14 January 2025

EFSA'S STAKEHOLDER WEBINAR ON THE RE-ASSESSMENT OF STYRENE SAFETY FOR USE IN FOOD CONTACT MATERIALS

EUROPEAN FOOD SAFETY AUTHORITY

AGENDA

15:00-15:10
Opening of the webinar
Sandra Rainieri

15:10-15:20
EC Mandate on Styrene, EFSA's approach and Risk Assessment methodologies
Zainab Al Harraq

15:20-15:55
Assessment of Genotoxicity of Styrene after oral exposure
Riccardo Crebelli

15:55-16:00
Closing of the webinar
Sandra Rainieri

HOUSEKEEPING RULES

- You are now connected to the audio/video broadcast. This is a **one-way audio/communication channel** (listen/view only mode).
- The event is held in **English**.
- The event is being **recorded**, and the recording will be published on EFSA's website, along with the presentations.
- After the event, attendees will receive a link to a **survey** to evaluate EFSA's event services.

EFSA'S STAKEHOLDER WEBINAR ON THE RE-ASSESSMENT OF STYRENE SAFETY FOR USE IN FOOD CONTACT MATERIALS

449 registrants
from **55** countries

EFSA'S STAKEHOLDER WEBINAR ON THE RE-ASSESSMENT OF STYRENE SAFETY FOR USE IN FOOD CONTACT MATERIALS

OBJECTIVES OF THE WEBINAR

- Explaining the **mandate**, the **methodology** followed for the re-assessment, and the **conclusions** reached in the draft opinion.
- Illustrating the **ongoing public consultation** and providing guidance on how stakeholders can contribute.
- Starting to **address questions and requests for clarification** received from stakeholders regarding the draft output.

QUESTIONS/COMMENTS SUBMITTED BY REGISTRANTS

11 questions/comments received:

- addressed in the following presentations;
- on the SML (measurement, calculation): information can be found in the EC regulation 10/2011[1] and the EFSA Note for Guidance for Food Contact Materials (EFSA CEF Panel, 2008)[2];
- on Risk Management measures: to be addressed by the EC;
- out of the scope of the webinar (not clear, recycling,..).

You are invited to submit your additional questions/comments on the draft opinion while it is in PC (until 28 January) and/or through Ask A Question service.

LINK: https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0lTk0000039j7R/pc1239

LINK: https://connect.efsa.europa.eu/RM/s/help

[1] EC regulation 10/2011 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0010

[2] EFSA Note for Guidance for the preparation of an application for the safety assessment of a substance to be used in plastic food contact materials (EFSA CEF Panel, 2008) https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.21r

AGENDA

15:10-15:20

EC Mandate on Styrene, EFSA’s approach and Risk Assessment methodologies

Zainab Al Harraq

BACKGROUND INFORMATION

Styrene is authorised without a migration limit or other restrictions for the manufacture of plastic FCM in accordance with Regulation No 10/2011.

>High priority group of substances needing re-evaluation.

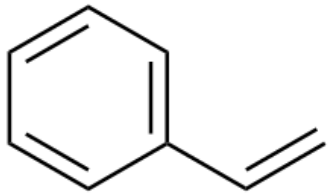
In 2018, the International Agency for Research on Cancer (**IARC**) classified styrene and its primary metabolite styrene-7,8-oxide "**probably carcinogenic to humans**". [**2019** IARC Monograph, vol.121]

Therefore, the Commission requested EFSA to re-evaluate whether the evidence examined by IARC could be of consequence to the safety of styrene in FCMs. [**2018** EC Mandate]

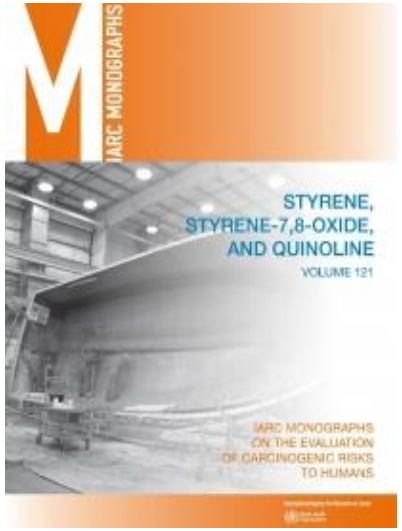
STYRENE RE-EVALUATION: 1ST PHASE

2020 EFSA opinion

- The IARC evaluation based on high-dose occupational exposure studies and animal studies by inhalation pertains to hazard identification.
- The implications of styrene oral exposure via FCM on the health of consumers should be evaluated based on a comprehensive analysis of the reliability and relevance of all available experimental and human findings on styrene genotoxicity. Toxicokinetic aspects, ultimately enabling a qualitative and quantitative genotoxic risk estimate associated with the oral exposure to styrene should be considered.



Styrene



EFSA Journal

SCIENTIFIC OPINION

ADOPTED: 9 September 2020

doi: 10.2903/j.efsa.2020.6247

Assessment of the impact of the IARC Monograph Vol. 121 on the safety of the substance styrene (FCM No 193) for its use in plastic food contact materials

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), Vittorio Silano, José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis, Holger Zorn, Laurence Castle, Emma Di Consiglio, Roland Franz, Nicole Hellwig, Maria Rosaria Milana, Karla Pfaff*, Maria Carfi, Ellen Van Haver and Gilles Rivière

Abstract

The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) was requested by the European Commission to re-evaluate the safety of styrene (FCM No 193) for use in plastic food contact materials (FCM) following the classification by the International Agency for Research on Cancer (IARC) as 'probably carcinogenic to humans'. The IARC Monograph pertains to hazard identification, based on studies on high-dose occupational exposures by inhalation and animal studies, also mainly by inhalation. The Panel considered that the IARC conclusions cannot be directly applied to the evaluation of risks for consumers from the oral exposure to styrene, but also concluded that, based on the data provided in the IARC Monograph and by the industry, a concern for genotoxicity associated with oral exposure to styrene cannot be excluded. The migration of styrene into foods packed in styrenic plastics is below 10 μg/kg for the majority of the foods, but up to 230 μg/kg was reported. Migration tends to be high for contact with fatty foods, and/or with high surface to volume ratios of the FCM. Dietary exposure of the consumers to styrene migrating from styrenic plastics was estimated in the order of 0.1 μg/kg body weight (bw) per day. It is in the same range as exposure from styrene present in foods as such. The dietary exposure (food component plus migration from styrenic plastics) is similar or lower than that by inhalation in the general population. Taking the human exposure data into account, the Panel concluded that a systematic review of genotoxicity and mechanistic data, comparative toxicokinetics and analysis of species differences is required for assessing the safety of styrene for its use in FCM.

CONCLUSIONS ON GENOTOXICITY OF STYRENE IN FCMS

2020 EFSA opinion

"The CEP Panel concluded that a concern for genotoxicity associated with oral exposure to styrene cannot be excluded.

Taking the human exposure data into account, a systematic review of genotoxicity and mechanistic data, comparative toxicokinetics and analysis of species differences is required for assessing the safety of styrene for its use in FCM".

STYRENE RE-EVALUATION: 2ND PHASE

2023 EC Mandate

TERMS OF REFERENCE

In accordance with Article 12(3) of Regulation (EC) No 1935/2004[4], the European Commission requests EFSA to provide an opinion, which addresses the following points:

- whether styrene is genotoxic following oral exposure and the relevance to human health, and,
- whether the use of styrene if authorised in accordance with Article 5 of Regulation (EU) No 10/2011 subject to the above mentioned SML of 40ppb, is in accordance with Article 3 of Regulation (EC) No 1935/2004.

To this purpose, EFSA shall take into account data provided by third parties during 2022 and 2023, which is already available to EFSA.

EFSA shall ensure the opinion is conclusive on the above points. If needed EFSA shall thereto:

- conclude on a lower SML or alternative restrictions under which the use could be considered safe, if any, if this would be needed to ensure Article 3 is met, and,
- in view of remaining uncertainties or knowledge gaps, or if the available data would not be suitable to support the SML of 40 ppb, request additional data from the applicant in accordance with Article 10 of Regulation (EC) No 1935/2004 ([5]).

In addition, should there be a risk for an inconclusive opinion due to insufficiency of available data over the toxicity of styrene, the Commission invites EFSA to explore all tools at its disposal for the collection of all relevant scientific data and studies, including the potential launch of new scientific studies to support the risk assessment.

With respect to the systematic review of genotoxicity and mechanistic data on which EFSA in its previous opinion concluded that it should be required, the Commission considers that such a review should be carried out only to the extent that EFSA considers it necessary for the purpose of addressing the mandate.

If, as part of its work on the mandate, the panel would observe variations between its reasoning and reasoning expressed by the WHO (on which the guidance value is based) or reasoning expressed in the 2019 IARC conclusion, these variations should be detailed and explained.

EFSA shall deliver its opinion within 15 months following reception of this mandate. In case EFSA would request data from third parties, launch a call for data, launch scientific studies or organise a public consultation on the resulting opinion, it may "stop the clock" to accommodate the required time. However, the total time for the delivery of the opinion shall not exceed 30 months following the reception of the mandate.

