The MAK Collection for Occupational Health and Safety

Diethylbenzene (all isomers)

MAK Value Documentation – Translation of the German version from 2018

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Keywords: diethylbenzene; MAK value; maximum workplace concentration; toxicity; peak limitation; developmental toxicity; developmental neurotoxicity; skin absorption

Citation Note: Hartwig A, MAK Commission. Diethylbenzene (all isomers). MAK Value Documentation – Translation of the German version from 2018. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Jul;4(3):1100-1129]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/mb13501isme6519_w

Republished (online): 08 Aug 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.mb13501isme6519

Manuscript completed: 22 Mar 2017

Published (online): 25 Jul 2019

The commission established rules and measures to avoid conflicts of interest.

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Diethylbenzene (all isomers)[1)]

MAK value documentation

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DOI: 10.1002/3527600418.mb13501isme6519

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated diethylbenzene as mixture of isomers [25340-17-4], as well as the individual isomers 1,2-diethylbenzene [135-01-3], 1,3-diethylbenzene [141-93-5] and 1,4-diethylbenzene [105-05-5] considering all toxicological end points. Available publications and unpublished studies are described in detail. 1,2-Diethylbenzene is the most toxic of the isomers, the critical effect being peripheral neurotoxicity which is due to a gamma-diketone formed metabolically. In subchronic inhalation and oral studies in rats with diethylbenzene mixtures containing 1,2-diethylbenzene, subclinical neurotoxicity is observed at all concentrations and doses used. From these studies a maximum concentration at the workplace (MAK value) of 1 ml/m³ is set for 1,2-diethylbenzene. For a diethylbenzene mixture containing 10% 1,2-diethylbenzene, the critical effect in rats is leuco- and lymphopenia. A MAK value of 5 ml/m³ is set for diethylbenzene mixtures and the MAK value for 1,2-diethylbenzene has to be observed additionally. In rats, critical end points for 1,3-diethylbenzene are increased liver and thyroid weight and for 1,4-diethylbenzene altered clinical chemical parameters and increased kidney weight. A MAK value of 5 ml/m³ for both isomers is derived.

Since systemic effects are critical, Peak Limitation Category II is designated for all isomers. As peripheral neurotoxicity is a cumulative effect, an excursion factor of 8 is set for 1,2-diethylbenzene. The default excursion factor of 2 for systemically acting substances is set for diethylbenzene mixtures as well as 1,3- diethylbenzene and 1,4-diethylbenzene as their half-lives are not known. Thus, the allowable peak exposures are lower than those of other alkyl benzenes and also prevent from irritation.

By analogy with acrylamide it is deduced that rats exposed in utero to 1,2-diethylbenzene are not more susceptible to peripheral neurotoxicity than adult animals. The oral NOAEL for developmental toxicity of 1,2-diethylbenzene in rats is scaled to a concentration of 17 ml/m³ at the workplace. Therefore, damage to the embryo or foetus is unlikely when the MAK value is observed and 1,2-diethylbenzene is assigned to Pregnancy Risk Group C. The oral NOAEL for developmental toxicity of a diethylbenzene mixture in rats is scaled to a concentration of 94 ml/m³ at the workplace. Developmental toxicity studies with 1,3-diethylbenzene and 1,4-diethylbenzene are lacking, however, these isomers are contained in the diethylbenzene mixture to about 60% and 30%, respectively. Therefore, damage to the embryo or foetus is unlikely when the MAK value is observed and diethylbenzene mixtures as well as 1,3-diethylbenzene and 1,4-diethylbenzene are assigned to Pregnancy Risk Group C.

Diethylbenzenes are not genotoxic in vitro and in vivo. In a carcinogenicity study dermal application of a diethylbenzene mixture induced a single squamous carcinoma in mice, which is judged not sufficient for classification as a carcinogen. According to skin absorption models, percutaneous absorption can contribute significantly to systemic toxicity and all diethylbenzene isomers are designated with an "H" notation. Limited data show no sensitization.

