



# **Reactie Gezondheidsraad op commentaar conceptadvies Styreen**

Response Health Council to comments  
draft report Styrene

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## 1 Reactie op commentaar FME

### Response to comments FME

Op 25 april 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van FME op het concept van het advies Styreen. De reactie staat hieronder.

*On April 25<sup>th</sup> 2025, the Health Council sent a letter to FME in response to the comments on the draft report on Styrene. The response is cited below, in the same language as the original comments (Dutch).*

'Geachte heer Halm,

Bedankt voor uw interesse in het conceptadvies over styreen. Via deze brief ontvangt u een reactie op uw commentaar, verstuurd per email op 20 november 2024.

In uw commentaar geeft u aan dat de conclusies uit het rapport in tegenspraak zijn met zichzelf. In het advies wordt aangegeven dat er niet voldaan is aan het criterium voor bewijs in dieren, terwijl de commissie desondanks een classificatie in categorie 1B adviseert. De commissie wijst erop dat de classificatie (conform CLP) is gebaseerd op een synthese van onderzoeken zowel in dieren als in mensen. De commissie beoordeelt het bewijs uit epidemiologische studies, die zoals u aangeeft beperkingen hebben, als beperkt (*'limited'*). Ondanks deze beperkingen is de commissie van mening dat deze epidemiologische studies, met relevante beroepsmatige blootstellingen, goed zijn uitgevoerd. Daarnaast houden deze studies zoveel mogelijk rekening met mogelijke versturende factoren en bias. Om die reden kunnen deze resultaten niet worden genegeerd bij de beoordeling van de carcinogeniteit van styreen. Op basis van de resultaten uit de epidemiologische studies in combinatie met de aanwijzingen voor carcinogeniteit uit de dierstudies acht de commissie classificatie in categorie 1b van toepassing.

Verder wijst u erop dat styreen door IARC wordt ingedeeld in groep 2A, waarbij u aangeeft dat deze categorie vergelijkbaar is met de EU-categorie 2. Indien deze categorieën vergeleken worden, dan staat een IARC-classificatie 2A (*probably carcinogenic*) dichterbij een EU classificatie 1B (*presumed to have carcinogenic potential for humans*) dan bij een EU classificatie 2 (*suspected to have carcinogenic potential for humans*). De commissie wijst erop dat de IARC-en EU-categorieën en de onderbouwing hiervan verschillen. De IARC classificeert styreen in categorie 2A op basis van *'limited evidence in humans'* en *'sufficient evidence in animals'*. De commissie classificeert styreen in categorie 1B op basis van *'limited evidence'* in mens en dier.

In de bijgevoegde e-mail vindt u een link naar het definitieve rapport over styreen. Alle commentaarbrieven en reacties daarop zijn beschikbaar via onze website: [www.gezondheidsraad.nl](http://www.gezondheidsraad.nl)

## 2 Reactie op commentaar NIOSH

### Response to comments NIOSH

Op 25 april 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van NIOSH op het concept van het advies Styreen. De reactie staat hieronder, in dezelfde taal als het oorspronkelijke commentaar (Engels).

*On April 25<sup>th</sup> 2025, the Health Council sent a letter to NIOSH in response to the comments on the draft report on Styrene. The response is cited below.*

'Dear Daniel J. Hardt,

Thank you for accepting the invitation to comment on the draft report 'Styrene' that the Health Council published for public review in September 2024. I am writing in response to your comments on behalf of the Council's Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The subcommittee appreciates your review of the report.

The subcommittee considered your comments and adopted your suggested changes in the final report. We are pleased that you agree with the proposed classification for styrene.

The accompanying e-mail contains a link to the final report on styrene.'

### 3 Reactie op commentaar NIOSH

#### Response to comments NIOSH

Op 25 april 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van NIOSH op het concept van het advies Styreen. De reactie staat hieronder, in dezelfde taal als het oorspronkelijke commentaar (Engels).

*On April 25<sup>th</sup> 2025, the Health Council sent a letter to NIOSH in response to the comments on the draft report on Styrene. The response is cited below.*

'Dear Dr. Bertke,

Thank you for accepting the invitation to comment on the draft report 'Styrene' that the Health Council published for public review in September 2024. I am writing in response to your comments, on behalf of the Council's Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The subcommittee appreciates your review of the report.

The majority of your comments deal with grammatical, editorial and punctuation errors. Where appropriate, the necessary corrections have been made. You further noted that human occupational studies may have been affected by healthy worker survivor bias as demonstrated in Bertke et al. (2021) where unhealthy workers select out due to acute irritation because of styrene exposure. This limitation is mentioned in Table B1.1 of our report.

We are pleased that you agree with the proposed classification for styrene.

The accompanying e-mail contains a link to the final report on styrene.'

#### 4 Reactie op commentaar EFSA

##### Response to comments EFSA

Op 21 februari 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van EFSA op het concept van het advies Styreen. De reactie staat hieronder, in dezelfde taal als het oorspronkelijke commentaar (Engels).

*On February 21<sup>st</sup> 2025, the Health Council sent a letter to EFSA in response to the comments on the draft report on Styrene. The response is cited below.*

'Dear Dr.,

Thank you for accepting the invitation to comment on the draft report *Styrene* by the Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert committee on Occupational Safety (DECOS) of the Health Council of the Netherlands, which was made available for public review in September 2024. The committee highly appreciates your comments on behalf of the EFSA FCM Panel, which enabled the committee to further improve its advisory report.

In reply to several comments received during the public consultation, including the comments from the EFSA FCM Panel, the committee added a paragraph to clarify the evidence synthesis for mutagenicity. In this additional text, the committee explains its weight-of-evidence approach more transparently, as is summarised below in a reflection to your comments.

The committee based its assessment of mutagenicity mainly on epidemiological studies in occupationally exposed individuals, supported by *in vitro* studies. Less emphasis was placed on rodent studies, because the metabolism of styrene in rodents does not fully align with its metabolism in humans (refs 6,7 of the draft report). Several human studies showed an association between styrene exposure and cytogenetic endpoints (refs 26-31,45,47), whereas other studies did not show an association (refs 19-25,27,46,48). The committee is of the opinion that the positive findings, despite study limitations, are suggestive for mutagenic properties of styrene and cannot be ignored.

The committee further considers the studies investigating DNA damage (including DNA adducts) in humans as supporting evidence. Since the majority of these studies were positive (refs 26,27,57-64,69-76), the committee concludes that an interaction between styrene (or its metabolites) and DNA is possible in humans. Suspected mutagenic properties are further supported by the predominantly positive effects from *in vitro* studies with styrene and styrene-7,8-oxide (SO).

The committee would like to emphasize an important distinction between EFSA's risk assessment and the committee's hazard assessment. If mutagenicity is observed in an *in vivo* test using intraperitoneal administration, it demonstrates that the substance has intrinsic mutagenic properties, which the committee considers relevant for hazard classification. This is in contrast to the FCM Panel's viewpoint, which considers the data using the intraperitoneal route of limited relevance.

Furthermore, the FCM Panel noted that the assessment of the *in vivo* genotoxic potential of styrene should only rely on the evidence provided by the testing of styrene. Since styrene can be metabolised to styrene-7,8-oxide (SO) once absorbed in humans, the committee considers both *in vivo* and *in vitro* studies on SO relevant for its hazard assessment of styrene. However, it should be noted that the data regarding the mutagenicity of SO is considered as supporting evidence only.

The committee decided to rely primarily on the 2019 IARC Monograph as the main data source. The two Collins meta-analyses were excluded, because the committee prefers to base its evaluation on individual studies, similar to the approach taken by IARC. However, in response to the comments of the FCM Panel, the committee reviewed the studies included in these meta-analyses that were not previously examined by IARC (Anwar 1995, Biro 2002, Van Hummelen 1994). While these studies were added to our report, their inclusion did not affect the conclusions on the recommended classification for mutagenicity of styrene.

Furthermore, the committee would like to emphasize that this report addresses the classification for hazardous properties of styrene in the occupational context (worker-related exposure). Exposure to styrene in food and subsequent health risks to consumers is beyond the scope of this report. We have now further clarified this distinction in the first chapter of our report.

Overall, based on the limited evidence for mutagenicity from human studies in combination with the supporting evidence from *in vitro* studies, the committee maintains its decision to recommend classifying styrene in Category 2 for mutagenicity.

The final advisory report *Styrene* will be published on the website of the Health Council probably in April 2025. All comments and replies will be made available to the public, including this letter.'

## 5 Reactie op commentaar Plastics Europe

### Response to comments Plastics Europe

Op 25 april 2025 heeft de Gezondheidsraad per brief gereageerd op het commentaar van Plastics Europe op het concept van het advies Styreen. De reactie staat hieronder, in dezelfde taal als het oorspronkelijke commentaar (Engels).

*On April 25<sup>th</sup> 2025, the Health Council sent a letter to Plastics Europe in response to the comments on the draft report on Styrene. The response is cited below.*

'Dear Dr. Block,

Thank you for accepting the invitation to comment on the draft report 'Styrene' that the Health Council published for public review in September 2024. I am writing in response to your comments, on behalf of the Council's Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The committee highly appreciates your comments on behalf of the Plastics Europe, which enabled the committee to further improve its advisory report.

In response to your comments related to the substantiation of the committee's recommendations, the committee added two paragraphs to its report to clarify the evidence synthesis for mutagenicity (section 3.4) and carcinogenicity (section 4.3). In this additional text, the committee explains its weight-of-evidence approach more transparently. The committee also further clarified its quality assessment and study result assessment for the mutagenicity and carcinogenicity of styrene in section 1.4. These sections should address most of your comments regarding the evidence synthesis and quality and study result assessment (see enclosed annex, comment 27, 28, 31,32, 40, 41, 45, 66, 67, 69, 70, 72-79, 84, 86, 89, 93-95).

You recommended that the Council should reconsider its decision to exclude the two meta-analyses by Collins and Moore (2019) and Collins and Moore (2021), and all of the studies therein, as they provide new information and analyses not previously considered, overall adding to the body of evidence supporting a lack of association between styrene exposure and increased micronuclei frequencies and chromosomal aberrations in workers. However, the committee decided to rely primarily on the 2019 IARC Monograph as the main data source. The two meta-analyses by Collins were excluded, because the committee prefers to base its evaluation on individual studies, similar to the approach taken by IARC. While meta-analyses can offer broader insights, a well-conducted individual study is considered more reliable and relevant for hazard identification in our case. This is because a well-conducted individual study with a clear positive result can be averaged out in a meta-analysis, causing its impact to be lost. Additionally, individual studies may provide a clearer view of scientific data than meta-analyses because they avoid issues such as study heterogeneity and the inclusion of low-quality research.

The committee reviewed the studies included in the two meta-analyses by Collins that were missing in our draft report (see comment 30, 31, 80). Additional relevant studies were evaluated by the committee and added to the final report (see comment 29, 81, 82). The outcome of these studies did not affect the recommended classification for mutagenicity of styrene.

Overall, the committee concludes that although the evidence for mutagenicity from human studies is limited, it is strongly supported by evidence from in vitro studies with human cells. The committee included studies investigating DNA damage (including DNA adducts) in exposed humans as supporting evidence. Since the majority of these studies were positive, the committee concludes that an interaction between styrene (or its metabolites) and DNA is possible in humans. Therefore, the committee maintains its decision to recommend classifying styrene in category 2 for mutagenicity. Regarding carcinogenicity, the committee considers classification in category 1B warranted based on the combination of the limited evidence for carcinogenicity from human studies and animal studies.

Other specific comments that were addressed in your response are addressed in the enclosed annex.

The accompanying e-mail contains a link to the final report on styrene. All comments and replies, including this letter, will be made available on our website: [www.healthcouncil.nl](http://www.healthcouncil.nl).'