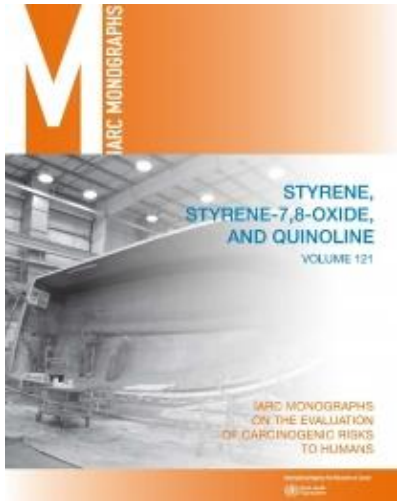
WHO (2003) Styrene in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. Geneva, World Health Organization (WHO/SDE/WSH/03.04/27).

EFSA Note for Guidance for the preparation of an application for the safety assessment of a substance to be used in plastic food contact materials (EFSA CEF Panel, 2008)
https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.21r

TO ADDRESS THE MANDATE

- Data to be analysed:
  - Genotoxicity
  - Toxicokinetics (Tks)
  - Human exposure (HBM+dietary)
- Sources of data:
  - IARC Monograph (2019)
  - Studies submitted by US SIRC after IARC Monograph publication
  - Literature search (last 7 years, 3392 papers)

SIRC Styrene Information & Research Center



PROTOCOL & METHODOLOGY

- Literature search methodology is based on standard EFSA guidance (EFSA, 2010)[1].
  - 3 databases (Pubmed, Scopus, Web of Science)
  - Inclusion/exclusion criteria (language, timeframe, type of evidence)
  - Search terms/keywords
  - Title & Abstract, Full Text screenings (DistillerSR tool)

- Genotoxicity studies were evaluated for their reliability and relevance according to harmonised EFSA criteria[2].

- Tks and human exposure studies were evaluated using a narrative approach.

[1] **EFSA, 2010**. Application of systematic review methodology to food and feed safety assessments to support decision making. EFSA Journal 2010;8(6):1637, 90 pp. doi: 10.2903/ j.efsa.2010.1637.
[2] **EFSA Technical Report, 2023**. Harmonised approach for reporting reliability and relevance of genotoxicity studies. EFSA Supporting publication 2023: EN-8270. doi:10.2903/sp.efsa.2023.EN-8270.

APPROACH FOR THE EVALUATION OF GENOTOXICITY STUDIES

- Genotoxicity studies were evaluated for their reliability and relevance according to harmonised EFSA criteria[1]
  - The **reliability of the studies** is based on Klimisch criteria, mainly related to the compliance with validated and internationally agreed experimental protocols (e.g. OECD TGs), including the adequacy of data presentation; [R. without restrictions, with restrictions, insufficient, not reliable].
  - The **relevance of the study results** (high, limited or low) considers both the reliability of the study and the relevance of the test system (e.g. apical vs indicator end-points) and other factors (e.g. the route of administration: oral vs non-oral).
- Study results with sufficient relevance (high or limited) were integrated in lines of evidence for each of the main genotoxicity endpoints in a **WoE** approach[1,2,3]. Information on MoA from in vitro studies, human biomonitoring and TK data were considered as supporting information in the WoE.

[1] **EFSA Technical Report, 2023**. Harmonised approach for reporting reliability and relevance of genotoxicity studies. EFSA Supporting publication 2023:EN-8270. doi: 10.2903/sp.efsa.2023.EN-8270.
[2] **EFSA Scientific Opinion, 2017a** Clarification of some aspects related to genotoxicity assessment. doi: https://doi.org/10.2903/j.efsa.2017.5113.
[3] **EFSA Scientific Opinion, 2017b**: Guidance on the use of the weight of evidence approach in scientific assessments. doi: 10.2903/j.efsa.2017.4971.

FORMAT FOR STUDY SUMMARY AND EVALUATION FROM STYRENE DRAFT OPINION 2024 - TABLE ON IN VIVO CA/MN TESTS

Test system/ Test object	**Exposure** conditions	Information on the characteristics of the **test substance**	**Results**	**Reliability**/ Comments	**Relevance** of the test system/ relevance of the results	Reference
Mammalian erythrocyte MN test Four male Chinese hamsters (two in the control group) OECD TG and GLP compliance: not reported	1000 mg/kg bw by single i.p. injection Vehicle olive oil Sampling 30 h after treatment MN scored in 2000 PCE and 2000 NCE/animal No positive control	Styrene stabil. > 99% (Fluka AG/Buchs SG)	Inconclusive No increase of MN with no evidence of bone marrow exposure Toxicity: Non-statistically significant decrease in the PCE/NCE ratio in styrene treated animals	2 reliable with restrictions This study was performed before the establishment of the OECD TG 474. The study results are adequately reported, but a limited protocol was applied, with few animals (4 treated and 2 vehicle control animals), a single dose and sampling time, and no positive control.	High/low because of the protocol limitations, the non-oral route of exposure and inconclusive results	Penttilä et al., 1980

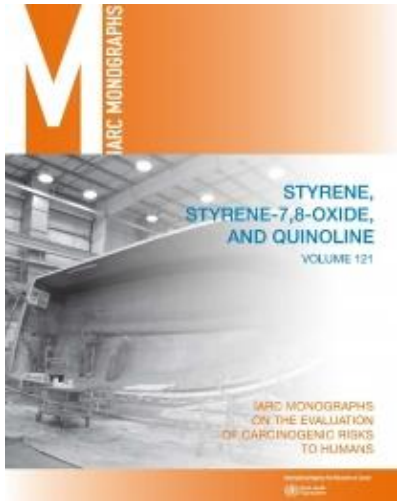
(OECD TG 474 and TG 475 were considered for the evaluation of reliability of MN and CA tests, respectively)

TO ADDRESS THE MANDATE

- Data to be analysed:
  - **Genotoxicity**
  - Toxicokinetics (Tks)
  - Human exposure (HBM+dietary)

- Sources of data:
  - ❑ IARC Monograph (2019)
  - ❑ Studies submitted by US SIRC after IARC Monograph publication
  - ❑ Literature search (last 7 years, 603 papers)

SIRC Styrene Information & Research Center



AGENDA

15:20-15:55

Assessment of Genotoxicity of Styrene after oral exposure

Riccardo Crebelli

GENOTOXICITY DATA, RESULTS AND EVALUATION

1. Published *in vivo* genotoxicity studies evaluated in the IARC Monograph 121, 2019

IN VIVO GENOTOXICITY STUDIES ON STYRENE EVALUATED IN THE IARC MONOGRAPH 121 – CHROMOSOME DAMAGE (CA AND MN)

Table 4.7 Genetic and related effects of styrene and styrene-7,8-oxide in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA strand breaks, micronuclei	Rat, F344 (M)	Leukocytes. peripheral blood reticulocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay and micronuclei evaluated at the 3rd and 20th day of exposure	Gaté et al. (2012)
DNA strand breaks (comet assay), chromosomal aberrations, micronuclei	Rat, F344 (F)	Lymphocytes	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
Chromosomal aberrations	Rat, Sprague-Dawley (M, F)	Bone marrow	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 1 yr		Sinha et al. (1983)
Chromosomal aberrations, sister-chromatid exchange	Rat, F344 (M)	Peripheral blood lymphocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk		Preston & Abernethy (1993)
Micronuclei	Rat, Porton (M)	Bone marrow (PCE)	–	3000 mg/kg	Intraperitoneal injection, 48 h after treatment		Simula & Priestly (1992)
Chromosomal aberrations, micronuclei	Mouse $B6C3F_1$ (F)	Lung, spleen	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
Chromosomal aberrations	Mouse, CD1 (M, F)	Bone marrow	–	1000 mg/kg	Gavage, single dose (1×), 24 h after treatment		Loprieno et al. (1978)
Chromosomal aberrations	Mouse, CD-1 (M)	Bone marrow	–	200 × 70, 500 × 4 mg/kg	Oral, 4 or 70 mg/kg per day		Sbrana et al. (1983)
Chromosomal aberrations, sister-chromatid exchange	Mouse, C57BL/6 (M)	Bone marrow	–	1000 mg/kg bw	Intraperitoneal injection, BrdU-labelled M1 cells 16 h after BrdU		Sharief et al. (1986)

CONTN'D

Table 4.7 Genetic and related effects of styrene and styrene-7,8-oxide in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Micronuclei	Mouse, NMRI (M)	Bone marrow	+/–	1500 mg/m³	Inhalation, 5 h/d, 7 d/wk, 1–21 d	2-fold higher than in control after 7 d (but not 21 d) of exposure	Vodička et al. (2001a)
Micronuclei	Mouse, NMRI (NR)	Bone marrow (PCE)	–	1500 mg/m³	Inhalation, 6 h/d, 1–21 d		Engelhardt et al. (2003)
Micronuclei	Mouse, LACA Swiss (M)	Bone marrow (PCE)	+	600 mg/kg	Intraperitoneal injection, 48 h after treatment		Simula & Priestly (1992)
Micronuclei	Mouse, C57BL/6 (M)	Bone marrow (PCE)	+	250 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Norppa (1981)
Chromosomal aberrations	Hamster, Chinese (M)	Bone marrow	–	300 ppm	Inhalation, 6 h/d, 5 d/wk, 4 d or 3 wk		Norppa et al. (1980)
Micronuclei	Hamster, Chinese (M)	Bone marrow	–	1000 mg/kg bw	Intraperitoneal injection, 30 h after treatment		Penttilä et al. (1980)

IN VIVO GENOTOXICITY STUDIES ON STYRENE EVALUATED IN THE IARC MONOGRAPH 121 – CHROMOSOME DAMAGE (CA AND MN)

Fifteen studies evaluated the induction of chromosomal damage in rodents following the exposure to styrene by various routes. **Ten** out of the fifteen studies were considered of sufficient (limited) relevance for inclusion in the WoE. **Nine** of these studies provided negative results. **One** study of limited relevance (Simula & Priestly, 1992) was evaluated as positive for the induction of MN in PCE of mice following i.p. administration of styrene.

