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Methyl styrene (vinyl toluene) (all isomers)

MAK Value Documentation, addendum – Translation of the German version from 2017

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Methyl styrene (vinyl toluene) (all isomers) / Ethenylmethylbenzene

MAK Value Documentation

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated methyl styrene [25013-15-4] considering all toxicological endpoints and revised the maximum concentration at the workplace (MAK value). Publications are described in detail.

In long-term inhalation studies with methyl styrene, inflammation and hyperplasia were observed in nasal tissue of rats and mice with LOAECs of 100 ml/m³ and 10 ml/m³, respectively. As was shown with styrene, the reason for mice being more susceptible than rats is differences in the rates of phase I and phase II metabolism. While the rate of oxidation to the epoxide is almost the same in rats and mice, the detoxification via epoxide hydrolase and GSH-transferase is about 10-fold faster in rats than in mice. In human nasal tissue in vitro, oxidation of styrene is minimal but metabolization via epoxide hydrolase and GSH-transferase occurs at almost the same rate as in rat nasal tissue. Therefore, humans are much less sensitive to nasal effects of styrene and this species difference is assumed to be the same for the similarly metabolized methyl styrene. The MAK value is derived from the LOAEC in rats and set at 20 ml methyl styrene/m³. Due to the local effect being critical, the substance is assigned to Peak Limitation Category I. As there was no sensory irritation in volunteers with methyl styrene at 50 ml/m³, the excursion factor is 2.

Methyl styrene has a limited genotoxic potential only at systemically toxic doses, and 2-year carcinogenicity studies were negative.

Dermal absorption does not contribute significantly to the systemic toxicity and investigations in humans and animals do not indicate a significant sensitizing effect.

Because 3 studies on the developmental toxicity of methyl styrene are only available as summaries and the reason for the differing effects cannot be evaluated, methyl styrene is assigned to Pregnancy Risk Group D.

Keywords

methyl styrene; methylvinylbenzene; tolylethylene ; vinyl toluene; ethenylmethylbenzene; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Methyl styrene (vinyl toluene) (all isomers)

[25013-15-4]

Supplement 2017

MAK value (2016)	**20 ml/m³ (ppm) ≙ 98 mg/m³**
Peak limitation (2002)	**Category I, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2016)	**Pregnancy Risk Group D**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	methylvinylbenzene tolylethylene vinyl toluene
Chemical name	ethenylmethylbenzene
CAS number: isomer mixture	25013-15-4
2-methyl styrene	611-15-4
3-methyl styrene	100-80-1
4-methyl styrene	622-97-9
Molar mass	118.18 g/mol
Melting point: isomer mixture	–76.7 °C (US EPA 2010)
3-methyl styrene	–86.3 °C (US EPA 2010)
4-methyl styrene	–34.1 °C (US EPA 2010)
Boiling point at 1013 hPa: isomer mixture	170–171 °C (US EPA 2010)
3-methyl styrene	164 °C (US EPA 2010)
4-methyl styrene	172.8 °C (US EPA 2010)

Density at 20 °C: isomer mixture	0.898 g/cm³ (IARC 1994)
2-methyl styrene	0.904 g/cm³ (IARC 1994)
3-methyl styrene and 4-methyl styrene	0.911 g/cm³ (IARC 1994)
Vapour pressure at 20 °C: isomer mixture	1.47–2 hPa (US EPA 2010)
3-methyl styrene	2.3 hPa (US EPA 2010)
4-methyl styrene	2.4 hPa (US EPA 2010)
log K_{OW}[1)]: isomer mixture	3.58 (US EPA 2003)
3-methyl styrene	3.35 (US EPA 2010)
4-methyl styrene	3.35 (US EPA 2010)
Solubility at 25 °C: isomer mixture	89 mg/l water (US EPA 2010)
3-methyl styrene	151 mg/l water (calculated; US EPA 2010)
4-methyl styrene	89 mg/l water (SRC 2015)
1 ml/m³ (ppm) ≙ 4.904 mg/m³	**1 mg/m³ ≙ 0.204 ml/m³ (ppm)**

For methyl styrene (all isomers) there is documentation available from 1976 (documentation "Vinyltoluol (alle Isomere)" 1976, available in German only) and a supplement from 2002 (supplement "Methylstyrol (Vinyltoluol) (alle Isomere)" 2002, available in German only), in which the MAK value from 1958 was confirmed and, in 2002, Peak Limitation Category I with an excursion factor of 2 was established for the substance without any further designations or classifications. Since the documentation from 1976 (documentation "Vinyltoluol (alle Isomere)" 1976, available in German only) further studies relevant for the evaluation have been published.

The methyl styrene defined under CAS number 25013-15-4 consists of about 60% to 70% 3-methyl styrene and of about 30% to 40% 4-methyl styrene (US EPA 2010).

This supplement is based mainly on the studies carried out under the NTP program (NTP 1990), and on the assessments of the IARC (1994), the US EPA (2003, 2010) and the publicly available registration data in the REACH database (ECHA 2015). In addition to methyl styrene (all isomers) (CAS number 25013-15-4), studies with the isomer 4-methyl styrene (CAS number 622-97-9) are described.

Methyl styrene monomers are used alone or in combination with other substances in the manufacture and processing of polymers, as adhesives and binders, synthetic resin, synthetic rubber, as surface coatings, dyes and pigments, for the production of organic chemicals and in insecticides. Under normal conditions methyl styrene isomers are present in liquid form with a very unpleasant odour (ECHA 2015; NTP 1990; US EPA 2010).

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1) octanol/water partition coefficient.

1 Toxic Effects and Mode of Action

Methyl styrene is absorbed by inhalation and ingestion and transformed by CYP450 monooxygenases to form presumably mainly vinyl toluene-7,8-oxide as the main reactive metabolite. In the urine, after intraperitoneal administration, about 55% of the dose was recovered in the form of metabolites.

In well documented studies, which, however, do not meet present day test guideline requirements, methyl styrene was found to be a moderate skin irritant and slightly irritating to the eye in rabbits.

In 2-year inhalation studies, methyl styrene had strong effects on the respiratory tract even at the lowest concentration tested of 10 ml/m^3 in mice and at 100 ml/m^3 in rats. In mice exposed to 10 ml/m^3 almost 100% of the animals were found to have inflammation and hyperplasia in the respiratory epithelium of the nose and 30% of the animals inflammation of the lungs or the bronchioles. In the olfactory epithelium of rats exposed to a concentration of 100 ml/m^3, erosion and hyperplasia were observed in 12% and 16% of the animals, respectively. Intraepithelial cysts were found in the respiratory epithelium in 26% of the animals. Rats are therefore less sensitive than mice by a factor of about 10. Reduced body weights were found in mice at a concentration of 25 ml/m^3 and in rats at 300 ml/m^3.

Skin sensitizing effects of methyl styrene have been reported only sporadically in patch tests. Experiments in guinea pigs with methyl styrene and the structurally very closely related styrene yielded negative or equivocal results for skin sensitization.

There are no studies available of the developmental toxicity of the methyl styrene isomer mixture; however, there are three developmental toxicity studies with 4-methyl styrene, which are cited in secondary sources, two of them in rats and one in rabbits. No developmental toxicity occurred in rabbits up to the highest dose tested of 150 mg/kg body weight and day and in rats up to 600 mg/kg body weight and day. In the other developmental toxicity study in rats, one case of meningocele, which is a rare malformation, was observed at the high dose of 600 mg/kg body weight and day. Since the original reports of the studies are not available, no conclusive assessment can be made as regards possible developmental toxicity of methyl styrene (all isomers).

In in vitro investigations, methyl styrene was not mutagenic in bacteria. In CHO cells (a cell line derived from Chinese hamster ovary) the induction of sister chromatid exchange (SCE) or chromosomal aberration (CA) was not observed; in human lymphocytes, on the other hand, SCE and CA were induced at much higher concentrations. In the studies of CA, no data for cytotoxicity are given. Methyl styrene was not found to be mutagenic in mammalian cells. In in vivo tests carried out at the same laboratory in which the in vitro studies with a positive result for clastogenicity were conducted, a micronucleus test yielded positive results, however at cytotoxic doses. Tests for SCE, CA and dominant lethal mutations produced no evidence of a genotoxic potential.

In carcinogenicity studies, no significantly increased tumour incidences occurred up to methyl styrene concentrations of 300 ml/m^3 in rats and up to 25 ml/m^3 in mice.

2 Mechanism of Action

There are no studies available for the mechanism of action of methyl styrene itself. For this reason, some investigations with styrene are described below.

Comparative data for methyl styrene and styrene

In a genotoxicity study carried out by Norppa and Vainio (1983), which determined the extent to which methyl styrene isomers and styrene caused the formation of SCE and CA in a culture of human whole blood lymphocytes, 3-methyl styrene and 4-methyl styrene had about the same effect as styrene, while 2-methyl styrene was somewhat less effective.