Keywords

diethyl benzene; DEB; o-diethylbenzene; m-diethylbenzene; p-diethylbenzene; 1,2-diethylbenzene; 1,3-diethylbenzene; 1,4-diethylbenzene; 1,2-DEB; 1,3-DEB; 1,4-DEB; DEB isomer mixture; 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; peripheral neurotoxicity; axonopathy

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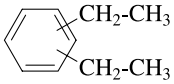
1) In addition to the MAK value for the mixture, the MAK value for 1,2-diethylbenzene has to be observed.

Diethylbenzene (all isomers)

MAK value (2017)	**diethylbenzene isomer mixture[1], 1,3-diethylbenzene, 1,4-diethylbenzene: 5 ml/m³ (ppm) ≙ 28 mg/m³**
	1,2-diethylbenzene: 1 ml/m³ (ppm) ≙ 5.6 mg/m³
Peak limitation (2017)	**diethylbenzene isomer mixture, 1,3-diethylbenzene, 1,4-diethylbenzene: Category II, excursion factor 2**
	1,2-diethylbenzene: Category II, excursion factor 8
Absorption through the skin (2017)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2017)	**diethylbenzene isomer mixture and all isomers: Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	DEB *o*-diethylbenzene *m*-diethylbenzene *p*-diethylbenzene
Chemical name	1,2-diethylbenzene 1,3-diethylbenzene 1,4-diethylbenzene
CAS number	1,2-DEB: 135-01-3 1,3-DEB: 141-93-5 1,4-DEB: 105-05-5 DEB isomer mixture: 25340-17-4

1) In addition to the MAK value for the mixture, the MAK value for 1,2-diethylbenzene has to be observed.

Structural formula	
Molecular formula	$C_{10}H_{14}$
Molar mass	134.22 g/mol
Melting point	1,2-DEB: –31.2 °C (SRC 2016) 1,3-DEB: –83.9 °C (SRC 2016) 1,4-DEB: –42.8 °C (SRC 2016)
Boiling point at 1013 hPa	1,2-DEB: 184 °C (SRC 2016) 1,3-DEB: 181.1 °C (SRC 2016) 1,4-DEB: 183.7 °C (ECHA 2016 a)
Density at 20 °C	1,2-DEB: 0.880 g/cm³ (Mayfield 1996) 1,3-DEB: 0.860 g/cm³ (Mayfield 1996) 1,4-DEB: 0.866 g/cm³ (ECHA 2016 a) DEB isomer mixture: 0.865 g/cm³ (calculated; ECHA 2016 b)
Vapour pressure at 25 °C	1,2-DEB: 1.4 hPa (SRC 2016) 1,3-DEB: 1.5 hPa (SRC 2016) 1,4-DEB: 1.4 hPa (SRC 2016)
log K_{OW}[2)]	1,2-DEB: 3.72 (SRC 2016) 1,3-DEB: 4.57 (SRC 2016) 1,4-DEB: 4.58 (SRC 2016); 4.06 at 25 °C (ECHA 2016 a)
Solubility in water at 20 °C	1,2-DEB: 71.1 mg/l (SRC 2016) 1,3-DEB: 24 mg/l (SRC 2016) 1,4-DEB: 24.8 mg/l (SRC 2016)
1 ml/m³ (ppm) ≙ 5.569 mg/m³	**1 mg/m³ ≙ 0.18 ml/m³ (ppm)**

Diethylbenzene is a by-product in the synthesis of ethylbenzene from ethylene and benzene. It may also be produced from ethylbenzene by disproportionation and occurs mainly as a mixture of the 3 position isomers. The ratio of the 1,2-isomer, 1,3-isomer and 1,4-isomer is 5:65:30 (NLM 2016). 1,4-Diethylbenzene can be produced from ethylbenzene by gas-phase alkylation with ethylene (NLM 2016; OECD 1994).