The studies evaluated as of low relevance included two publications reported as positive in the IARC Monograph 121:

- Norppa, 1981: low relevance because of the inadequate protocol and inconclusive results.
- Vodicka et al, 2001: low relevance because of the insufficient reliability and the demonstrated lack of reproducibility of the results.

IN VIVO GENOTOXICITY STUDIES ON STYRENE EVALUATED IN THE IARC MONOGRAPH 121 – DNA DAMAGE (COMET ASSAY)

Table 4.7 Genetic and related effects of styrene and styrene-7,8-oxide in non-human mammals in vivo

End-point	Species, strain, (sex)	Tissue	Results[a]	Concentration (LEC or HIC) or dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Oxidative damage to DNA (comet assay with Fpg sensitive sites)	Rat, F344 (M)	Leukocytes	+/–	75 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay assessed at 3rd and 20th days; in the presence of Fpg, positive result was observed at 3rd but not at 20th day	Gaté et al. (2012)
DNA strand breaks, micronuclei	Rat, F344 (M)	Leukocytes. peripheral blood reticulocytes	–	1000 ppm	Inhalation, 6 h/d, 5 d/wk, 4 wk	Comet assay and micronuclei evaluated at the 3rd and 20th day of exposure	Gaté et al. (2012)
DNA strand breaks (comet assay), chromosomal	Rat, F344 (F)	Lymphocytes	–	500 ppm	Inhalation, 6 h/d, 14 d		Kligerman et al. (1993)
DNA strand breaks (comet assay)	Mouse, C57BL/6 (M)	Lymphocytes, liver, kidney, bone marrow	+	250 mg/kg	Intraperitoneal injection, 4 h after treatment	LED is 350 mg/kg bw for bone marrow	Vaghef & Hellman (1998)
DNA strand breaks (comet assay without EndoIII) and oxidative damage to DNA (comet with EndoIII)	Mouse, NMRI (M)	Lymphocytes, bone marrow	+/–	750 mg/m³	Inhalation, 5 h/d, 7 d/wk, 1–21 d	Positive in bone marrow for EndoII-sensitive sites after 21 d	Vodička et al. (2001a)
DNA strand breaks (comet assay without EndoIII) and oxidative damage to DNA (comet with EndoIII)	Mouse, NMRI (M)	Liver	–	1500 mg/m³	Inhalation, 5 h/d, 7 d/wk, 1–21 d	Negative results with and without EndoIII	Vodička et al. (2001a)

IN VIVO GENOTOXICITY STUDIES ON STYRENE EVALUATED IN THE IARC MONOGRAPH 121 – DNA DAMAGE (COMET ASSAY)

Four studies evaluated the induction of primary DNA damage (ssb) by the alkaline comet assay in rodents, following inhalational or i.p. exposure to styrene. **Three** were considered of sufficient (limited) relevance for inclusion in the WoE. **One** study showed positive results in mice treated by i.p. (Vaghef & Hellman, 1998). Negative results were reported in **two** inhalational studies in rat leukocytes.

One study evaluated of low relevance was reported as positive in the IARC Monograph 121:

- Vodicka et al., 2001: low relevance because of the inadequate protocol and inconclusive results.

Other in vivo genotoxicity studies evaluated in the 2019 IARC Monograph include a negative rat liver unscheduled DNA synthesis and SCEs tests in rats and mice with mixed results. These studies were considered of less relevance in the WoE.

GENOTOXICITY DATA, RESULTS AND EVALUATION

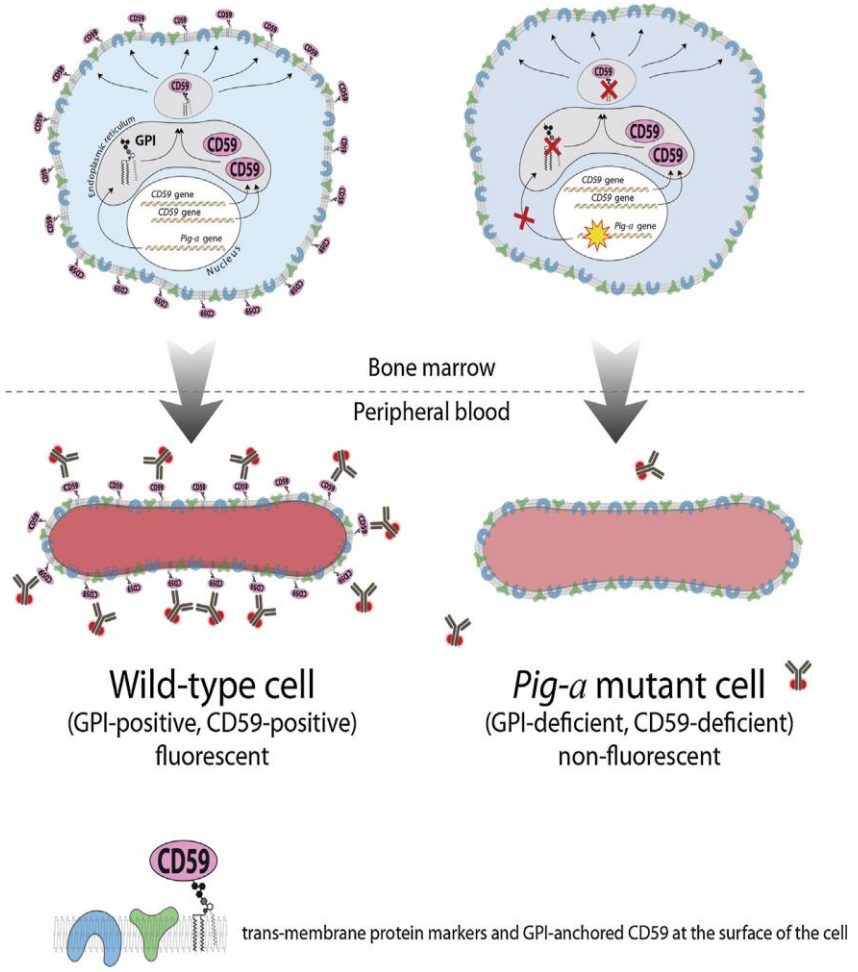
2. Unpublished studies submitted by the US Styrene Information and Research Center (SIRC)

IN VIVO GENOTOXICITY STUDIES SUBMITTED BY US SIRC

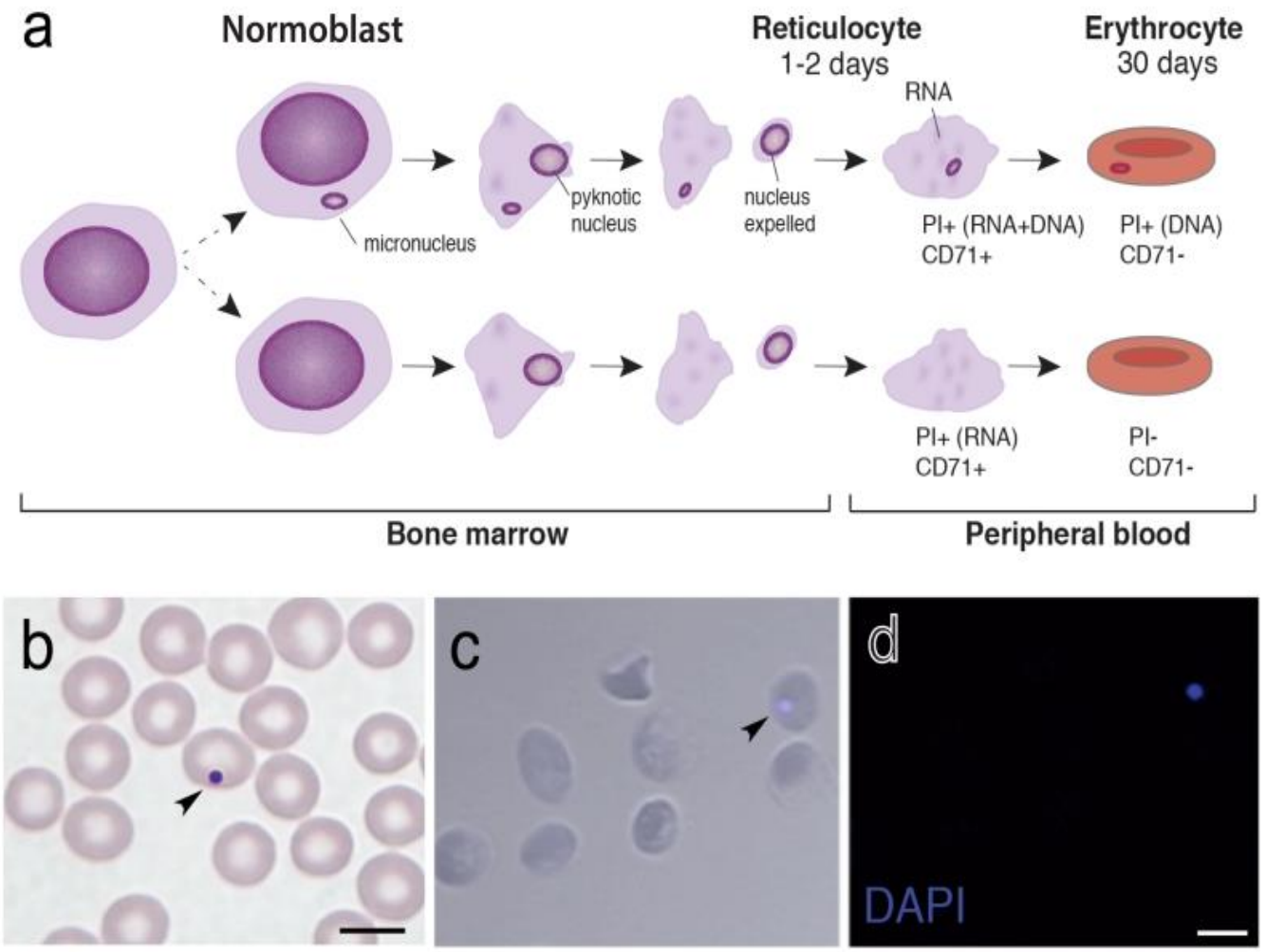
1. Combined Pig-a, Micronucleus, and Comet Study in B6C3F1 Mice After Oral Administration of Styrene for 28 Days
2. Combined Pig-a, Micronucleus, and Comet Study in Fischer 344 Rats After Oral Administration of Styrene for 28 Days
3. Styrene: mammalian alkaline comet study in male Fischer 344 rats via oral gavage administration for 28 days
4. An Oral Gavage In Vivo Mutation Assay of Styrene at the cII Locus in Transgenic Big Blue® Hemizygous B6C3F1 Mice

