V, Vestergaard JM, Iversen IB, et al. *Sinonasal adenocarcinoma following styrene exposure in the reinforced plastics industry*. Occup Environ Med 2018; 75(6): 412-414.

176 Coggon D. *Epidemiological studies of styrene-exposed populations*. Crit Rev Toxicol 1994; 24 Suppl: S107-115.

177 Coggon D, Ntani G, Harris EC, Palmer KT. *Risk of cancer in workers exposed to styrene at eight British companies making glass-reinforced plastics*. Occup Environ Med 2015; 72(3): 165-170.

178 Bond GG, Bodner KM, Olsen GW, Cook RR. *Mortality among workers engaged in the development or manufacture of styrene-based products--an update*. Scand J Work Environ Health 1992; 18(3): 145-154.

179 Graff JJ, Sathiakumar N, Macaluso M, Maldonado G, Matthews R, Delzell E. *Chemical exposures in the synthetic rubber industry and lymphohematopoietic cancer mortality*. J Occup Environ Med 2005; 47(9): 916-932.

180 Sathiakumar N, Graff J, Macaluso M, Maldonado G, Matthews R, Delzell E. *An updated study of mortality among North American synthetic rubber industry workers*. Occup Environ Med 2005; 62(12): 822-829.

181 Cruzan G, Cushman JR, Andrews LS, Granville GC, Miller RR, Hardy CJ, et al. *Subchronic inhalation studies of styrene in CD rats and CD-1 mice*. Fundamental and Applied Toxicology 1997; 35(2): 152-165.

182 Cruzan G, Bus J, Hotchkiss J, Sura R, Moore C, Yost G, et al. *Studies of styrene, styrene oxide and 4-hydroxystyrene toxicity in CYP2F2 knockout and CYP2F1 humanized mice support lack of human relevance for mouse lung tumors*. Regul Toxicol Pharmacol 2013; 66(1): 24-29.

183 Conti B, Maltoni C, Perino G, Ciliberti A. *Long-term carcinogenicity bioassays on styrene administered by inhalation, ingestion and injection and styrene oxide administered by ingestion in Sprague-Dawley rats, and para-methylstyrene administered by ingestion in Sprague-Dawley rats and Swiss mice*. Ann N Y Acad Sci 1988; 534: 203-234.

184 Brunnemann KD, Rivenson A, Cheng SC, Saa V, Hoffmann D. *A study of tobacco carcinogenesis XLVII. Bioassys of vinylpyridines for genotoxicity and for tumorigenicity in A/J mice*. Cancer Lett 1992; 65(2): 107-113.

185 Maltoni C, Failla G, Kassapidis G. *First experimental demonstration of the carcinogenic effects of styrene oxide; long-term bioassays on Sprague-Dawley rats by oral administration*. Med Lav 1979; 70(5): 358-362.

186 Maltoni C, Ciliberti A, Carretti D. *Experimental contributions in identifying brain potential carcinogens in the petrochemical industry*. Ann N Y Acad Sci 1982; 381: 216-249.

187 National Cancer Institute. *Bioassay of a solution of beta-nitrostyrene and styrene for possible carcinogenicity*. National Institute of Health; US Dep. of Health, Education, and Welfare; Public Health Service, Maryland, Technical Report Series No. 170, NCI-CG-TR-170, 1979.

188 National Cancer Institute. *Bioassay of styrene for possible carcinogenicity*. National Institute of Health; US Dep. of Health, Education, and Welfare; Public Health Service, Maryland, Technical Report Series No. 170, NCI-CG-TR-185, 1979.

189 Ponomarkov V, Tomatis L. *Effects of long-term oral administration of styrene to mice and rats*. Scand J Work Environ Health 1978; 4 Suppl 2: 127-135.

190 Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Bevan C, et al. *Chronic toxicity/oncogenicity study of styrene in CD-1 mice by inhalation exposure for 104 weeks*. J Appl Toxicol 2001; 21(3): 185-198.

191 Cruzan G, Bus JS, Banton MI, Sarang SS, Waites R, Layko DB, et al. *Editor's Highlight: Complete Attenuation of Mouse Lung Cell Proliferation and Tumorigenicity in CYP2F2 Knockout and CYP2F1 Humanized Mice Exposed to Inhaled Styrene for up to 2 Years Supports a Lack of Human Relevance*. Toxicol Sci 2017; 159(2): 413-421.

192 Beliles RP, Butala JH, Stack CR, Makris S. *Chronic toxicity and three-generation reproduction study of styrene monomer in the drinking water of rats*. Toxicological Sciences 1985; 5(5): 855-868.

193 National Toxicology Program. *Final report on carcinogens background document for styrene*. 2008; 2151-3805 (Electronic)

2151-3805 (Linking). https://www.ncbi.nlm.nih.gov/pubmed/20737009.

194 Cruzan G, Cushman JR, Andrews LS, Granville GC, Johnson KA, Hardy CJ, et al. *Chronic toxicity/oncogenicity study of styrene in CD rats by inhalation exposure for 104 weeks*. Toxicol Sci 1998; 46(2): 266-281.

1   195   Weil CS, Condra N, Haun C, Striegel JA. *Experimental Carcinogenicity and Acute*
2   *Toxicity of Representative Epoxides*. Am Ind Hyg Assoc J 1963; 24: 305-325.

3   196   Lijinsky W. *Rat and mouse forestomach tumors induced by chronic oral administration*
4   *of styrene oxide*. J Natl Cancer Inst 1986; 77(2): 471-476.

5   197   Ponomarkov V, Cabral JR, Wahrendorf J, Galendo D. *A carcinogenicity study of*
6   *styrene-7,8-oxide in rats*. Cancer Lett 1984; 24(1): 95-101.

7   198   Smit C, van Raaij M. *Factsheets for the (eco) toxicological risk assessment strategy of*
8   *the National Institute for Public Health and the Environment-Part IV*. 2005:

9   199   IARC. *Predictive value of rodent forestomach and gastric neuroendocrine tumours in*
10   *evaluating carcinogenic risks to humans*. 2003:

11   200   Ruder AM, Ward EM, Dong M, Okun AH, Davis-King K. *Mortality patterns among*
12   *workers exposed to styrene in the reinforced plastic boatbuilding industry: an update*.
13   Am J Ind Med 2004; 45(2): 165-176.

14   201   Coggon D, Osmond C, Pannett B, Simmonds S, Winter PD, Acheson ED. *Mortality of*
15   *workers exposed to styrene in the manufacture of glass-reinforced plastics*. Scand J
16   Work Environ Health 1987; 13(2): 94-99.

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Annex A

Annex A1 Summary table of mutagenicity in humans after styrene exposure

Table A1.1 Chromosomal aberration in humans after styrene exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Forni et al. (1988)	Milan, Italy, 1985–1986	Factory A, reinforced plastic laminates and insulating polymers; Factory B, small plastic boats manufacture	Peripheral blood	Factory A, 32 exposed; Factory B, 8 exposed, 40 controls	A, 123–249 (up to 1978), 1.7–17.0 (after 1978) mg/m3; B, 41–198 (after 1978) mg/ m3	Smoking, age, other exposures to mutagenic chemicals	+/– (Factory A, P < 0.001; Factory B, P < 0.05)	No clear results. No overall effect, only with high dosage (nested case-control)
Oberheitmann et al. (2001)	Germany, NR	Boat manufacturing	Peripheral blood	14 exposed, 7 controls	< 100 mg/m3 35 (1.5–211) µg/L styrene in blood	Smoking	+/–	Small study. Elevated, but not statistically significant effect
Jablonická et al. (1988)	Czechia, NR	Laminators of various kinds of sport utensils, boats, and containers	Peripheral blood	11 exposed, 11 controls	253 (118–582) mg/m3 NR (214–711) µL/mmol creatinine MA NR (50–175) µL/mmol creatinine PGA	Smoking, sex, alcohol consumption, drug intake, X-ray examination, rtg. therapy	–	Small study. No evidence for association

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Sorsa et al. (1991)	Finland, before 1991	Reinforced plastics production	Peripheral blood	109 exposed, 54 controls 70 exposed, 31 controls 50 exposed, 37 controls	Laminators, 43 (5– 182) ppm Others, 11 (1– 133) ppm (8 h TWA); laminators, 2.2 (SD, 2.4) nmol/L MA+PGA in urine	Age, smoking	– (P > 0.05)	Study report not accessible.
Hagmar et al. (1989)	Sweden, 1985–1986	Reinforced plastics production	Peripheral blood	11 exposed, 14 controls 20 exposed, 22 controls	43–221 mg/m3, 4–551 mg/m3 (1974–1986); 128 (< 6–317) mmol/mol creatinine, MA+PGA in urine (in 1985)	Smoking, age	– (P > 0.5)	Small study. No statistically significant effect
Mäki-Paakkanen (1987)	Finland, 1987	Reinforced plastics workers	Peripheral blood	21 exposed, 21 controls	98 (34–263) mg/m3; 1.6 (< LOD–7) mmol/L MA in urine	Smoking, sex	–	

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Pohlová & Srám (1985)	Czechia, before 1985	Two polystyrene plants: A, food vessel manufacturing; B, boat manufacturing	Peripheral blood	A, 36 exposed, 19 controls; B, 22 exposed, 22 controls	A, 70–150 (5.6–982.8) mg/ m3; B, ~200 (39–548) mg/ m3	Smoking, acute viral diseases, sex, drug intake The above plus X-ray examinations, alcohol	–	
Hansteen et al. (1984)	Norway, before 1984	Reinforced plastics production	Peripheral blood	(i) 11 exposed, (ii) 7 exposed; 9 controls	(i) 7.5 (2–13) ppm; (ii) 22.3 (14–44) ppm	Smoking, sex, age	– (P > 0.1)	Small study. No association
Thiess & Fleig (1978)	Germany, 1975	Polystyrene production plant	Peripheral blood	12 exposed, 12 controls	GM, 0.23 (0.02–46.92) ppm; NR (< 10–100) mg/L MA in urine	Age, sex, smoking, drug intake, acute viral diseases, X-ray examinations, vaccinations	–	Small study. No association
Somorovská et al. (1999); see also Vodička et al. (2001b)	Czech Republic, 1999	Reinforced plastics workers	Peripheral blood	17 high concentration (I), 12 medium concentration (II), 15 low concentration (III), 19 controls	I: 199.1 (SD, 101.6) mg/m3 II: 55.0 (SD, 22.9) mg/m3 III: 27.3.1 (SD, 25.1) mg/m3	Smoking	I: + (P < 0.001) II: + (P<0.004) III: + (P=0.0001) Frequency I: 3.75 ± 1.13 II: 3.27 ± 0.70 III: 2.50± 0.85	Small study. Effects in three different exposure groups with highest frequency of CA in high exposure group.