**Annex Response to Plastics Europe Comments on: Draft Advisory Report for Public Review | September 2, 2024 | Styrene | The Health Council of the Netherlands**

NUMBER	LOCATION (CHAPTER, PAGE, LINE)	COMMENTS REVIEWER	REPLY COMMITTEE
<b>Comments on Animal Carcinogenicity</b>			
1	Comment 1	The Council's draft advisory report's evaluation that lung tumors found in mice exposed to styrene are not relevant to humans is supported by a large body of information that is only briefly and incompletely summarized. The advisory report would benefit from additional discussion of this information.	<b>Regarding comments 1-10:</b> The committee agrees that lung tumors in mice are not relevant. There are sufficient arguments to support this, but the committee does not find it necessary to cite all literature that is not specifically related to carcinogenicity. Instead, we refer to the following two papers: - 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. - 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.
2	Comment 1.1 Page 33, Line 11 to Page 36, Line 9 (Report Section 4.2.1)	The majority of the mouse carcinogenicity studies are appropriately and accurately described in the draft advisory report and the analysis of the Council is consistent with conclusions published by others, however, a couple of the study summaries and analyses lack some fundamental details that should be included in the final report.	
3	Comment 1.1.1 Page 35, Line 11-25 (Report Section 4.2.1, Inhalation Studies)	This study summary does not mention the key Cruzan et al. (2001) conclusion that no tumors were observed in the 52- and 78-week interim sacrifice groups, i.e., increased tumors were late developing and apparent only at 22.5 months and thereafter. This latter observation is important in that the late appearance of tumors at a single site and in a single species is consistent with a weakly active and non-genotoxic carcinogen, a key consideration (ultimately overlooked) in the overall evaluation of the mode of action (MOA) of styrene carcinogenicity.	This involves only 10 animals per group, evaluated at 52 and 78 weeks, making the study not particularly robust for drawing and emphasizing such strong conclusions.
4	Comment 1.1.2 Page 35, Line 33-34 to Page 36, Line 1-9 (Report Section 4.2.1, Inhalation Studies)	The summary of the styrene transgenic mouse study (Cruzan et al., 2017) provides an accurate accounting of only some of the key findings from this important mechanistic study. Cruzan et al. note that the lung neoplastic findings in the styrene CD-1 mice exhibited an approximate 25% increase in the incidence of the bronchoalveolar adenoma and adenocarcinoma compared to the CD-1 control mice but did not find in their statistical analyses that the lung tumor incidence to be statistically different between the four tested strains of mice (CD-1, WT, KO, TG); however, it is notable that IARC (2019) performed its own analyses of these results and reported that the incidence of bronchioloalveolar carcinoma was significantly increased in CD-1 mice [P < 0.05] exposed to styrene (17/67) compared with the CD-1 control mice (7/67) and confirmed there were no statistically increased tumors in the genetically modified mice (KO, TG). As discussions of the other mouse studies' findings do note non-statistical, statistical, and trend results, for completeness and consistency, this study should be similarly described.	This has been adjusted in the report.
5	Comment 1.1.3 Page 35, Line 33-34 to Page	In addition, a brief summary of the non-neoplastic lesions that were observed for the four mouse strains in the Cruzan et al. (2017) study is recommended as these results are relevant to	

	36, Line 1-9 (Report Section 4.2.1, Inhalation Studies)	the overall styrene lung responses in these animals. Reference to the Annex table alone is insufficient. In particular, there was degeneration of epithelial cells in terminal bronchioles observed in the CD-1 and WT styrene treated mice and NONE found in of the KO and TG styrene treated mice, there was increase in cell proliferation (statistically significant) in the CD-1 and WT styrene treated mice and comparable levels of cell proliferation in the KO and TG styrene treated mice, and there was an increase in hyperplasia (statistically significant) in the CD-1 and WT styrene treated mice and NO increases in hyperplasia in the KO and TG styrene treated mice. These findings are important evidence that supports the proposed MOA that the mouse lung tumors arise through CYP2F2 metabolism and warrant specific identification and discussion in the final advisory report.	
6	<b>Comment 1.2</b> Page 41, Line 14-21 (Section 4.3)	The Council's advisory report warrants a detailed review of the available MOA information to inform the evaluation of styrene-induced mouse lung tumors which are the only tumors that have been found to be increased in the over a dozen chronic/cancer bioassays that have been performed for styrene. The draft advisory report lacks detailed discussion of the important large body of mechanistic information that has been developed and assessed to inform on the animal carcinogenicity of styrene and the potential relevance of animal cancer to human cancer concerns.	
7	<b>Comment 1.2.1</b> Page 41, Lines 14-21 (Section 4.3)	In addition to the numerous mechanistic and toxicokinetic/toxicodynamic studies that have been published regarding the mouse and styrene exposures (including studies by Andersen et al., 2017, 2018; Boogard et al., 2000; Carlson, 1997, 2004, 2008, 2012; Carlson et al., 2000, 2002; Cruzan et al., 2002, 2005, 2009, 2012; Csanady et al., 2003; Filser and Gelbke, 2016; Filser et al., 1992; Gadberry et al., 1996; Green et al., 2001; Hoffman et al., 2006; Hynes et al., 1999; Johanson et al., 2000; Nakajima et al., 1994; Sarangapani et al., 2002; Shen et al., 2010, 2014; Zhang et al., 2011), several reviews and assessments have been published that synthesize this information into a well-supported MOA (Cruzan et al., 2018; Banton et al., 2019; Meek et al., 2013, 2014; Frank and Meek, 2024; see below).  This information robustly supports a conclusion that styrene mouse lung toxicity and tumorigenicity are not mediated by styrene oxide, but rather through additional mouse-lung specific metabolism of styrene and/or styrene oxide by cytochrome P450-2F2 (CYP2F2) to ring-oxidized metabolites. Importantly, the human isoform of mouse CYP2F2, CYP2F1, has substantially reduced or questionable metabolic capacity for metabolism of styrene to ring-oxidized metabolites, and CYP2E1, which is primarily responsible for the extensive metabolism of styrene to styrene oxide in rodents and humans, also has very limited if any capability for ring-oxidation.	
8	<b>Comment 1.2.2</b> Page 41, Lines 14-21 (Section 4.3)	The styrene mouse lung tumor MOA analysis has been integrated into multiple published weight-of-evidence (WoE) styrene and styrene oxide framework MOA analyses that include the above cited studies that examine MOA data collected at multiple timepoints and doses across a breadth of styrene exposure durations, up to and including chronic lifetime styrene inhalation exposures in mice. These data reviews provide comprehensive integrated evidence revealing that styrene oxide is not the mechanistically proximate metabolite responsible for styrene-initiated lung tumors.  The Council is urged to review the available primary study references and the MOA assessments that are published for styrene and include in the final advisory report a more detailed and comprehensive assessment that styrene induced mouse lung tumors are not relevant to humans.	
9	<b>Comment 1.2.2.1</b>	Cruzan et al. (2018) and Banton et al. (2019) developed a comprehensive human relevance analysis of styrene mouse lung	

		<p>tumor using data integration standards promoted in multiple MOA analysis framework proposals (Meek et al., 2013, 2014). The styrene analysis proposed a primary non-genotoxic MOA action hypothesis based on CYP2F2-mediated formation of mitogenic/cytotoxic ring-oxidized metabolite(s), and contrasted the data-driven merits of that hypothesis against the alternative genotoxic styrene reactive-metabolite mediated MOA as focused on in Cohen et al. (2002). The primary MOA hypothesis identified a key (molecular) initiating event and three subsequent key events as accounting for how styrene exposure in mice ultimately progresses to lung tumors.</p> <p>Those events are:</p> <p>(1) metabolism of styrene to ring-oxidized metabolite(s) by CYP2F2 in mouse lung terminal bronchiole club cells;</p> <p>(2) immediate changes in lung gene expression responses reflecting mitogenic signals and later-in-life changes consistent with shifts in circadian clock genes linked to mouse lung tumorigenesis;</p> <p>(3) initiation of mitogenic cell proliferative response as a primary event in mouse lung terminal bronchiolar club cells with cell cytotoxicity as a minor contributor to cell proliferation; and</p> <p>(4) development of persistent terminal bronchiole hyperplasia with ultimate progression to localized lung tumors.</p>	
10	<b>Comment 1.2.2.2</b>	<p>Independently, scientists at NIOSH and a Canadian university (Frank and Meek, 2024) published an analysis that used the MOA human relevance framework to assess the likelihood that bronchiolar lung tumors observed in mice chronically exposed to styrene represented a plausible tumor risk in humans. Using the available datasets for styrene, they analyze the weight-of-evidence 1) that styrene-induced tumors in mice occur through a MOA based on metabolism of styrene by CYP2F2; and 2) whether the hypothesized key event relationships are likely to occur in humans. Their assessment described how the five modified Hill causality considerations support that a CYP2F2-dependent MOA causing lung tumors is active in mice, but only results in tumorigenicity in susceptible strains. They compared the key event relationships assessed in the mouse to an analogous MOA hypothesis staged in the human lung and found that while some biological concordance was recognized between key events in mice and humans, the MOA as hypothesized in the mouse appears unlikely in humans due to quantitative differences in the metabolic capacity of the airways and qualitative uncertainties in the toxicological and prognostic concordance of pre-neoplastic and neoplastic lesions arising in either species. Their analysis provides a rigorous demonstration of the framework's utility in increasing transparency and consistency in evidence-based assessment of MOA hypotheses in toxicological models and determining relevance to human health.</p>	
11	<b>Comment 2</b>	<p>The Council's draft advisory report's evaluation that the increases in female rat mammary and lymphohematopoietic (LH) cancers were observed in an unreliable study is supported by the available information, however, the advisory report would benefit from additional discussion of this and information from the other styrene rat studies.</p>	<p><b>Regarding comments 11-17:</b> The committee added a paragraph to clarify the evidence synthesis for carcinogenicity. In this additional text, the committee explains its weight-of-evidence approach more transparently and included the most important study limitations.</p>
12	<b>Comment 2.1</b> Page 37, Line 24 to Page 38, Line 11 (Section 4.2.2, Inhalation Studies)	<p>The Jersey et al. (1978) styrene rat inhalation study is an early pre-guideline study, has confounding factors, and is poorly reported, hence this study is appropriately concluded to be unreliable in the draft advisory report. Limitations of this study include:</p>	

		<p>2.1.1. This study has not been published in detail and the information on this study is only available from secondary source reviews that cannot be independently verified.</p> <p>2.1.2. The study was conducted prior to standardized toxicity testing and Good Laboratory Practice and OECD test guidelines and used a minimal experimental design. Only two styrene exposure concentrations were tested and the highest test concentration (1200 ppm) had to be decreased (to 1000 ppm) after two months into the study due to excessive mortality. The design of the study did not appear to include evaluations for chronic toxicity (hematology, clinical chemistry) nor were results of non-neoplastic pathology findings identified which can be informative and supportive on neoplastic findings.</p> <p>2.1.3. The rats in this study were afflicted with chronic murine pneumonia which confounds interpretation of the study findings. McConnell and Swenberg (1994) identified that the rats in this study had chronic murine pneumonia, also now known as murine respiratory mycoplasmosis caused by the highly contagious bacteria <i>Mycoplasma pulmonis</i>, which resulted in a high rate of mortality in the males but also affected the females. The increase in mortality observed in this study impacted the number of animals available to have cancers induced but mycoplasma infections are also a risk factor in the development of cancers through their causing cell transformations (Yacoub et al., 2019) and hence may have influenced the cancer incidences that were found in the Jersey et al. (1978) study.</p> <p>2.1.4. Although there were two neoplastic incidence increases reported in the female rats in the study, analyses support that these were likely not related to styrene exposure. An increase in mammary adenocarcinomas occurred in the low dose group (600 ppm) but these were determined not to be related to styrene treatment because mammary adenocarcinoma did not occur in the high-dose group, the incidence of mammary adenocarcinoma in the control group was low compared with historical controls, and the range among historical controls contained the rate observed in the treatment group. The other neoplastic increase was in combined incidences of lymphosarcoma and leukemia (LH cancer). This increase was not statistically significant compared with the concurrent controls but was significant when compared with historical controls. Of note, these tumors are not typically combined for analyses in carcinogenicity studies.</p> <p>While this study cannot be considered as reliable evidence of rat carcinogenicity, it is notable that there was no lung cancers observed following the high styrene inhalation exposures in the study.</p>	
13	<b>Comment 2.2</b> Page 36, Line 11 to Page 38, Line 30 (Section 4.2.2)	The increases in mammary and LH cancers observed in the Jersey et al. (1978) study were not replicated in the other five rat studies that evaluated styrene inhalation and oral exposure.	
14	<b>Comment 2.2.1</b> Page 38, Line 12-24 (Section 4.2.2, Inhalation Studies)	The study of rats by Cruzan et al. (1998) evaluated the chronic/carcinogenicity potential of styrene inhalation exposure using contemporary testing guidelines/experimental designs and GLP practices and hence is a highly reliable study. This study also tested very high inhalation exposures up to 1000 ppm administered up to 104 weeks, which was longer than the exposure duration in the Jersey study for females of 18.3 months, and this study found no increases in either LH or mammary gland cancers and rather a treatment-related decrease in mammary gland tumors were found.	
15	<b>Comment 2.2.2</b> Page 36, Line 17 to Page 37,	Two NCI studies (NCI, 1979) examined chronic rat oral exposures to styrene (one study tested a mixture of styrene and B-nitro styrene) were also of limited experimental designs but	