GENOTOXICITY END-POINTS INVESTIGATED

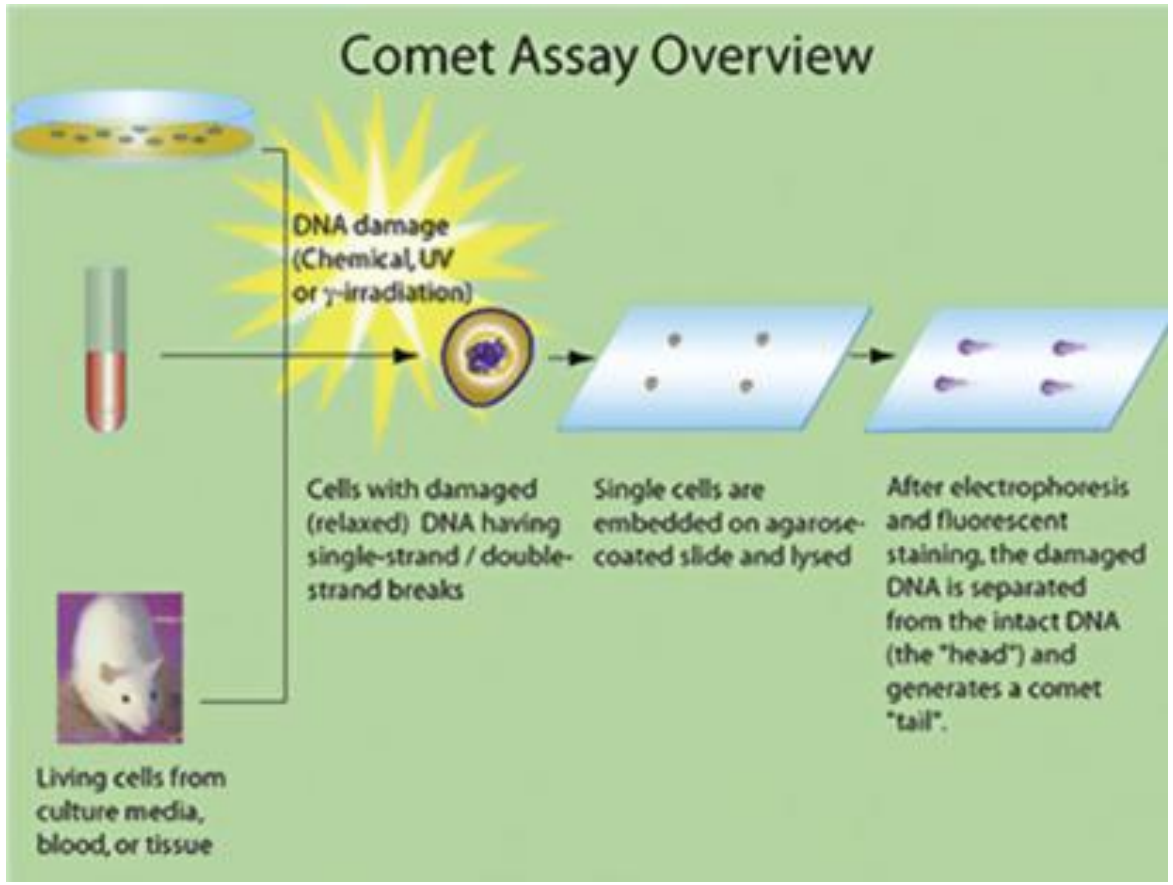
PIG-A

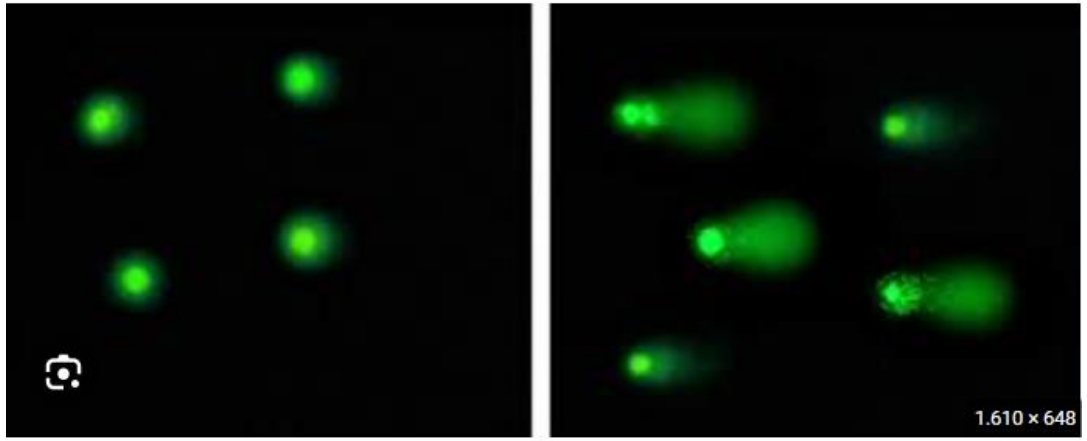


Micronucleus test



GENOTOXICITY END-POINTS INVESTIGATED





1. COMBINED PIG-A, MICRONUCLEUS, AND COMET STUDY IN B6C3F1 MICE AFTER ORAL ADMINISTRATION OF STYRENE

Study protocol: according to OECD TG 470 (Pig-A), TG 474 (MN), TG 489 (comet)

GLP: yes

Chemical: Styrene monomer (CAS RN 100-42-5) purity 99.95%

Animals: 8 male B6C3F1 mice (8-12 weeks of age)/group (6 analysed)

Treatment: 29 daily oral (gavage) administrations of 75, 150, 300 mg/kg/day

Vehicle: corn oil

Positive controls: ENU daily for the first 3 days (days 1-3) for Pig-a

EMS for the last 3 days (days 27-29) for micronucleus test and comet assay

Dose-range finding study: 50, 250, and 500 (350) mg/kg bw/day for 28 days.

Whole blood collected 15 minutes after dosing on day 21 for bioanalysis.

Animals sacrificed from 3-3.5 hours after the final dose.

Reliability: reliable without restrictions

1. COMBINED PIG-A, MICRONUCLEUS, AND COMET STUDY IN B6C3F1 MICE AFTER ORAL ADMINISTRATION OF STYRENE

Results:

Pig-A: **Negative** with no bone marrow toxicity. Systemic exposure demonstrated by plasma bioanalysis. Study design compliant with OECD TG 470 and results consistent with the laboratory historical data. Relevance of the result: **high.**

Micronucleus: **Negative** with no bone marrow toxicity. Systemic exposure demonstrated by plasma bioanalysis. Study design compliant with OECD TG 474 and results consistent with laboratory historical negative and positive control data and with literature data. Relevance of the result: **high.**

Comet: **Negative** in liver, lung, kidney and stomach. Study protocol compliant with OECD TG 489. Adequate response of positive control. Relevance of the result: **high.**

Inconclusive in duodenum for the high baseline value. Relevance of the result: **low**

2. COMBINED PIG-A, MICRONUCLEUS, AND COMET STUDY IN FISCHER 344 RATS AFTER ORAL ADMINISTRATION OF STYRENE

Study protocol: according to OECD TG 470 (Pig-A), TG 474 (MN), TG 489 (comet)

GLP: yes

Chemical: Styrene monomer (CAS RN 100-42-5) purity 99.95%

Animals: 8 male Fisher 344 rats (8-12 weeks of age)/group (6 analysed)

Treatment: 28 daily oral (gavage) administrations of 100, 250, or 500 mg/kg/day

Vehicle: corn oil

Positive controls: ENU daily for the first 3 days (days 1-3) for Pig-a

EMS for the last 3 days (days 27-29) for micronucleus test and comet assay

Dose-range finding study: 100, 500 and 1000 mg/kg bw/day for 28 days.