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Vodička et al. (2004)	Czech Republic, 2004	Reinforced plastics workers	Peripheral blood	86 exposed, 42 controls	81.3 (SD, 56.3) mg/m3		–	Adequate size. No association. Low exposure, but comparable to Somorovská et al (1999)
Helal & Elshafy (2013)	Egypt (El Oboor City), before 2013	Reinforced plastics production	Peripheral blood	40 exposed, 50 controls	1117 (SD, 64.52) µg/L in blood 246 (SD, 21.60) µmol/L MA in urine	Smoking, sex, socioeconomic status, age	+ (P < 0.001)	High level exposure
Camurri et al. (1983)	Italy, 1983	Reinforced plastics industries (six plants)	Peripheral blood	25 exposed, 22 controls	NR (30–400) mg/m3	Age, sex, smoking	+ (P < 0.005 for all 6 plants)	High level exposure
Andersson et al. (1980)	Sweden, 1978	Factory making boats from fibreglass-reinforced plastics	Peripheral blood	36 exposed, 37 controls 20 exposed, 21 controls	Low concentration, 137 (6–283) mg/m3; high concentration, 1204 (710–1589) mg/m3	Age, sex	+ (P < 0.001)	High level exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Tomanin et al. (1992)	Italy, 1990	Reinforced plastics production factories (Group 1, fibreglass tanks; Group 2, small boat production)	Peripheral blood	Group 1, 7 exposed, 7 controls; Group 2, 12 exposed, 12 controls	Group 1, NR (21–100); Group 2, NR (112–435) mg/m3 Group 1, 186 (46–345); Group 2 725 (423– 1325) mg/g creatinine MA in urine	Smoking, age, sex	II: + (P < 0.05) I: -	High level exposure. Less clear results
Nordenson & Beckman (1984)	Sweden, 1980	Fibreglass-reinforced polyester factory	Peripheral blood	15 exposed, 13 controls 12 exposed, 12 controls	24 ppm NR (< 2) mmol/L MA in urine	Sex, smoking	– (P > 0.05)	High level exposure.
Watanabe et al. (1983)	Japan, before 1983	Boat manufacturing	Peripheral blood	18 exposed, 6 controls	40–50 (NR) ppm	Smoking, age, sex	+/–	High level exposure
Artuso et al. (1995)	Italy, Viareggio, 1988–1990	Fibre-reinforced plastic boat factory	Peripheral blood	(i) 23 low concentration; (ii) 23 high concentration, 51 controls	(i) NR, 2–120; (ii) NR, 86–1389 mg/m3	NR	(+) (P < 0.01)	Disregard

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Mäki-Paakkanen et al. (1991)	Finland, before 1991	Reinforced plastics production in a plant manufacturing containers	Peripheral blood	17 exposed, 17 controls	300 (NR) mg/m3 (based on ACGIH conversion); 9.4 (< 1–21.5) mmol/L MA in urine	Age, sex, smoking, viral infections, vaccinations, other exposures to mutagenic chemicals, alcohol consumption, drug intake	+/– (one-sided P < 0.02)	Disregard
Meretoja et al. (1977)	Finland, 1977	Plants manufacturing polyester plastic products	Peripheral blood	10 exposed, 5 controls	NR	Sex	(+)	Disregard. Small study, no dose-response relationship
Dolmierski et al. (1983)	Poland, before 1983	Laminated styrene plates production	Peripheral blood	37 exposed, 2 controls	NR (< 100) mg/m3		+/–	Disregard. Small control group and lack of control for confounders
Meretoja et al. (1978)	Finland, 1976–1977	Reinforced plastics production, two plants	Peripheral blood	16 exposed, 6 controls	569.8 (55–3257) mg/g creatinine MA in urine in 1976 329.3 (53–1646) mg/g creatinine MA in urine in 1977	Smoking	+ (P < 0.001)	Disregard

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Fleig & Thiess (1978)	Finland, NR	Three plants: (i) styrene manufacturing; (ii) polystyrene production; (iii) unsaturated polyester resins processing	Peripheral blood	(i) 5 exposed; (ii) 12 exposed; (iii) 14 exposed, 20 controls	(i) NR (19–40) mg/L MA in urine; (ii) NR (< 5–100) mg/L MA in urine; (iii) NR (102 to > 1500) mg/L MA in urine		+/–	Disregard. Small study, lack of control for confounders. Suggestion of a dose-response relationship.
Smejkalová et al. (1989)	Czech Republic, 1989?	Workers occupationally exposed to styrene	Peripheral blood	13 women exposed, 6 women controls	225 (83–366) mg/m3	Sex	+	Disregard. Small study
Högstedt et al. (1979)	Sweden, 1977	Plant manufacturing polyester resin boats	Peripheral blood	6 exposed, 6 controls	115 (50–400) mg/m3	Sex, age, smoking	(+) (P = 0.001)	Disregard. Small study
Mierauskiene et al. (1993)	Lithuania, before 1993	Chemical plant	Capillary blood	109 exposed, 64 controls	NR (< 1.9 ppm) in year before sampling	Sex, smoking	(+) (P < 0.01)	Disregard
Lazutka et al. (1999)	Lithuania, (i) 1983–1984; (ii) 1985–1986	Two plants: (i) carpet production; (ii) plastics production	Peripheral blood	(i) 79 exposed; (ii) 97 exposed, 90 controls	(i) NR (0.13–1.4) mg/m3; (ii) NR (4.4–6.2) mg/m3	Smoking, age	(+) (P < 0.0001)	Disregard

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Tates et. al (1994)	Germany, 1990	Container and board manufacturing (plus dichloromethane exposure)	Peripheral blood	46 exposed, 23 controls 46 exposed, 22 controls 46 exposed, 23 controls 45 exposed, 5 of 23 controls	70 (0–598) mg/m3 (8 h TWA)	Smoking, age, sex	(+) (P <0.0001)	Disregard. Co-exposure

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

2

1   *Table A1.2 Micronuclei in humans after styrene exposure*

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Migliore et al. (2006)	Italy, NR	Reinforced plastics production	Peripheral blood	92 exposed, 98 controls	37.1 (2–535) mg/m3; 300.0 (10.2–1856) mg/g creatinine MA+PGA in urine	Smoking, sex, age	+ (P < 0.001)	Adequate study size with statistically significant association
Yager et al. (1993)	USA, before 1993	Reinforced plastic boat manufacturing facility	Peripheral blood	48 exposed	64.2 (0.88–235.35) mg/m3 (8 h TWA)	Smoking, sex, age	– (P > 0.05)	No association. Subjects are own control (before/after exposure)
Högstedt (1984)	Sweden, 1983	Reinforced plastics and polyester resins workers	Peripheral blood	38 exposed, 20 controls	13 (1–40) ppm (8 h TWA)	Sex	+ (P = 0.005)	Positive association. Small study.
Sorsa et al. (1991)	Finland, before 1991	Reinforced plastics production	Peripheral blood	109 exposed, 54 controls 70 exposed, 31 controls 50 exposed, 37 controls	Laminators, 43 (5– 182) ppm Others, 11 (1–133) ppm (8 h TWA); laminators, 2.2 (SD, 2.4) nmol/L MA+PGA in urine	Age, smoking	–	No association. High exposure

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Hagmar et al. (1989)	Sweden, 1985–1986	Reinforced plastics production	Peripheral blood	11 exposed, 14 controls 20 exposed, 22 controls	43–221 mg/m3, 4–551 mg/m3 (1974–1986); 128 (< 6–317) mmol/mol creatinine, MA+PGA in urine (in 1985)	Smoking, age	– (P > 0.5)	No association. Small study.
Mäki-Paakkanen (1987)	Finland, 1987	Reinforced plastics workers	Peripheral blood	21 exposed, 21 controls	98 (34–263) mg/m3; 1.6 (< LOD–7) mmol/L MA in urine	Smoking, sex	–	No association. Small study.
Vodička et al. (2004)	Czech Republic, 2004	Reinforced plastics workers	Peripheral blood	86 exposed, 42 controls	81.3 (SD, 56.3) mg/m3		+ (P = 0.002)	
Hanova et al. (2010)	Czech Republic, 2010	Reinforced plastics workers	Peripheral blood	62 exposed, 50 controls	50.3 (0–238) mg/m3	Smoking	– (P > 0.05)	No association
Godderis et al. (2004)	Belgium, 2000–2001	Reinforced plastics industries	Peripheral blood, nasal mucosa	38 exposed, 41 controls (blood); 23 exposed, 17 controls (nasal mucosa)	9.5 (SD, 9.6) ppm (converted from urine)	Smoking, alcohol consumption, age	+ (P < 0.05)	

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Tomanin et al. (1992)	Italy, 1990	Reinforced plastics production factories (Group 1, fibreglass tanks; Group 2, small boat production)	Peripheral blood	Group 1, 7 exposed, 7 controls; Group 2, 12 exposed, 12 controls	Group 1, NR (21–100); Group 2, NR (112–435) mg/m3 Group 1, 186 (46–345); Group 2 725 (423– 1325) mg/g creatinine MA in urine	Smoking, age, sex	– (P > 0.05)	High Level exposure. No statistically significant association in both groups
Nordenson & Beckman (1984)	Sweden, 1980	Fibreglass-reinforced polyester factory	Peripheral blood	15 exposed, 13 controls 12 exposed, 12 controls	24 ppm NR (< 2) mmol/L MA in urine	Sex, smoking	+ (one-sided P = 0.00017)	Effect was seen in the group of 12 exposed workers.
Mäki-Paakkanen et al. (1991)	Finland, before 1991	Reinforced plastics production in a plant manufacturing containers	Peripheral blood	17 exposed, 17 controls	300 (NR) mg/m3 (based on ACGIH conversion); 9.4 (< 1–21.5) mmol/L MA in urine	Age, sex, smoking, viral infections, vaccinations, other exposures to mutagenic chemicals, alcohol consumption, drug intake	–	Small study

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Results[a]	Comments committee
Tates et. al (1994)	Germany, 1990	Container and board manufacturing (plus dichloromethane exposure)	Peripheral blood	46 exposed, 23 controls 46 exposed, 22 controls 46 exposed, 23 controls 45 exposed, 5 of 23 controls	70 (0–598) mg/m3 (8 h TWA)	Smoking, age, sex	(+) (P <0.0001)	Disregard. Co-exposure, styrene secondary exposure

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

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

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Comments committee
Naccarati et al. (2003)	Italy, Tuscany, before 2002	Reinforced plastics production	Semen	18 out of 46 exposed, 13 out of 27 controls	292.5 (20.8–947.8) mg/g creatinine MA in urine	Smoking, age, alcohol consumption	+/– (P > 0.05)	No overall association, positive association among exposed non-smokers (n=6)

2   [a]+/–, equivocal (variable response in several experiments within an adequate study).
3
4   *Tabel A1.4 Gene mutation in humans after styrene exposure*

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Comments committee
Compton-Quintana et al. (1993)	Berkeley, USA, 1993	Boat manufacturing and maintenance workers	Peripheral blood	15 high concentration 22 low concentration	32 ppm 1.2 ppm (8 h TWA)		+ (P = 0.028)	Association effected by smoking.
Bigbee et al. (1996)	Finland, 1996	Reinforced plastics workers	Peripheral blood	47 exposed, 47 controls	37 (6–114) ppm (8 h TWA)	Age, smoking, sex	– (P = 0.058) + (p=0.036)	No overall association. Statistically significant association among highly exposed.
Vodička et al. (2001b)	Czechia, 1999	Reinforced plastics workers	Peripheral blood	19 exposed, 19 controls	101.2 (SD, 102.4) mg/m$_3$		+/– (P > 0.05)	Two outliers in exposed group.

Reference	Location, date	Setting	Sampling matrix	No. of subjects	Mean (range)	Covariates controlled	Result (significance)[a]	Comments committee
Vodička et al. (1999)	Czechia, 1995	Reinforced plastics workers	Peripheral blood	13 exposed, 13 controls	68.0 (15–156) mg/m$_3$	Smoking	(+) (P = 0.039)	
Vodička et al. (1995)	Czechia, 1993–1994	Hand-lamination workers	Peripheral blood	9 exposed, 15 controls	91 (25–250) mg/m$_3$	Smoking	+ (P = 0.021)	No association compared to factory controls, but association found compared to laboratory controls.