Sensory irritation and CNS effects are the critical end points for the derivation of a threshold limit value in air for styrene. According to an earlier volunteer study, there are no significant differences between styrene and methyl styrene with regard to sensory irritation. For color vision discrimination, the most sensitive end point of styrene (EU 2008), there are no studies available for methyl styrene. Ototoxicity, another important end point of styrene, does not occur with methyl styrene (Gagnaire and Langlais 2005).

Differences in the sensitivity of the rat and mouse to methyl styrene

In 2-year inhalation studies, mice were approximately 10 times more sensitive to methyl styrene than rats as regards effects in the nasal cavity. Also intraperitoneal injection of methyl styrene led to a more pronounced reduction in the glutathione content in the liver of mice than that in rats. In rats, the hepatic CYP450 enzymes were not affected by methyl styrene, whereas in mice they were markedly reduced. The exact mechanism that accounts for this difference in sensitivity to methyl styrene between the species is not known (NTP 1990).

The results of the investigations of the mode of action of styrene and of the species-specific quantitative difference in the metabolism of the substance between rats and mice can presumably be applied to methyl styrene as well.

Sensitivity to styrene of the rat and mouse compared with that of humans

Styrene is oxidized to an epoxide which is detoxified by epoxide hydrolase and GSH transferase. While the oxidation in the nose of rats and mice takes place at about the same speed, the detoxification is more rapid in rats than in mice by a factor of about 10. An in vitro comparison with nasal tissue of humans shows that almost no oxidation takes place there, whereas the activities of epoxide hydrolase and GSH transferase are about the same as those in the rat. The human nose is thus less sensitive than the nose of rats and mice (Green et al. 2001). Therefore, in the EU Risk Assessment Report for styrene, the findings in the nose are not used as a starting point for risk assessment (EU 2008).

In the lungs of mice, oxidation of styrene takes place in Clara cells, which are present to a much lesser extent in the lungs of humans. Here as well, humans are less sensitive than mice (EU 2008).

Likewise, humans are less sensitive than mice with regard to the liver, which is confirmed by the fact that deaths occurred in mice at styrene concentrations to which workers had been exposed for years without any occurrence of liver toxicity or mortality (EU 2008).

Similar differences in sensitivity due to a quantitative difference in the metabolism of the substance are to be assumed for methyl styrene as well.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

There are no in vivo data available for oral, dermal and inhalation absorption and the distribution of methyl styrene in the body. The systemic effects mainly in the liver of rats and mice after inhalation and oral exposure suggest good absorption.

In rats given single intraperitoneal doses of methyl styrene of 50 mg/kg body weight, 55% of the dose was recovered as urinary metabolites; the percentage was slightly lower at higher doses. Elimination took place mainly within the first six hours (ECHA 2015; NTP 1990; US EPA 2003).

Human skin was used in an in vitro static diffusion cell model with 5 ml receptor fluid and an exposure area of 0.64 cm^2. To determine the permeability coefficient, methyl styrene (purity > 99%, about 55% 3-methyl styrene and 45% 4-methyl styrene) was applied in concentrations of 100 µl/cm^2 to 6 skin samples from 4 donors and the amount of methyl styrene in the receptor fluid was determined after 1 hour and 2, 4, 12, 24, 36 and 48 hours. The steady-state velocity at the concentration of 894.6 mg/cm^3 (which was given as its density) was 203.3 ± 120 µg per cm^2 and hour. The permeability coefficient was calculated to be $2.27 \pm 1.34 \times 10^{-4}$ cm per hour. For the short-term absorption experiments, 12 skin samples from 3 human subjects were loaded with methyl styrene at a concentration of 10 µl/cm^2 and the chambers sealed with parafilm for 10 or 60 minutes. The chambers were opened after the end of the exposure period, the epidermis was washed and the methyl styrene was determined in the receptor fluid and the skin. After 10 minutes, 3.39 ± 2.42 µg methyl styrene was determined in the receptor fluid and 3.79 ± 1.8 µg in the skin, after 60-minute exposure 59.8 ± 39.5 µg in the receptor fluid and 6.86 ± 3.18 µg in the skin. For 10-minute and 60-minute exposure periods the respective absorption rates were 66.0 (± 29.9) and 104.2 (± 63.0) µg per cm^2 and hour (Fasano and Baer 2006). From the latter value, an absorption of 208 mg is calculated for an exposed skin surface of 2000 cm^2 and an exposure time of one hour.

3.2 Metabolism

In rats given single intraperitoneal doses of methyl styrene of 50 mg/kg body weight, 55% of the dose was eliminated as urinary metabolites (see Figure 1). These were 25% thioether, 5.7% 4-methyl mandelic acid, 11.9% 4-methylphenyl glyoxalic acid, 9.3% 4-methylbenzoyl glycine, 2.5% 4-methylphenylacetyl glycine and 1% 4-vinylbenzoyl glycine. This means that most of the identified metabolites were formed via the epoxide pathway and 1% via oxidation of the methyl group. Elimination of these metabolites was prevented by pretreatment with an inhibitor of the CYP450 monooxygenases. Single intraperitoneal doses of methyl styrene led in rats to decreased glutathione levels in the liver and kidneys. These findings suggest that the metabolism of methyl styrene is catalyzed by CYP450, producing vinyl toluene-7,8-oxide

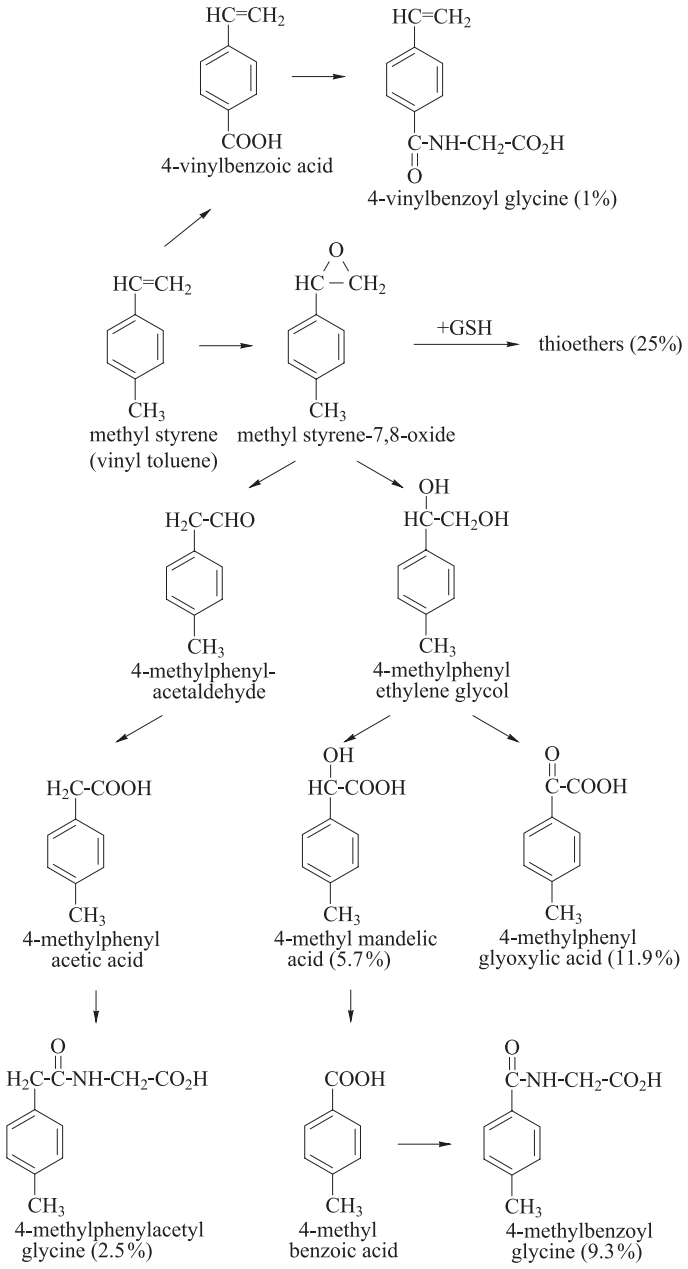

Figure 1 Metabolism of methyl styrene in rats (NTP 1990)

as the main reactive intermediate with subsequent conjugation to glutathione or hydration to a diol. A dose and time-dependent decrease in the glutathione levels in the liver and kidneys of rats, mice and hamsters occurred also after exposure to concentrations of 50, 100 or 300 ml/m³ for 6 hours a day, on 5 days per week, for 15 weeks (ECHA 2015; IARC 1994; NTP 1990; US EPA 2003).