	Line 2 (Section 4.2.2, Oral Studies)	tested gavage dosages up to 1000 mg/kg bw/day and found no significant increases in tumor incidences.	
16	<b>Comment 2.2.3</b> Page 37, Line 3-12 (Section 4.2.2, Oral Studies)	A drinking water combined chronic and reproductive toxicity study in rats (Beliles et al., 1985) tested styrene concentrations up to 250 ppm and found no significant increases in tumor incidences. This study, although of a non-standard study design, provides supportive information on rat non-carcinogenicity.	
17	<b>Comment 2.2.4</b> Page 37, Line 13-22 (Section 4.2.2, Oral Studies)	In another non-standard study design (Ponomarkov and Tomatis, 1978), styrene was tested for carcinogenicity in offspring of rat dams that had received a single high oral dosage of styrene (1350 mg/kg bw) and then the offspring from the time of weaning through their lifespan received 500 mg/kg bw/day styrene by oral gavage. This study also found no increases in tumor incidences.	
18	<b>Comment 3</b>	The Council's draft advisory report's evaluation that the increases in rat forestomach tumors in oral studies of styrene oxide is supported by the available information, however, the advisory report would benefit from additional discussion that supports the lack of human relevance of the forestomach tumors and discussion that styrene oxide is not the mechanistic driver of styrene's rodent carcinogenicity.	<b>Regarding comments 18-23:</b> The committee agrees that forestomach tumors in rats are not relevant. There are sufficient arguments to support this, but the committee does not find it necessary to cite all literature that is not specifically related to carcinogenicity. Instead, we refer to the following two references: - Smit C, van Raaij M. <i>Factsheets for the (eco) toxicological risk assessment strategy of the National Institute for Public Health and the Environment-Part IV. 2005:</i> - IARC. <i>Predictive value of rodent forestomach and gastric neuroendocrine tumours in evaluating carcinogenic risks to humans. 2003:</i>
19	<b>Comment 3.1</b> Page 38, Line 32 to Page 40, Line 29 (Sections 4.2.3 and 4.2.4) and Page 41, Lines 31-35 (Section 4.3)	The only consistent increase in tumor incidence in animal studies for styrene oxide exposure were to forestomach tumors. The draft advisory report appropriately concludes these tumors lack human relevance based on a WoE assessment, however, details of this assessment would be useful to include in the final advisory report.	
20	<b>Comment 3.1.1</b>	Forestomach tumors were increased in both sexes in both rats and mice in studies that administered high chronic dosages of styrene oxide orally (Lijinsky, 1986; Ponomarkov et al., 1984). A WoE evidence evaluation was apparently performed by the Council but the details of this evaluation are not presented in the draft advisory report. Inclusion of the WoE report should be provided to improve transparency of the evaluation.	
21	<b>Comment 3.1.2</b>	A useful discussion of the human relevance of styrene oxide-induced forestomach tumors for human hazard assessment was also provided in the review published by McConnell and Swenberg (1994) (cited but not referenced in the draft advisory report). They noted that: (1) the route of exposure is not relevant to the human situation, where the primary route of exposure is inhalation and to a lesser extent dermal, and (2) styrene given by the oral route would not be converted to styrene oxide in the stomach because this requires a metabolic reaction that occurs primarily in the liver.	
22	<b>Comment 3.1.3</b>	The lack of forestomach toxicity associated with styrene exposure is also demonstrated in the available rat and mouse oral carcinogenicity studies that failed to find any evidence of forestomach tumor increases.	
23	<b>Comment 3.1.4</b>	Further support is provided in the recently reported styrene genotoxicity studies conducted in rats and mice that did not find	

		evidence of DNA damage in the rodent's forestomaches (as tested by the Comet assay) following oral gavage exposures (Gollapudi, 2023, 2024).	
24	<b>Comment 3.2</b> Page 41, Lines 31-35 (Section 4.3)	In addition to the styrene oxide animal cancer studies, the styrene and styrene oxide toxicokinetic data supports that styrene oxide is not a plausible driver in the styrene animal carcinogenicity studies. The advisory report would benefit from inclusion of this information.	<b>Regarding comment 24-26</b> Since styrene can be metabolised to styrene-7,8-oxide (SO) once absorbed in humans, the committee considers both <i>in vivo</i> and <i>in vitro</i> studies with SO relevant for its hazard assessment of styrene. However, it should be noted that the data of SO is considered as supporting evidence only, as mentioned in our report.
25	<b>Comment 3.2.1</b>	The Cruzan et al. (1998, 2001) rat and mouse chronic studies measured blood concentrations of styrene and styrene oxide from blood collected from rats and mice sacrificed after the end of exposure week 74 (mice) and week 95 (rat) and found that the styrene blood concentrations did not correlate with the comparative mouse and rat tumor responses, i.e., despite substantially higher styrene oxide blood concentrations in rats, no tumors were observed at any site. The styrene oxide blood concentrations in male rats exposed to the top, non-tumorigenic 1000 ppm inhalation exposure to styrene was 185 ng/mL which was 5.5-fold higher than the styrene oxide blood concentrations in male mice of 33.5 ng/mL which was found at the top lung tumor inducing exposure of 160 ppm.	
26	<b>Comment 3.2.2</b>	<p>Further perspectives on the toxicokinetic relationship of styrene and styrene oxide in rats and humans was described in an evaluation by Filser and Gelbke (2016). They concluded: "Due to saturation of metabolism of styrene to SO following inhalation or oral treatment, it was estimated that the maximum blood concentrations of SO cannot exceed 0.33 µg/ml in rats and 0.036 µg/ml in humans. Interestingly, mice and rats administered high gavage doses of SO (500 mg/kg mice; 200 mg/kg rats; Filser et al., 1992) had brief peak SO blood concentrations of approximately 7 and 3.6 µg/ml, respectively. These doses were only tumorigenic to the site of contact in the stomach, indicating that even relatively high systemic SO concentrations are not carcinogenic. Compared to these maximum SO concentrations, exposure of volunteers for 2 h to 50 ppm styrene representative of occupational exposures resulted in blood SO concentrations ranging from 0.0003 to 0.0015 µg/ml (mean=0.0008 µg/ml) (Johanson et al., 2000). The volunteer SO concentrations were consistent with PBPK model estimates of end of shift SO concentrations following 75 ppm styrene exposures of 0.0048 µg/ml (Csanady et al., 2003). Importantly, all of the animal and human blood SO concentrations, with exception of oral SO, are substantively less than SO concentrations necessary to elicit genotoxicity and mutations. Of the 39 clearly positive SO mutagenicity studies described by IARC (1994; 2002), 35 had lowest effect level concentrations (LEC) of ≥24 µg/ml. The lowest SO LECs were 12, 3 and 1.2 µg/ml for in vitro human cell micronucleus formation, chromosomal aberrations and DNA strand breaks, respectively (IARC, 1994), while 24 µg/ml was the LEC reported for in vitro gene mutations in human cells (IARC, 2002). Overall, the very large disparity between maximally achievable blood concentrations compared to in vitro concentrations necessary to elicit genotoxicity and mutagenicity indicates the SO toxicokinetic data are inconsistent with the hypothesis that SO is a biologically plausible genotoxicant in animals or humans exposed to styrene."</p> <p>These points of Filser and Gelbke (2016) are critically important to the assessment of styrene oxide potential role in the animal carcinogenicity of styrene.</p>	

Comments on Mutagenicity			
27	<p><b>Comment Section 1.4</b> Page 12, line 16 (Quality Assessment)</p>	<p>Page 12, line 20 – “...the committee only evaluated the original sources of the studies when these were considered most relevant in assessing the mutagenicity and carcinogenicity of styrene.”</p> <p>It would be informative to specify what factors are considered in making the assessment that the studies are “most relevant.”</p> <p>Page 12, lines 22-24 – “For mutagenicity, the committee only evaluated the quality of the original studies with clastogenic and aneuploidic outcome measures, as these are considered most important for the assessment of mutagenicity.” The exclusion of gene mutation endpoint outcomes in the committee’s evaluation seems at odds with the general scientific standards and contradictory to the statement on page 13, lines 2-5 that read “[m]utagenicity 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.”</p> <p>The committee determined the study quality based on the Health Council of the Netherlands “Guideline for the classification of carcinogenic substances” (cited as reference 1 in the committee’s document). It is commendable that adherence to the OECD test guidelines/assessment criteria is mentioned in determining the acceptability of a genotoxicity study. It is rather unfortunate that the publication by Moore <i>et al.</i> (2019; Environ Mol Mutagen. 60(7):624-663. doi: 10.1002/em.22278) which critically evaluated styrene genotoxicity literature for compliance with the OECD test guidelines was not identified in the committee’s literature search which would have helped the committee in their evaluations.</p>	<p>See the general comment in the response letter.</p> <p>The explanation under section 1.4 is adapted accordingly.</p> <p>The committee excluded studies without original data. Therefore, Moore <i>et al.</i> (2019) was not included.</p>
28	<p><b>Comment Section 3</b> Page 18, line (Mutagenicity)</p>	<p>This section should include some detail on the decision logic used by the committee in their evidence synthesis. Transparency and documentation would be improved by inclusion of a flowchart describing the relative weights given to various study types and endpoints along with any adjustments made for deficiencies in the study quality. The Health Council of the Netherlands “Guideline for the classification of carcinogenic substances” (2023; cited as reference 1 in the document) does not provide the needed details on this topic.</p>	<p>See the general comment in the response letter.</p>
29	<p><b>Comment Section 3.1.</b> (Human data) Page 18, lines 11-12</p>	<p>“No new studies on mutagenicity in humans were published after the IARC monograph.” There are at least 2 new studies that have been published since the IARC report: Cavallo <i>et al.</i>, 2018 and Ladeira <i>et al.</i>, 2020.</p>	<p>Cavallo <i>et al.</i>, 2018 and Ladeira <i>et al.</i>, 2020 are included in the report.</p>
30	<p><b>Comment Section 3.1.</b> (Human data) Page 18, lines 12-15</p>	<p>“Two meta-analysis by Collins <i>et al.</i> were published after the IARC-monograph, which also did not report new data. As both publications did not contain new information, they were not taken into account by the committee.” These two studies by Collins <i>et al.</i> did indeed produce new information by them being meta-analyses and as such the committee should have considered the valuable information derived from these carefully conducted studies. Based on their analyses, Collins <i>et al.</i> concluded that the published data are insufficient to support the conclusion that styrene exposure increases either micronucleus or chromosomal aberration frequencies in occupationally exposed workers.</p>	<p>See the general comment in the response letter.</p>
31	<p><b>Comment Section 3.1.1</b> Page 18, line 18 (Chromosomal aberration)</p>	<p>The 28 studies reviewed by the committee are listed in Annex A, Table A1.1 of the document. However, this table only includes cursory comments on a few studies regarding the study quality. Based on this information, it is not possible to assess how the committee classified 11 studies as being adequate quality and 11 studies of low quality. Ideally, the table should include all the information that the committee considered in terms of their quality judgment on the study quality. For example, the publications by Collins <i>et al.</i> (cited in the document) do an</p>	<p>See the general comment in the response letter.</p>

		excellent job of providing detailed study quality information to enable independent assessment. Alternatively, a link can be provided in the document where information can be found on the scoring of each study for various quality parameters.	
32	<b>Comment Section 3.1.1</b> Page 19, line 1 (Micronuclei)	Like the comments above on chromosome aberration studies, it is not possible to assess how the committee classified the studies listed in Annex Table A1.2 as adequate and moderate quality based on the cursory information provided in the table. It is suggested that additional details on the reasons used to judge the quality of each of the reviewed studies be provided in the table.	See the general comment in the response letter.
33	<b>Comment Section 3.1.1</b>	The committee makes a statement on line 5, page 19 that " <i>four studies of adequate quality found an association</i> ", but gives only three references, <i>i.e.</i> , 27, 45, and 47. This discrepancy needs to be corrected.	Discrepancy has been corrected
34	<b>Comment Section 3.1.1</b>	The report should acknowledge that the Migliore <i>et al.</i> study (cited as reference 45 in the report) also examined chromosomal aberrations in the peripheral blood lymphocytes of the same group of people and no significant difference between styrene-exposed and unexposed groups was evident despite a significantly higher incidence of micronuclei in the exposed group. This is an intriguing observation since both endpoints should have yielded similar outcomes especially given that the increased incidence of micronuclei in the exposed group was reported to be due to both chromosomal breakage and whole chromosome loss (and not preferentially due to the latter).	This has been adjusted in the report.
35	<b>Comment Section 3.1.1</b>	Vodicka <i>et al.</i> (reference 27 in the report) studied micronucleus frequencies in styrene-exposed subjects from 3 plants (Plants A, B, and C). The plant controls (PC) were derived from Plant B whereas the external controls (EC) were selected from the employees of the Regional Hygienic Station. The authors reported a significant increase in micronucleus frequency in styrene-exposed subjects of Plant A compared to the PC, but not to the EC. There was no significant difference when the micronucleus frequency from all exposed groups was compared to either PC or EC. The authors reported an association between elevated micronucleus frequencies with higher index of cumulative styrene exposure. Interestingly, like Migliore <i>et al.</i> study reviewed above, there was no increase in chromosomal aberration frequency in any of the exposed groups compared to PC or EC. Collectively, these results do not demonstrate convincing evidence for the increased MN frequency in styrene-exposed workers and thus the positive association currently stated in in Table A1.2 on page 70 of the report should be changed to negative or to at least equivocal.	This has been adjusted in the report to +/-. for MN: + (PC controls and exposure response provides strongest evidence, for CA: -
36	<b>Comment Section 3.1.1</b>	The report states that the study by Hogstedt (reference 47 in the report) found a statistically significant effect of styrene exposure on micronucleus frequency in the peripheral blood lymphocytes in a smaller setting. However, this is more like a method development study rather than a study designed to evaluate the effect of styrene exposure by employing an established laboratory protocol. As such, this study should have been given a lower weight and the results listed with a designation of "(+)" ( <i>i.e.</i> , positive in a study of limited quality) in Table A1.2 on page 69 of the report.	Although it is a small study with 38 exposed and 20 controls, the committee still adds weight to the evidence. As such, the result for this study will be left as it is.
37	<b>Comment Section 3.1.1</b>	The study by Gedderis <i>et al.</i> (reference 49 in the document) was listed as positive in Table A1.2 on page 70 of the report. A critical analysis of this study by Collins and Moore (reference 14 in the document) stated the following: " <i>There is a large variability in the frequency of MN in blood for both the controls and workers. In the absence of the detailed data by individual, it is difficult to interpret the results. The range of values for the controls and workers is not very different. Both show a low of 0. The controls had a high of 10 and the workers had a high of 13.50. MN data (for lymphocytes) were only presented for the XRCC1 codon 399 genotypes; while the workers had slightly higher MN frequencies than the controls for both identified genotypes, there was substantial variability and although the</i>	The committee considers this study positive, as the micronuclei are positive in mononucleated cells, binucleated cells and nasal cells. The difference in MN BC is small but significant in a group of 41 workers ( $p < 0.02$ ).