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

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

Table A2.1 Chromosomal aberration in animals after styrene exposure

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Kligerman et al. (1993)	Rat, F344 (F)	Lymphocytes	500 ppm	Inhalation, 6 h/d, 14 d	–
Sinha et al. (1983)	Rat, Sprague- Dawley (M, F)	Bone marrow	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 1 yr	–
Preston & Abernethy (1993)	Rat, F344 (M)	Peripheral blood lymphocytes	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	–
Kligerman et al. (1993)	Mouse B6C3F$_1$ (F)	Lung, spleen	500 ppm	Inhalation, 6 h/d, 14 d	–
Loprieno et al. (1978)	Mouse, CD1 (M, F)	Bone marrow	1000 mg/kg	Gavage, single dose (1×), 24 h after treatment	–
Sbrana et al. (1983)	Mouse, CD-1 (M)	Bone marrow	200 × 70, 500 × 4 mg/kg	Oral, 4 or 70 mg/kg per day	–
Sharief et al. (1986)	Mouse, C57BL/6 (M)	Bone marrow	1000 mg/kg bw	Intraperitoneal injection, BrdU-labelled M1 cells 16 h after BrdU implantation	–
Norppa et al. (1980)	Hamster, Chinese (M)	Bone marrow	300 ppm	Inhalation, 6 h/d, 5 d/wk, 4 d or 3 wk	–

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

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

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Fabry et al. (1978)	Mouse, BALB/c (M)	Fetus	250 mg/kg bw	Intraperitoneal injection, mate after 1–3 wk	–

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

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

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3   *Table A2.3 Micronuclei in animals after styrene exposure*

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

Reference	Species, strain, (sex)	Tissue	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Results[a]
Engelhardt et al. (2003)	Mouse, NMRI (NR)	Bone marrow (PCE)	1500 mg/m3	Inhalation, 6 h/d, 1–21 d	–
Simula & Priestly (1992)	Mouse, LACA Swiss (M)	Bone marrow (PCE)	600 mg/kg	Intraperitoneal injection, 48 h after treatment	+
Norppa (1981)	Mouse, C57BL/6 (M)	Bone marrow (PCE)	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment	+
Penttilä et al. (1980)	Hamster, Chinese (M)	Bone marrow	1000 mg/kg bw	Intraperitoneal injection, 30 h after treatment	–

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

2

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

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

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

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

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

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

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

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

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

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

4

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

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

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

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

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

2   [a] +, positive; the level of significance was set at $P < 0.05$ in all cases.
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4   *Table A3.5 Gene mutation in in vitro human cells after styrene-7,8-oxide exposure*

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)
Bastlová et al. (1995), Bastlová & Podlutsky (1996)	Human lymphocytes, peripheral blood mononuclear cells, and T-lymphocytes	0.2 mM [24 µg/mL]	+, Hprt locus

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

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

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
Ishidate & Yoshikawa (1980)	Chinese hamster, lung cells	100 µg/mL	-	(+)
Matsuoka et al. (1979)	Chinese hamster, lung cells	250 µg/mL	-	(+)

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

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

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

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

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

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

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

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1   *Table A3.8 Gene mutation in in vitro mammal cells after styrene exposure*

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

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

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5   *Table A3.9 Gene mutation in in vitro mammal cells after styrene-7,8-oxide exposure*

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

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

8

1 *Table A3.10 Chromosomal aberration in in vitro micro-organism cells after styrene exposure*

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

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

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4 *Table A3.11 Chromosomal aberration in in vitro micro-organism cells after styrene-7,8-oxide exposure*

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

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

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7 *Table A3.12 Aneuploidy in in vitro micro-organism cells after styrene exposure*

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

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

9

1   *Table A3.13 Gene mutation in in vitro micro-organism cells after styrene exposure*

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

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
Bauer et al. (1980)	Saccharomyces pombe P1	2080 µg/mL	Forward mutation		-
Del Carratore et al. (1983)	Saccharomyces cerevisiae D7	104 µg/mL	Reverse mutation	+	
Vainio et al. (1976)	Salmonella typhimurium TA100	52 µg/mL	Reverse mutation	(+)	(+)
De Meester et al. (1977)	Salmonella typhimurium TA100, TA1537, TA1538, and TA98	100 µmol/plate [5200 µg/mL]	Reverse mutation	-	-
Stoltz & Whitey (1977)	Salmonella typhimurium TA100, TA1535, TA1537, TA1538, and TA98	500 µg/mL	Reverse mutation	-	-
Watabe et al. (1978)	Salmonella typhimurium TA100, TA1535, TA1537, TA1538, and TA98	250 µg/mL	Reverse mutation		-
Busk (1979)	Salmonella typhimurium TA100, TA1535, TA1537, TA1538, and TA98	104 µg/mL	Reverse mutation	-	-
De Flora (1979)	Salmonella typhimurium TA100, TA1535, TA1538, and TA98	250 µg/mL	Reverse mutation	-	-
Florin et al. (1980)	Salmonella typhimurium TA100, TA1535, TA1537, and TA98	312 µg/mL	Reverse mutation	-	-
De Meester et al. (1981)	Salmonella typhimurium TA100	1000 µg/mL	Reverse mutation	-	+
Brams et al. (1987)	Salmonella typhimurium TA100 and TA98	500 µg/mL	Reverse mutation	-	-

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
De Meester et al. (1981)	Salmonella typhimurium TA1530	0.02 µg/mL	Reverse mutation	+	+
Vainio et al. (1976)	Salmonella typhimurium TA1535	0.5 µg/mL	Reverse mutation	-	+
De Meester et al. (1977)	Salmonella typhimurium TA1535	52 µg/mL	Reverse mutation	-	+
Poncelet et al. (1980)	Salmonella typhimurium TA1535	521 µg/mL	Reverse mutation	NT	+
De Meester et al. (1981)	Salmonella typhimurium TA1535	1000 µg/mL	Reverse mutation	-	+
Vainio et al. (1976)	Salmonella typhimurium TA1537, TA1538, and TA98	52 µg/mL	Reverse mutation	-	-
De Meester et al. (1981)	Salmonella typhimurium TA1537, TA1538, and TA98	1000 µg/mL	Reverse mutation	-	-
Zeiger et al. (1988)	Salmonella typhimurium TA97, TA98, TA100, TA1535, and TA1537	1666 µg/plate	Reverse mutation	-	-

1 [a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+), positive/negative in a study of limited quality (e.g. only a single dose
2 tested; data or methods not fully reported); the level of significance was set at P < 0.05 in all cases.
3 [b] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+), positive/negative in a study of limited quality (e.g. only a single dose
4 tested; data or methods not fully reported); the level of significance was set at P < 0.05 in all cases.
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1   *Table A3.14 Gene mutation in in vitro micro-organism cells after styrene-7,8-oxide exposure*

Reference	Tissue, cell line	Concentration (LEC or HIC) or dose (LED or HID)	Endpoint	Results[a] (no metabolic activation)	Results[b] (with metabolic activation)
Donner et al. (1979)	Drosophila melanogaster	1000 µg/mL, inhalation	Sex-linked recessive lethal mutations	+	NA
Loprieno et al. (1976)	Saccharomyces cerevisiae	1200 µg/mL	Gene conversion	+	NT
Loprieno et al. (1976)	Schizosaccharomyces pombe	600 µg/mL	Forward mutation	+	NT
Voogd et al. (1981)	Klebsiella pneumoniae	120 µg/mL	Forward mutation	+	NT
Milvy & Garro (1976)	Salmonella typhimurium TA100	200 µg/mL	Reverse mutation	+	NT
Vainio et al. (1976)	Salmonella typhimurium TA100 and TA1535	0.6 µg/mL	Reverse mutation	+	+
de Meester et al. (1977)	Salmonella typhimurium TA100	60 µg/mL	Reverse mutation	+	+
Watabe et al. (1978)	Salmonella typhimurium TA100	250 µg/mL	Reverse mutation	+	NT
Busk (1979)	Salmonella typhimurium TA100	120 µg/mL	Reverse mutation	+	+
Yoshikawa et al. (1980)	Salmonella typhimurium TA100	240 µg/mL	Reverse mutation	+	+
De Flora (1979)	Salmonella typhimurium TA100 and TA1535	NR	Reverse mutation	+	+
Sugiura & Goto (1981)	Salmonella typhimurium TA100	144 µg/mL	Reverse mutation	+	NT

Turchi et al. (1981)	Salmonella typhimurium TA100	120 µg/mL	Mutation	+	NT
Pagano et al. (1982)	Salmonella typhimurium TA100	48 µg/mL	Reverse mutation	+	NT
Glatt et al. (1983)	Salmonella typhimurium TA100	60 µg/mL	Reverse mutation	+	NT
Hughes et al. (1987)	Salmonella typhimurium TA100	500 µg/mL	Reverse mutation	+	+
Einistö et al. (1993)	Salmonella typhimurium TA100	60 µg/mL	Reverse mutation	+	NT
Sinsheimer et al. (1993)	Salmonella typhimurium TA100	120 µg/mL	Reverse mutation	+	NT
Brams et al. (1987)	Salmonella typhimurium TA100	300 µg/mL	Reverse mutation	+	NT
de Meester et al. (1981)	Salmonella typhimurium TA100, TA1530, and TA1535	768 µg/mL	Reverse mutation	+	+
Zeiger et al. (1992)	Salmonella typhimurium TA104	100 µg/plate	Reverse mutation	+	+
Guyonnet et al. (2001)	Salmonella typhimurium TA100	1200 µg/plate	Reverse mutation	+	+
Einistö et al. (1993)	Salmonella typhimurium TA104	120 µg/mL	Reverse mutation	+	NT
Milvy & Garro (1976)	Salmonella typhimurium TA1535, TA1537, TA1538, and TA98	5000 µg/mL	Reverse mutation	+	NT
Vainio et al. (1976)	Salmonella typhimurium TA1535	0.60 µg/mL	Reverse mutation	+	+

de Meester et al. (1977)	Salmonella typhimurium TA1535	24 µg/mL	Reverse mutation	+	+
Stoltz & Whitey (1977)	Salmonella typhimurium TA1535	125 µg/mL	Reverse mutation	+	+
Loprieno et al. (1978)	Salmonella typhimurium TA1535	60 µg/mL	Reverse mutation	+	+
Wade et al. (1978)	Salmonella typhimurium TA1535	250 µg/mL	Reverse mutation	(+)	NT
Watabe et al. (1978)	Salmonella typhimurium TA1535	50 µg/mL	Reverse mutation	+	NT
Busk (1979)	Salmonella typhimurium TA1535	60 µg/mL	Reverse mutation	+	+
El-Tantawy & Hammock (1980)	Salmonella typhimurium TA1535	60 µg/mL	Reverse mutation	+	NT
De Flora (1981)	Salmonella typhimurium TA1535	NR	Reverse mutation	+	+
de Meester et al. (1981)	Salmonella typhimurium TA1535	768 µg/mL	Reverse mutation	+	+
Milvy & Garro (1976)	Salmonella typhimurium TA1537, TA1538, and TA98	5000 µg/mL	Reverse mutation	-	NT
Vainio et al. (1976)	Salmonella typhimurium TA1537	600 µg/mL	Reverse mutation	-	-
de Meester et al. (1977)	Salmonella typhimurium TA1537	6000 µg/mL	Reverse mutation	-	-
Wade et al. (1978)	Salmonella typhimurium TA1537 and TA98	NR	Reverse mutation	-	NT