In vitro, 4-methyl styrene is oxidized to the diol by CYP450 enzymes at the vinyl group just as rapidly as styrene. The binding to proteins in vitro is also very similar (IARC 1994).

4 Effects in Humans

The only studies available in humans investigated irritation and sensitization.

Irritation

In a study in test persons (no other details) the odour threshold was 50 ml methyl styrene/m³. Strong odour without excess annoyance was described for a concentration of 200 ml/m³, and, at ≥ 400 ml/m³, strong irritation of the eyes and nose occurred (Wolf et al. 1956). The NOAEC (no observed adverse effect concentration) for irritation is 50 to 100 ml/m³. The study does not meet present-day requirements for a study with test persons and is poorly documented. As, however, styrene was also investigated and, at similar concentrations, induced effects like those found for methyl styrene, the study gives at least a qualitative indication that the sensory irritation of methyl styrene corresponds to that of the better investigated styrene.

Sensitization

No commercially available test preparations are available for patch tests with methyl styrene or styrene. There are no case reports of contact sensitization to methyl styrene. One report of positive patch test results indicated cross-reactivity in a patient with sensitization to styrene. This person developed vesicular dermatitis on the hand after repeated application of an unsaturated polyester resin containing styrene and a hardener containing dibenzoyl peroxide. In the patch tests, strong reactions to the resin and to styrene (tested as 10% and 5% concentrations, respectively, in 2-butanone) and a weak irritant reaction to 5% dibenzoylperoxide were found. In further patch tests the patient reacted once again to styrene down to a concentration of 0.001% and to a mixture of 3-methyl styrene and 4-methyl styrene at a concentration of 0.11% in ethanol. This mixture produced no reaction in 16 patients tested as a control group, and also no reaction in 303 patients tested with 5% styrene as a control (Sjöborg et al. 1982). The patient also reacted to the individual methyl styrene isomers, as well as to styrene epoxide (0.1% in ethanol) and to 4-hydroxystyrene (Sjöborg et al. 1984).

Although, in addition, other publications reported reactions to styrene in 4 of 45 tested patients with contact dermatitis from shoes (test concentration not specified; Grimalt and Romaguera 1975) as well as in a jeweller with skin reactions on the fingers after exposure to resins and positive patch test results to 1% styrene (in olive oil as well as in 2-butanone; Condé-Salazar et al. 1989), evidently no tests with methyl styrene were carried out in these investigations.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

There were no data available for inhalation exposure for the documentation of 1976 (documentation "Vinyltoluol (alle Isomere)" 1976, available in German only).

During the 6-hour whole-body exposure of groups of 5 male and 5 female Sprague Dawley rats to methyl styrene concentrations of 1510 or 1960 ml/m³ ("MCTR-161-79", no other details) and a 14-day recovery period, none of the female animals died; at the high concentration, one male animal died. At the low concentration, reduced activity, lacrimation, closed eyes, extreme salivation and laboured breathing were observed during the exposure. After the end of the exposure and up to the end of that day, ataxia, impaired righting reflex and hunched posture were observed. On the day following the exposure the behaviour of the animals was normal. At the high concentration, the signs were stronger during the exposure, and, additionally, ataxia, very fast breathing or gasping, prostration, and twitching and swollen extremities were seen. Even after the end of the exposure the motor difficulties continued for another two days. Thereafter, behaviour was normal (ECHA 2015). The LC_{50} for rats in this study was greater than 1960 ml/m³.

In another study with 4-hour whole-body exposure of 5 male and 5 female Sprague Dawley rats to 3500 ml/m³ (16 891 mg/m³, "MCTR-142-79", no other details) no deaths occurred. During the exposure, all animals exhibited respiratory abnormalities and, after about 15 minutes and later, discharge from the eyes, nose and ears. After the animals were taken out of the exposure atmosphere, neuromuscular effects such as muscular weakness, loss of reflexes, spontaneous muscle activities, uncoordinated movements and tremor were observed. The discharge decreased within 4 hours after the end of the exposure, whereas the neuromuscular abnormalities and respiratory difficulties increased. One day after the end of the exposure these effects had disappeared, whereas the rales during breathing persisted until the third day after the end of the exposure (ECHA 2015). The LC_{50} for rats in this study was greater than 3500 ml/m³.

5.1.2 Oral administration

The oral LD_{50} for male Wistar rats, determined in 42 animals, was about 4000 mg/kg body weight (about 55%–70% 3-methyl styrene and 30%–45% 4-methyl styrene; documentation "Vinyltoluol (alle Isomere)" 1976, available in German only; NTP 1990; US EPA 2010).

Groups of 10 male Sprague Dawley rats were given gavage doses of undiluted methyl styrene ("MCTR-243-77", no other details) of 0.6, 1.3, 2.5 or 5 ml/kg body weight. The calculated LD_{50} was 3.68 ml/kg body weight, from which an LD_{50} between 2000 and 5000 mg/kg body weight was derived. In rats given 2.5 ml/kg body weight and more, tremor, increased respiratory rate and slight apathy were observed, all persisting for up to 3 days. Bloody lungs, bloody small intestine, colourless liquid in the bladder and red discharge in the mouth, nose and eyes were found in the deceased animals (ECHA 2015).

Groups of 2 male and 2 female beagle dogs were given a single gavage dose of undiluted methyl styrene of 4000 or 5000 mg/kg body weight ("MCTR-88-79", no other details) and a single dose of 4000 mg/kg body weight as a 50% solution. The animals were observed for 14 days. All animals vomited within the first one or two hours after substance administration, and trembling was observed; at the dose of 5000 mg/kg body weight, additional salivation was observed. Some animals of the low dose group and all animals of the high dose group lost 100 to 400 g in body weight after administration of the substance; no histopathological changes or deaths occurred (ECHA 2015). The LD_{50} for dogs in this study was greater than 5000 mg/kg body weight.

5.1.3 Dermal application

In a study from 1977 with New Zealand White rabbits, 4-methyl styrene of unknown purity was applied in doses of 0.5, 1, 2, 4 or 5 ml/kg body weight (450, 900, 1800, 3600, 4500 mg/kg body weight) to the dorsal skin. One male and one female animal per dose group had abraded skin, one male and one female animal per dose group intact skin. The site of application was not covered during the 24-hour exposure period, ingestion, however, was prevented. A 14-day recovery period followed. Neither mortality nor signs of systemic toxicity occurred. The local effects were slight to moderate erythema, oedema and leathery skin, and were dependent on the dose. In the high dose group, these signs were still present even at the end of the recovery period. In the lower dose groups they had subsided within one week. No histopathological findings occurred. The dermal LD_{50} was therefore greater than 4500 mg/kg KG (ECHA 2015).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In a 2-year inhalation study with exposure of F344 rats to methyl styrene concentrations of 0, 100 or 300 ml/m^3, cysts were found in the respiratory and olfactory epithelium, and hyperplasia of the respiratory epithelium of the nose was observed even at the low dose (see Table 1 and 2). The latter finding was diffuse and characterized by an increased number of goblet cells and thickening of the epithelium. The cysts were small, intraepithelial, gland-like structures distended with mucus. The lesions in the olfactory epithelium occurred mainly in the anterior region along the dorsal meatus. The olfactory epithelium was focally eroded; the underlying Bowman's glands were cystically dilated and the glandular epithelium replaced by ciliated columnar cells. In some male rats there was also respiratory metaplasia of the olfactory epithelium. In the female animals, mainly eosinophilic hyperplasia of the olfactory epithelium occurred, which was presumably due to the intracytoplasmic accumulation of secretory material. In 2 male rats of the 300 ml/m^3 group, lipomas of the kidney and likewise in 2 male animals, papillomas of the bladder were found (NTP 1990). Borderline neuropathy as described in other inhalation studies at a concentration of 300 ml/m^3 in rats did not occur (NTP 1990). In this study, no local NOAEC was obtained. The LOAEC (lowest observed adverse effect concentration) for effects in the nose was 100 ml methyl styrene/m^3.

The most striking effect in rats is the replacement of the olfactory epithelium by respiratory epithelium mainly along the dorsal nasal passage and the replacement of the Bowman's glands by ciliated columnar cells. These changes have been seen also after exposure to other irritants. Hyperplasia of the respiratory epithelium often occurs in connection with inflammation (NTP 1990).