		<i>authors indicate a difference for these genotypes for the genotoxicity response, the differences for MN do not appear to be significant</i> ". The committee should consider this critique and change the outcome of the study to equivocal instead of positive in Table A1.2 on page 70 of the report.	
38	<b>Comment Section 3.1.1</b> Page 19, line 12 <b>(Aneuploidy and diploidy)</b>	The assessment of the quality of a single study identified under this category in Annex Table A1.3 was not provided. This study by Naccarati <i>et al.</i> (reference 51 in the document) concluded that in the sperm of workers exposed to styrene "...no important events of aneuploidy may occur..." The overall aneuploidy frequency (disomy and nullisomy) in exposed subjects ( $0.64 \pm 0.13$ ) was not significantly different from that of unexposed controls ( $0.62 \pm 0.13$ ). The authors reported that the difference between the nullisomy and disomy sperm in the group of styrene-exposed non-smokers was significantly different from the expected zero and this was only true for the sex chromosomes, but not the other autosome ( <i>i.e.</i> , chromosome 2) analyzed. According to the multifactor analysis of variance reported by the authors in Table 3 of their publication, styrene exposure itself had no significant effect on the excess of nullisomy sperm. The authors, nevertheless, speculated that the statistically significant excess of nullisomy sperm compared to the disomy sperm in the subgroup of styrene-exposed non-smokers "... could be somehow associated with styrene exposure". However, given the small number of subjects in this subgroup ( <i>i.e.</i> , $N = 8$ ), the authors theorized that their finding might be due to stochastic fluctuation and came to the overall conclusion that there was a lack of striking association between exposure to styrene and sperm aneuploidies. Currently, the statement on page 19, lines 17-18 of the committee's document which reads "[t]he only statistically significant finding was an excess of nullisomy in the exposed non-smokers" needs to be revised to clarify that the excess was not relative to the styrene-unexposed subjects. Furthermore, in Table A1.3 (page 73) under the column "Comments committee", the statement "positive association among exposed non-smokers ( $n=6$ )" needs to be revised to clarify what the positive association meant lest give the impression that it is associated with styrene exposure and also " $n=6$ " needs to be revised to $n=8$ to reflect the number in Table 3 of Naccarati <i>et al.</i> publication. Finally, the committee's assessment of the results under the column "Result (significance)" in Table A1.3 (page 73) should be changed from "+/-" to "-" to reflect that the study does not provide any evidence for aneuploidy or diploidy.	The committee agrees that the study population was small. The committee already stated in its report that this study did not provide sufficient evidence for mutagenicity.
39	<b>Comment Section 3.1.1</b> Page 19, line 19 <b>(Gene mutation)</b>	The five studies listed in Annex Table A1.4 were judged by the committee as being adequate quality, but the supporting information underpinning this judgment was not provided. Some of these studies had either a very small sample size (Vodicka <i>et al.</i> , 1995, 1999, references 54 and 54 in the document) or used an endpoint (Glycophorin A variant frequency; Compton-Quintana <i>et al.</i> , 1993 (reference 52 in the document) and Bigbee <i>et al.</i> , 1996 (reference 53 in the document) that has questionable reliability as a biomarker of mutagenicity. It is also uncertain whether the Vodicka <i>et al.</i> , 1999 study is simply a follow-up to their 1995 publication with a slightly larger sample size rather than being an independent study. It also appears that the committee might have missed the following two studies in their evaluation which reported <i>HPRT</i> mutant frequencies in styrene-exposed workers: Tate <i>et al.</i> , 1994 and Lambert <i>et al.</i> , 1995.	Tate <i>et al.</i> , 1994 has been disregarded due to co-exposure (See Table A1.1 Chromosomal aberration in humans after styrene exposure)  Lambert <i>et al.</i> , 1995 has been excluded due to the use of an irrelevant control group.
40	<b>Comment Section 3.1.2</b> Page 19, line 22 <b>(Miscellaneous)</b>	Other than providing citations of the studies reviewed, this section provides very little information on the evidence synthesis conducted by the committee in reaching their conclusions on the association between styrene exposure and the occurrence of DNA damage, sister-chromatid exchanges (SCE), DNA adducts and the "rate" of gaps. At a minimum, there should be tables in Annex A listing the evaluation of these studies by the committee. In Section 3 (page 18), it is stated that the quality assessment of the IARC monograph was followed for these endpoints.	See the general comment in the response letter.

		<p>However, it is not clear whether the committee also relied upon IARC's assessment of the studies' outcome. Nevertheless, it is commendable that the committee considered only chromosome aberrations, micronuclei, aneuploidy and gene mutations as most important endpoints for the assessment of mutagenicity (as stated in Section 3, page 18). The document should reinforce the above statement by acknowledging that the miscellaneous endpoints in Section 3.1.2 were given a lower weight in their evidence synthesis since DNA adducts/damage and SCE are essentially markers of exposure rather than an irreversible effect and [chromosome] gaps are not considered as <i>bona fide</i> indicators of genotoxic effect as the OECD test guidelines recommend their exclusion in the calculation of total aberration frequency. e.g., Wilson, 2007 and Eastmond, 2014.</p>	
41	<p><b>Comment Section 3.2</b> Page 20, line 1 <b>(Animal Data)</b></p>	<p>The document states that "[a]n overview of the mutagenic data subtracted from the IARC Monograph considered most important can be found in Table A2 in Annex A." It will be informative if the document provides some detail on what constituted the basis for considering a study/data "most important."</p>	<p>See the general comment in the response letter.</p>
42	<p><b>Comment Section 3.2.1</b> Page 20, line 8 <b>(Chromosomal aberration)</b></p>	<p>The document provides few details on the critical evaluation of the data, other than showing "-", "+", or "(+)" on the studies' outcome in Table A2.2. The studies by Loprieno <i>et al.</i> and Sinsheimer <i>et al.</i> (references 82 and 85, respectively, in the document) on styrene oxide were listed as positive. A closer look at the Loprieno <i>et al.</i> study reveals that the study does not comply with the relevant OECD test guideline (TG475) in that 1) insufficient number of cells were evaluated per animal, 2) gaps were included in calculating total aberrations which is not acceptable, 3) a relatively large fraction of aberrations scored were listed under the category "others" without specifying what exactly they were, and 4) it is not clear which of the several endpoints listed in Table 3 of the publication was used for the statistical analysis. Incidentally, there appears to be an error in the calculation of aberrations/damaged cell for the 1000 mg/kg styrene oxide treated group (0.07 aberrations/damaged cell vs. 1.22 for the control). Given these deficiencies, this study should have been listed as uninterpretable. The study by Sinsheimer <i>et al.</i> was critically reviewed by Moore <i>et al.</i> (2019; doi: 10.1002/em.22278) and the authors concluded that this study was also uninterpretable due to significant deviations from the OECD TG 475 due to the use of an irrelevant route of administration (<i>i.e.</i>, intraperitoneal), use of a single dose level and scoring of insufficient number of cells for aberrations. An additional study by Norppa <i>et al.</i> (reference 90 in the document) was listed as equivocal for "both cytogenetic tests" without specifying what the two cytogenetic tests were since Table A2.2 lists only chromosome aberration endpoint. The use of the irrelevant route of administration (<i>i.e.</i>, intraperitoneal) renders this study uninterpretable in addition to its being of "limited quality" as interpreted in the document. A critical review of this study can be found in Moore <i>et al.</i> (2019; doi: 10.1002/em.22278).</p>	<p>The committee would like to stress that studies that do not fully adhere to OECD guidelines may still provide valuable insights for assessing a substance's mutagenicity and carcinogenicity. These studies were therefore also taken into account. <i>Evaluation of a larger number of cells improves the statistical power and helps in detecting rare genotoxic events and thus increases the sensitivity of the assay. The exact number of cells evaluated is of less relevance in case a positive response is already detected.</i> Moreover, we agree that the <i>i.p.</i> route of exposure may be of less relevance, but it does add to the evidence about the intrinsic capacity of a substance to induce genotoxic effects. Therefore, <i>ip</i> studies were given less weight, but were not ignored. Finally, we would like to refer to our new knowledge synthesis paragraph, in which we make clear that the classification of styrene is predominantly based on studies in humans and <i>in vitro</i> studies with human derived cells, because of the differences in styrene metabolism between rodents and humans.</p>
43	<p><b>Comment Section 3.2.1</b> Page 21, line 1 <b>(Micronuclei)</b></p>	<p>Table A2.3 of the document lists the studies conducted by Simula and Priestly (1992) and Norppa (1981) as positive for styrene-induced micronuclei. However, both these studies used the intraperitoneal route of administration, which is no longer considered a relevant route of exposure (OECD TG 474). In addition, there are other test guideline deviations such as the number of cells enumerated for micronuclei and excessive (50%) mortality at the top (and only) dose eliciting a significant MN response in the Simula and Priestly study. Thus, these studies are of limited value and should not have been classified as positive.</p>	<p>If mutagenicity is observed in an <i>in vivo</i> test using intraperitoneal administration, it demonstrates that the substance has intrinsic mutagenic properties, which the committee considers relevant for hazard classification. Overall, the committee prioritized the human studies over rodent studies for the classification, placing less emphasis on the latter.</p>

44	<b>Comment</b> <b>Section 3.2.2</b> Page 21, line 18 <b>(Miscellaneous)</b>	Few details were provided on the critical analysis of the literature covering DNA damage, SCE, unscheduled DNA synthesis, and DNA adducts. At a minimum, there should be tables in Annex A of the document listing these studies and their critical evaluation by the committee. As commented earlier under Section 3.1.2, it is not clear whether the committee relied not only on the IARC's quality assessment but also on their critical assessment of the studies' outcome. Given that all the endpoints covered under this section are primarily measures of exposure rather than irreversible genotoxic effects, the committee's recognition that they are given lower weight in their evidence synthesis is commended.	The committee takes note that the endpoints DNA damage, SCE, unscheduled DNA synthesis, and DNA adducts are not irreversible effects. However, it does involve DNA damage. Therefore, these outcome measures are still considered relevant supporting evidence due to their interaction with DNA.
45	<b>Comment</b> <b>Section 3.2.3</b> Page 22, line 27 <b>(Recent Studies)</b>	The recent studies reviewed in this section were all conducted in compliance with relevant OECD test guidelines and addressed the potential for styrene to induce gene mutations, cytogenetic effects (chromosomal aberrations/aneuploidy) and DNA damage (using the comet assay). Accordingly, results from these studies should be regarded as most reliable to inform the <i>in vivo</i> genotoxic potential of styrene.	See the general comment in the response letter.
46	<b>Comment</b> <b>Section 3.2</b>	<p>Overall, the assessment of these studies by the committee is balanced. The committee, however, stated that no firm conclusion can be drawn on the mutagenicity of styrene based on the liver mutant frequency of the transgenic Big Blue mouse study (reference 78 in the document) because i) the robustness of the historical vehicle control data is questionable, and ii) the data did not meet the criteria for clear negative results per OECD TG 488 criteria. Each of these points is discussed below.</p> <p>OECD TG 488 states:  <i>"When first acquiring data for a historical negative control distribution, concurrent negative controls should be consistent with published data where they exist."</i> It goes on to state, <i>"The laboratory's historical negative control database should be compiled, analysed and regularly updated according to literature recommendations. This should include: consideration of the minimum number of data sets required to establish a robust distribution (a minimum of 30 animals is desirable)."</i> <i>"Where the laboratory does not complete a sufficient number of experiments to establish a statistically robust negative control distribution during the proficiency investigations, it is acceptable that the distribution can be built during the first routine tests. This approach should follow the recommendations set out in the literature and the negative control results obtained in these experiments should remain consistent with published negative control data"</i>.</p> <p>Gentronix, the contract research organization that conducted the Big Blue study, acquired the rights to the Big Blue TGR model following the closure of a laboratory in the USA that previously provided these services. This acquisition happened around the time of commissioning the styrene study. The <i>cII</i> gene mutant frequency data derived at Gentronix was compared with the robust historical data of the laboratory in the USA that previously conducted regulatory Big Blue mutation studies. Gentronix's historical data based on the limited number of animals was highly consistent with the larger historical data of the US laboratory (see Gollapudi <i>et al.</i>, 2024). Thus, there should be little concern regarding the historical control data generated at Gentronix.</p> <p>The committee correctly stated that the liver data did not meet the criteria for a clear negative result per OECD TG 488 stipulations. However, TG 488 (para 66) also gives the option for expert scientific judgment of the data. The small, 1.3-fold increases in the mutant frequency in the liver were not biologically relevant since they were not dose-related and were within the historical vehicle control distribution. Furthermore, liver is not a tumor target tissue in styrene-treated mice and styrene did not increase DNA damage in this tissue in the comet assay</p>	We would like to refer to our new knowledge synthesis paragraph, in which we clarify that the classification of styrene is predominantly based on studies in humans and <i>in vitro</i> studies with human derived cells, because of the differences in styrene metabolism between humans and rodents. We acknowledge the comments regarding the animal mutagenicity study using transgenic rodents. However, in our weight of evidence approach, we have given more weight to the human data. Consequently, we feel it is not essential to elaborate further on the details of this study as it would not affect the evidence found in humans and our classification.