Watabe et al. (1978)	Salmonella typhimurium TA1537	250 µg/mL	Reverse mutation	(+)	NT
El-Tantawy & Hammock (1980)	Salmonella typhimurium TA1537 and TA98	500 µg/mL	Reverse mutation	-	NT
de Meester et al. (1981)	Salmonella typhimurium TA1537	1150 µg/mL	Reverse mutation	-	-
Vainio et al. (1976)	Salmonella typhimurium TA1538	6 µg/mL	Reverse mutation	-	+
de Meester et al. (1977)	Salmonella typhimurium TA1538 and TA98	6000 µg/mL	Reverse mutation	-	-
Watabe et al. (1978)	Salmonella typhimurium TA1538 and TA98	250 µg/mL	Reverse mutation	-	NT
De Flora (1981)	Salmonella typhimurium TA1537, TA1538, and TA98	NR	Reverse mutation	-	-
de Meester et al. (1981)	Salmonella typhimurium TA1538 and TA98	1150 µg/mL	Reverse mutation	-	-
Vainio et al. (1976)	Salmonella typhimurium TA98	600 µg/mL	Reverse mutation	-	-
Ueno et al. (1978)	Salmonella typhimurium TA98	250 µg/mL	Reverse mutation	-	-
Zeiger et al. (1992)	Salmonella typhimurium TA98	3333 µg/plate	Reverse mutation	-	-
Brams et al. (1987)	Salmonella typhimurium TA97	300 µg/mL	Reverse mutation	+	NT
Einistö et al (1993)	Salmonella typhimurium TA4001	240 µg/mL	Reverse mutation	+	NT

Einistö et al. (1993)	Salmonella typhimurium TA4006	960 µg/mL	Reverse mutation	(+)	NT
Sugiura et al. (1978)	Escherichia coli WP2 uvrA	720 µg/mL	Reverse mutation	+	NT
Sugiura & Goto (1981)	Escherichia coli WP2 uvrA	480 µg/mL	Reverse mutation	+	NT

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

4

1 Annex B

2 Annex B1 Summary table of carcinogenicity in humans after styrene exposure
3 *Table B1.1 Boat builders study in Washington State, USA.*

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Bertke et al. (2021), [161] Daniels et al. (2020), [162] Bertke et al. (2018), [160] Ruder et al. (2017), [159] Ruder et al. (2016), [158] Ruder et al. (2004), [200] Okun et al. (1985), [157] 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.	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,	Job exposure was also possible to acetone (TWA 50.6 ppm plat A and 54.3 ppm plant B), fibrous glass (not measured), and at much lower concentrations (no quantitative data) to glycols, anhydrides, cobalt hapthenate, and methyl ethyl ketone peroxide or benzoyl peroxide, in the high exposure departments; in other departments exposure was possible to wood dusts, paints, ergonomic stress, and solvents such as toluene, xylenes, and naphtas, and

Left censoring: 1959 (use of styrene started in 1957) Right censoring: end 1978 for exposure and work histories Inclusion criteria: employed ≥ 1 day in glass fiber-reinforced plastic and composites boat manufacturing between 1959 and 1978. Study population: 5,163 boatbuilders working at one of two boat building facilities in Kelso (plant A) and Bellingham (plant B), Washington, USA. Reference population : general population in the state Washington Number of exposed and non-exposed; total amount of person-years;	plant B. Low exposure estimated at 5 ppm/day Job histories and demographic data were extracted from company personnel records Classification of jobs based on level of styrene exposure as evaluated based on in-depth industrial hygiene surveys Cumulative exposure calculated with life table analysis system Statistical analyses: Mostly calculation of standardised mortality ratios (SMR), both for overall mortality and cause-specific (cancer) deaths, and 95% CI's based on Poisson distribution	For cancer incidence, see specific studies		solvents, wood dust, or wood finishing agents No information on hospitalisation Left truncation in 1959, but use of styrene in plants started only in 1957 Work-history records did not indicate specific job titles, with a large range of exposures among jobs classified as high exposure. Therefore misclassification of exposure not to be excluded	isocyanates. These exposures were not assessed, but mentioned as being possible at the job Information on exposure of cohort members since 1978 not available. In 1978 at time of job data collection 772 workers were still employed
Ruder et al. (2017), [159] See general information above Study population:	See general information above for exposure assessment	Health outcomes: Cancer incidence evaluated as standardised incidence	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-	See also general information above Healthy worker effect not assessed	See also general information above Regarding this study:

3,704 out of 5,203 workers;
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 various sources, was created in order to ascertain residence in Washington State during 1991-2007.
580 classified as potentially high exposure group
Censoring:
Workers who left Washington State or died before end 2007 censored at date of migration or death.
Reference population:
Age and calendar specific cancer incidence rates of Washington State from the Washington State Cancer Registry.
Follow-up:

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

Statistical analyses:
See also general information above.
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

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 bladder cancers. Diagnosis dates assigned to June 30 if only year known (only two cases).

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:
Cancer trachea, bronchus, lung SIR 1.11 (0.89-1.37). In high exposure SIR 1.42 (1.00-1.95)
Lymphatic and haematopoetic cancers SIR overall 1.03 (0.77-1.35); SIR high exposure 0.99 (0.59-1.57); low exposure 1.05 (0.73-1.46)
Ovarian cancer in high exposure SIR 2.26 (0.62-5.78)

High exposure versus low exposure:
All cancers together increased, but none of specific cancers, except buccal and pharyngeal cancer:
All cancers SRR 1.28 (1.05-155)
Trachea, bronchus and lung cancer SRR 1.41 (0.87-2.29)

Workers > 1 year employment:
Trachea, bronchus and lung cancer SRR 0.66 (0.33-1.34)

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

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 power)
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

Vital status through end 2011					follow-up. Together with relatively small sample size, this implies power to detect excess cancer incidence low Analyses were performed using the NIOSH LTAS.NET life table analysis system
Bertke et al. (2018), [160] See general information above Study population: 5,201 workers (after 2 removed for missing birth date resp. duplicate entry), of whom 1960 in high exposure group Reference population: General population of Washington State, 1960-2014. Censoring: Exposure person-time for workers still active in 1978 truncated at October 1, 1988 Follow-up:	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 Two exposure metrics used: Ever/never worked in high exposure job Employment duration (administrative jobs excluded). Exposure duration lagged 10 years and for workers still employed in 1978 exposure truncated at	Health outcomes: See information above, with follow-up extended to 2016. 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 1979 (start NDI) and 19 emigrants classified as 'vital status unknown' and censored at date last observed	Mortality: Total person-years at risk 203,404 with 2111 deaths (41% of cohort) 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) Lung cancer SMR 1.37 (1.19-1.57) whole cohort; employment ≥1 year SMR 1.20 (0.95-1.51) Cox regression *Exposed versus not exposed:*	See also general information above 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 plasticians workers) As above, no information on lifestyle-related factors, in particular smoking and alcohol. For internal analyses, persons employed in the administrative group removed because potentially confounding	See also general information above Regarding this study: 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 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

Additional follow-up since 2011 through 2016 using the (US) National Death Index (NDI)	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) Statistical analyses: See also general information above. Calculation of Standardised mortality ratios (SMRs) as ratio of expected versus observed 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	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) *Duration employed* in high exposure group (log-linear): 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) Duration employed in high exposure group (2 piece spline, RRs for slope of first piece of spline): 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)	lifestyle and socioeconomic factors 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 spline	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 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)). 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

	(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 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), [162] See general information above Study population: 5,163 workers (after removal of 38 workers with inadequate information), 87% male and 93% Caucasian. Of those, 1958 working directly with styrene Reference population: General US population. Censoring: Date last observed or December 31, 2016; Exposure person-time	See further general information above for exposure assessment. For this study exposure assessment extended to a job-exposure matrix describing individual cumulative exposure as continuous variable reflecting changes in exposure potential over time: Exposure scientists blinded to case status Work history based on job titles and department	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, 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)	See also general information above Healthy worker effect not assessed Those working directly with styrene on average worked shorter (1.18 years versus 1.85 years) Cumulative exposures (unlagged) were highly positively skewed (mean 31 versus median 5.7 ppm-years). This might have detracted from validity of model As above, no information on lifestyle-related	Compared to previous studies, this one used more detailed employment records and exposure assessment 46 workers (< 1%) lost to follow-up Average age at end of follow-up 68 years and average length of employment < 2 years, with 68% employed < 1 year The 'unit' of 50 ppm-years the HRs were expressed in was based on the NIOSH

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	assignments and linked to exposure levels Exposure levels measured as described above (general information) Individual jobs and departments categorised into similar exposure groups by plant based on expert judgement (19 for plant A, 13 for plant B) Individual cumulative exposure calculated in ppm-years by summing product of exposure (group-specific mean styrene airborne concentrations) and duration spent in each group Statistical analysis: *Cox proportional hazards regression* Hazard ratios (HRs) per) expressed as per 50 ppm-years with zero exposure as reference; risk-sets matched on race, gender, birth data (5 years margin),	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	Pancreas 0.84 (0.43-1.13) Respiratory (overall) 0.87 (0.71-1.02) Lung 0.87 (0.70-1.02) Urinary tract (overall) 1.18 (0.97-1.37) Kidney 1.12 (0.80-1.37) Bladder and other urinary 1.27 (0.95-1.61) Lymphatic and haematopoietic (overall) 1.19 (0.99-1.37) Non-Hodgkin 1.10 (0.58-1.51) Multiple myeloma 1.18 (0.80-1.56) Leukemia 1.21 (0.93-1.49) Myeloid leukemia 1.33 (0.86-1.83) Same as above with SES adjustment Only minor differences *Analyses restricted to exposure < 500 ppm-years* Of note (without SES adjustment): Urinary tract overall 1.43 (1.11-1.79) Bladder and other urinary 1.64 (1.14-2.33) Lymphatic and haemopoietic cancers overall 1.37 (1.09-1.69) Leukemia 1.46 (1.04-1.97)	factors. Potential effect of smoking was explored by considering smoking-associated cancers: no association observed To avoid overestimation of risk at higher exposures, the linear slope between 0-50 ppm-years was used for risk projection. This might have resulted in underestimation of effect size In general: this study strongly depended on modelling and underlying assumptions To account for mortality from competing sources life table analysis was used, under assumption that relative risk is independent of age. Assumption might be incorrect Further modelling assumption was that increased leukemia risk is persistent, proportional to	Recommended Exposure Limit SES was not included in previous studies. Here it was approximated by category of first job held, related to an occupational prestige score (range 0-100) Analyses were performed using the NIOSH LTAS.NET life table analysis system Relatively small study (low statistical power)

and employment duration
(< 1 year versus ≥ 1 years).
Timescale was age
Exposure-response relation
modelled with restricted
cubic splines, and full and
trimmed loglinear models
Exposure lagged 10 years
Only outcomes with at least
10 deaths modelled
Models adjusted for
attained age, sex, race, 5-
year birth cohort,
employment duration. In
sensitivity analysis also
adjustment for
socioeconomic status
(SES).
95% CIs based on profile
likelihood

*Working lifetime leukemia
risks estimation*
Done with a hypothetical
model using the derived
leukemia HR and a few
assumptions (see further).
Risk expressed as styrene
concentration causing one
extra leukemia case per

(no cases in persons with
cumulative exposure ≥ 500 ppm-
years)

Restricted cubic spline models at
50 ppm-years (95% CI):
Urinary 2.39 (1.92-3.25)
Kidney 2.39 (1.92-3.83)
Bladder 6.20 (3.93-11.83)
Lymphatic and haematopoietic
4.32 (3.00-6.56)
Non-Hodgkin 0.01 (Not calculable-
3.52)
Multiple myeloma 34 (14.08-
96.94)
Leukemia 4.10 (2.88-7.29)
Myeloid leukemia 11.67 (6.31-
30.76)

Furthermore, these models
showed much higher risks at low
exposures than did loglinear
models

Sensitivity analyses:
Model estimates without lag
similar to those with 10-year lag
Leukemia findings not appreciably
different when person-time for
active workers after 1978 included

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

10,000 workers exposed over a working lifetime.