In a 2-year inhalation study with exposure of B6C3F1 mice to methyl styrene concentrations of 0, 10 or 25 ml/m^3, high incidences of hyperplasia and chronic inflammation of the respiratory epithelium of the nose and chronic inflammation in the bronchioles or the lungs were found even at the low concentration (see Table 1 and 2). The severity of the findings in the nose was mild to moderate at the concentration of 10 ml/m^3 and moderate to marked at 25 ml/m^3. The effects occurred mainly in the respiratory epithelium in the middle and posterior regions of the dorsal meatus. The inflammation was characterized by focal infiltration of the mucosa by neutrophils and mononuclear cells. The hyperplasia of the respiratory epithelium consisted of thickening of the epithelium, downgrowth of ciliated columnar cells into the submucosal glands, the formation of intraepithelial gland-like structures and the extension of the respiratory epithelium into areas of the olfactory epithelium. In the bronchioles of the lungs minimal to moderate chronic inflammation with the accumulation of neutrophils, macrophages and lymphocytes within the walls of the bronchioles and of the interstitium of adjacent alveoli were seen. The epithelium of the affected alveoli was found to have an increased number of cuboidal cells; the lumina contained inflammatory cells, proteinaceous material and, in places more severely affected, also eosinophilic crystals and cholesterol clefts. The incidence of neoplasms in the bronchioles and lungs, the haematopoetic system and the liver was lower in the exposed animals than in the control group (see Section 5.2.7). A local NOAEC was not obtained in this study. The LOAEC for local effects in the respiratory tract is 10 ml/m^3.

In Wistar rats exposed to methyl styrene for 6 hours a day, on 5 days per week, for 15 weeks, a reduced motor nerve conduction velocity in the tail nerve and a reduced amplitude of the evoked motor action potential were found at concentrations of 100 ml/m^3 and above; the concentration of 50 ml/m^3 had no electrophysiological effects (Seppäläinen and Savolainen 1982). A similar study in Sprague Dawley rats with 21-week exposure yielded a corresponding NOAEC of 100 ml/m^3 with a LOAEC of 300 ml/m^3 for motor and sensory nerve conduction velocities (US EPA 2010).

Summary: In 2-year inhalation studies, methyl styrene caused inflammation and hyperplasia of the respiratory epithelium and inflammation of the lungs or of the bronchioles in mice even at the lowest concentration tested of 10 ml/m^3, as well as cysts and hyperplasia of the olfactory and respiratory epithelia of rats at concentrations of 100 ml/m^3 and above. The systemic NOAEC was 10 ml/m^3 for mice and 100 ml/m^3 for rats, because, at the next-higher concentrations of 25 and 300 ml/m^3, respectively, body weight gains were reduced.

Table 1 Effects of methyl styrene after repeated inhalation

Species, strain, number per group	Exposure	Findings	References
rat, F344/N, 5 ♂, 5 ♀	**15 days**, 0, 200, 400, 800, 1300 ml/m³ isomer mixture (65%–71% 3-methyl styrene and 32%–35% 4-methyl styrene), whole-body exposure, 6 hours/day, 5 days/week, purity 99%	histopathological examination only of animals of the 1300 ml/m³ group; **400 ml/m³ and above**: body weights ♂ 13%–19% ↓ and body weights ♀ 10%–13% ↓; **1300 ml/m³**: lethargy, excessive lacrimation, red discoloration around the nose and mouth, dysplasia of the bronchial epithelium, chronic bronchitis, lymphoid hyperplasia of the lungs, absolute and relative liver weights ↑, ♂: centrilobular necrosis and focal inflammatory cell infiltration of the liver in 4/5, ♀: minimal to slight centrilobular vacuolization in the liver in 5/5	NTP 1990
rat, F344/N, 10 ♂, 10 ♀	**13 weeks**, 0, 25, 60, 160, 400, 1000 ml/m³ isomer mixture (65%–71% 3-methyl styrene and 32%–35% 4-methyl styrene), whole-body exposure, 6 hours/day, 5 days/week, purity 99%	histopathological examination only of animals of the 1000 ml/m³ group and the control group; **160 ml/m³ and above**: body weights ♂ 6% ↓, ♀ 5% ↓, ♂: increase in the severity of nephropathy (which was present in all ♂ animals); **400 ml/m³ and above**: body weights ♂ 8% ↓, ♀ 6% ↓; **1000 ml/m³**: excessive lacrimation, palpebral closure, rough coat, ♂: body weights 19% ↓, relative liver weights 34% ↑, ♀: body weights 12% ↓, relative liver weights 27% ↑ (not absolute), in ♀ no substance-related histopatholigical findings	NTP 1990
rat, Sprague Dawley, 15 ♂, 15 ♀	**13 weeks**, 0, 100, 500, 1600/1300 ml/m³, 6 hours/day, 5 days/week, 97% **4-methyl styrene** (3% 3-methyl styrene)	incomplete study report, **100 ml/m³**: no local effects, no histopathological data; **500 ml/m³ and above**: excessive lacrimation, swollen eyes, discoloured fur in the anogenital region; **1600 ml/m³**: mortality: 2 ♀ in week 1 (were replaced) then: 3 ♀ and 1 ♂, tremor, loss of balance, debilitation, dull coat, increased activity with coordination problems, reduction to **1300 ml/m³**: dull coat, alkaline phosphatase ↑, liver weights ↑ without histopathological correlate, ♀: white blood cell count ↓	ECHA 2015

Table 1 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 20 ♂, 12 weeks old	**15 weeks**, 0, 50, 100, 300 ml/m³ isomer mixture, 6 hours/day, 5 days/week, purity not specified	**exposed in darkness**, determination of the motor conduction velocity (MCV) of the tail nerve of immobilized animals at the start of the study and after 4, 8, 12 and 15 weeks, amplitude of the evoked motor action potential, examination of the protein composition of myelin-deprived nerves from the spinal cord, **100 ml/m³ and above**: from week 12: MCV ↓, potential half as high as in the control animals and in the animals of the 50 ml/m³ group; **300 ml/m³**: animals inactive during exposure, body weights ↓	Seppäläinen and Savolainen 1982; US EPA 2010
rat, Sprague Dawley, 10 ♂, 6 weeks old	**21 weeks**, 0, 100, 300 ml/m³ isomer mixture, 6 hours/day, 5 days/week, purity not specified	**100 ml/m³: NOAEC;** **300 ml/m³**: body weights ↓ (not significant), from week 15: motor and sensory conduction velocity of the tail nerve ↓, histopathology normal	Gagnaire et al. 1986
rat, Wistar, 10–25 ♂, 10–25 ♀	**28 weeks**, 0, 580, 1130, 1350 ml/m³, 7–8 hours/day, 5 days/week, 139 days, purity not specified	**no precise details of study,** examinations: histopathology and weight of selected organs and tissue, haematological parameters, blood urea nitrogen, bone marrow; **1130 ml/m³ and above**: body weight gains ↓, liver weights ↑, fatty degeneration of the midzonal and central cells of the liver lobules; **1350 ml/m³**: "moderate mortality" (no other details)	Wolf et al. 1956
rat, F344/N, 50 ♂, 50 ♀	**2 years**, 0, 100, 300 ml/m³ isomer mixture (65%–71% 3-methyl styrene and 32%–35% 4-methyl styrene), whole-body exposure, 6 hours/day, 5 days/week, purity 99%	surviving animals at the end of the study (controls, 100 ml/m³, 300 ml/m³): ♂: 19/49, 17/50, 19/50; ♀: 31/50, 28/50, 26/50, no clinical signs of toxicity; **100 ml/m³ and above**: findings in the nasal turbinates, see Table 2, ♀: body weights 5% ↓; **300 ml/m³**: body weights ♂ 4%–8% ↓ and ♀ 11% ↓, lipoma in kidney (♂ 2/50), papilloma in bladder (♂ 2/50)	NTP 1990

Table 1 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, B6C3F1, 5 ♂, 5 ♀	**15 days**, 0, 10, 25, 50, 100, 200 ml/m³ isomer mixture (65%–71% 3-methyl styrene and 32%–35% 4-methyl styrene), whole-body exposure, 6 hours/day, 5 days/week, purity 99%	histopathological examination only in 200 ml/m³ group and 1 ♂ and 1 ♀ animal of the control group; **100 ml/m³**: ataxia; **200 ml/m³**: lethargy, palpebral closure, absolute and relative liver weights ↑, ♂: mortality 3/5 (of which 1 with hyperaemia and haemorrhage of pulmonary parenchyma, 2 with interstitial pneumonia, moderate to severe hepatocellular necrosis in 4/5), ♀: hyperplasia of the epithelium of the intrapulmonary bronchi in 5/5, centrilobular necrosis, vacuolization and inflammatory cell infiltrates in the liver in 5/5	NTP 1990
mouse, B6C3F1, 10 ♂, 10 ♀	**13 weeks**, 0, 10, 25, 60, 160 ml/m³ isomer mixture (65%–71% 3-methyl styrene and 32%–35% 4-methyl styrene), whole-body exposure, 6 hours/day, 5 days/week, purity 99%	histopathological examination of all animals of the 25, 60, 160 ml/m³ groups and the control group; **0 ml/m³**: inflammation in the lungs and metaplasia of the respiratory epithelium, see Table 3; **10 ml/m³ and above**: metaplasia of the respiratory epithelium, see Table 3; **25 ml/m³**: body weights ♂ 12% ↓, ♀ 13% ↓; **60 ml/m³**: lethargy, body weights ♂ 12% ↓, ♀ 14% ↓, inflammation of lungs, see Table 3; **160 ml/m³**: lethargy, palpebral closure, body weights ♂ 20% ↓, ♀ 16% ↓, inflammation of lungs, see Table 3	NTP 1990
mouse, B6C3F1, 50 ♂, 50 ♀	**2 years**, 0, 10, 25 ml/m³ isomer mixture (65%–71% 3-methyl styrene and 32%–35% 4-methyl styrene), whole-body exposure, 6 hours/day, 5 days/week, purity 99%	surviving animals at the end of the study (controls, 10 ml/m³, 25 ml/m³): ♂: 33/50, 30/50, 41/50; ♀: 36/50, 37/50, 34/50, **10 ml/m³ and above**: body weights 5%–14% ↓, hyperplasia and inflammation of the nasal mucosa (Table 2), inflammation of bronchioles (Table 2); **25 ml/m³**: body weight 10%–23% ↓	NTP 1990