Whole blood collected 15 minutes after dosing on day 21 for bioanalysis.

Animals sacrificed from 3-3.5 hours after the final dose.

Reliability: reliable without restrictions

2. COMBINED PIG-A, MICRONUCLEUS, AND COMET STUDY IN FISCHER 344 RATS AFTER ORAL ADMINISTRATION OF STYRENE

Results:

Pig-A: **Negative** with no bone marrow toxicity. Systemic exposure demonstrated by plasma bioanalysis. Study design compliant with OECD TG 470 and results consistent with the laboratory historical data. Relevance of the result: **high**.

Micronucleus: **Negative** with no bone marrow toxicity. Systemic exposure demonstrated by plasma bioanalysis. Study design compliant with OECD TG 474 and results consistent with laboratory historical negative and positive control data and with literature data. Relevance of the result: **high.**

Comet: **Negative** in liver and lung. Study protocol compliant with OECD TG 489; adequate response of positive control. Relevance of the result: **high.**

Inconclusive in duodenum, stomach and kidney due the high baseline values and/or inadequate response of the positive control. Relevance of the results: **low.**

3. STYRENE: MAMMALIAN ALKALINE COMET STUDY IN MALE FISCHER 344 RATS VIA ORAL GAVAGE ADMINISTRATION FOR 28 DAYS

Study protocol: according to OECD TG 489

GLP: yes

Chemical: Styrene (99.86%)

Animals: male Fischer 344 rats (6 per group)

Treatment: once daily by gavage for 28 (styrene) of 100, 250, 500 mg/kg bw: (based on previous findings);

Vehicle: corn oil

Positive control: EMS (200 mg/kg bw) for the last two days

Animals sacrificed 3-4 h after last administration.

Lung, liver, kidney, glandular stomach and duodenum isolated for comet analysis.

Reliability: reliable without restrictions

3. STYRENE: MAMMALIAN ALKALINE COMET STUDY IN MALE FISCHER 344 RATS VIA ORAL GAVAGE ADMINISTRATION FOR 28 DAYS

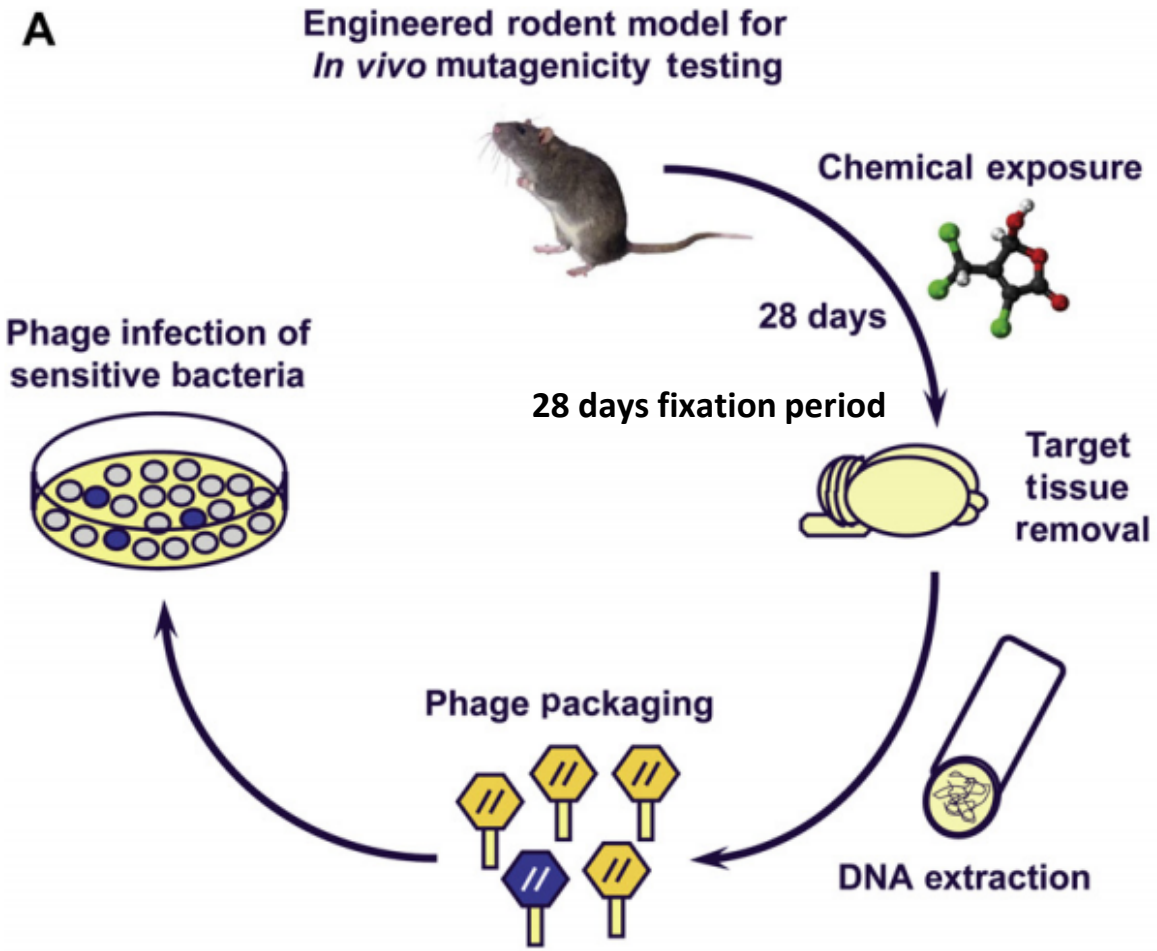
Results:

Negative in liver, lung and kidney. Relevance of the results: **high** for liver and lung, **limited** for kidney (negative control slightly lower than HIC)

Equivocal in stomach (statistically significant increase, not dose related and within the HIC limits). Relevance of the result: **limited**

Inconclusive in duodenum (exceedingly high baseline). Relevance of the results: **low**

MUTATION ASSAY OF STYRENE AT THE CII LOCUS IN TRANSGENIC BIG BLUE® HEMIZYGOUS B6C3F1 MICE



Big Blue® mice model system carry multiple copies of a recoverable lambda bacteriophage shuttle vector in every cell of the body.
***cII* reporter gene**

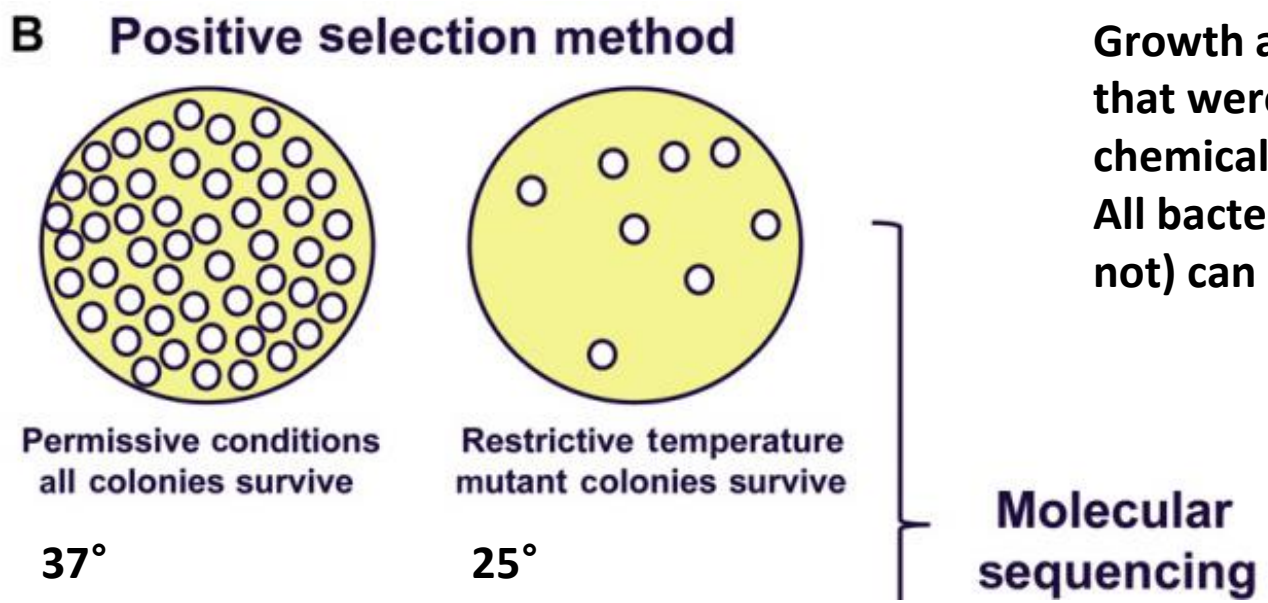
glandular stomach, lung and liver duodenum, bone marrow, kidney, and testes

Isolated DNA was processed using packaging extract to isolate the recoverable lambda shuttle DNA vectors from the genomic DNA and to package the lambda shuttle vector DNA into empty phage capsids creating infectious phage particles.