		(reference 79 in the document). In general, the comet and the transgenic gene mutation assays have been shown to identify the same substances as genotoxic in the liver (Kirkland <i>et al.</i> , 2019).	
47	<b>Comment Section 3.2</b>	In reviewing the comet assay results of a rat study (reference 80 in the document), the committee noted that the percentage Tail DNA in the vehicle control group was above historical vehicle control distribution for the duodenum and kidney. It is noteworthy that the inter-study variability in percent Tail DNA values in the negative controls is quite high at most laboratories, and this is likely attributable to the confounders associated with the processing of the tissue samples (Dertinger <i>et al.</i> , 2023). Because of this, expert judgment is often used in accepting the concurrent vehicle control values of a given study even when they fall outside of the laboratory historical data.	
48	<b>Comment Section 3.3</b> Page 27 (In vitro data)	Several studies in Table A3 of Annex 3 did not appear to have gone through a critical evaluation. Examples are provided below.	<b>Regarding comments 49-54</b> Limitations were added to the report
49	<b>Comment Section 3.3</b> Page 78, <b>Table A3.1</b>	The study by Linnainmaa <i>et al.</i> (1978a) was listed as positive for the induction of chromosomal aberrations. However, a critical examination of this study indicates several limitations such as the lack of concurrent cytotoxicity data and inappropriate endpoint scoring making the results of this study uninterpretable.	Added limitations to the report.
50	<b>Comment Section 3.3</b> Page 78, <b>Table A3.1</b>	The study by Pohlová <i>et al.</i> (1984) which was listed as positive is more appropriately classified as uninterpretable because there was no measure of cytotoxicity raising the question of whether the concentration evaluated exceeded the recommended cytotoxicity upper limit for these assays.	Added to the report that cytotoxicity data is missing.
51	<b>Comment Section 3.3</b> Page 79, <b>Table A3.2</b>	The study by Fabry <i>et al.</i> (1978) should be listed as uninterpretable rather than positive because there was no concurrent measure of cytotoxicity in the study raising the possibility of excess toxicity leading to misleading positive results. For similar reasons, the studies by Linnainmaa <i>et al.</i> (1978a) and Pohlova <i>et al.</i> (1984) should have been listed as uninterpretable.	Added to the report that cytotoxicity data is missing.
52	<b>Comment Section 3.3</b> Page 80, <b>Table A3.4</b>	The Godderis <i>et al.</i> (2006) study should be classified as uninterpretable rather than positive since no concurrent measure of cytotoxicity was reported leading to the uncertainty of whether the response is related to an unacceptable level of cytotoxicity.	Added to the report that cytotoxicity data is missing.
53	<b>Comment Section 3.3</b> Page 80, <b>Table A3.5</b>	The results of the study by Bastlová <i>et al.</i> (1995) are more accurately characterized as uninterpretable due to inadequacies in the number of cells treated as well as the cell survival following treatment.	Added limitations to the report.
54	<b>Comment Section 3.3</b> Page 81, <b>Table A3.6</b>	Both studies listed in this table should be better characterized as uninterpretable rather than “ <i>positive in a limited quality study</i> ” due to limitations on data reporting (Ishidate and Yoshikawa, 1980) or lack of concurrent measure of cytotoxicity (Matsuoka <i>et al.</i> , 1979).	Added limitations to the report.
55	<b>Comment Section 3.3</b> Page 81, <b>Table A3.7</b>	Turchi <i>et al.</i> (1981) study was not cited in the references and thus the data cannot be verified.	Added reference to the report.
56	<b>Comment Section 3.3</b> Page 81, <b>Table A3.8</b>	Turchi <i>et al.</i> (1981) was not cited in the references and thus the data could not be verified.	Added reference to the report.
57	<b>Comment Section 3.3</b> Page 82, <b>Table A3.8</b>	The title for this table was listed as <i>Gene mutation in in vitro mammal cells after styrene exposure</i> whereas a table with the same number on Page 81 was listed as <i>Micronuclei in in vitro mammal cells after styrene-7,8-oxide exposure</i> . Given that the two studies listed in Table A3.8 on page 82 ( <i>i.e.</i> , Loprieno <i>et al.</i> , 1976, and Beije and Jenssen, 1982) did indeed examine gene mutations, it is assumed that there is a typographical error	Table numbering adjusted in the report.

		in table numbering. The studies by Loprieno <i>et al.</i> and Beije and Jenssen have several deficiencies vis-à-vis the OECD test guideline 476 and are thus more appropriately classified as uninterpretable rather than positive/negative.	
58	<b>Comment Section 3.3</b> Page 82, <b>Table A3.9</b>	All the studies listed in this table are better characterized as uninterpretable rather than positive due to significant deviations from the OECD test guideline 476.	The committee does not agree that studies that deviate from the OECD guidelines are automatically uninterpretable for the evaluation of mutagenicity. It is a quality criterion, but not the only factor to consider.
59	<b>Comment Section 3.3</b> Page 83, <b>Tables A3.10, A3.11 and A3.12</b>	These tables are incorrectly labeled as <i>in vitro</i> tests in microorganisms whereas the species tested were not microorganisms (plant <i>Allium cepa</i> and fruit fly <i>Drosophila melanogaster</i> ).	Adjusted labelling to 'non-mammalian experimental systems' in the report.
60	<b>Comment Section 3.3</b> Page 84, <b>Table A3.13</b>	Studies conducted with the fruit fly <i>Drosophila melanogaster</i> were erroneously listed under the title <i>Gene mutation in in vitro micro-organism cells after styrene exposure</i> .	Adjusted labelling to 'non-mammalian experimental systems' in the report.
61	<b>Comment Section 3.3</b> Page 87, <b>Table A3.14</b>	Tests conducted by Donner <i>et al.</i> (1979) in the fruit fly <i>Drosophila melanogaster</i> were erroneously listed under the title " <i>Gene mutation in in vitro micro-organism cells after styrene-7,8-oxide exposure.</i> "	Adjusted labelling to 'non-mammalian experimental systems' in the report.
62	<b>Comment Section 3.3</b> Page 27	While the division of the studies between mammalian and non-mammalian cells is logical, it is not clear why studies using human cells need to be listed on their own. OECD test guidelines do not make such a distinction between cells/cell lines derived from human origin and other mammalian origins. It gives the impression that results from studies employing human cells are given a different weight than the other mammalian cell cultures and if so, such a differential weighing is not scientifically justified.	To maintain consistency, the committee followed the layout of the IARC, which explains why this distinction was made.
63	<b>Comment Section 3.3</b> Page 27	A critical evaluation of the studies conducted by Moore <i>et al.</i> (2019) indicated that most of the studies conducted using mammalian (including human) cell cultures were uninterpretable because of deficiencies vis-à-vis the relevant OECD guideline requirements. However, the critical review by Moore <i>et al.</i> (2019) also found interpretable studies with positive results to enable a conclusion that styrene/styrene oxide can induce chromosomal damage <i>in vitro</i> . None of the studies that investigated gene mutations in mammalian cell cultures were interpretable in the critical review of Moore <i>et al.</i> (2019) except for a single study using mouse lymphoma cells that showed evidence for the induction of gene mutations when treated with styrene-7,8-oxide.	The committee excluded studies without original data. Therefore, Moore <i>et al.</i> (2019) was not included.  The committee does not agree that studies that deviate from the OECD guidelines are automatically uninterpretable for the evaluation of mutagenicity. It is a quality criterion, but not the only factor to consider.
64	<b>Comment Section 3.3</b> Page 28, line 16 <b>(Non-mammalian cells)</b>	Inclusion of studies employing the fruit fly <i>Drosophila melanogaster</i> under <i>in vitro</i> non-mammalian cell category is not valid since these studies are in fact <i>in vivo</i> studies using non-mammalian species.	See comment 59
65	<b>Comment Section 3.4</b> Page 29, line 3 <b>(Evaluation of mutagenicity)</b>	The committee's conclusion that styrene should not be classified to 1A or 1B for germ cell mutagenicity is justified based on the available data and the criteria specified in the Netherlands Health Council's guideline for the classification of carcinogenic substances (2023; cited as reference 1 in the document).	
66	<b>Comment Section 3.4</b>	In their consideration for classifying styrene for Category 2 for germ cell mutagenicity, the committee observed that "[i]n general, studies on rodents exposed to styrene or styrene-7,8-oxide yielded either negative or inconclusive outcomes regarding cytogenetic effects". However, the weight-of-the-evidence conclusion by Moore <i>et al.</i> (2019) based on their critical review of the <i>in vivo</i> cytogenetic data indicated that neither styrene nor styrene-7,8-oxide induces cytogenetic damage. In addition, the recent OECD test guideline-compliant studies cited by the committee in Section 3.2.3 of the document clearly indicated that styrene is not capable of inducing cytogenetic damage in the	See the general comment in the response letter.

		mouse and rat bone marrow cells. Thus, based on the weight of the evidence, there is a compelling argument for stating that styrene (and styrene-7,8-oxide) yielded negative outcomes in rodents regarding cytogenetic effects.	
67	<b>Comment Section 3.4</b>	The committee states "... <i>epidemiological studies showed limited evidence for chromosomal aberrations and micronuclei</i> " (page 29, lines 22-23). However, the recent meta-analyses conducted by Collins <i>et al.</i> (cited as references 14 and 15 in the document) found insufficient evidence to support the conclusion that styrene exposure increases either micronucleus or chromosomal aberration frequencies in occupationally exposed workers. Thus, the available epidemiology data supports the characterization of styrene as showing no evidence for chromosomal aberrations and micronuclei.	See the general comment in the response letter.
68	<b>Comment Section 3.4</b>	The committee also makes the statement "... <i>in vitro studies in human cells have consistently shown that both styrene and its metabolite, styrene-7,8-oxide, cause genotoxic effects</i> " (page 29, lines 23-25). This statement gives the impression that the results of studies employing human cells are somehow treated differently from the other mammalian cell culture studies. There is no data to justify such a distinction and mammalian cell culture studies, whether originating from humans or rodents, should be treated similarly.	To maintain consistency, the committee followed the layout of the IARC, which explains why this distinction was made. The committee does not treat the <i>in vitro</i> studies in human cells differently.
69	<b>Comment Section 3.4</b>	In summary, the available data on styrene and styrene-7,8-oxide is best characterized as showing evidence for mutagenicity and/or cytogenetic effects in the <i>in vitro</i> cell systems, but no such evidence can be found in epidemiological or experimental animal studies. Accordingly, germ cell mutagenicity in humans is unlikely. Although it is acknowledged that the classification of styrene as Category 2 mutagen is based on the Netherlands Health Council's guideline for the classification of carcinogenic substances (2023; cited as reference 1 in the document), there is a compelling scientific rationale to support styrene as Category 4 mutagen (mutagenicity in man is unlikely) given that there is sufficient data from both epidemiological studies and experimental animal studies to indicate that mutagenicity in man is unlikely.	See the general comment in the response letter.
70	<b>Comment Section 3.5</b> Page 29, line 27 <b>(Recommendation on the classification for mutagenicity)</b>	In contrast to the conclusion made by the Committee, in our opinion, a weight of evidence assessment of the comprehensive database on the genotoxicity of styrene justifies a different outcome. As discussed above, styrene-induced mutagenic and cytogenetic effects are only evident in <i>in vitro</i> test systems, whereas epidemiological and experimental animal studies show no convincing evidence that styrene possesses significant mutagenic/clastogenic potential <i>in vivo</i> . Consequently, the genotoxicity profile of styrene is better justified by a recommendation to classify it as a Category 4 mutagen (mutagenicity in man is unlikely) in accordance with the Netherlands Health Council's guideline for the classification (cited as reference 1 in the document). Furthermore, and to avoid any possible confusion, this assessment and conclusion would not support classification of styrene for mutagenicity according to EU-criteria (67/548/EEC) and to GHS-criteria (1272/2008/EC).	See the general comment in the response letter.
<b>1 DHC Scope and Methodology</b>			
71	<b>Comment 1</b>	The Council's draft advisory report starts with an overview of the procedure, data, and quality assessment approach that were used in its evaluation of the carcinogenicity and mutagenicity of styrene in humans. However, this section lacks critical details that facilitate the understanding of the Council's discussions and conclusions that are made in the following sections. For transparency and clarity, we suggest that a few key elements be added to this section.	See the general comment in the response letter.
72	<b>Comment 1.1</b> Page 11, Line 13 to Page 12, Line 1 ( <b>Report</b> )	The Council states that "A literature summary published by the National Institute for Public Health and the Environment (RIVM), which was prepared at the request of the Health Council, was used as a starting point for the evaluation. <sup>2</sup> Another important	See the general comment in the response letter.