Diethylbenzene is almost exclusively converted to divinylbenzene, an intermediate in the production of synthetic rubber and synthetic resins. Less than 5% is used as heat transfer fluid (Gagnaire and Boucard 2014; Payan et al. 1999). 1,2-Diethylbenzene can be dehydrated and cyclized to form naphthalene. 1,3-Diethylbenzene can be converted to ethylvinylbenzene (NLM 2016). 1,4-Diethylbenzene is used as a solvent in closed systems (NLM 2016; OECD 1994) to desorb *p*-xylene in the Parex process (NLM 2016).

2) octanol/water partition coefficient

The 3 isomers can be found in JP-8 military jet fuel with average concentrations of 500 to 1500 mg/l (Mayfield 1996). JP-5 fuel contains 0.61% w/w 1,3-diethylbenzene and 0.77% w/w 1,4-diethylbenzene, while 1,2-diethylbenzene concentrations of up to 0.41% w/w were found in Jet-A fuel (ATSDR 2016). High-octane petrol contains 0.1% 1,2-diethylbenzene (NLM 2016).

This documentation is partly based on the publicly available REACH registration data for 1,4-diethylbenzene (ECHA 2016 a) and diethylbenzene isomer mixtures (ECHA 2016 b). As of 2017, 1,2-diethylbenzene and 1,3-diethylbenzene were pre-registered. The toxicological studies from the 1950s used mixtures that contained 25% 1,2-diethylbenzene. This high percentage was presumably due to the production process and no longer corresponds to the specifications for the diethylbenzene isomer mixtures that are used today (ECHA 2016 b). Studies from the 1990s used mixtures with 6% to 10% 1,2-diethylbenzene (Gagnaire et al. 1990, 1992 a; Monsanto 1992, 1993).

1 Toxic Effects and Mode of Action

Unlike the two other isomers, 1,2-diethylbenzene not only is CNS-depressive, but also toxic to the peripheral nerves, as is also known from *n*-hexane. The latter effect is caused by 1,2-diacetylbenzene, which is a protein-reactive gamma-diketone that forms from 1,2-diethylbenzene during metabolism. However, the two glucuronides of the stereo-isomeric 1-(2′-ethylphenyl)ethanol are the main metabolites. After subchronic exposure of rats to a diethylbenzene isomer mixture by inhalation, leukopenia and lymphopenia were observed at concentrations of 110 ml/m^3 and above. At 496 ml/m^3 and above, decreases in nerve conduction velocity and action potential amplitudes were the most sensitive neurotoxic effects caused by 1,2-diethylbenzene in the mixture (the LOAEC (lowest observed adverse effect concentration) of 496 ml/m^3 corresponds to a 1,2-diethylbenzene concentration of about 30 ml/m^3). Histopathological lesions of motor axons, adverse effects on body weights and clinical signs of nerve damage ranging from unsteady gait to paralysis were observed in rats at higher concentrations.

When rats were given gavage doses of 1,3-diethylbenzene of 1000 mg/kg body weight and day for 4 weeks, the liver weights were increased and centrilobular hypertrophy was observed presumably as a result of the induction of metabolizing enzymes of the liver. This affected the thyroid hormones and increased the thyroid weights. In male rats, alpha2u-protein deposits were observed in the renal epithelial cells.

In a screening study carried out according to OECD Test Guideline 422, 1,4-diethylbenzene induced changes in blood urea nitrogen levels, alanine aminotransferase and gamma-glutamyl transpeptidase activity and led to increased kidney weights in male rats at 150 mg/kg body weight and day and above.

The diethylbenzene isomers cause irritation of the skin, but not of the eyes.

There is no evidence that the diethylbenzene isomers have sensitizing effects on the skin or respiratory tract.

After the exposure of rats to 1,2-diethylbenzene, maternal and foetal weights were decreased as a result of reduced feed consumption at 15 mg/kg body weight and above, but no specific effects on development were observed up to 35 mg/kg body

weight and day. In rats, a diethylbenzene isomer mixture caused maternal toxicity at 100 mg/kg body weight and day and above; this led to reduced foetal body weights, but specific effects on development were not observed. 1,4-Diethylbenzene did not impair the fertility of rats up to 750 mg/kg body weight and day.