Table 1 (continued)

Species, strain, number per group	Exposure	Findings	References
guinea pig, albino, 5–10 ♂, 5–10 ♀	**28 weeks**, 0, 580, 1130, 1350 ml/m^3, 7–8 hours/day, 5 days/week, 139 days, purity not specified	**no precise details given for study,** examinations: histopathology and weights of selected organs and tissue, haematological parameters, blood urea nitrogen, bone marrow; **1130 ml/m^3 and above**: body weight gains ↓, kidney weights ↑, fatty degeneration of the midzonal and central cells of the liver lobules; **1350 ml/m^3**: liver weights ↑	US EPA 2010
rabbit, albino, 1–2 ♂, 1–2 ♀	**28 weeks**, 0, 580, 1130, 1350 ml/m^3, 7–8 hours/day, 5 days/week, 139 days, purity not specified	**no precise details given for study,** examinations: histopathology and weights of selected organs and tissue, haematological parameters, blood urea nitrogen, bone marrow; **1130 ml/m^3 and above**: kidney weights slightly ↑; **1350 ml/m^3**: fatty degeneration of the midzonal and central cells of the liver lobules	US EPA 2010
monkey, rhesus, 1–2 ♂, 1–2 ♀	**28 weeks**, 0, 580, 1130, 1350 ml/m^3, 7–8 hours/day, 5 days/week, 139 days, purity not specified	**no precise details given for study,** examinations: histopathology and weight of selected organs and tissue, haematological parameters, blood urea nitrogen, bone marrow; no substance-related findings	US EPA 2010

Table 2 Findings in the respiratory tract of F344 rats and B6C3F1 mice obtained in 2-year inhalation studies with methyl styrene (NTP 1990)

Rats, 2 years	0 ml/m³	100 ml/m³	300 ml/m³	0 ml/m³	100 ml/m³	300 ml/m³
nose	**male animals**			**female animals**		
olfactory epithelium						
cysts	0/48	4/50	6/50*	0/50	5/49*	13/50**
focal erosion	0/48	8/50**	1/50	0/50	3/49	4/50
eosinophilic hyperplasia	1/48	0/50	0/50	2/50	9/49*	21/50**
metaplasia	0/48	6/50*	4/50	0/50	1/49	0/50
respiratory epithelium						
intraepithelial cysts	2/48	13/50**	9/50*	0/50	6/49*	10/50**
diffuse hyperplasia	12/48	24/50*	28/50**	8/50	19/49**	19/50**
mice, 2 years	0 ml/m³	10 ml/m³	25 ml/m³	0 ml/m³	10 ml/m³	25 ml/m³
nose	**male animals**			**female animals**		
respiratory epithelium						
chronic active inflammation	2/50	47/48**	48/49**	3/48	49/49**	47/48**
hyperplasia	5/50	48/48**	49/49**	5/48	49/49**	47/48**
lungs/bronchioles						
chronic active inflammation	0/50	15/49**	30/49**	0/48	14/49**	37/49**

*: $p < 0.05$ (one-sided) (NTP 1990); **: $p < 0.01$ (one-sided) (NTP 1990)

Table 3 Findings in the respiratory tract of B6C3F1 mice after 13-week inhalation exposure to methyl styrene (NTP 1990)

13 weeks	0 ml/m³	10 ml/m³	25 ml/m³	60 ml/m³	160 ml/m³
acute inflammation and metaplasia of the respiratory epithelium of the nasal turbinates					
♂	0/10	3/10 p = 0.105	8/ 9** p = 0.0001	7/ 8** p = 0.0002	7/10** p = 0.0015
♀	1/10	4/10 p = 0.152	9/10** p = 0.0005	10/10** p = 0.0000	9/ 9** p = 0.0001
inflammation of the lungs					
♂	0/10	0/10	0/ 9	4/ 9* p = 0.032	5/10* p = 0.0162
♀	1/10	0/10	0/10	2/10 p = 0.5	3/ 9 p = 0.25

*: $p < 0.05$ (one-sided) (NTP 1990); **: $p < 0.01$ (one-sided) (NTP 1990)

5.2.2 Oral administration

The three isomers 2-methyl styrene, 3-methyl styrene and 4-methyl styrene were not ototoxic in Sprague Dawley rats given doses of 8.47 mmol/kg body weight and day (1001 mg/kg body weight and day) for 14 days (Gagnaire and Langlais 2005).

According to the ECHA registration database, a 90-day study was carried out in 1979 with 4-methyl styrene (purity 99.7%) in F344 rats. Deviating from OECD Test Guideline 408, no specific functional observational tests were performed, fewer clinico-pathological parameters were determined and markedly fewer organs were examined histopathologically. Groups of 15 male and 15 female animals were given daily gavage doses of 4-methyl styrene of 0, 50, 100, 300, 700 or 1500 mg/kg body weight and day in olive oil. The weights of the following organs were determined: brain, heart, kidneys, liver, lungs, testes with epididymis, and ovaries. Gross-pathological examinations were carried out on the organs brain, pituitary gland, spinal cord, eyes, mandibular salivary gland, thyroid gland, thymus, trachea, oesophagus, lungs, heart, liver, spleen, kidneys, adrenal glands, stomach, pancreas, duodenum, jejunum, ileum, colon, caecum, mesenteric lymph nodes, bladder, testes with epididymis, prostate gland, ovaries, uterus, femur, bone marrow and all gross lesions. The lungs, liver, kidneys, testes, ovaries, prostate gland and uterus were examined histopathologically. Deaths occurred at doses of 300 mg/kg body weight and day and above: 1 of 30 animals at 300 mg/kg body weight and day, 3 of 30 animals at 700 mg/kg body weight and day and some animals ("notable mortality", no other details) at 1500 mg/kg body weight and day. The high dose was thus higher than the maximum tolerated dose. No other signs of clinical toxicity were observed. The body weights of the male animals of all dose groups were decreased in comparison with the weights of the control animals (no other details); this effect did not occur in the female animals. At 700 mg/kg body weight and day and above, the haemotocrit value, the erythrocyte count, and blood protein and albumin were increased. These pathological effects are, according to the registration database, more characteristic of dehydration than of a toxicological effect. The urinary parameters were normal. Occasionally, slight increases in blood urea nitrogen levels were found, which were, however, independent of the dose. The organ weights of the treated male animals differed in some cases significantly from those of the control animals, which, however, was considered to be more likely due to the reduced body weights. The liver weights were increased in animals given 300 mg/kg body weight and day and above; the kidney weights were increased at 700 mg/kg body weight and day and above (no other details). Histopathological changes occurred only in the lungs, but here even at the lowest dose of 50 mg/kg body weight and day. The irritation of the lungs was characterized by multifocal chronic pneumonitis and focal hyperplasia of bronchial and bronchiolar epithelium with histiocyte accumulation at the affected location, peribronchial fibrosis, focal bronchiectasis (irreversible sack-like dilation of the bronchioles), and hypersecretion with severe mucus formation in the alveoli. At 700 mg/kg body weight and day and above, in addition perivascular and peribronchial accumulation of eosinophils were observed. In the control animals, inflammatory sites of minimal to slight severity were found; in the treated animals these findings were more frequent and of moderate severity. A NOAEL (no observed adverse effect level) was not obtained, as even at the lowest dose tested of 50 mg/kg body weight

and day all animals were found to have higher incidences of multifocal chronic inflammatory sites in the lungs and focal hyperplasia of the bronchi and bronchioles, and the body weights of the male animals were reduced (ECHA 2015). The original study was not available.