	<b>Section 1.3 Data)</b>	<p>source of information was the evaluation by the International Agency for Research on Cancer (IARC).”<sup>2</sup></p> <p>For example, regarding study quality assessment, the Council states in <b>Report Section 1.4 Quality assessment</b> that: For mutagenicity, the committee only evaluated the quality of the original studies with clastogenic and aneugenic outcome measures, as these are considered most important for the assessment of mutagenicity. For the studies with miscellaneous outcome measures, the committee followed the quality assessment of the IARC. For carcinogenicity, the committee evaluated all the selected carcinogenicity studies on their quality.</p> <p>However, in its evaluation of human data on mutagenicity (<b>Section 3.1</b>), the Council presented in Table A1 “An overview of the data subtracted from the IARC Monograph considered most important”; in its evaluation of human data on carcinogenicity (<b>Section 4.1</b>), the Council presented in Table B1 “An overview of the carcinogenicity data in humans” that appear to be extracted from the RIVM report.</p>	
73	<b>Comment 1.2</b> Page 11, Lines 1 and 10 ( <b>Report Section 1.2 Committee and procedure</b> )	<p>While the Council introduced the meaning of different categories for the classifications of mutagenicity and carcinogenicity of styrene in a table and cited the <i>Guideline for the classification of carcinogenic substances</i>, it is unclear whether the criteria for classification into the different categories listed in the cited guideline were followed strictly or modified in any way for the Council's assessment. We recommend that the Council present the exact criteria that were considered in its styrene assessment in this section.</p> <p>More specifically, in the draft advisory report, the Council lists 1A, 1B, and 2 as possible categories for mutagenicity and 1A, 1B, 2, and “no classification” as possible categories for carcinogenicity. In contrast, the <i>Guideline for the classification of carcinogenic substances</i> cited by the Council lists 1A, 1B, 2, 3, and 4 as possible categories for both mutagenicity and carcinogenicity. The Council should explain the requirements for these categories and the basis for each study's classification.</p>	See the general comment in the response letter.
74	<b>Comment 1.3</b>	<p>Page 11, Line 3-9 (<b>Report Section 1.2 Committee and procedure</b>) – The Council states, “The classification systems on mutagenicity and carcinogenicity are based on a weight of evidence assessment, in which more weight is given to evidence obtained from human data than to evidence obtained from animal studies or laboratory data. Furthermore, the weight of evidence depends on the number of reliable studies that show clear associations between exposure and the occurrence of mutagenicity or carcinogenicity. This implies that studies with significant shortcomings contribute to a lesser extent to the overall weight of evidence.”</p> <p>The “weight of evidence assessment” appears to have played a crucial role in the Council's classification of mutagenicity and carcinogenicity of styrene. However, it is unclear what this assessment entailed. <b>Section 04 strength of evidence for causality</b> of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i><sup>3</sup> discusses “[s]ome considerations and tools” that can be used for a weight of evidence assessment, including the “Bradford Hill's considerations” and the “GRADE framework.” For transparency, it is important that the Council describe in detail its “weight of evidence assessment,” specifying any tools used or any reasons for not using one.</p>	See the general comment in the response letter.
75	<b>Comment 1.4</b> Page 12, Lines 27-34 ( <b>Report Section 1.4 Quality assessment</b> )	<p>The Council very briefly describes the study quality assessment that was performed by stating, “Study quality may vary, and therefore, the committee assessed the quality of the study based on reliability (quality of methodology and reporting), on the relevance for the purpose of the assessment, and on adequacy (usefulness), according to the current views in the scientific community. The quality evaluation was performed to assess the</p>	See the general comment in the response letter.

		<p>weight of evidence for an association between substance exposure and mutagenicity and/or risk of cancer development. The committee's considerations for determining the quality of a study can be found in the Guideline for the classification of carcinogenic substances.”</p> <p>It is unclear whether the guideline that the Council cites was followed strictly or modified in any way for its study quality assessment. However, there appear to be inconsistencies. The <i>Guideline for the classification of carcinogenic substances</i> cited by the Council refers to another source for study quality assessment, namely the <i>Guidance for recommending classifications and health-based occupational exposure limits</i>, which states that “each study is assessed for quality by judging its relevance, reliability, and validity.” “[A]dequacy (usefulness),” as noted in the draft advisory report does not appear to be part of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i>. Furthermore, “quality of methodology and reporting,” which the Council noted for reliability is also a consideration for validity according to the <i>Guidance for recommending classifications and health-based occupational exposure limits</i>.</p> <p>We suggest that the Council add to this section the specific criteria for study quality assessment that were used in its styrene assessment, and that the basis for each study's quality judgment be explicit.</p>	
<b>2 Mutagenicity in Humans</b>			
76	<b>Comment 2.1</b> Pages 18, Lines 2-6 ( <b>Report Section 3 Mutagenicity</b> )	The draft advisory report states that “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.” However, details regarding how this quality assessment was performed and the individual results for each study are not provided.	See the general comment in the response letter.
77	<b>Comment 2.1</b> pages 18-19 ( <b>Report Section 3.1.1 Clastogenic and aneugenic effects</b> )	<p>Similarly, on pages 18-19 (<b>Report Section 3.1.1 Clastogenic and aneugenic effects</b>), in its discussions of epidemiology studies of styrene exposure and clastogenic and aneugenic effects, the Council uses the terms “adequate quality”, “moderate quality”, and “low quality” to refer to the quality of various groups of cited studies, but does not provide definitions for these categories or the criteria that was used to place individual studies in a particular category. In addition, the study tables in <b>Annex A1 (Summary table of mutagenicity in humans after styrene exposure)</b> do not provide the individual quality scores for each study.</p> <p>For example, the draft advisory report noted the Naccarati <i>et al.</i> (2003) study was the “[o]nly one study of adequate quality” (emphasis added) that “studied frequencies of sperm cells with aneuploidy and diploidy in individuals occupationally exposed to styrene.” However, no further information related to the Council's determination that the quality of this study was “adequate” is provided; Naccarati <i>et al.</i> (2003) was comprised of a small group of volunteer workers in Tuscany, Italy, who provided specimens for cytogenetic analysis (including 18 exposed and 13 unexposed individuals), thus its findings may be biased and not generalizable to other workers.</p>	See the general comment in the response letter.
78	<b>Comment 2.1</b>	It should be noted that all study quality assessments involve some degree of subjectivity. For transparency, it is important that a detailed documentation of the Council's quality assessments of individual studies is made available. We note that <b>Section 3.3 Expert judgement</b> of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i> states, “The evaluation of study quality is inherently reliant on expert judgement. This may lead to differences in conclusions among experts and among expert committees. To obtain scientifically robust hazard and risk assessments, the Health	See the general comment in the response letter.

		Council strives to use a systematic and transparent evaluation, so that third parties can find out how the quality of the individual studies was addressed. This means that the advisory reports contain objective summaries of the available studies, accompanied by a comprehensive evaluation of the quality of the study."	
79	<b>Comment 2.1</b>	We ask that the Council provide this critical information regarding the study quality assessment that was performed for the mutagenicity studies. This information should include the specific criteria and definitions used by the Council to determine each overall study quality level, along with the individual quality level determined for each mutagenicity study it evaluated.	See the general comment in the response letter.
80	<b>Comment 2.2</b> Page 18, Lines 10-15 ( <b>Report Section 3.1 Human data</b> )	<p>The draft advisory report states, "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-analyses by Collins <i>et al.</i> 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."</p> <p>This statement is inaccurate, as the two meta-analyses by Collins <i>et al.</i> contain additional studies and information not considered in the draft advisory report. Specifically:</p> <p>- Collins and Moore (2019) is an update to a prior meta-analysis of micronucleus frequencies in workers exposed to styrene by Costa <i>et al.</i> (2016). The authors used additional studies not previously included and also eliminated the double counting of study subjects appearing in more than one publication, along with an increased consideration of levels of styrene exposure, publication bias, and consistency of findings. Of the 12 studies included in Collins and Moore (2019) that evaluated micronucleus frequencies in styrene workers, four (Van Hummelen <i>et al.</i>, 1994; Anwar <i>et al.</i>, 1995; Teixeira <i>et al.</i>, 2004, 2010) are not referenced in the draft advisory report, with two of these (Van Hummelen <i>et al.</i>, 1994; Anwar <i>et al.</i>, 1995) also not previously considered by IARC in the 2018 monograph (IARC, 2018). Three additional studies (Holz <i>et al.</i>, 1995; Laffon <i>et al.</i>, 2002; Costa <i>et al.</i>, 2012) are discussed in the draft advisory report, but their micronucleus results are not. Details of these seven studies are discussed further below (in Comment 2.3).</p> <p>Overall, Collins and Moore (2019) concluded, given the lack of consistency across studies and the equivocal findings on exposure response, that the data overall are insufficient to support a conclusion that increased micronucleus frequencies in workers is associated with styrene exposure.</p> <p>- Collins and Moore (2021) is a critical review and meta-analysis of epidemiology studies of occupationally exposed styrene workers evaluated for chromosomal aberrations. The authors used a systematic approach to assess data and study quality, consistency, strength of the association, and exposure-response, also considering the potential for co-exposures, confounding exposures, and publication bias. Of the 18 studies included that examined the incidence of chromosomal aberrations in workers exposed to styrene, three (Thiess <i>et al.</i>, 1980; Anwar <i>et al.</i>, 1995; Biro <i>et al.</i>, 2002) are not referenced in the draft advisory report. Two of these studies (Anwar <i>et al.</i>, 1995; Biro <i>et al.</i>, 2002) were also not previously considered by IARC in the 2018 monograph. An additional study, Migliore <i>et al.</i> (2006), is discussed in the draft advisory report (in Section 3.1.1 <b>Clastogenic and aneugenic effects</b>) and in the 2018 IARC monograph for its micronucleus results, but not its chromosomal aberration results. Details of these four studies are discussed further below (in Comment 2.4).</p>	See the general comment in the response letter.

		<p>Overall, Collins and Moore (2021) found a lack of consistency across studies and the absence of an exposure-response relationship, along with several study limitations, with the authors concluding that, while studies of styrene workers overall had a slight increase in chromosomal aberrations relative to comparison groups, there was insufficient evidence to support a conclusion that styrene exposure increases chromosome aberration frequencies in styrene workers.</p> <p>We recommend that the Council reconsider its decision to exclude the two meta-analyses by Collins and Moore (2019) and Collins and Moore (2021), and all of the studies therein, as they provide new information and analyses not previously considered, overall adding to the body of evidence supporting a lack of association between styrene exposure and increased micronuclei frequencies and chromosomal aberrations in workers.</p>	
81	<b>Comment 2.3</b> Pages 69-72 <b>(Report Table A1.2 Micronuclei in humans after styrene exposure)</b>	<p>In Table A1.2, the draft advisory report lists 13 epidemiology studies identified that evaluated micronuclei levels humans after styrene exposure. These 13 studies are also discussed on Page 19, lines 1-11 (<b>Report Section 3.1.1 Clastogenic and aneugenic effects</b>). It is recommended that DHC add seven additional studies to Table A1.2 that have also evaluated micronuclei in humans after styrene exposure; these studies should also be included in the discussion of micronuclei studies in Section 3.1.1 (Page 19, lines 1-11). These studies are Van Hummelen <i>et al.</i> (1994), Anwar <i>et al.</i> (1995), Teixeira <i>et al.</i> (2004) and (2010), Holz <i>et al.</i> (1995), Laffon <i>et al.</i> (2002), and Costa <i>et al.</i> (2012) (Table 1). Van Hummelen <i>et al.</i> (1994) and Anwar <i>et al.</i> (1995) were also not previously considered by IARC in the 2018 monograph (IARC, 2018). Overall, these seven studies provide important information and add to the body of evidence supporting the lack of an association between increased micronuclei frequency and styrene exposure in human populations.</p>	See the general comment in the response letter.
82	<b>Comment 2.4</b>	<p>Pages 61-68 (<b>Report Table A1.1 Chromosomal aberration in humans after styrene exposure</b>) – In Table A1.1, the draft advisory report lists 28 epidemiology studies that evaluated chromosomal aberrations in humans after styrene exposure. These 28 studies are also discussed on Page 18, Lines 18-31 (<b>Report Section 3.1.1 Clastogenic and aneugenic effects</b>). It is recommended that DHC add four additional studies to Table A1.1 that have also evaluated chromosomal aberrations in humans after styrene exposure; these studies should also be included in the discussion of chromosomal aberration studies in Section 3.1.1 (Page 18, Lines 18-31). These studies are Thiess <i>et al.</i> (1980), Anwar <i>et al.</i> (1995), Biro <i>et al.</i> (2002), and Migliore <i>et al.</i> (2006) (Table 2). Anwar <i>et al.</i> (1995) and Biro <i>et al.</i> (2002) also are not referenced by IARC in the 2018 monograph (IARC, 2018). In addition, while the Migliore <i>et al.</i> (2006) study is discussed in the draft advisory report (in <b>Section 3.1.1 Clastogenic and aneugenic effects</b>) and in the 2018 IARC monograph for its micronucleus results, its chromosomal aberration results were not discussed. With respect to the Thiess <i>et al.</i> (1980) study, IARC has previously noted that this study did not identify a statistically significant increase in chromosomal aberration rates in workers compared with unexposed controls (IARC, 2018). Overall, these four studies provide important</p>	See the general comment in the response letter.