Subgroup analyses:
Outcomes in a major category with indication of positive exposure-response association
Separate analysis restricted to male baseline mortality and incidence rates
Separate analysis in those with exposure < 500 ppm-years

Latency analysis:
Models without exposure lag
Grid search over a range of lags (2-40 years)
Time since last exposure among cases, using restricted cubic splines

Sensitivity analysis:
Leukemia models without person-time truncation

Latency analysis
Best-fitted lags > 10 years for all cancers; longest lags for non-Hodgkin and multiple myelomas (both 40 years), shortest for kidney cancer (33 years)
Median time since last exposure (TSLE) ranged from 28 years (kidney cancer) to 35 years (multiple myeloma)

Risk projection
Estimate of leukemia risk under 10-year lag with trimmed data: linear slope 0.0088 per ppm-year, corresponding to extra risk of 1/10,000 for a 45-year continuous exposure to 0.05 ppm styrene (sex-averaged rate) or 0.03 ppm (male only rates)

1

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

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Loomis et al. (2019), [169] Christensen et al. (2018), [174] Nissen et al. (2018), [175] Coggon et al. (2015), [177] Boffetta et al. (1998), [168] Kolstad et al. (1995), [170] (1 of 6 countries) Kogevinas et al. (1994), [167] Kolstad et al. (1994) , [171](1 of 6 cohorts countries) Kogevinas et al. (1993), [166] Coggon et al. (1987), [201] (1 of 6 countries) Study population: 37,021-40,688 (all cohorts combined)) workers at reinforced plastics production plants in the 6 countries, organised into 8 subcohorts. Inclusion criteria:	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
Ever employed at one of the plants included in the eight subcohorts					
Loomis et al. (2019), [169] Retrospective cohort study See Kogevinas et al. (1994). Here only differences mentioned. Study population: 37,021 reinforced plastics workers at reinforced plastics production plants in the 5 countries. The cohort from Norway (had contributed 9% of person-time) was excluded due to new privacy protection legislation. Furthermore, no new mortality data were added for the English and Danish cohorts Reference population:	See Kogevinas et al. (1994). Here only differences mentioned. Exposure categories Exposed (laminators, production workers with mixed tasks or in small plants, and workers who regularly entered areas where styrene was handled) versus unexposed Measurements: -In addition to first study, here mentioned around 18,000 measurements of styrene metabolites mandelic and phenoglyoxylic acid in urine. Exposures before 1965 set equal to Denmark data at 200 ppm and then linearly declining	Health outcomes: Mortality from specific cancers. Health assessment ICD 8 and 9 codes of previous study Kogevinas et al. (1994) were regrouped into WHO classification. 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	Total number of person-years 506,459, of which 407,459 in exposed jobs, and 61,514 of those with exposure duration ≥ 5 years. *Exposed versus unexposed workers*: All-cause mortality RR 1.01 (95% CI 0.89-1.14) All cancer mortality RR 1.01 (0.81-1.17) Oesophageal cancer mortality RR 3.50 (0.46-26.82) Prostate cancer mortality RR 1.85 (0.81-6.15) Other cancers RR round 1	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
Unexposed jobs in the cohort Follow-up: Varied per country. Overall: 1945-1991. Mean follow-up 12.8 years. Lost to follow-up approximately 3% Left censoring: First data for which complete payroll records were available for those already employed at start follow-up	to arithmetic mean of earliest measurement -Mean exposure estimated at 63.1 ppm (in exposed jobs) and mean cumulative exposure at 158.0 ppm-years using the job exposure matrix. Statistical analysis: Poisson regression, ungrouped form (equal to discrete time hazard model), to calculate (hazard) rate ratios (RRs) with likelihood-based 95% CIs. -Follow-up time as time axis (person-year). -Adjustment for age, calendar time, sex, country (all categorically) length of follow-up and time since first exposure (both continuous), with retainment in model of those that changed RR 'appreciably' (not specified). Various exposure indicators were used: exposed versus unexposed, employment as	and histiocytic tissue (202) and chronic lymphoid leukemia (201.1) and multiple myeloma (203) were aggregated under non-Hodgkins' lymphoma. Acute and chronic myeloid leukemia (ICD 8/9 205.0 and 205.1) were combined.	*Most highly exposed workers (laminators) versus unexposed* Oesophageal cancer mortality RR 2.71 (1.00-7.37) Pancreas cancer mortality RR 1.18 (0.53-2.61) Prostate cancer mortality RR 1.85 (0.64-5.36) *Exposed workers employed 2-< 5 years or > 5 years versus those employed , <2 years* Non-Hodgkin's lymphoma (NHL) mortality RR 1.40 (0.51-3.79) Pancreas cancer mortality RR 2.12 (0.93-4.38) No increase in mortality > 5 years, except for prostate cancer mortality RR 1.35 (0.57 to 3.16) and lung cancer Lung cancer:		

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

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			RR 1.89 (1.17-3.06) per 100 ppm. Sensitivity analysis: Exclusion of workers exposed before 1970 resulted in lung cancer mortality RR, cumulative exposure, 1.11 (1.02-120)		

1

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

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
General information cohort study in Collins et al. (2013), [165] Wong et al. (1994), [164] Wong et al. (1990), [163] Retrospective cohort study US wide study 15,908 workers (number for first study, Wong et al. (1990)) at 30 reinforced plastics manufacturing plants, selected based on	Exposure assessment based on work histories and occasional measurements Work history assessment: Based on employment records, "record job title lists" were generated for each cohort member. Jobs were grouped according to similar exposure potential, taking into account	Health outcomes: Mortality and cancer-Deaths and cause specific mortality. Health assessment Deaths among active employees and annuitants identified through company records Vital status of ex-employees through social security administration	See individual studies	Risk of exposure misclassification is difficult to evaluate, as exposure measurements are not described No information for the whole cohort on other potential toxic exposures (including smoking), during employment, outside work, during follow-up, and prior to follow-up	Most workers exposed relatively shortly: only 22.1% employed at least 5 years Regarding exposure assessment: this was done by a consulting firm, but no information is provided on the measurements performed. Entire cohort was assumed to be white (only 1.3% non-white)

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
study feasibility; 24.4% women Period of exposure 1948-1977 Inclusion criteria: Having worked in an area with potential styrene exposure at any of the 30 plants for at least six months, in the period 1948-1977 Reference population: General white US population (information on race missing for most of cohort, therefore assumed to be white) Follow-up: Latest 2008 (Collins et al.) Censoring: Left: 1 January 1948 Right: those lost to follow-up were censored at last	weighted average exposure values (ppm, categorised into 10 ppm increments) and peak range exposures (ppm). The final result was a grouping of jobs in 173 exposure categories Exposure assessment: Not clear how this was performed: 'with help of a consultancy firm', who visited individual plants around 1980 and performed measurements. Wong et al. (1994) mentions that time weighted average exposures for jobs ranged from 1-200 ppm. In addition to the consultancy firm there was routine exposure monitoring Measures of exposure: Cumulative exposure grouped into tertiles: 5-	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)		Also no information on socioeconomic status, which could be a confounder (would lead to more expected deaths) No assessment of HWSB	

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
date of contact (mostly end of employment)	<500 ppm; ≥500-<5,000 ppm; ≥5,000 ppm Duration of exposure Peak exposures Mean exposure Time since first exposure (employment) Statistical analyses: Calculation of (age, sex and calendar year) standardised mortality ratios (SMR) (as percentages) Cause-specific deaths standardised for age, race, and five-year periods (1948-1977) Mortality in relation to exposure				
Collins et al. (2013), [165] See general information above Number of cohort members reduced to 15,826 after removal of duplicates and revision of work histories	See general information above In addition: Four measures of exposure were examined:	See general information above Deaths were in addition identified from Social Security data, the National	Total person-years 561,530, 5,026 (32%) deaths identified Whole cohort All-cause mortality SMR 1.08 (95% CI 1.05-1.11)	See general information above	See general information above For this study also information was used that at 19 plants asbestos was used (but exposure levels or area specific usage

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
Follow-up: End of 2008	Cumulative exposure: Mean time-weighted average exposure for an 8-hour workday estimated at 28 ppm. Peak exposure was set at 100 ppm and 15 minutes of the working day above that limit, and days with at least one peak counted. 100 ppm based on lowest level at which irritation occurs. Mean number of peaks across workers was 113; 6% had > 5 years of cumulative peak exposures. Mean duration of exposure was 4.3 years. Average exposure: the arithmetic mean of average exposure was obtained by dividing total cumulative exposure by total cumulative duration. Statistical analysis: Cox proportional hazards for	Center for Health Statistics and a commercial bureau Causes of death coded by a nosologist according ICD version in effect at time of death.	All cancers SMR 1.12 (1.05-1.18) All lymphatic and haematopoietic cancers SMR 0.84 (0.69-1.02) Respiratory system cancers (ICD10 C30-C39) SMR 1.34 (1.23-1.45) Non-Hodgkin's lymphoma SMR 0.72 (0.50-1.00) Leukemia SMR 0.84 (0.60-1.14) Pancreatic cancer SMR 0.96 (0.73-1.22) Lung cancer SMR 1.34 (1.23-1.46) Diabetes mellitus SMR 1.29 (1.09-1.51) Ischaemic heart disease SMR 1.08 (1.02-1.15) Nonmalignant respiratory disease SMR 1.15 (1.05-1.27) Restricted to at least 15-year latency similar results (only minor changes in SMRs)		patterns not known). Seems no effect on lung cancer Lost to follow-up reduced to < 1 % Average exposure were lower in 1977 (25 ppm) than a decade earlier (35 ppm) Entire cohort was assumed to be white (only 1.3% non-white)?? No nested case-control study to examine cigarette smoking as potential causes of excess of death, but lung cancer deaths and other deaths commonly related to cigarette smoking including bladder cancer; kidney cancer; bronchitis, emphysema, and asthma; and heart disease were examined in more detail

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
	cumulative time-weighted averages (units of 100 ppm-months), adjusted for sex, year of hire and year of birth, with age as time scale Exposure-response trend for smoking related cancers		Subgroup analysis according to asbestos use at plant showed somewhat higher SMRs for lung cancer at asbestos using versus not asbestos using plants: SMR 1.35 (1.23-1.48) versus 1.30 (1.05-1.58). Similarly for bronchitis, emphysema and asthma: 1.42 (1.21-1.65) versus 1.04 (0.69-1.51) Analysis per cumulative exposure categories (with cut-offs 150 ppm-months, 400, and 1,200 ppm-months (only P-values shown for significant trends): Lung cancer: P trend = 0.003 Kidney cancer: P trend = 0.045 All heart diseases: P trend = 0.028		

Study design and population	Exposure assessment	Health assessment	Results	Bias/confounding	Remarks
			Cox proportional hazards: Pancreatic cancer HR 1.008 (1.002-1.015), but poor model fit (P=0.196) Kidney cancer HR 1.009 (1.000-1.017) Analysis per peak exposure categories There are no major differences among the risk estimates of the four exposure categories. No trends with peak exposures are seen.		