MUTATION ASSAY OF STYRENE AT THE CII LOCUS IN TRANSGENIC BIG BLUE® HEMIZYGOUS B6C3F1 MICE

Packaged phage were adsorbed onto E. coli G1250 suspension cultures and the cells subsequently plated onto bottom agar plates. Plates were incubated overnight at 37°C ± 2.0°C, then scored for plaque formation and titre determination

***cII* reporter gene**



Growth at 25° C is limited to those E. coli colonies that were infected by phages that had packaged chemically mutated cII reporter genes
All bacteria carrying the reporter gene (mutated or not) can survive at 37° C

$$\text{Mutant frequency} = \frac{\text{number of mutant plaques}}{\text{total number of plaques screened}}$$

4. ORAL GAVAGE IN VIVO MUTATION ASSAY OF STYRENE AT THE CII LOCUS IN TRANSGENIC BIG BLUE® HEMIZYGOUS B6C3F1 MICE

Study protocol:	according to OECD TG 488
GLP:	yes
Chemical:	Styrene (99.86%)
Animals:	male transgenic Big Blue® hemizygous B6C3F1 mice (8 per group)
Treatment:	once daily by gavage for 28 (styrene) of 75, 150 and 300 mg/kg bw (based on previous findings)
Vehicle:	corn oil
Positive control:	ENU (40 mg/kg bw) on days 1, 2 and 3

Animals sacrificed at day 56 (28 days after 28 days treatment)

Glandular stomach, duodenum, lung and liver isolated for the analysis of mutations in the *cII* transgene

Reliability: reliable with restrictions (because of the limited laboratory HIC data)

4. ORAL GAVAGE IN VIVO MUTATION ASSAY OF STYRENE AT THE CII LOCUS IN TRANSGENIC BIG BLUE® HEMIZYGOUS B6C3F1 MICE

Results:

Negative in lung, glandular stomach and duodenum (no statistically significant increase in mutation frequency (MF) at any dose, and no dose-related trend). The relevance of the results is **limited**, because of the restricted reliability of the study.

Inconclusive in liver. A marginal (1.3-fold) and not dose-related increase in MF was reported; the result could not be further evaluated due to the lack of adequate historical control data. Relevance of the result: **low**.

GENOTOXICITY DATA, RESULTS AND EVALUATION

3. New literature on *in vivo* genotoxicity of styrene (published between Jan. 2018 and Oct. 2024)

OUTCOME OF THE LITERATURE SEARCH

Out of **603** original publications retrieved, **57** passed the first screening based on title and abstract content. A further screening based on full text identified **5** publications reporting original data on styrene genotoxicity, not considered in the previous EFSA evaluation (2020):

- Cavallo et al. Toxicol. Lett 298 (2018) 53-59; human biomonitoring study (buccal micronucleus test): inconclusive (limited protocol, few subject with inadequate matching): **low relevance**
- Ladeira et al., Regul.Toxicol.Pharmacol. 116 (2020) 104726; human biomonitoring study (MN and comet in HULY): inconclusive (mixed exposure): **low relevance**
- Gollapudi, Environm. Molec. Mutagen. 64 (2023) 282-290; published version of the combined PigA/micronucleus/comet study in B6C3F1 mice submitted to the EFSA by SIRC.
- Gollapudi, Environm. Molec. Mutagen 65 (2024) 67–75; published version of the combined PigA/micronucleus/comet study in Fisher 344 rats submitted to the EFSA by SIRC.
- **Murata et al., Genes and Environment 45 (2023) 12; In vivo mutagenicity assessment of styrene in MutaMouse liver and lung**

3. PUBLISHED STUDIES RETRIEVED AFTER THE EXTENSIVE LITERATURE SEARCH

Murata *et al. Genes and Environment* (2023) 45:12
https://doi.org/10.1186/s41021-023-00270-9

Genes and Environment

RESEARCH Open Access

In vivo mutagenicity assessment of styrene in MutaMouse liver and lung

Yasumasa Murata[1], Masakatsu Natsume[2], Takako Iso[1], Yoshiyuki Shigeta[1], Nozomu Hirose[1], Takaaki Umano[1], Katsuyoshi Horibata[3], Kei-ichi Sugiyama[3], Kenichi Masumura[1], Akihiko Hirose[1,4] and Mariko Matsumoto[1*]

1 Division of Risk Assessment, National Institute of Health Sciences, Kanagawa, Japan

MURATA ET AL., GENES AND ENVIRONMENT 45:12 (2023)

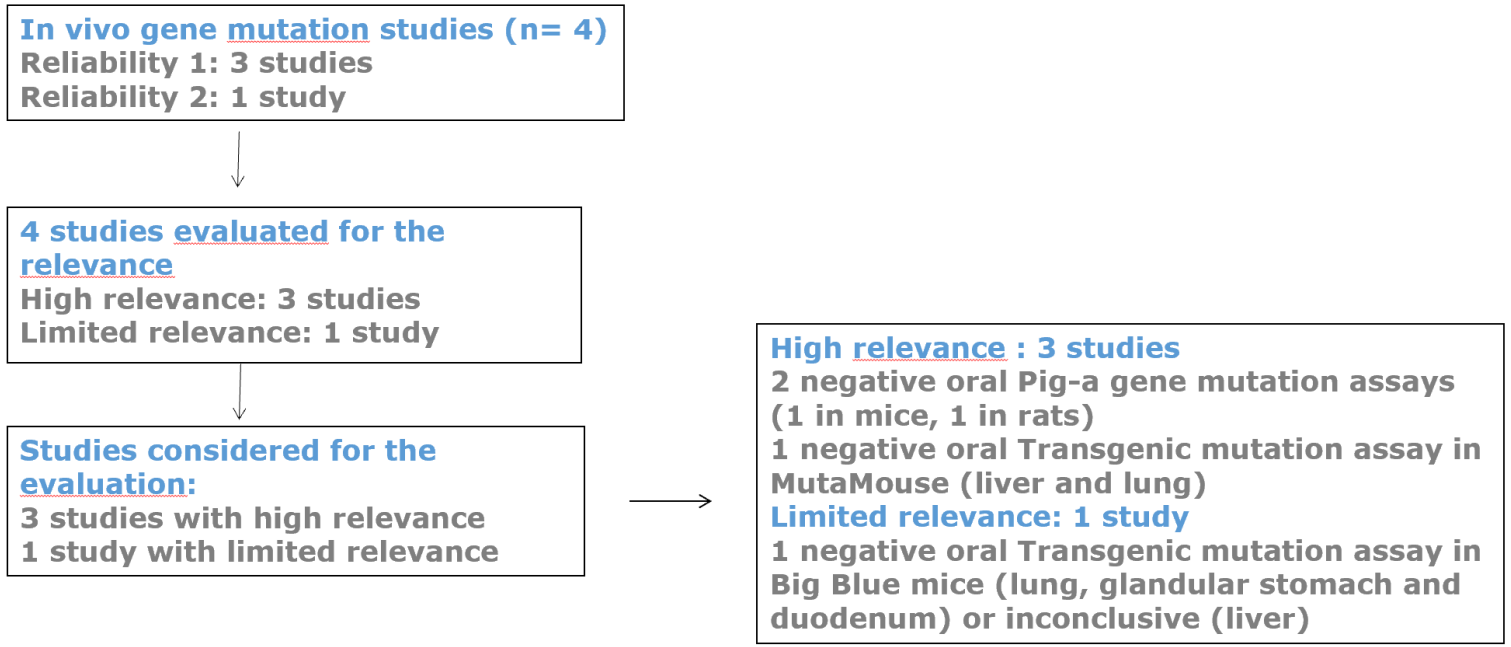
Test system/ Test object	Exposure conditions	Information on the characteristics of the test substance	Result	Reference
Oral exposure				
Transgenic mutation assay in MutaMouse *lacZ* system Treated animals (6-8 males/group); mutations in the *lacZ* transgene determined in DNA isolated from liver and lung of five animals. ENU was given twice by i.p. as positive control GLP compliance: not reported	Daily administration by gavage of 75, 150 or 300 mg styrene/kg bw for 28 days Vehicle corn oil sacrifice 3 days after last administration	Styrene 100% pure	**Negative** In the preliminary dose range finding experiment, high toxicity and lethality were observed at 1000 mg/kg bw (> MTD). In the main experiment, at the highest dose (300 mg/kg bw) there were no clinical signs of toxicity but gross pathological changes in the liver (liver darkening in 3/8 animals). A single animal in the low dose group displayed extremely high mutation frequencies (MF) in liver and lung, attributed to clonal expansion; it was replaced by an additional animal for mutation analysis in this group. When the outlier animal was excluded. mean group MF in liver and lung DNA were not statistically different in styrene treated mice and vehicle controls, and within the range of historical negative control data.	Murata et al., 2023

Evaluation: reliable without restriction; relevance of the results: high

SUMMARY OF AVAILABLE IN VIVO GENOTOXICITY STUDIES ON STYRENE (1)