		information and add to the body of evidence supporting the lack of an association between increased chromosomal aberrations and styrene exposure in human populations.	
83	<b>Comment 2.5</b> Page 19, Lines 12-18 ( <b>Report Section 3.1.1 Clastogenic and aneugenic effects</b> )	<p>The Council identified one epidemiology study that analyzed frequencies of aneuploidy and diploidy in the sperm cells of individuals occupationally exposed to styrene (Naccarati <i>et al.</i>, 2003; this study is also listed in Table A1.3, Page 73). As stated by the Council, this study did not show a statistically significant difference in the incidence of aneuploidy and diploidy between the group of exposed workers and the unexposed controls; however, the Council further states that "The only statistically significant finding was an excess of nullisomy in the exposed non-smokers" (Page 19, Lines 17-18).</p> <p>Naccarati <i>et al.</i> (2003 reported no significant difference in the frequencies of aneuploidy (disomy + nullisomy) between styrene-exposed subjects (<math>0.64 \pm 0.13</math>) and unexposed controls (<math>0.62 \pm 0.13</math>). [These frequencies are consistent with those of other sperm-FISH studies examining aneuploidy in normal populations (Zhu <i>et al.</i>, 2022).] However, Naccarati <i>et al.</i> (2003) state, in an "attempt to extend" their analysis, excess nullisomy was evaluated for based on the rationale that each event during meiosis yielding a disomic sperm produces a nullisomic sperm as well, thus the frequency of nullisomy should be similar to that of disomy and "the difference between nullisomies and disomies should not be statistically significantly different from zero." From this analysis, the authors reported that the difference between nullisomy and disomy was significantly different from zero for sex chromosomes in exposed non-smokers, stating that "this groups showed 'excess' of nullisomy, behaving differently than expected." However, the underlying rationale of this analysis (<i>i.e.</i>, that sperm with chromosome losses and those with gains should be equally common) is not supported by the results of recent studies comparing the incidence rates of nullisomy and disomy in normal populations. These studies, including those that have studied the genomes of thousands of individual sperm, have reported that the average incidence of nullisomy per chromosome is approximately twice that of disomy (Zhu <i>et al.</i> 2022; Bell <i>et al.</i> 2020). Moreover, Naccarati <i>et al.</i> (2003) stated that the finding of excess nullisomy in the exposed non-smokers "might be due to stochastic fluctuations, because only 8 subjects belonged to the sub-group of exposed non-smokers" and that the effect was only observed for the sex chromosomes and not for chromosome 2, further concluding that "Overall, we can conclude that there is a lack of a striking association between exposure to styrene and both sperm aneuploidies and semen parameters."</p> <p>We recommend that the Council refine its reporting and interpretation of the results of the Naccarati <i>et al.</i> (2003) study, incorporating the above-mentioned caveats and others that may have been identified from its study quality assessment.</p>	See comment 38
84	<b>Comment 2.6</b> Page 29, Lines 4-30 ( <b>Report Section 3.4 Evaluation of mutagenicity and Section 3.5 Recommendation on the Classification for Mutagenicity</b> )	<p>Overall, the Council's summary of the evaluation of mutagenicity and its ultimate recommendation for classification is confusing, with the logic hard to follow without the aforementioned requested details concerning the processes and criteria that were used. While the Council acknowledges that sufficient evidence of an effect was not present in the one epidemiological study on mutagenicity in germ cells (Naccarati <i>et al.</i>, 2003), concluding overall that styrene should not be classified in Category 1A, the Council appears to use what it terms "limited evidence" shown in epidemiological studies for chromosomal aberrations and micronuclei to support its recommended classification of styrene in Category 2. However, the body of evidence from epidemiology studies examining these endpoints indicates there are no associations.</p> <p>We ask that the Council reassess the totality of the epidemiology literature evaluating the association between styrene exposure</p>	See the general comment in the response letter.

		and micronuclei frequency and chromosomal aberrations in human populations, with consideration of the additional studies provided above (in Comments 2.2-2.4), which overall do not support an association between styrene exposure and increased micronuclei frequency or chromosomal aberrations. We also ask that the Council provide further details and clarification in its evaluation and recommendation for classification by addressing our comments above regarding the various processes (e.g., study selection, study quality assessment, weight of evidence assessment, classification) introduced in Section 1 <b>Scope</b> and later used in Section 3 <b>Mutagenicity</b> .	
<b>3 Carcinogenicity in Humans</b>			
85	<b>Comment 3.1</b> Page 30, Lines 5-9 ( <b>Report Section 4.1 Human data</b> )	The Council states, "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 Council should justify why these were selected as key studies.	The committee selected one publication for each of the three cohorts, presenting the most recent and complete study results. These studies are called key publications.
86	<b>Comment 3.3</b> Page 30, Line 5 to Page 32, Line 11 ( <b>Report Section 4.1 Human data</b> )	<ul style="list-style-type: none"> <li>▪ Regarding the quality of the boatbuilder cohort in Washington State, USA, the Council states, "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.<sup>3</sup> The committee agrees with the conclusions of IARC on the quality of the studies within this large cohort."</li> <li>▪ Regarding the quality of the cohort of workers in the reinforced plastics and composites industry in the USA, the Council states, "IARC concluded that the strengths of this study were the long follow-up, the high number of cases, the high concentrations of styrene exposure, and the lack of known carcinogenic occupational co-exposures within the industry. Quantitative styrene exposure metrics were applied but information on the exposure assessment was sparse; no styrene intensity information was apparently available for a substantial part of the exposure period, namely between 1948 and 1976, and for 27% of the cohort exposure data after 1977 was missing.<sup>3</sup>"</li> <li>▪ Regarding the quality of the six-country cohort of workers at reinforced plastics production plants in Europe, the Council states, "IARC noted that the strengths of this study were the large study population of workers of small- and medium-sized companies, with expected homogeneous and high-concentration exposure to styrene, and a long and almost complete follow-up. The limitations were the lack of quantitative estimates of exposure to styrene or any information on the prevalence of smoking.<sup>3</sup>"</li> </ul> <p>It appears that the Council refers largely to IARC's conclusions in its discussions of the quality of the three main cohorts. Since the Council "evaluated all the selected carcinogenicity studies on their quality," we suggest that the Council's own detailed study quality assessments be presented for these cohorts, along with a discussion of where it agrees or disagrees with IARC's evaluation.</p> <p>The Council also notes the quality of each cohort without discussing the impact of study quality on the validity and interpretation of the study findings it reports. This defeats the purpose of performing a study quality assessment. As noted in <b>Section 03 quality of the individual studies</b> of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i>, "After scientific literature has been collected, each study is assessed for quality by judging its relevance, reliability, and validity.... Validity refers to whether results really</p>	See the general comment in the response letter.

		<p>represent what they are supposed to measure (accuracy). The committees make a distinction between external and internal validity. Internal validity is the extent to which a method or study design provides results as close to the truth as possible, while alternative explanations are ruled out. External validity is the extent to which the results of a study can be generalized to other situations, populations, or organisms." The Council should incorporate its study quality assessment results into its interpretations of the study findings.</p>	
<p>87</p>	<p><b>Comment 3.4</b> Page 30, Line 31 (<b>Report Section 4.1 Human Data</b>)</p>	<p>The draft advisory report states that "the committee selected two key publications" for the boatbuilder cohort, namely Bertke <i>et al.</i> (2018) and Daniels <i>et al.</i> (2020). However, in the corresponding table (<b>Table B1.1 Boat builders study in Washington State, USA</b>), a third study by Ruder and Bertke (2017) is presented in detail and an additional study, Bertke <i>et al.</i> (2021), is listed in the table but not discussed in the text. Important limitations to these studies are described below.</p> <p>Ruder and Bertke (2017) used incidence rates from the Surveillance, Epidemiology, and End Results Program (SEER) as the reference, which may have biased the reported standardized incidence ratios (SIRs) due to confounding related to the specific geographic regions from which the SEER data are collected. In addition, the exposure assessment used by the authors was crude and based only on departmental assignment, <i>i.e.</i>, workers were classified as having high styrene exposure if they were ever employed in the fibrous or lamination departments and classified as having low styrene exposure if they were employed in any of the other departments. The cohort evaluated by Ruder and Bertke (2017) was also relatively young, with a median age &lt; 65 at the end of follow-up, which is the median age of cancer diagnosis in the US. This younger median age likely made it difficult to detect cancer incidence in the cohort. The effect estimates in Ruder and Bertke (2017) from the authors' internal analyses, comparing workers classified as having high styrene exposure to those having low styrene exposure, were above the null, which is in contrast with the authors' external analyses. This pattern may be the result of bias and not indicative of a causal mechanism. Finally, the validity of the Ruder and Bertke (2017) study is also hampered by a small sample size and potentially by confounding from lifestyle factors such as smoking.</p> <p>The Council states that it "selected key publications within these three [boat builders] cohorts presenting the most recent and complete study results." Given that the cohort follow-up in Ruder and Bertke (2017) was through 2011, which is earlier than the 2016 date listed for the Bertke <i>et al.</i> (2018) and Daniels <i>et al.</i> (2020) studies, we suspect that Ruder and Bertke (2017) was left in <b>Table B1.1</b> by mistake. We recommend that the Ruder and Bertke (2017) study be deleted from <b>Table B1.1 Boat builders study in Washington State, USA</b>, so only the two key studies (Bertke <i>et al.</i>, 2018; Daniels <i>et al.</i>, 2020) selected by the Council and discussed in the text are in the tabulated information provided in <b>Annex B</b>.</p> <p>As noted above, Bertke <i>et al.</i> (2021) is also listed in <b>Table B1.1 Boat builders study in Washington State, USA</b>, but not discussed in the main text of the draft advisory report. This study examined healthy worker survival bias using lung cancer mortality and styrene exposure; however, it may have been subjected to selection bias because the deaths observed from lung cancer would only have been those workers whose survival times were less than the follow-up period, and the survival times may have been impacted by exposure levels. Because a worker's survival status may have been determined by their exposure status, there is unlikely to have been a healthy worker survival bias, but rather survival times related to different styrene</p>	<p>The committee choose the study of Daniels as key study as it uses the most recent mortality figures and extended the analyses by making fuller use of available employment information and exposure measurement data Ruder and Bertke (2017) and (Bertke <i>et al.</i>, 2018 have been deleted from the table.</p>

		exposure levels. We recommend that the potential for bias be acknowledged by the Council if it continues to list the Bertke <i>et al.</i> (2021) study in <b>Table B1.1</b> .	
88	<b>Comment 3.5</b> Page 30, Lines 20-24 ( <b>Report Section 4.1 Human Data</b> )	<p>In its description of the exposure assessment performed in the boatbuilder cohort, the Council states, “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.”</p> <p>This description is inaccurate, as the two key publications of this cohort used different exposure assessment approaches. The Council's description is consistent with the exposure assessment approach used in Daniels <i>et al.</i> (2020), which notes that its analysis was “the first to make full use of available employment information and exposure measurement data to construct a job-exposure matrix describing career cumulative exposures for each worker as a continuous variable that accounted for changes in exposure potential over time.” In contrast, Bertke <i>et al.</i> (2018) only measured workers' styrene exposures in terms of ever/never worked in “highly exposed jobs” (<i>i.e.</i>, “Fiberglass or plasticians, comprising mostly skilled labor directly working with styrene”) and employment duration in such jobs.</p> <p>The exposure assessment approach plays a crucial role in determining the validity of study results, as noted in section <b>03 Quality of the individual studies</b> of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i>. We suggest that the Council specify the exposure assessment approach used in each publication for clarity. Considering that Bertke <i>et al.</i> (2018) did not estimate workers' styrene exposure concentrations and that both Daniels <i>et al.</i> (2020) and Bertke <i>et al.</i> (2018) followed the cohort through 2016, we further suggest that the Council reconsider its choice of Bertke <i>et al.</i> (2018) as a key publication of the cohort.</p>	See comment 87
89	<b>Comment 3.6</b> Page 30, Lines 25-29 ( <b>Section 4.1 Human data</b> )	<p>Regarding the quality of the boatbuilder cohort in Washington State, USA, the Council states, “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. The committee agrees with the conclusions of IARC on the quality of the studies within this large cohort.”</p> <p>We note that the comments from the IARC Monograph that the Council refers to only apply to Bertke <i>et al.</i> (2018) and not necessarily the other key publication, Daniels <i>et al.</i> (2020), or any of the remaining publications of the boatbuilder cohort. In fact, Daniels <i>et al.</i> (2020) was published after the IARC Monograph. It is important that the Council discuss the quality of each publication based on its quality assessment.</p> <p>Bertke <i>et al.</i> (2018) acknowledged that a “study limitation is the use of mortality as opposed cancer incidence, especially given the high survival rates of [lymphohaematopoietic cancers].” In other words, the study results are subject to information bias due to outcome misclassification. Although not noted in the IARC Monograph, we suggest that the Council add this study limitation to the draft advisory report, as it is important to the validity and interpretation of the study results.</p> <p>Daniels <i>et al.</i> (2020) noted as their study strengths that: For this analysis, individual estimates of cumulative styrene exposures were derived from employment records and personal air sampling data not used in previous examinations. The employment records also provided for estimating SES; an unmeasured potential confounder in prior exposure–response assessments. Other strengths include lengthy follow-up and</p>	See comment 87 and the general comment in the response letter.