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Annex B2. Summary table of carcinogenicity tests with styrene in animals

Table B2.1 Oral studies with styrene in mice

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality

NCI, 1979a [187]	Mice B6C3F1
	
	Males and females
	Controls: 20/sex
	Exposed:
	50/sex/dose group
		Test item:
		Styrene (70%) and β-nitrostyrene (30%) in corn oil
		
		Oral gavage
		3 times/week
		78 weeks
		
		Dosing males/females (expressed in β-nitrostyrene)
		0 (vehicle)
		87.5 mg/kg bw
		175 mg/kg bw
		
		Endpoint:
		14 weeks after the treatment
		
		Observations:
		Full necropsies and histopathological examinations were performed on all animals.
			Survival and body weight:
			- In males: a dose-response relation for mortality (P=0.007).
			- in females: mean body weight was decreased in 175 mg/kg bw group
			
			Non-neoplastic lesions:
			- haemorrhage and necrosis in the liver of males:
			  0 mg/kg: 1/20
			  87.5 mg/kg: 3/50
			  175 mg/kg: 16/50
			
			Neoplastic lesions:
			- Combined lung alveolar/bronchiolar carcinoma or adenomas in <u>males</u>:
			  0 mg/kg: 0/20
			  87.5 mg/kg: 11/49 (P=0.016)
			  175 mg/kg: 2/36
				Statistical analyses:
				- Survival: Kaplan Meier
				- Dose-response relations: Cox method with Tarone's extension
				- Tumour incidence: Fisher exact test (with Bonferroni correction)
				- Cochran-Armitage test for linear trend in proportions
				
				Authors report one-tailed p-values
					Non-GLP.
					Non-guideline.
					
					Authors conclude that there is no convincing evidence for carcinogenicity in mice as lung tumors increased at medium dose, not at highest dose.
					
					Lower tumour incidence in highest dose group is probably due to high mortality rate.
					
					For mice, the Fisher Exact was significant; the Cochran-Armitage not significant
					
					Not clear what happened to 14 males lost in high dose group.
					
					Only mice surviving at least 52 weeks included.
					Survival till end was respectively:

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
					18/20, 43/50, 33/55

NCI 1979b [188]	Mice B6C3F1
	
	Males and females
	Controls: 20/sex
	Exposed:
	50/sex/dose group
		Test item:
		Styrene (in corn oil).
		Purity not mentioned.
		
		Oral gavage
		5 days/week
		78 weeks
		
		Dosing males/females
		0 (vehicle)
		150 mg/kg bw
		300 mg/kg bw
		
		Endpoint:
		13 weeks after treatment
		
		Observations:
		Full necropsies and histopathological examinations were performed on all animals.
			Survival and body weight:
			- In males: mortality was increased in all dose groups. –
			In females: slight dose-related mean body weight depression, mortality was not affected.
			
			Neoplastic lesions:
			- Combined lung alveolar/bronchiolar carcinoma or adenomas in <u>males</u>:
			0 mg/kg bw: 0/20
			150 mg/kg bw: 6/44
			300 mg/kg bw: 9/43 (P=0.024)
			
			- Hepatocellular adenomas in <u>females</u>:
			  0 mg/kg: 0/20
			  150 mg/kg: 1/44
			  300 mg/kg: 5/43 (P=0.034)
				Survival: Kaplan Meier
				- Dose-response relations: Cox method with Tarone's extension
				- Tumour incidence: Fisher exact test (with Bonferroni correction)
				- Cochran-Armitage test for linear trend in proportions
				
				Authors report one-tailed p-values.
					Non-GLP.
					Non-guideline.
					
					The study authors note a large variation and higher incidence in occurrence of lung tumours in untreated historical control male mice compared to the vehicle controls in the current study.

Ponomarkov et al., 1978 [189]	Mice O20 Pregnant dams Control: 9	Test item: Styrene (in olive oil) Purity: 99% Oral gavage	Survival: - Preweaning mortality was higher in the styrene group (43% versus 22% in olive oil controls).	No details on statistics. Percentage of tumour bearing animals expressed in relation to	Non-GLP. Non-guideline. An increase in lung tumours was observed,

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	Exposed: 29 Offspring males Control: 20 Exposed: 45 Offspring females Control: 22 Exposed: 39 Extra control group Males: 54 Females: 47	Single administration on day 17 of gestation (pregnant dams) Weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan. Dosing: 0 (olive oil or untreated) 1350 mg/kg bw Endpoint: 120 weeks Observations: Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned. Treatment of offspring was suspended after 16 weeks due to toxicity.	- High mortality in styrene progeny group: at 20 weeks, 50% of <u>males</u> and 20% of <u>females</u> died. Observed lesions: liver necrosis, spleen hypoplasia, congestion of lungs. - Average age of death: 32 weeks (males, styrene), 49 weeks (females, styrene), 88 weeks (vehicle males), 85 weeks (vehicle females). Observed lesions (survival <45 weeks): liver inflammation around necrosis area, bronchitis and peribronchitis. Observed lesions (survival>45 weeks): abscess cavities in liver, calcium deposits. Neoplastic lesions: -Increased incidence in total tumour bearing animals in offspring of styrene-treated dams in <u>males</u> (styrene: 89%, vehicle: 52%) and <u>females</u> (styrene: 100%, vehicle: 67%).	the effective number of animals.	although the background level of lung tumours in these mice is already high. These mice are highly susceptible to developing lung tumours. Lung tumours were more likely to occur in the group treated with styrene (effective number). The average age of animals with lung tumour was lower in the progeny treated with styrene.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			- Increase in lung tumours in treated offspring of styrene-treated dams in <u>males</u> (styrene: 89%, vehicle: 42%) and <u>females</u> (styrene: 100%, vehicle: 67%), P<0.01 for both sexes. - Incidence adenocarcinomas in <u>male progeny:</u> Untreated: 12/53 (22.6%) Olive oil: 4/19 (21.1%) Styrene: 8/23 (34.8%) - Incidence adenocarcinomas in <u>female progeny:</u> 14/47 (29.8%) 4/8 (50.0%) 4/21 (19.0%), 7/20 (35.0%) 18/32 (56.2%)* - Lung tumours occurred earlier in styrene-treated group compared to control. Average age of death in mice with lung tumours differed: <u>males</u> (styrene: 49 weeks, vehicle: 84 weeks) and <u>females</u> (styrene: 58 weeks, vehicle: 85 weeks).		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Ponomarkov et al., 1978 [189]	Mice C57 BL Pregnant dams Control: 5 Exposed: 15 Offspring males Control: 12 Exposed: 27 Offspring females Control: 13 Exposed: 27 Extra control group Males: 51 Females: 49	Test item: Styrene (in olive oil) Purity: 99% Oral gavage Single administration on day 17 of gestation (pregnant dams), weekly administration to offspring from the time of weaning. Offspring treated for whole lifespan. Dosing: 0 (olive oil or untreated) 300 mg/kg bw Endpoint: 120 weeks Observations: Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.	Litter size, preweaning mortality, offspring mortality and body weights did not differ between the groups. Neoplastic lesions: - Increased incidence in tumour-bearing females receiving a single styrene administration during pregnancy. - Lymphomas in <u>females</u> styrene: 10/12 olive oil: 3/5 untreated: 20/47; not statistically significant). - Hepatocellular carcinomas or adenoma in <u>males</u>: styrene: 3/24; olive oil: 1/12; untreated: 1/47	No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non-GLP. Non-guideline. Increased incidence of lymphomas is not statistically significant. Dosage is much higher in the 020 mice than in the BL mice. Only one dose tested, which does not provide enough information about a dose-response relationship.

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2 *Table B2.2 Inhalation studies with styrene in mice*

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Cruzan et al., 2001 190	Mice CD-1 Chronic/oncogenicity study and a follow-up study 70/sex/group Males 104 weeks Females 98 weeks Follow-up study: 55 males	Test item: Styrene (Purity: >99.5%) Inhalation, styrene vapour, whole body, 6h/day 5 days/week for 104 weeks (males), 98 (females) weeks or 13 weeks (males, follow-up). Dosing: 0, 20, 40, 80, and 160 ppm (equivalent of 0, 85, 170, 341, and 682 mg/m3)a Follow up study: 0, 40, and 80 ppm (equivalent of 0, 170, and 341 mg/m3)a Interim kills: 10 animals/sex/group terminated at week 52 and 78.	Blood levels of styrene and styrene-7,8-oxide were proportional to the exposure concentration. Survival, observations and body weight: - At 160 ppm, 1 female died during the first week and a second died in the second week (both with hepatocyte necrosis). Inhalation of styrene had no effect on survival of male mice. - No effects of styrene exposure on the appearance, behaviour or clinical observations. - Weight gain was decreased in males (80 ppm: -23%; 160 ppm: -31%) and females (160 ppm: -15%). Food consumption decreased in these groups. - No effect on water consumption. Neoplastic lesions:	Tumour incidence was analysed using methodology described by IARC (1980). Other pathologic data were analysed using Fisher's exact test.	GLP-study. Lung: - Increased incidence in areas of bronchioloalveolar hyperplasia in males (40, 80 and 160 ppm) ppm and in females (all exposures) after 24 months. However, the dose-response varies between de groups.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Full necropsies and full histopathological examinations were performed on all control and 160 ppm animals.	- No effects at week 52 and 78 interim necropsies. Terminal necropsy: - Total number of tumour bearing mice in females Control: 27 20 ppm: 34 40 ppm: 37 (P<0.05) 80 ppm: 28 160 ppm: 37 (P<0.05) - Incidence of bronchioloalveolar adenomas in males Control: 15/50 20 ppm: 21/50 40 ppm: 35/50 (P<0.05) 80 ppm: 30/50 (P<0.05) 160 ppm: 33/50 (P<0.05) - Incidence of bronchioloalveolar adenomas in females Control: 6/50 20 ppm: 16/50 (P<0.05) 40 ppm: 16/50 (P<0.05); 80 ppm: 11/50 160 ppm (24/50).		