In a 90-day study from 1979 carried out in a similar way with a similarly limited scope of examinations, as described above, groups of 15 male and 15 female Sprague Dawley rats were given 4-methyl styrene doses of 0, 0.1, 0.3 or 0.6 ml/kg body weight and day (0, about 91, 273, 547 mg/kg body weight and day) by gavage on 5 days per week, for 13 weeks. No substance-related mortality occurred. After administration of the substance, salivation and the motor activity of the animals were increased; in some cases, they trembled slightly. As signs of the irritant effect of the substance, the animals were found to have abrasions at the application site on the snout, and pneumonia was caused by aspiration of the substance. The body weights of the male animals were decreased in a dose-dependent manner (ECHA 2015). The original was not available.

Groups of 60 or 90 male and female Sprague Dawley rats were given methyl styrene (96.8% 4-methyl styrene and 3% 3-methyl styrene, purity > 99%) doses of 0, 10, 50, 250 or 500 mg/kg body weight and day in olive oil by gavage on 5 days per week, for 108 weeks. The study was terminated after 123 weeks, when, at least in one group, survival had dropped below 50%. An interim examination was carried out with 5 male and 5 female animals after 54 and 107 weeks. The survival of male animals was reduced at 250 mg/kg body weight and day and above (no other details). There was no substance-related increase or decrease in tumour incidences (no other details; IARC 1994).

Groups of 10 male and 10 female CFW mice were treated with 4-methyl styrene at dose levels of 250, 500, 1000, 2000 or 4000 mg/kg body weight and day for 4 weeks. There was no mention of a control group. The animals given 1000 mg/kg body weight and day and more died after the first dose. In the two low dose groups, a moist yellowish substance on the neck and thorax, decreased motor activity, laboured breathing and "ventral masses" were observed (ECHA 2015). The original study was not available. The study description is inadequate, but shows nevertheless that the NOAEL for 4-methyl styrene in mice is below 250 mg/kg body weight and day.

Summary: In the studies described here, the originals which are not available and in some cases have been inadequately reported, no NOAEL was obtained after 90-day administration of 4-methyl styrene by gavage to rats because body weight gains were reduced in all treated groups. After administration of a mixture of 96.8% 4-methyl styrene and 3% 3-methyl styrene for 108 weeks, tumour incidences were not increased. Increased mortality was observed in a 4-week study with mice at 1000 mg/kg body weight and day and above.

5.2.3 Dermal application

Undiluted 4-methyl styrene was applied non-occlusively to 25% of the total skin area of 3 male and 3 female New Zealand White rabbits daily for 21 days in severely irritant doses (0.5 or 2 ml/kg body weight). Ingestion was prevented. After the 6-hour application period, the shaved skin site was washed and dried. The study report, according to information provided in the ECHA registration database, is incomplete.

There were no signs of systemic effects. As the substance caused severe skin irritation even after a few applications, which according to current test guidelines would have prohibited further treatment, the pain occurring on substance application, the reduced body weights and the increased number of white blood cells are presumably due to the irritant effect (ECHA 2015). This study can therefore not be used for the assessment of the systemic effects of the substance.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

Methyl styrene ("MCTR-109-77"; no other details) in undiluted form or as a 10% solution in butyl carbitol acetate was applied to the ear or shaved dorsal skin of one rabbit (no other details) in each case for 24 hours. In another study, undiluted methyl styrene was applied 10 times within 13 days to one ear or the shaved dorsal skin of one rabbit (no other details) in each case. Moderate irritation at the application site occurred after all applications, and the skin had not completely healed 10 days after the last application. A similar study with application of 1% or 10% methyl styrene in butyl carbinol acetate resulted in very slight irritation; healing was rapid and complete. Methyl styrene was assessed as slightly to moderately irritating (US EPA 2010).

In a study from 1977, which according to information given in the ECHA registration database was only available as an abstract, 0.5 ml 4-methyl styrene applied non-occlusively to the intact and abraded skin of 3 male and 3 female New Zealand White rabbits for 24 hours caused slight irritation. After 24 hours, erythema with a score of 2 was found in 3 of 6 animals and with a score of 1 in the other 3 animals, so that a mean primary irritation index of 1.5 on a scale of a maximum 8 was obtained (ECHA 2015).

In another study from 1977, which according to information given in the ECHA registration database was likewise only available as an abstract, 0.5 ml 4-methyl styrene applied occlusively to the shaved or shaved and abraded skin of 3 male and 3 female New Zealand White rabbits for 24 hours caused slight irritation with a primary irritation index of 1.1 on a scale of a maximum 8 (ECHA 2015).

When methyl styrene (55%–70% 3-methyl styrene, 30%–45% 4-methyl styrene) was applied to the ear or the shaved skin of the body of white rabbits (no other details) for 2 to 4 weeks or 10 to 20 times, perceptible defined erythema, oedema, superficial necrosis and blistering occurred. Methyl styrene was assessed as moderately irritating in this study (US EPA 2010).

5.3.2 Eyes

Two drops (about 90 mg) of methyl styrene (55%–70% 3-methyl styrene, 30%–45% 4-methyl styrene) instilled into one conjunctival sac of white rabbits (no other details) caused slight irritation of the conjunctiva (US EPA 2010).

In a study from 1977, which, according to information provided in the ECHA registration database, was available as an abstract only, 0.1 ml 4-methyl styrene instilled into one eye of New Zealand White rabbits (n = 6) (no details as to whether the eye was rinsed) had a very slight irritant effect. Immediately after the instillation of the

test substance, the animals closed their eyes and vocalized; slight erythema and some discharge were observed, which were reversible after 4 days (ECHA 2015). According to GHS (Globally Harmonized System of Classification and Labeling of Chemicals), this finding does not require the substance to be classified as irritating to the eyes.

Summary: In the cited studies, which did not meet present-day test guideline standards, methyl styrene was moderately irritating to the skin and slightly irritating to the eye in rabbits.

5.4 Allergenic effects

5.4.1 Sensitization of the skin

A negative result was reported for a maximization test in 15 guinea pigs with a mixture of 3-methyl styrene and 4-methyl styrene. The intradermal and the topical induction were carried out with 2.5% and 5% of the mixture in acetone, respectively. None of the 15 animals reacted during the challenge treatment with 0.5% of the mixture. The same result was obtained in a maximization test with styrene (intradermal induction with 10%, topical induction with 20% and challenge treatment with 2% styrene in acetone) (Sjöborg et al. 1982).

Taking into account the number of animals that reacted and the severity of the reactions, a borderline to negative result was obtained in an incompletely documented modified maximization test with styrene. In this study the intradermal and the topical induction treatment were carried out with 5% styrene (vehicle not specified). Prior to the topical induction treatment, a 24-hour non-occlusive application of a 10% preparation of sodium dodecylsulfate in petrolatum was performed. During the challenge treatment carried out with 1% styrene, 3 of the 9 animals produced a slight reaction (grade 1 on a scale of 0–3). Cinnamyl alcohol, which was tested in the same way, did not cause a reaction in any of 10 animals, while 10 of 10 and 2 of 9 animals, respectively, reacted to cinnamaldehyde and α-amyl cinnamaldehyde. The authors give a sensitization incidence of 11% for α-amyl cinnamaldehyde and styrene (Senma et al. 1978), which is evidently derived, however, from the ratio of the observed and maximum possible reaction severity.

5.4.2 Sensitization of the airways

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In the 13-week and 2-year inhalation studies with methyl styrene isomers (see Section 5.2.1) no substance-related effects on the reproductive organs were observed either in rats up to a concentration of 300 ml/m^3 or in mice up to a concentration of 25 ml/m^3 (NTP 1990; US EPA 2010).

In a 2-generation study, which according to information given in the ECHA registration database was not available in the original, 4-methyl styrene doses of 0, 25, 200, 500 or 600 mg/kg body weight and day were administered to Sprague Dawley rats by gavage for 404 days. The NOAEL for toxicity in the parents and offspring was 200 mg/kg body weight and day. At 500 mg/kg body weight and day increased mortality and decreased body weight gains in the parental animals and a slight increase in the mortality of the offspring of the F1 generation were observed. There were no effects on mating, fertility, gestation, and birth, nor on the lactation index (ECHA 2015; US EPA 2003).

5.5.2 Developmental toxicity

There are no studies available for the developmental toxicity of the methyl styrene isomer mixture.

Gavage doses of 4-methyl styrene of 0, 60, 190 or 600 mg/kg body weight and day were administered to groups of 20 pregnant Sprague Dawley rats from days 6 to 15 of gestation. No substance-related findings were seen in the dams. In the foetuses of the 60 and 190 mg/kg groups, incomplete ossification of the vertebrae was found; in the low dose group, the incidence of rudimentary ribs was increased, and in the middle dose group also the number of supernumerary ribs. In the foetuses of the 600 mg/kg group, no substance-related findings were observed (no other details; US EPA 2010). The NOAELs for developmental toxicity and maternal toxicity are higher than 600 mg 4-methyl styrene/kg body weight and day.