		<p>relatively high styrene inhalation exposures without concomitant exposure to other occupational carcinogens.</p> <p>Daniels <i>et al.</i> (2020) noted as their study limitations that: First, although improvements were made to exposure assessment, the validity in exposure estimates is still uncertain. Without validation, bias in risk estimates from measurement error cannot be ruled out. Second, the potential for bias from other sources common to occupational studies, such as [healthy worker survival effects] and residual confounding remains.... Third, the study lacks adequate information to appropriately examine tumor-specific risks (e.g., leukemia subtypes) which may exhibit different exposure–response patterns. Fourth, this study is relatively small; therefore, is disadvantaged by low statistical power. Lastly, the mode of action for styrene carcinogenicity remains unclear. Lacking these data, this study assumed a linear relationship between leukemia and cumulative exposure at low exposure levels, although a true exposure–response, if present, may be sublinear or may have a threshold.</p> <p>Daniels <i>et al.</i> (2020) also studied cancer mortality, so their results are similarly subject to information bias due to outcome misclassification because they didn't use incidence data. In addition, the distribution of exposure estimates was markedly right-skewed, with a mean and median of 31 and 5.7 ppm-years, respectively. As with other studies based on the Washington boatbuilders cohort, study validity is limited by the relatively small cohort size and short duration of worker employment. The authors also claimed that, based on their risk projections, a working lifetime exposure to 0.05 ppm styrene would correspond to one extra leukemia death per 10,000 workers; however, when extrapolated to typical occupational limits of styrene exposure (<i>i.e.</i>, 10-20 ppm), the resulting leukemia incidence rates are not consistent with what has been observed.</p> <p>In the cubic spline models used in Daniels <i>et al.</i> (2020) to estimate cumulative risk from styrene exposure, the highest risks for both bladder cancer and leukemia were not observed at the highest exposure levels, with the authors arguing that the observed attenuation in risk at higher levels of exposure could be the result of measurement error, the healthy worker effect, or biological saturation. However, it could be argued that exposure misclassification among workers with the highest level of exposure to styrene is minimal. Given that the strongest effects should have been observed at the highest levels of exposure if styrene were causal, the study findings do not support causality. Finally, as shown in Daniels <i>et al.</i> (2020), the boatbuilder cohort is “predominately Caucasian (93%) and male (87%),” limiting the generalizability of the results to women and people who are not Caucasian. The Council should take into consideration the above-mentioned strengths and limitations of the Daniels <i>et al.</i> (2020) study in its evaluation.</p>	
90	<p><b>Comment 3.7</b> Page 31, Lines 18-25 (<b>Report Section 4.1 Human data</b>)</p>	<p>Regarding the quality of the cohort of workers in the reinforced plastics and composites industry in the USA, the Council states, “IARC concluded that the strengths of this study were the long follow-up, the high number of cases, the high concentrations of styrene exposure, and the lack of known carcinogenic occupational co-exposures within the industry. Quantitative styrene exposure metrics were applied but information on the exposure assessment was sparse; no styrene intensity information was apparently available for a substantial part of the exposure period, namely between 1948 and 1976, and for 27% of the cohort exposure data after 1977 was missing. The committee noticed that the cohort was formed with aid of the industry.”</p> <p>We note that the comments from the IARC Monograph to which the Council refers only apply to Collins <i>et al.</i> (2013) and not necessarily any of the other publications of the same cohort. It is</p>	<p>Only the most relevant limitations determined by the committee are discussed. The committee is of the opinion that this limitation does not need to be explicitly mentioned.</p>

		<p>important that the Council discuss the quality of each publication based on its quality assessment.</p> <p>Collins <i>et al.</i> (2013) acknowledged, "Among the limitations of this study is the reliance on data from death certificates for investigating some cancer types, especially cancers of the lymphatic and hematopoietic tissues, where misclassification sometimes occurs." Although not noted in the IARC Monograph, we suggest that the Council add this study limitation to the draft advisory report, as it is important to the validity and interpretation of the study results.</p>	
91	<p><b>Comment 3.8</b> Page 32, Lines 7-11 (<b>Report Section 4.1 Human data</b>)</p>	<p>Regarding the quality of the six-country cohort of workers at reinforced plastics production plants in Europe, the Council states, "IARC noted that the strengths of this study were the large study population of workers of small- and medium-sized companies, with expected homogeneous and high-concentration exposure to styrene, and a long and almost complete follow-up. The limitations were the lack of quantitative estimates of exposure to styrene or any information on the prevalence of smoking." We note that the comments from the IARC Monograph to which the Council refers appear to apply to a study by Christensen <i>et al.</i> (2017) that is cited as one of the "six publications only on the Danish cohort" in the draft advisory report but not listed in <b>Table B1.2 Six-country study on workers at reinforced plastics production plants</b>. This should be corrected.</p>	<p>Christensen <i>et al.</i> (2017) has been added to table B1.2.</p>
92		<p>With respect to Loomis <i>et al.</i> (2019), which is considered to be a key publication, the Council discusses several strengths and limitations noted by IARC. There are several additional limitations and interpretation issues that the Council should mention. For example, the mean duration of exposure was only 2.2 years, with 60% of the workers exposed for less than 2 years. Increased cancer risks were only found among workers who had been exposed 2-5 years, and not among those who were exposed longer. Also, the effect estimates were imprecise, as most have large confidence intervals. Finally, most of the statistically significant findings were only observed when exposure was defined as the mean level of styrene exposure. When evaluating cancer outcomes, cumulative exposure, which accounts for intensity and duration, may be a more appropriate metric.</p> <p>IARC's comments on the Loomis <i>et al.</i> (2019) study substantially differ from those on the Christensen <i>et al.</i> (2017) study, with respect to the availability of quantitative estimates of styrene exposure and length of follow-up. It is thus important that the Council provide adequate details on its quality assessment for each study. IARC's comments on the Christensen <i>et al.</i> (2017) and/or Loomis <i>et al.</i> (2019) study alone do not necessarily apply to any of the other publications of the six-country cohort. However, the existence of residual confounding should be acknowledged, with Loomis <i>et al.</i> (2019) failing to account for lifestyle confounders such as smoking.</p> <p>IARC noted the available quantitative measures of styrene exposure in Loomis <i>et al.</i> (2019) as a strength. However, it is important to note that Loomis <i>et al.</i> (2019) noted as a limitation the possibility that "some risk estimates were affected by exposure measurement error" because (1) "all workers in Denmark were assigned to an unspecified job category presumed to involve exposure to styrene," (2) "heavily exposed short-term workers were excluded from the cohort", or (3) "The estimation of exposure before 1970" was performed "by extrapolating from measurements in Denmark." Loomis <i>et al.</i> (2019) also acknowledged a few other limitations that were not noted in the IARC Monograph, including the lack of "information on tobacco smoking and other personal risk factors," "the deletion of data from Norway," "the relatively short duration of employment in the industry," the "small numbers of deaths for</p>	<p>Only the most relevant limitations determined by the committee are discussed, while recognizing that some degree of residual confounding is always present.</p>

		some of the cancers of interest,” and potential “healthy worker survivor bias.” Furthermore, we note that the results of the Loomis <i>et al.</i> (2019) study are also subject to information bias due to outcome misclassification, given its examination of cancer mortality as opposed to cancer incidence. It is important that the Council take these facts into consideration in its discussion.	
93	<b>Comment 3.9.1</b> Page 40, Line 31 to Page 42 Line 5 ( <b>Report Section 4.3 Evaluation of carcinogenicity</b> )	The Council's summary of the evaluation and classification of carcinogenicity of styrene is hard to follow without detailed descriptions upfront of the processes and criteria that were relied upon. We suggest that the Council clarify this section after addressing our comments above on the various processes (e.g., study selection, study quality assessment, weight of evidence assessment, classification) in <b>Section 1 Scope</b> .	See the general comment in the response letter.
94	<b>Comment 3.9.2</b> Page 40, Line 31 to Page 42 Line 5 ( <b>Report Section 4.3 Evaluation of carcinogenicity</b> )	The draft advisory report states, “There are several epidemiological studies available that are large and well performed, although none of the studies performed in workers are without flaws. The committee considers the evidence from the boatbuilder study in Washington State and the European cohort as most relevant as they present dose-response relationships within exposed workers only as this reduces the impact of bias. The study of Daniels <i>et al.</i> (2020) showed an elevated risk for leukemia and bladder cancer and the study of Loomis <i>et al.</i> (2019) showed an elevated risk for non-Hodgkin-lymphoma, oesophageal and pancreatic cancer. Overall, the committee concludes that there is limited evidence of carcinogenicity from human studies, and bias and confounding cannot be excluded.” The Council's synthesis of epidemiology evidence is brief and only highlights the boatbuilder cohort in Washington State (specifically, Daniels <i>et al.</i> 2020) and the six-country cohort of reinforced plastics production workers in Europe (specifically, Loomis <i>et al.</i> 2019), two of the three “main” cohorts discussed in <b>Section 4.1 Human data</b> . It should be clarified whether and how results from other available epidemiology studies are weighed in arriving at the Council's overall conclusion. We would also recommend a revision to the Council's statement in <b>Section 4.3 (Evaluation of carcinogenicity)</b> that “none of the studies performed in workers are without flaws” to one that reflects that all of the available studies have “limitations”. We believe the word “limitations” is a more appropriate term to describe the issues with these studies. Considering that the draft advisory report does not include many of the available epidemiology studies (as commented above), it is questionable whether the Council's evidence synthesis followed a “weight of evidence” approach as noted in <b>Section 1.2 Committee and procedure</b> of the draft advisory report. It is crucial this is clarified, as <b>Section 04 Strength of evidence for causality</b> of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i> states, “The committees, however, want to weigh the strength of evidence by integrating the findings of all the individual epidemiological studies combined (a form of triangulation). That is why the committees support the concept of triangulation. Others have suggested such an approach for environmental and occupational observational epidemiology. It extends Bradford Hill's approach by considering evidence from all relevant epidemiological studies, irrespective of the design or context, so that not only the overall strength of evidence can be evaluated, but also the overall direction of various possible biases.” Furthermore, there is no indication that the Council's synthesis of epidemiology evidence was performed regarding each cancer type separately, which is crucial because the underlying pathophysiological process varies by cancer type. We note that <b>Section 04 Strength of evidence for causality</b> of the <i>Guidance for recommending classifications and health-based occupational exposure limits</i> states, “For each potential adverse effect, the	See the general comment in the response letter.

		committees search for several lines of evidence, which they then integrate to determine the overall weight of evidence for each effect observed." Both the IARC Monograph on which the draft advisory report relied and a recent systematic review by Collins and Dezell (2018) synthesized epidemiology evidence on styrene carcinogenicity by cancer type. We urge that the Council clarify, and adjust if needed, its approach for evidence synthesis of the epidemiology studies.	
95	<b>Comment 3.9.3</b> Page 40, Line 31 to Page 42 Line 5 ( <b>Report Section 4.3 Evaluation of carcinogenicity</b> )	<p>The draft advisory report states, "Although no significant increase of relevant malignant tumours in at least two experimental animal species or studies was found after styrene exposure, the committee considers classification in category 1B warranted based on the limited evidence of carcinogenicity in epidemiological studies and limited evidence of carcinogenicity in animal studies."</p> <p>We commented above that it is unclear whether the criteria listed in the <i>Guideline for the classification of carcinogenic substances</i> were followed strictly or modified in any way for the Council's assessment. It appears in this section that the Council's evaluation of carcinogenicity of styrene strictly followed <b>Table 3 Classification table on assessment of human and experimental animal data</b> of this guideline. However, it is important to note that categories of epidemiology evidence (<i>i.e.</i>, sufficient evidence of carcinogenicity, limited evidence of carcinogenicity, inadequate evidence regarding carcinogenicity, and evidence suggesting lack of carcinogenicity) that are applied to this table were defined "[t]o assess the overall evidence of the available studies" (as opposed to select evidence, say from key publications of main cohorts).</p> <p>We urge the Council to clarify its classification process and update its classification according to any changes made to its evidence synthesis of the epidemiology studies. Importantly, we note that most of the cancer types (<i>i.e.</i>, leukemia, non-Hodgkin-Lymphoma, oesophageal cancer, and pancreatic cancer) that the Council highlights to have been positively associated with styrene exposure in Daniels <i>et al.</i> (2020) and Loomis <i>et al.</i> (2019) were meta-analyzed by Collins and Dezell (2018) and shown not to be statistically significantly associated with styrene exposure when considering the epidemiology literature as a whole.</p>	See the general comment in the response letter.