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

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			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. Follow-up study: - No effects in lungs at all exposures. 80 ppm: - After single exposure: single-cell necrosis in olfactory epithelium of mice.		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			- After 2, 4 and 7 exposures, increase in degree of lesions and changes in the Bowman's glands. - After 40 or 65 exposures: more pronounced atrophy and disorganization leading to respiratory metaplasia. - No recovery occurred. 40 ppm: - Minimal focal changes to the olfactory epithelium; the effects became slightly more severe.		
Cruzan et al., 2017 191	Mice CD-1 Mice C57BL/6 wild-type (WT) Mice CYP2F2(–/–) (KO) Mice 2B6-transgenic (TG), CYP2F2(–/–) 2F1,2A13, 6-7 weeks old	Test item: Styrene monomer PO-11 Bulk Grade (CAS No. 100-42-5, 99.95% pure) Inhalation 6h/day, 5 days/week, except holidays Dosing: 0, 120 ppm (equivalent to 0, 511 mg/m3)a styrene vapor	- No signs of styrene-induced toxicity in any of the 4 strains of mice based on general observations of behavior or activity. - CD-1, WT and KO mice exposed to styrene weighed less than controls (2-13%; 2-10%; up to 7% respectively). No difference with TG mice. - Mean body weights were lower compared to control at 1, 52 and	Body weight: one-way ANOVA Survival: Kaplan and Meier procedure Lung neoplasms and nonneoplastic lesions: Fisher's Exact test	Non-GLP. Non-guideline. An inhibitor of styrene polymer formation, t-butyl catechol, was added to the styrene by the producer at 10–15 ppm. Not clear how long exposure took place. In this study, no increased tumour incidence was found in CD-1 mice, which contradicts the findings of

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	75 animals per group males only Chronic/oncogenicity study (focussing on lung)		78 weeks (CD-1 mice P<0.05), at 1, 24, 52 and 78 weeks (WT mice P<0.05) and at 24 and 78 weeks (KO mice P<0.05). - Cell proliferation in terminal bronchioles was 4- to 5-fold increased at week 1 in exposed CD-1 and WT mice (P<0.05). Non-neoplastic lesions: - Increased incidence of epithelial cell degeneration in terminal bronchioles occurred in WT and CD-1 mice at 1 and 26 weeks (3, 4 or 5 out of 5 mice) and in WT mice at 52 and 78 weeks (1 out of 5 mice). Overall, the incidence up to 104 weeks of exposure CD-1 mice:10/53 WT mice: 34/50 - Hyperplasia occurred in terminal bronchioles in exposed CD-1 mice exposed at week 1, 26, 78 or 104 (P<0.05 at this time point). Overall incidence		the previous study conducted by Cruzan et al. in 2001.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			Control: 0/67 Exposed: 50/67 - Hyperplasia occurred in the terminal bronchioles in WT mice at week 1, 26, 52, 78 and 104 ($P<0.05$ at this time point). Overall incidence Control: 0/69 Exposed: 55/70 Neoplastic lesions: No statistical significant increase in lung adenomas or adenocarcinomas		

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2 *Table B2.3 Oral studies with styrene in rats*

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Beliles et al., 1985 192	Rats Charles River COBS (SD) BR Male: Control: 76 Exposure: 50	Test item: Styrene (in deionised water) Purity: 98.9% Oral, drinking water	Weekly analytical mean concentrations were approximately 90% of nominal concentrations. Survival: not significantly different from controls.	- No statistics for tumour incidences - Dunnet's t-test or Wilcoxon Rank sum test for other parameters	Non-GLP. Non-guideline. Only the results of the chronic toxicity segment are reported in this table and the text below.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	Female: Control: 106 Exposure: 70	Continuous exposure for 2 years Nominal dose 0 (vehicle) 8.9 mg/kg bw/day 17.9 mg/kg bw/day Chronic toxicity (and three-generation reproduction study) Males (10-15) and females (20-30) from each group were mated after 90 days and returned to chronic toxicity study after weaning; Endpoint: At 52 weeks, 10 rats/sex/group were sacrificed.	Clinical findings: decreased mean terminal body weight and increased relative brain weight (250 ppm females; $P<0.05$), water consumption decreased (125 ppm and 250 ppm males and females; $P<0.05$; dose-response effect). Non-neoplastic lesions: non-treatment related pathological changes across all groups, no details reported. Neoplastic lesions: no significant increase in treatment-related tumour incidences in rats treated for two years.		No symptoms were reported. This study is negative but not very informative. Applied dose levels were not high enough due to lack of toxicity. There is no reduced survival due to exposure, which was the case in the oral gavage study in mice.
NCI, 1979a 187	Rats F344	Test item: Styrene (70%) and β-	Survival was not affected by styrene.	- Survival: Kaplan Meier	Non-GLP. Non-guideline.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	Males and females Control: 20/sex Exposed: 50/sex/dose group	nitrostyrene (30%) (in corn oil). Oral, via gavage 3 times/week 79 weeks Dosing: Males: 0 (vehicle) 150 mg/kg bw 300 mg/kg bw Females: 0 (vehicle) 75 mg/kg bw 150 mg/kg bw Endpoint: Animals were sacrificed 29 weeks after the treatment period. Full necropsies and histopathological examinations were performed on all animals.	Mean body weight was decreased in male rats (300 mg/kg bw) compared to control. No significant effects in tumour incidences	- Dose-response relations: Cox method with Tarone's extension - Tumour incidence: Fisher exact test (with Bonferroni correction) - Cochran-Armitage test for linear trend in proportions	Authors report one-tailed p-values. No effects were found in rats exposed to mixture of styrene (70%) and β-nitrostyrene (30%). These results suggest that rats may be less sensitive to the effects of styrene compared to mice.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
NCI 1979b 188	Rats F344 Males and females Control: 2 control groups of 20/sex Exposed: 50/sex/dose group	Test item: Styrene (in corn oil). Purity not mentioned. Oral exposure via gavage. Dosing: 0 (two groups), 500, 1000 and 2000 mg/kg bw, 5 days per week Exposure for 78 weeks for 0 (first control), 1000 and 2000 mg/kg bw group, for 103 weeks for 0 (second control) and 500 mg/kg bw rats. Endpoint: Rats were sacrificed at 27 weeks (1000 and 2000 mg/kg bw) or 1 week (500 mg/kg bw) after the exposure period.	Mortality was significantly higher in male and female rats compared to control (both P<0.001, 2000 mg/kg bw). Slight dose-related mean body weight depression was observed in males. Neoplastic lesions: There was no significant increase in tumour incidences.	- Survival: Kaplan Meier - Dose-response relations: Cox method with Tarone's extension - Tumour incidence: Fisher exact test (with Bonferroni correction) - Cochran-Armitage test for linear trend in proportions	Non-GLP. Non-guideline. Authors report one-tailed p-values. No effects were found in rats exposed to styrene. These results suggest that rats may be less sensitive to the effects of styrene compared to mice.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Initially groups were 60/sex/dose, this was reduced to 50 due to excessive mortality in week 8 of the study. The 500 mg/kg bw group and extra control group were added later. Full necropsies and histopathological examinations were performed on all animals.			
Ponomarkov et al., 1978 189	Rats BD IV Pregnant dams Control:10 Exposed: 21 Offspring males Control: 36 Exposed: 73 Offspring females Control: 39 Exposed: 71	Test item: Styrene (in olive oil) Purity: 99% Oral, via gavage Dosing: 0 (vehicle), and 1350 mg/kg bw (dams) or 500 mg/kg bw (offspring) Single administration on day 17 of gestation (pregnant dams), weekly	Survival and body weights: Preweaning mortality in offspring of styrene-treated pregnant females increased (offspring, styrene: 10%; offspring, olive oil: 2.5%). No differences in survival or body weights. Non-neoplastic lesions: Several lesions in all animals such as congestion of lung and kidney and necrotic areas in liver, forestomach and kidney.	No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non-GLP. Non-guideline. There is no increased incidence of tumourgenis observed in rats, unlike the observations in 020 mice. This indicates strain dependency.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		administration to offspring from the time of weaning. Offspring treated for whole lifespan. Endpoint: All animals were sacrificed at 120 weeks Full necropsies and histopathological examinations were performed on all animals.	Neoplastic lesions: - 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.		
Maltoni et al., 1982 186	Rat Sprague-Dawley Males and females: 40/sex/group	Test item: Styrene (purity not stated, in olive oil) Oral gavage 4-5 days/week 52 weeks	Total brain tumour bearing animals in males: Control: 0/40; 50 mg/kg bw/day: 1/40 250 mg/kg bw/day: 1/40 Total brain tumour bearing animals in females:	Statistics not reported.	Non-GLP Non-guideline Limited reporting on data and methods.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Dosing 0 (vehicle) 50 mg/kg bw/day 250 mg/kg bw/day Endpoint: All animals included until spontaneous death	Control: 1/40 50 mg/kg bw/day: 4/40 250 mg/kg bw/day: 1/40		No incidence of brain tumours. Results are not significant. Survival data is missing. The committee excludes this study from the evaluation of carcinogenicity due to its poor quality.
Conti et al., 1988 183	Rats Sprague-Dawley Males and females, 40/sex/dose group	Test item: Styrene (in olive oil) Purity: 99.8% Oral, via gavage for 4-5 days per week for 52 weeks Dosing: 0 (vehicle), 50 mg/kg bw/day 250 mg/kg bw/day Endpoint: Males and females, included until spontaneous death.	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). - Percentage total benign and malignant mammary tumours in females: Control: 60 50 mg/kg bw/day: 75 250 mg/kg bw/day: 37.5	No details on statistical analyses reported.	Non-GLP. Non-guideline. Limited reporting on the data. A higher mortality rate was observed in females at 250 mg/kg bw. It is possible that these deaths may be attributed to other factors, potentially preventing them from having sufficient time to develop tumours. Overall, there is no increase in tumour incidence. The committee excludes this study from the evaluation of

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Full necropsies and histopathological examinations were performed on all animals.	- Percentage malignant mammary tumours in females: Control: 12.5 50 mg/kg bw/day: 15.0 250 mg/kg bw/day: 12.5		carcinogenicity due to its poor quality.

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2 *Table B2.4 Inhalation studies with styrene in rats*