Groups of 25 pregnant COBS-CD rats were given gavage doses of 4-methyl styrene of 0, 50, 300 or 600 mg/kg body weight and day from days 6 to 19 of gestation. The body weight gains of the dams and the mean body weights of the foetuses were decreased in a dose-dependent manner. In the registration dossier of the ECHA, the statistically significant reduction in body weights of the foetuses was attributed to the unusually high values of the control animals, which were higher than those of the historical controls. The US EPA, however, regards this finding as substance-related, as the comparison must be made also with the concurrent control animals. In a foetus of the high dose group meningocele was found. The number of corpora lutea was reduced in the animals of the high dose group. As ovulation and implantation took place before the administration of the substance, this effect was not regarded as substance-related. Due to the reduced body weight gains of the dams and the reduced body weights of the foetuses, compared with the weights of the control animals, the lowest dose tested of 50 mg/kg body weight and day was regarded as the LOAEL (lowest observed adverse effect level) (ECHA 2015; US EPA 2010). A NOAEL cannot be derived. The result of this study therefore contradicts the result of the rat study described before. The studies differed with regard to the different rat strains used, the different treatment duration and possibly different testing laboratories. Conspicuous is the occurrence of meningocele at 600 mg/kg body weight and day, which is found relatively rarely in CD rats. According to Hood (2011) spontaneous meningocele occurred in 59 744 foetuses in 165 studies in the period from 1998 to 2010, and, according to MARTA (1993) in 88 270 foetuses in 352 studies in the period up to 1993 (not specified from which year). Studies on chemical-induced meningocele showed

that this occurred together with other malformations and the incidences were, as a rule, found to be relatively high (Khera 1973; Nolen 1969; Saillenfait et al. 1991). Differing sensitivity of the rat strains with regard to the formation of meningocele was also observed (Nolen 1969). The occurrence of only one case of meningocele in 25 foetuses is therefore regarded as an incidental finding.

Groups of 16 pregnant Dutch rabbits were given gavage doses of 4-methyl styrene of 0, 50, 100 or 150 mg/kg body weight and day from days 6 to 27 of gestation. The abstract of the study reports that no biological effects and no teratogenic response were observed (no other details; US EPA 2010). The NOAELs for developmental and maternal toxicity in this study were therefore the highest dose tested of 150 mg 4-methyl styrene/kg body weight and day.

The three studies described above were not obtainable in the original and can therefore not be used for this evaluation.

Female rats given intraperitoneal doses of 3750 mg/kg body weight and day of the methyl styrene isomer mixture from days 1 to 15 of gestation were found to have postimplantation losses and stunted foetuses. No foetotoxicity was observed at 250 mg/kg body weight and day (no other details; US EPA 2003). Since, with intraperitoneal administration, a direct effect on the foetus cannot be excluded, this study is not used for the evaluation.

The inhalation of 6 ppm methyl styrene isomers for a period of four months or 6200 ppm for one month had teratogenic effects in guinea pigs. The authors note that the studies were not correctly referenced in Patty's Industrial Hygiene and Toxicology, 4th Edition, 1991, and were thus not obtainable (no other details; US EPA 2003).

5.6 Genotoxicity

5.6.1 In vitro

In the presence and absence of a rat or hamster liver metabolic activation system, methyl styrene concentrations of up to 1000 µg/plate did not induce mutations in the Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537. Methyl styrene did not induce sister chromatid exchange (SCE) or chromosomal aberration (CA) in CHO cells in the presence and absence of a rat liver metabolic activation system (see Table 4; NTP 1990).

The studies carried out by Norppa (Norppa 1981 a; Norppa et al. 1981; Norppa and Vainio 1983) in contrast demonstrate the induction of SCE and chromosomal aberrations in human lymphocytes. In these studies, however, positive controls were not used and data for cytotoxicity were not given. The concentrations used were higher than those in the NTP program.

A positive result was obtained in 2 of 3 tests in the $TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells at the highest non lethal, cytotoxic concentration (60 µg/ml; relative growth < 10%) in the absence of a metabolic activation system. This test was not carried out in the presence of a metabolic activation system, and no differentiation was made between large and small colonies (see Table 4; NTP 1990).

Table 4 Genotoxicity of methyl styrene in vitro

End point	Test system	Concentration	Effective concentration[a)]	Cytotoxicity[a)]	Result		References
					–m.a.	+m.a.	
Gene mutation	S. typhimurium TA98, TA100, TA1535, TA1537	0, 1, 3.3, 10, 33, 100, 333, 1000 µg/plate	–	at 333 and above or at 1000 µg/plate (depending on the strain tested; US EPA 2010)	–	–	NTP 1990; US EPA 2010
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	0.01–10 µmol/plate	–	no data	–	–	Norppa et al. 1981
SCE	CHO cells	– m.a.: test 1: 1.6, 5, 16, 50 µg/ml s.o.; test 2: 1, 5, 10, 25, 50, 75, 100, 150 µg/ml; test 3: 25, 50, 75, 100 µg/ml s.o. + m.a.: test 1: 5, 16, 50 µg/ml; test 2: 10, 25, 50, 75 µg/ml;	–	no data	–	–	NTP 1990
	human lymphocytes	0, 0.33, 1.0, 2.7, 4.0 mM (0, 39, 118, 319, 472 µg/ml)	0.33 mM	no data	+	n.i.	Norppa 1981 a; Norppa and Vainio 1983; Norppa et al. 1981
CA	CHO cells	– m.a.: 1.6, 5, 16, 50 µg/ml, + m.a.: 10, 25, 50, 75 µg/ml	–	–	–	–	NTP 1990
	human lymphocytes	0, 0.33, 1.0, 2.7, 4.0 mM (0, 39, 118, 319, 472 µg/ml)	2.7 mM	no data	+	n.i.	Norppa 1981 a; Norppa et al. 1981

Table 4 (continued)

End point	Test system	Concentration	Effective concentration[a)]	Cytotoxicity[a)]	Result		References
					–m.a.	+m.a.	
Gene mutation $TK^{+/-}$	mouse lymphoma cells L5178Y, no differentiation between large and small colonies	test 1: 12.5, 25, 50, 100 µg/ml; test 2: 1, 10, 20, 40, 60, 80 µg/ml s.o.; test 3: 40, 45, 50, 55, 60, 65 µg/ml	60 µg/ml-	test 1) 100 µg/ml: lethal test 2) and 3) 60 µg/ml: RTG < 10%; 80 and 65 µg/ml, respectively: lethal	only + at cytotoxic levels	n.i.	McGregor et al. 1988; NTP 1990

n.i.: not investigated; RTG: relative total growth; –m.a.: without metabolic activation +m.a.: with metabolic activation

5.6.2 In vivo

Methyl styrene was not mutagenic in the Drosophila sex-linked recessive lethal assay (NTP 1990) with male animals after 5-day exposure to 0, 50, 100, 300 ml/m^3 or after 24 or 72-hour feeding with 0, 200, 500 mg/l liquid (no other details; Norppa et al. 1981).

Groups of 4 to 5 male C57BL/6 mice were given single intraperitoneal injections of methyl styrene of 0, 100, 200, 300 or 500 mg/kg body weight. After 30 hours, bone marrow was collected and examined for micronuclei. At 500 mg/kg body weight 3 of 5 animals died. In the lower dose groups the ratio of polychromatic to normochromatic erythrocytes was significantly reduced compared with that in the control animals. At 200 mg/kg body weight and higher a significant increase in the number of micronucleated polychromatic erythrocytes was observed. Micronuclei were not observed in normochromatic erythrocytes (Norppa 1981 b; US EPA 2010).

Chromosomal aberrations in the bone marrow were not observed after single daily 4-methyl styrene doses of up to 1.34 g/kg body weight and day administered for 5 days by gavage to 5 male Sprague Dawley rats (ECHA 2015).

A dominant lethal test with 4-methyl styrene, in which male Sprague Dawley rats were exposed to up to 1.5 ml/kg body weight and day once a day for 5 days and then mated for 7 weeks with untreated females, yielded negative results. The body weight gains of the animals in the two upper dose groups were reduced (ECHA 2015).

Summary: In in vitro studies, methyl styrene was not mutagenic in bacteria. In CHO cells, there was no induction of sister chromatid exchange or chromosomal aberrations; in human lymphocytes, on the other hand, SCE and CA were induced at much higher concentrations. In the studies of chromosomal aberrations, no data are given for cytotoxicity. In mammalian cells, methyl styrene was not mutagenic. In in vivo tests carried out in the same laboratory in which in vitro studies with a positive result for clastogenicity were conducted, a micronucleus test yielded a positive result, however at cytotoxic doses. Tests for CA and dominant lethal mutations produced no evidence of a genotoxic potential.