Genotoxicity was not observed with 1,4-diethylbenzene or diethylbenzene isomer mixtures in vitro in Salmonella mutagenicity tests, in HPRT tests or in chromosomal aberration tests. A diethylbenzene isomer mixture did not cause clastogenic effects in mice in the micronucleus test with intraperitoneal injection. A squamous cell carcinoma was observed at the application site after lifetime epicutaneous application of a diethylbenzene isomer mixture in mice.

2 Mechanism of Action

The neurotoxicity of 1,2-diethylbenzene probably results from the reaction of the neurotoxic gamma-diketone metabolite1,2-diacetylbenzene (Gagnaire et al. 1991) with epsilon-amino or sulfhydryl groups of cytoskeletal neuroproteins in axons. This leads to the segregation of cytoskeletal elements with a clustering of microtubules and organelles and the accumulation of maloriented neurofilaments in the proximal part of the axon by cross-linking. Axonal swelling occurs, resulting in reduced nerve conduction velocity and reduced amplitudes of the sensory action potential. Later, this leads to demyelination of nerve fibres. The mechanism corresponds to that of 2,5-hexanedione, which is the gamma-diketone of *n*-hexane. The cause of the electrophysiological findings is still not known. Other studies found that 1,2-diacetylbenzene increases or reduces the expression of 22 proteins in the lumbosacral spinal cord including reduced expression of the protein disulfide isomerase, which is involved in protein folding, and gelsolin, which regulates the formation and degradation of actin filaments (Tshala-Katumbay et al. 2005, 2008).

1,2-Diacetylbenzene is 1000 times as protein-reactive as 2,5-hexanedione. It reacts particularly with lysine and glycine to form purplish-blue chromophores. Lysine-rich subunits of neurofilament proteins are thus more susceptible than lysine-poor ones (Kim et al. 2002).

The motor proteins kinesin and dynein, the cytoskeletal protein NF-M and the microtubule-associated tau protein from the sciatic nerves and spinal cord of rats formed adducts with 1,2-diacetylbenzene in vitro to varying degrees. After the administration of 1,2-diacetylbenzene doses of 20 mg/kg body weight to male Sprague Dawley rats, NF-M was reduced by 75% in the sciatic nerve and by 13% in the spinal cord, kinesin was reduced by 75% in the sciatic nerve and by 10% in the spinal cord, dynein was reduced by 57% in the sciatic nerve and by 16% in the spinal cord, and tau protein was reduced by 21% in the sciatic nerve and by 8% in the spinal cord (Sabri et al. 2007).

Unlike 1,3-diacetylbenzene,1,2-diacetylbenzene induced oxidative stress (detected with the fluorescent dye 2′,7′-dichlorofluoresceine diacetate), cytotoxicity and apoptosis in the human neuroblastoma SHSY5Y cell line. Administration of glutathione or *N*-acetylcysteine blocked the oxidative stress and cytotoxicity (Kim et al. 2008).

The morphological pattern of axonopathy caused by gamma-diketones is similar to the early stage of amyotrophic lateral sclerosis (Llorens 2013).

1,2-Diethylbenzene is about 5 times as neurotoxic as 2,5-hexanedione (Gagnaire et al. 1990). However, presumably only a very small fraction of 1,2-diethylbenzene is metabolized to form the neurotoxic metabolite 1,2-diacetylbenzene.

Rats (Kim et al. 2001) were more sensitive to the neurotoxicity of 1,2-diacetylbenzene than mice (Tshala-Katumbay et al. 2005). Guinea pigs were not sensitive to 1,2-diethylbenzene, nor did the substance induce blue discoloration of tissues in this species. However, quantitative data were not reported (Gagnaire et al. 1990). The reasons for these differences are not known. There are no data for effects in humans. Extensive studies with *n*-hexane have shown that humans form 2,5-hexanedione and are sensitive to it (documentation "n-Hexane" 1992). Therefore, 1,2-diethylbenzene or 1,2-diacetylbenzene are expected to have neurotoxic effects in humans.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Data for absorption by inhalation are not available. In the REACH dossier, 30% absorption by inhalation was assumed for the calculation of the DNEL (derived no effect level) for 1,4-diethylbenzene (ECHA 2016 a). However, this value is not supported by the data contained in the dossier. About 50% to 64% of the structurally similar ethylbenzene is absorbed in the respiratory tract of humans (supplement "Ethylbenzene" 2012). Therefore, 60% absorption by inhalation is assumed for diethylbenzenes.