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Jersey et al., 1978 Not published, based on secondary sources Described by NTP in 2008. 193	Rat, Sprague-Dawley 7-8 weeks old 96/97 males/group and 96 females/group Carcinogenicity study	Test item: Styrene Purity: 99.5% Inhalation, 6h/day, 5 days/week for 18.3 months (males) or 20.7 months (females). Dosing: 0, 600 or 1000 ppm (first 2 months at 1200 ppm) (corresponding to: 0, 2556 or 4260 mg/m3)a Endpoint:	After 2 months, excessive toxicity in 1200 ppm group. The dose was reduced to 1000 ppm. Survival males Control: 5 600 ppm: 18 1000 ppm: 6 Survival females Control: 30 600 ppm: 30 1000 ppm: 22 Neoplastic lesions:	Cochran-Armitage exact trend test on tumour incidences, conducted by NTP.	Non-GLP. Non-guideline. Secondary sources (McConnell and Swenberg, 1994) noted that "this study was seriously flawed by the presence of chronic murine pneumonia, which caused a high rate of mortality in both control and exposed male rat." Survival was lower in males (attributed to chronic murine pneumonia) than in females.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Interim sacrifices of 5/6 animals/sex/group after 6 and 12 months. Exposure until 50% mortality. Observation until death or 24 months. No further details about observation.	- Incidence of mammary adenocarcinoma in females Control: 1.2% 600 ppm: 8.2% No increase compared to historical control (mean 5.8%, range 0-9%). Trend: P=0.002 - Combined incidence of lymphosarcoma and leukemia in females Controls: 1.2% 600 ppm: 7.1% 1000 ppm: 7.1%) Statistically significant increase in females compared to incidence in historical controls (no details in original paper, 1.36% (range 0-2.64%) according to NTP) but not with concurrent controls. Trend: P=0.035 - Combined incidence of lymphosarcoma and leukemia in males Controls: 1.2% 600 ppm: 5.8%		Not clear whether nose-only or whole body inhalation was applied. In females the incidence of mammary adenocarcinoma was increased at 600 ppm compared to control, but not when compared to historical controls. The P-value for trend was 0.002. 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.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			1000 ppm: 1.2%		
Cruzan et al., 1998 194	Rat, CD 4 weeks of age 70/sex/group Chronic toxicity/oncogenicity study	Test item: Styrene (purity: 99.5-99.7%) Inhalation, styrene vapour, whole body, 6h/day 5 days/week for 104 weeks (520 exposures) Dosing: 0, 50, 200, 500, or 1000 ppm (corresponding to 0, 213, 852, 2130 or 4260 mg/m3) Endpoint: intermittent kills: 9-10 rats/sex/group sacrificed after 52 weeks Full necropsies and full histopathological examinations were performed on all control and 1000 ppm animals.	Analytical concentrations were within 1% of the target concentrations. Levels of styrene and styrene-7,8-oxide in blood at week 95 after exposure were proportional to exposure concentration (with smaller increase for the oxide). Survival:[a] - No effect on survival of male rats. Dose-related increase in survival of female rats (500 or 1000 ppm). Body weights, food and water consumption: -Males (50 ppm): increased weight gain (15%) compared to control. - Males (500 and 1000 ppm): decreased weight gain in males (500 and 1000 ppm) compared to controls (10% and 17% respectively after 1 year) and less food consumption during the first	Tumour incidence was analysed using methodology described by IARC (1980). Other pathologic data were analysed using Fisher's exact test.	GLP-study No tumours were found. Tumour reduction seen in three tumour types compared with control.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		Histopathologic examination of the nasal passages, lungs, liver, kidneys, testes/epididymides, and macroscopic abnormalities was performed on the animals of all lower exposure levels.	26 weeks. The weight differences were less at study termination. In the last 6 months the exposed males lost less weight than controls. There was a dose related increase in water consumption compared to controls (121 and 127% during whole study). - Females (200, 500 and 1000 ppm): decreased weight gain compared to controls during the first year (10, 29 and 34% less, respectively). The 500 and 1000 ppm group continued to gain less weight throughout the study and consumed 10% less food than controls. Also the 500 and 1000ppm group consumed more water compared to controls in the first 6 months. - Males and females (200 ppm): increased water consumption in the first month (112% of control). Clinical observations, clinical pathology and necropsy:		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			- Clinical signs only observed during exposure: salivation with restlessness, hunched posture. - No adverse effects on clinical pathology - No adverse effects on organ weights - No effects at interim necropsy - Terminal necropsy: increased incidences of testis masses (500 ppm and 1000 ppm males), decreased incidences of enlarged pituitary (500 and 1000 ppm females), increased incidence of pale foci in lung (1000 ppm females). Non-neoplastic lesions[b]: - Treatment-related effects on olfactory epithelium of the nasal passages: - Increased incidence in atrophic and/or degenerative changes in epithelium, number of affected animals increases with increasing dose. - Increased incidence of changes in the Bowman's glands, number		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			of affected animals increases with increasing dose.		
			Neoplastic lesions: - No statistically significant increase in the number of tumours.		
			- Incidence of testes interstitial cell tumours Control: 2/60 50 ppm: 2/60 200 ppm: 2/60 500 ppm: 4/54 1000 ppm: 6/52), but incidences were within historical range.		
			- 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).		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			- Treatment-related decrease in mammary adenocarcinomas in females Control: 20/60 50 ppm: 13/44 200 ppm: 9/43 500 ppm: 2/38 1000 ppm: 2/59 - Treatment-related decrease in mammary fibroadenomas in females Control: 21/60 50 ppm: 16/44 200 ppm: 13/43 500 ppm: 18/38 1000 ppm: 17/59 Of the female rats that survived 2 years the incidence was 38% (control), 64% (50 ppm), 58% (200 ppm), 61% (500 ppm), and 33% (1000 ppm).		
Maltoni et al., 1982 186	Rat, Sprague-Dawley 13 weeks old Males and females (styrene): 30/sex/group	Test item: Styrene (purity not stated)	Incidence in total brain tumour bearing animals in males Controls: 0/60 25 ppm: 1/30 100 ppm: 1/30	Statistics not reported.	Non-GLP. Non-guideline Limited reporting on data and methods.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	Control: 60/sex/group Carcinogenicity study (brain tumours)	Inhalation, styrene in air, 4 hours/day, 5 days/week for 52 weeks. Dosing: 0 (control), 25, 50, 100, 200 and 300 ppm (corresponding to: 0, 106, 213, 426, 852, 1278 mg/m3)a. Endpoint: All animals included until spontaneous death. Observations: Examination of animals on gross changes every two weeks. Full autopsy and histopathology on each animal. Extra examination of brain.	Incidence in total brain tumour bearing animals in females Controls: 0/60 25 ppm: 1/30 100 ppm: 3/30		Not clear whether nose-only or whole body inhalation applied There was no significant increase in brain tumours. However, little information is given on how the study was conducted. The committee excludes this study from the evaluation of carcinogenicity due to its poor quality.

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

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

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

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

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Lijinsky, 1986 [196]	Mouse, B6C3F1 7 weeks old Males and females: 52/sex/group Chronic study	Test item: Styrene-7,8-oxide (in corn oil) Purity: 96.6% Oral gavage 3 times per week, 104 weeks Dosing: 0 (vehicle), 375 and 750 mg/kg bw/day, Endpoint: Animals sacrificed at 107 or 108 weeks. Full necropsies and full histopathological examinations on all animals.	Survival of animals (750 mg/kg bw) was lower compared to control, half of the group died by 60 weeks. Reduced weight gain in males females (375 and 750 mg/kg bw). Weight loss in males after week 75 (375 and 750 mg/kg bw). Non-neoplastic lesions: - Lipoid degeneration, focal necrosis and haemorrhage of liver in males (750 mg/kg bw, no incidences reported). - Incidence of hyperplasia in forestomach in <u>males</u> Control: 0/51 375 mg/kg bw: 2/51 750 mg/bw: 2/52 - Incidence of hyperplasia in forestomach in <u>females</u> Control: 1/51 375 mg/kg bw: 6/50 750 mg/bw: 3/51	Statistics: Fisher exact test and Cochran-Armitage test	Non-GLP, Non-guideline 3.3% of the styrene-7,8-oxide solution consisted of benzaldehyde, benzene and one other unspecified compound

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			Neoplastic lesions: - Increased liver carcinomas + adenomas in <u>males</u> Control: 12/51 375 mg/kg bw: 28/52 (P<0.001) 750 mg/kg bw: 15/52) - Increased forestomach carcinomas + papillomas in <u>males</u> Control: 2/51 375 mg/kg bw: 37/51 (P<0.001) 750 mg/kg bw: 21/52 (P<0.001) - Increased forestomach carcinomas + papillomas in <u>females</u> Control: 0/51 375 mg/kg bw: 24/50 (P<0.001) 750 mg/kg bw: 20/51 (P<0.001) - Incidence of carcinomas of the forestomach in <u>males</u> Control: 0/51 375 mg/kg bw: 16/51 750 mg/bw: 15/52 - Incidence of carcinomas of the forestomach in <u>females</u> Control: 0/51 375 mg/kg bw: 10/50		

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

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

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
Lijinsky, 1986 196	Rat, F344 9 weeks old	Test item: styrene-7,8-oxide (in corn oil)	Survival of animals (550 mg/kg bw) was lower compared to control.	Statistics:	Non-GLP, non-guideline

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
	Males and females: 52/sex/group Chronic study	Purity: 96.6% Oral gavage 3 times per week, 104 weeks Dosing: 0 (vehicle), 275 and 550 mg/kg bw/day Endpoint: Animals sacrificed at 107 or 108 weeks. Observations: - Twice daily mortality checks. - Body weight was recorded once a week (first 4 months), every two weeks (next 4 months) and once every 4 weeks (rest of study). Full necropsies and full histopathological	Lower weight gain of animals (550 mg/kg bw). Small weight loss in males after 75 weeks (550 mg/kg bw). Non-neoplastic lesions: - Increased incidence of hyperplasia in forestomach in males Control: 2/52 275 mg/kg bw: 10/52 550 mg/kg bw: 9/51 - Non-neoplastic lesions: Increased incidence of hyperplasia in forestomach in females Control: 0/52 275 mg/kg bw: 8/52 550 mg/kg bw: 9/52 Neoplastic lesions: - Increased incidence of combined carcinomas and papillomas in forestomach in males Control: 1/52 275 mg/kg bw: 50/52 (P<0.001) 550 mg/kg bw: 50/51 - Increased incidence of combined carcinomas and papillomas in forestomach in females	Fisher exact test and Cochran-Armitage test	3.3% of the styrene-7,8-oxide solution consisted of benzaldehyde, benzene and one other unspecified compound

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		examinations on all animals.	Control: 0/52 275 mg/kg bw: 46/52 550 mg/kg bw: 50/52) - Increased incidence of carcinomas of the forestomach in males Control: 0/52 275 mg/kg bw: 35/52 550 mg/kg bw: 43/51 - Increased incidence of carcinomas of the forestomach in females Control: 0/52 275 mg/kg bw: 32/52 550 mg/kg bw: 36/51 - Increased incidence of papillomas of the forestomach in males Control: 1/52 275 mg/kg bw: 23/52 550 mg/kg bw: 18/51 - Increased incidence of papillomas of the forestomach in females Control: 0/52 275 mg/kg bw: 21/52 550 mg/kg bw: 24/51		

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
			- Decreased incidence of leukemia in males and females (both 550 mg/kg bw).		
Ponomarkov et al., 1984 197	Rat, BDIV 14 exposed dams and their offspring (62 females and 42 males). 14 control dams and their offspring (55 female and 49 male). Carcinogenicity study	Test item: Styrene-7,8-oxide (in olive oil) Purity: 97% Oral, via gavage Dosing: Pregnant dams: 0 (olive oil) and 200 mg/kg bw Single administration on day 17 of gestation Offspring: 0 (olive oil) and 100-150 mg/kg bw, 96 weekly doses from 4 weeks of age (weaning) until termination of experiment Endpoint:	Litter size, preweaning mortality, offspring mortality and body weights did not differ between the groups. Non-neoplastic and neoplastic lesions: Incidence in tumour-bearing pregnant dams was 57% (controls) and 31% (styrene-7,8-oxide). Effects in offspring: -Incidence in tumour-bearing animals in treated rats was 77% (females) and 52% (males) and in controls 58% (females) and 20% (males). Increased incidence in forestomach tumours: - Papillomas in males (control: 0/49; styrene-7,8-oxide: 7/42, P<0.003) - Carcinoma in situ in females (control: 0/55; 200 mg/kg: 6/60, P<0.02) and	Statistics: No details on statistics. Percentage of tumour bearing animals expressed in relation to the effective number of animals.	Non GLP, Non guideline.

Reference	Species/ strain/ experimental design	Exposure	Results	Statistics	Remarks and quality
		All animals were sacrificed at 120 weeks of the experiment. Observations: Full necropsies and histopathological examinations were performed on all animals. No further details on observations are mentioned.	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). Early changes of squamous epithelium frequently observed in styrene-7,8-oxide groups (though not statistically significant): - Incidence in nervous system tumours in males (control: 1/49; styrene-7,8-oxide: 3/42). - Incidence in lung tumours in females (control: 1/55; styrene-7,8-oxide: 6/60).		

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