5.7 Carcinogenicity

Inhalation

In 2-year inhalation studies, groups of 50 male and 50 female Fischer-344 rats were exposed to methyl styrene concentrations of 0, 100 or 300 ml/m^3 and groups of 50 male and 50 female B6C3F1 mice to 0, 10 or 25 ml/m^3. There were local findings even at the low concentration (see Section 5.2.1), however no substance-related, significant increase in tumour incidences (NTP 1990).

In 2 male rats of the 300 ml/m^3 group, lipomas were observed in the kidneys, and benign mesenchymal neoplasia was found, which occurred in untreated historical control F344 rats with an incidence of 2 in 1590 animals (0.1%) and of 0 in 346 chamber control animals (exposed in a chamber to air). The lipomas of the kidney were regarded as an incidental finding. In addition, papillomas of the bladder were observed in two male rats of the 300 ml/m^3 group, the incidence of which in untreated historical controls was 1 of 1552 (0.1%) and 3 of 339 (0.9%) in chamber control

animals. Due to the lack of preneoplastic lesions (hyperplasia), these findings in the bladder were also considered to be unrelated to the treatment (NTP 1990).

The incidence of neoplasms in the lungs of exposed mice was lower than that in the control group. The incidence of adenomas and carcinomas in the lungs of male mice was 12/50 in the chamber control animals, 5/49 in the animals of the 10 ml/m^3 group and 2/49 in the animals of the 25 ml/m^3 group; no lung tumours occurred in the female animals. Likewise, the incidence of lymphomas of the haematopoetic system was highest in the male control animals with 7/50, and decreased with increasing concentration from 3/50 at 10 ml/m^3 to 0/50 at 25 ml/m^3. A similar trend was found in the female mice with incidences of 16/48, 9/49 and 8/50, respectively. In the liver, the incidence of hepatocellular adenomas and carcinomas was likewise significantly decreased in the female animals at 25 ml/m^3 compared with that in the control animals: 9/48 (controls), 5/16 (10 ml/m^3), 2/49 (25 ml/m^3) (NTP 1990).

Oral

Methyl styrene (purity > 99%, 96.8% 4-methyl styrene and 3% 3-methyl styrene) was administered by gavage in doses of 0, 10, 50, 250 or 500 mg/kg body weight and day for 123 weeks to groups of 60 or 90 male and female Sprague Dawley rats, and in doses of 0, 10, 50 or 250 mg/kg body weight and day for 83 weeks to groups of 60 male and 60 female Swiss mice. The incidences of benign or malignant tumours per animal and the percentage of animals with benign or malignant tumours were not significantly increased as a result of the treatment. In the male rats survival was reduced at dose levels of 250 mg/kg body weight and day and above, and in the male mice in all treated groups (no other details; IARC 1994).

6 Manifesto (MAK value/classification)

The most sensitive effect in rats and mice is irritation of the nose after inhalation exposure.

MAK value. In 2-year inhalation studies, in mice methyl styrene caused inflammation and hyperplasia of the respiratory epithelium and inflammation in the lungs or the bronchioles even at the lowest concentration tested of 10 ml/m^3, and in rats, cysts and hyperplasia of the olfactory and respiratory epithelium at 100 ml/m^3 and above. The systemic NOAEC was 10 ml/m^3 for mice and 100 ml/m^3 for rats, as body weight gains were reduced at the next-higher concentration of 25 and 300 ml/m^3, respectively.

The metabolism of methyl styrene is similar to that of styrene. As described in the case of styrene, oxidation of styrene to the epoxide takes place in the nose of rats and mice at about the same speed, whereas detoxification of the epoxide by hydrolase and glutathione is faster in rats by a factor of about 10 (see Section 2; EU 2008). An in vitro comparison with human nasal tissue showed that in humans almost no oxidation takes place, whereas the activities of epoxide hydrolase and GSH transferase are about the same as those in the rat. Humans are therefore less sensitive for effects in the nose than rats and mice (Green et al. 2001). These species differences are to be assumed for methyl styrene as well. On the basis of the LOAEC of 100 ml methyl

styrene/m³ for local effects in the rat, a NAEC (no adverse effect concentration) of 33 ml/m³ is calculated. This study was a long-term study; an increase in effects over time does not therefore need to be taken into account. The human nose is presumably markedly less sensitive than that of the rat. Therefore, the NAEC is not divided by 2 in this case. Using the preferred value approach, a MAK value of 20 ml methyl styrene (all isomers)/m³ is thus obtained from the NAEC of 33 ml/m³. In a study in test persons from 1956, methyl styrene and styrene were found to have a strong irritant effect at a concentration of 400 ml/m³, but did not lead to excessive annoyance at 200 ml/m³; for both substances the odour threshold is 50 ml/m³. From this, it can be concluded that the sensory irritation caused by styrene and methyl styrene in humans is similar. The fact that the MAK value for styrene is likewise 20 ml/m³ lends support to the establishment of a MAK value for methyl styrene at this level.

Peak limitation. Because the MAK value has been derived on the basis of irritation in the respiratory tract, methyl styrene remains assigned to Peak Limitation Category I. No sensory irritation occurred in the study with test persons at a concentration of 50 ml/m³ either with methyl styrene or with styrene (Wolf et al. 1956). Since irritation of the nose and eyes were observed in rats after medium to long-term exposure to 1300 ml styrene/m³ in each case, but not with methyl styrene (Wolf et al. 1956), the sensory irritation caused by methyl styrene is obviously less severe than that caused by styrene. For styrene there are other studies with test persons available which suggest a higher NOAEC than 50 ml/m³ (EU 2008). Therefore the previous excursion factor of 2 is confirmed for methyl styrene (all isomers).

Prenatal toxicity. Developmental toxicity studies are available only for 4-methyl styrene.

In a developmental toxicity study in Sprague Dawley rats, there was no developmental toxicity or maternal toxicity up to the highest 4-methyl styrene dose tested of 600 mg/kg body weight and day except for delays in development, which, however, were not dose-dependent (US EPA 2010).

In contrast, in another developmental toxicity study in COBS-CD rats, reduced body weight gains were observed in the dams and reduced body weights in the foetuses even at the lowest 4-methyl styrene dose tested of 50 mg/kg body weight and day (US EPA 2010). The occurrence of meningocele in 1 of 25 foetuses is considered to be an incidental finding.

In a developmental toxicity study with Dutch rabbits, no developmental toxicity or maternal toxicity was observed up to the highest 4-methyl styrene dose tested of 150 mg/kg body weight (US EPA 2010).

As the three studies described are not available in the original and a detailed assessment of the effects cannot be made, methyl styrene (all isomers) has been assigned to Pregnancy Risk Group D.

Carcinogenicity. In 2-year inhalation studies with F344 rats and B6C3F1 mice, no treatment-related, significant increases in tumour incidences occurred. As before, the substance has not been classified in one of the categories for carcinogens.

Germ cell mutagenicity. In in vitro studies, methyl styrene was not found to be mutagenic in bacteria. In CHO cells, there was no induction of sister chromatid exchange or chromosomal aberrations; in human lymphocytes, on the other hand,

SCE and CA were induced at much higher concentrations. In the studies of CA, no data are given for cytotoxicity. In mammalian cells methyl styrene was not mutagenic. In in vivo tests carried out at the same laboratory in which the in vitro studies with a positive result for clastogenicity were conducted, a micronucleus test yielded a positive result, however at cytotoxic doses. Tests for SCE, chromosomal abberations and dominant lethal mutations produced no evidence of a genotoxic potential. Methyl styrene (all isomers) has not been classified in one of the categories for germ cell mutagens.

Absorption through the skin. From an in vitro study, it can be estimated that one-hour exposure of the hands and forearms (2000 cm^2) to undiluted methyl styrene results in the total absorption of about 208 mg (± 126 mg). From a 2-year study in F344 rats, a systemic NOAEC of 100 ml/m^3 (490.5 mg/m^3) can be derived. Taking into consideration the extrapolation of findings from animal experiments to humans (1:2) and the increased respiratory volume at the workplace (1:2), the total amount absorbed after the inhalation of 10 m^3 is 1225 mg. The amount absorbed dermally is thus less than 25% of that absorbed by inhalation, so that methyl styrene has not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Very few reports are available with positive patch test results for methyl styrene. Experiments in guinea pigs with methyl styrene and the structurally closely-related styrene yielded negative or equivocal results for skin sensitization. Since the supplement of 2002 (supplement "Methylstyrol (Vinyltoluol) (alle Isomere)" 2002, available in German only) no additional data have been published which would make a re-assessment of the sensitizing effects of the substance necessary. Therefore, the substance is still not designated with either "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

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