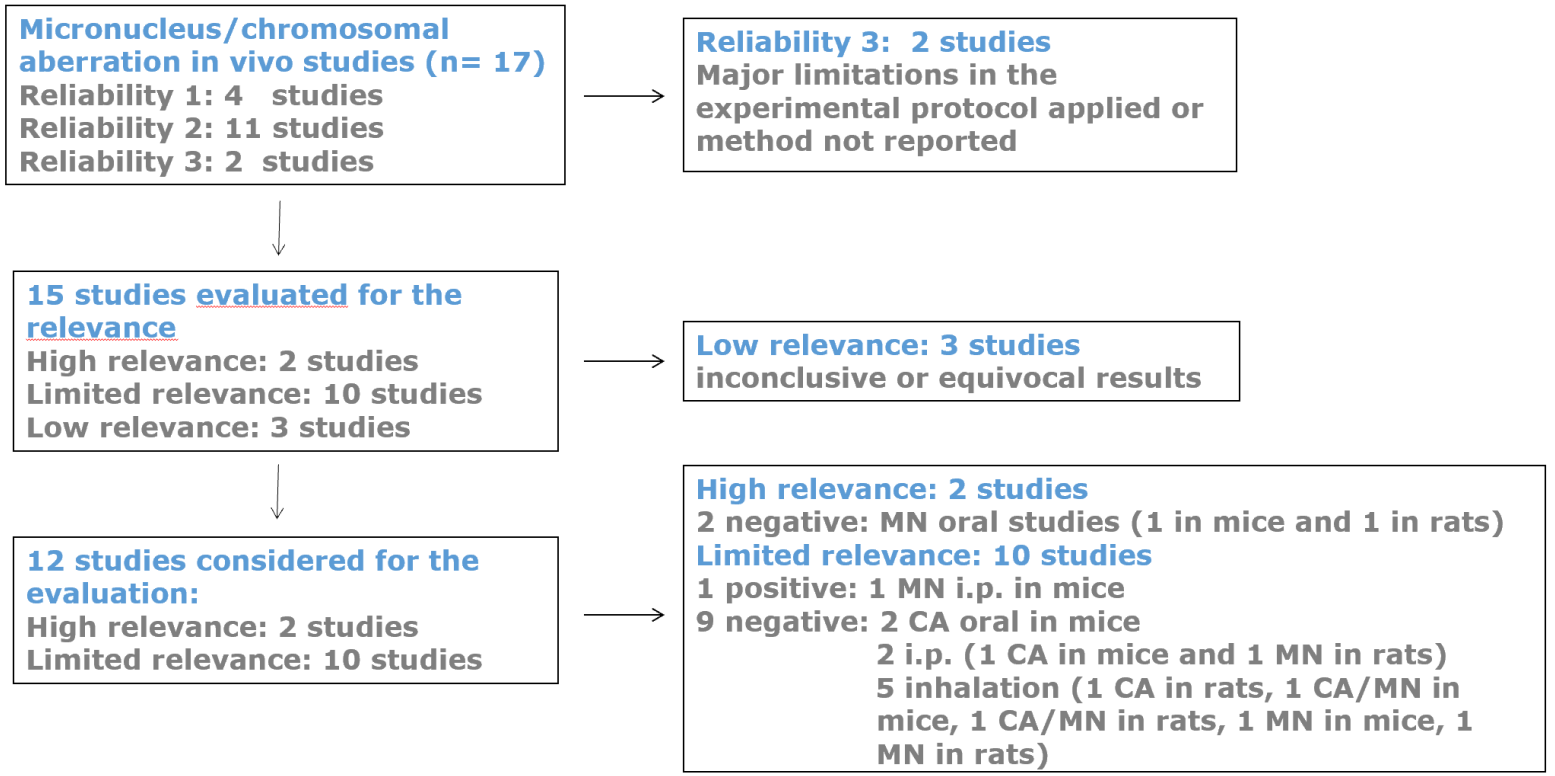
The results from all studies, published and unpublished, were integrated in lines of evidence for each genetic endpoint (gene mutation, chromosome damage and primary DNA damage evaluated by comet assay) in a WoE approach

1. **Gene mutation**: no evidence in **three** highly relevant oral studies (Pig-A in mice and rats, and mutations in liver and lung in transgenic MutaMice); negative (lung) or inconclusive (liver) results in one limited study in BigBlue Mice



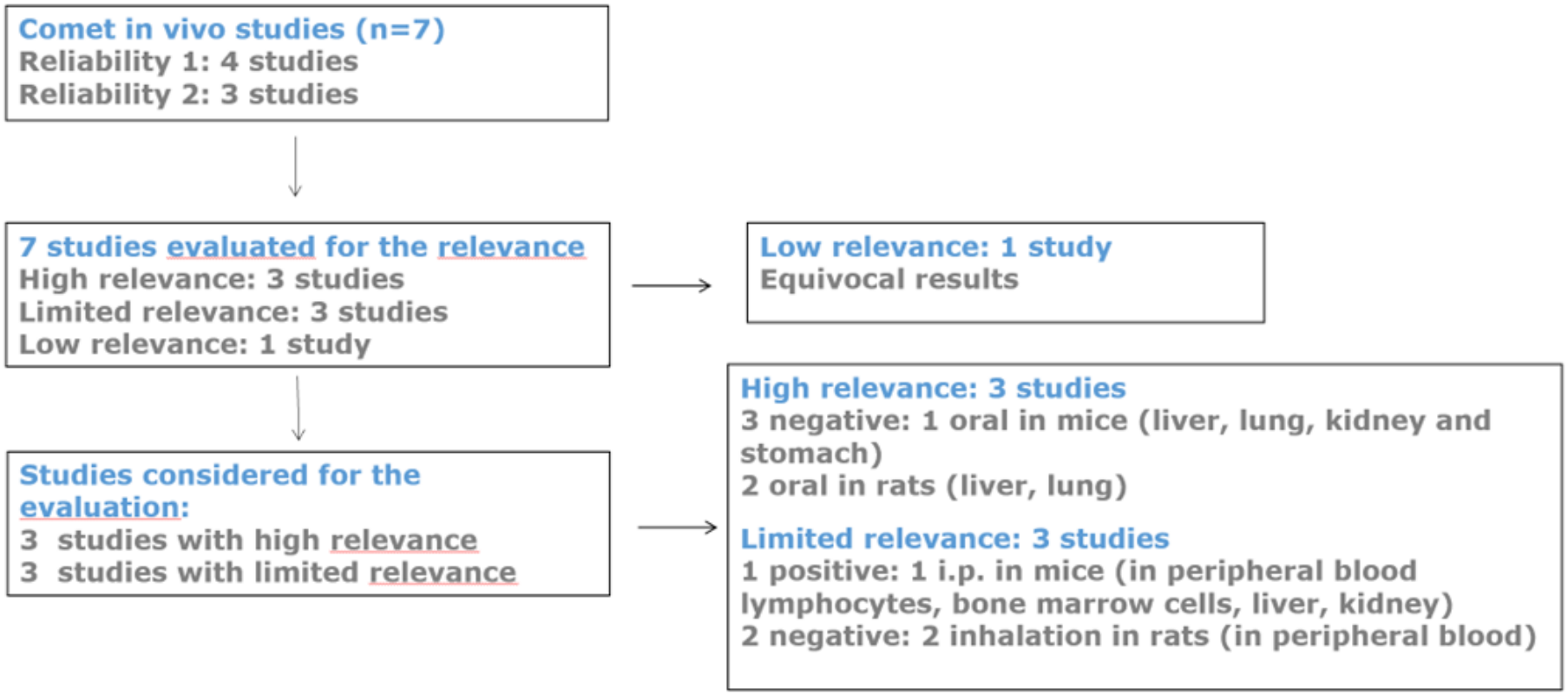
SUMMARY OF AVAILABLE IN VIVO GENOTOXICITY STUDIES ON STYRENE (2)

2. **Chromosome damage (structural aberrations and micronuclei)**: no evidence in **two** oral studies evaluated of high relevance and **nine** (oral, inhalational and i.p.) studies of limited relevance. **One** positive result in mice following i.p. injection in a study of limited relevance



SUMMARY OF AVAILABLE IN VIVO GENOTOXICITY STUDIES ON STYRENE (3)

Primary DNA damage (comet assay): no evidence in **five** studies following oral or inhalational exposure in studies of high (3 oral) and limited (2 inhalational) relevance. **One** positive result in mice following i.p. injection in a study of limited relevance.



SUMMARY OF RESULTS FROM ORAL IN VIVO GENOTOXICITY ASSAYS WITH STYRENE

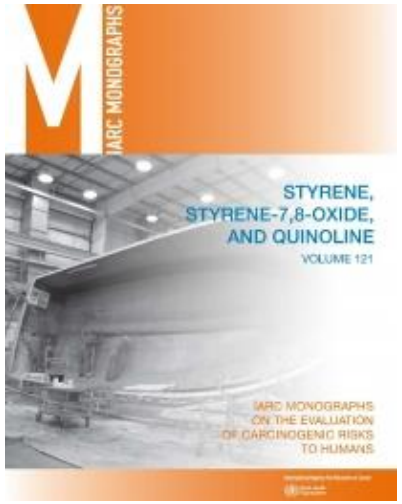
End-point/Test system	Species	Result	Reference
Chromosomal aberrations	CD-1 mice	**Negative**	Loprieno 1978; Sbrana 1983
Gene mutation (Pig-A)	Mouse B6C3F1	**Negative**	SIRC, Oct 2022
Micronucleus test	Mouse B6C3F1	**Negative**	SIRC, Oct 2022
Comet assay	Mouse B6C3F1	**Negative (liver, lung, stomach, kidney)**; inconclusive (duodenum)	SIRC, Oct 2022
Gene mutation (Pig-A)	Fischer 344 rat	**Negative**	SIRC, Dec 2022
Micronucleus test	Fischer 344 rat	**Negative**	SIRC, Dec 2022
Comet assay	Fischer 344 rat	**Negative (liver, lung)**; inconclusive (stomach, duodenum, kidney)	SIRC, Dec 2022
Comet assay	Fischer 344 rat	**Negative** (**liver, lung and kidney**); inconcl (duodenum)/equiv (stomach)	SIRC, Oct 2023
TGR mutation (*lac Z*)	MutaMouse	**Negative (liver, lung)**	Murata et al 2023
TGR mutation (*cII*)	Big Blue mice	**Negative (lung, stomach, duodenum);** inconclusive (liver)	SIRC, April 2024

TO ADDRESS THE MANDATE

- Data to be analysed:
  - Genotoxicity
  - **Toxicokinetics (Tks)**
  - Human exposure (HBM+dietary)

- Sources of data:
  - ❑ IARC Monograph (2019)
  - ❑ Literature search (last 7 years, 2001 papers)

SIRC Styrene Information & Research Center



TOXICOKINETICS DATA – CONCLUSIONS

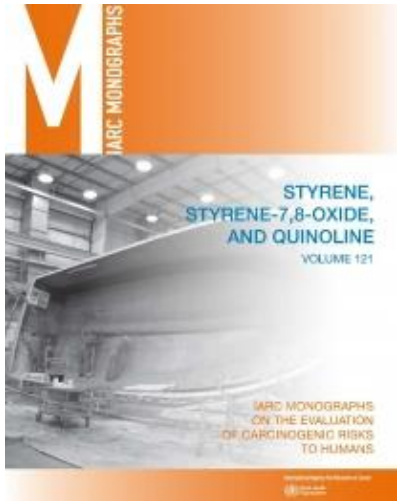
- Liver is the primary tissue responsible for styrene metabolism after oral exposure. The side chain oxidation, with the generation of styrene oxide (SO), is the first metabolic step in animals and humans. Extrahepatic metabolism has a minor role, if any, on styrene biotransformation.
- The systemic availability of SO is reduced by an efficient hepatic first-pass hydrolysis in situ.
- Compared to rodents, humans are less sensitive because of their lower capacity to form SO and their higher detoxification efficiency.
- Low (micromolar) concentrations of SO, rapidly cleared, were measured in the blood of rats after oral exposure to styrene (500 mg/kg bw).
- Measured and estimated (by PBK modeling) concentrations of SO in human blood following inhalational of dietary exposure were in the nanomolar range.

TO ADDRESS THE MANDATE

- Data to be analysed:
  - Genotoxicity
  - Toxicokinetics (Tks)
  - **Human exposure (HBM+dietary)**

- Sources of data:
  - ❑ IARC Monograph (2019)
  - ❑ Literature search (last 7 years, 788 papers)

SIRC Styrene Information & Research Center



HUMAN STUDIES - CONCLUSIONS

- The FCM Panel noted several limitations in the biomonitoring studies using genotoxicity biomarkers:
  - large variability and uncertainties in the extent and profile of exposure
  - possible co-exposure to other genotoxicants
  - lack of consistency across studies
  - absence of an exposure response
  - lack of control for relevant confounding factors
- Overall, taking also into account the conclusions of the two recent meta-analyses (Collins and Moore, 2019; 20121) the Panel concluded that these studies do not provide sufficient evidence to support an association between styrene exposure and genotoxic damage in humans.
- Studies on human exposure to styrene indicate that the most important exposure sources throughout the different age groups are food and air (similar contribution). For adult smokers, inhalation (air plus tobacco smoke) is the major exposure route.
- HBM data in the general population support the predicted kinetics (in particular metabolism and elimination) in humans after oral exposure.

OVERALL CONCLUSIONS ON STYRENE GENOTOXICITY

- The evidence provided by the available studies indicates that styrene, although genotoxic in some in vitro systems, does not show genotoxic effects in vivo following physiological routes of exposure (i.e. no i.p.). In particular, no indication of genotoxicity was observed in reliable studies using the oral route in mice and rats.
- Human biomonitoring studies in workers occupationally exposed to styrene did not provide relevant information for the assessment of the genotoxic hazard posed by oral exposure.
- Considering the available data, the Panel concluded that there is no scientific evidence that styrene is genotoxic following oral exposure.
- For substances demonstrated to be non-genotoxic, according to the EFSA Note for Guidance for Food Contact Materials (EFSA CEF Panel, 2008), a SML up to 50 µg/kg food would not be of safety concern. In relation to the ToR, this implies that the use of styrene in the manufacture of FCM with a SML of 40 µg/kg food is not of safety concern.

AGENDA

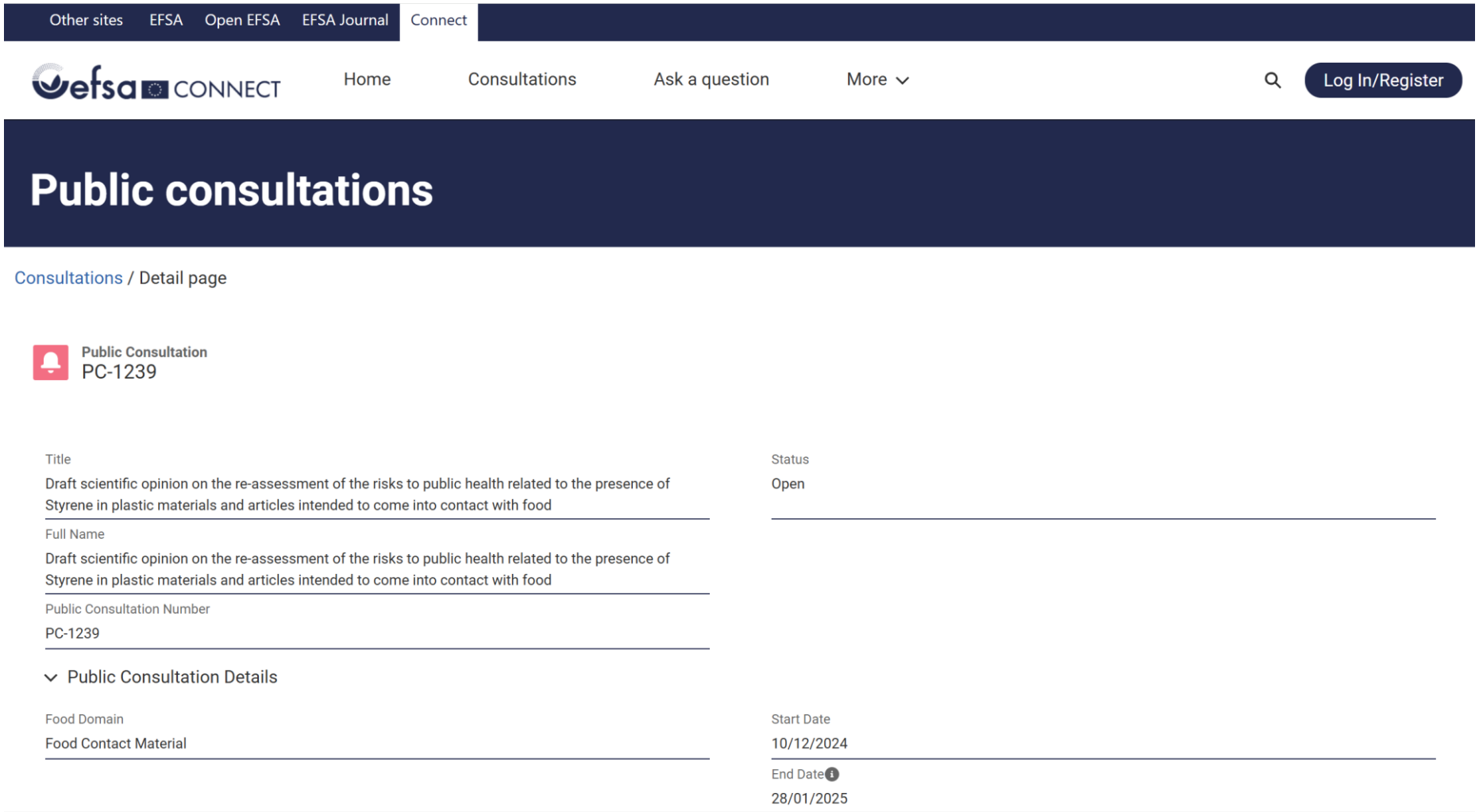
15:55-16:00

Closing of the webinar

Sandra Rainieri

PUBLIC CONSULTATION

LINK: https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0lTk0000039j7R/pc1239



Start date:
10 December 2024

End date:
28 January 2025

PUBLIC CONSULTATION

LINK: https://connect.efsa.europa.eu/RM/s/consultations/publicconsultation2/a0ITk0000039j7R/pc1239

Documents for consultation	File type
Annex A	PDF
Annex B	PDF
Draft_scientific_opinion_ Styrene_forpublicconsultation	PDF

Respond to consultation

Log in / Register To save your comments and keep track of your submissions.

or

Comment as guest

For technical questions please contact EFSA's IT support at servicedesk@efsa.europa.eu

NEXT STEPS

March 2025

- Possible adoption of the scientific opinion by the EFSA FCM Panel (18-20 March 2025)

  Link to register: 6th Plenary meeting of the Panel on Food Contact Materials (FCM) - Open for Observers | EFSA

April-May 2025

- Opinion publication

After publication:

- Risk Management Action

THANK YOU FOR ATTENDING THIS WEBINAR!

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