Radioactively labelled 1,2-diethylbenzene given once to male Sprague Dawley rats in gavage doses of 1 or 100 mg/kg body weight led to oral absorption of 90%, as 10% of the dose was found in the faeces of bile duct-cannulated rats. A fraction of about 55% underwent enterohepatic circulation and was finally eliminated with the urine. In non-cannulated rats, 65% to 75% of the administered dose of 1 or 100 mg/kg body weight was found in the urine, 17% to 23% was recovered in the faeces, and 2% to 3% was exhaled or remained in the body. The elimination of the substance was similar after intravenous injection of 1 mg/kg body weight. Within 24 hours, 90% of the total radioactivity eliminated after 168 hours was found in the urine, faeces and exhaled air (Payan et al. 1999).

In male F344 rats given 1,2-diethylbenzene doses of 100 to 120 mg/kg body weight by intraperitoneal injection, the highest concentration in the blood of about 15 µM (about 0.1% of the administered dose) was reached 2 hours after injection of the substance and then rapidly decreased. The concentration in the urine was highest at the first collection interval up to 12 hours after injection and then decreased rapidly. A total amount of 0.06 µmol was eliminated unchanged within 48 hours. In the brain, the highest concentration of 17.4 µM was found 2 hours after injection. However, the metabolite 1,2-diacetylbenzene was detected in the blood at relatively constant concentrations up to 24 hours after injection; the highest concentration was observed after 12 hours (about 3.7 µM). The concentration in the urine was highest 24 hours after injection and decreased only slightly over the course of the following 24 hours. A total amount of 0.13 µmol 1,2-diacetylbenzene was eliminated within 48 hours. There was no evidence of 1,2-diacetylbenzene in the brain (Thrall et al. 2007).

After male Sprague Dawley rats were given a radioactively labelled 1,2-diethylbenzene dose of 20 mg/kg body weight by intravenous injection, the highest radioactivity was determined in the nasal cavity and kidneys by whole-body autoradiography 5 minutes after injection. Notable radioactivity was found in the intestinal tract, which confirms the rapid biliary elimination. The concentrations were lower in the brain and spinal cord than in the plasma. Both after 1 hour and after 4 hours, the concentrations were 10 times as high in the nasal cavity as in the plasma and liver. After 4 hours, the concentrations were 1.4 to 10 times as high as after 24 hours, which suggests rapid elimination. After rats were given intravenous injections of a radioactively labelled 1,2-diethylbenzene dose of 1 mg/kg body weight, the ^{14}C concentrations decreased bi-exponentially in various tissue homogenates. The terminal half-lives were 558 minutes in the nose, 719 minutes in the kidneys, 945 minutes in the testes, 1101 minutes in the brain, 1406 minutes in the plasma, 1591 minutes in the liver, 3282 minutes in the blood, 11 315 minutes in brown adipose tissue and 49 348 minutes in the lungs. The initial half-lives were below 1 hour for all tissues except for the adipose tissue, lungs and intestinal tract. The half-lives of unchanged 1,2-diethylbenzene and 1-(2′-ethylphenyl)ethanol in plasma were 5 and 11 minutes. The corresponding half-lives in the brain were 10.7 and 16.7 minutes, respectively. The concentration of unchanged 1,2-diethylbenzene was 10 times as high in blood cells as in plasma. The half-life in whole blood and in blood cells was 14 minutes. 1,2-Diethylbenzene was not metabolized by blood cells and was only slightly metabolized by S9 mix from the brain. However, it was rapidly metabolized by S9 mix from the liver and lungs (Payan et al. 2008).

Rats were given an oral, radioactively labelled 1,2-diethylbenzene dose of 25 mg/kg body weight on day 18 of gestation. The highest radioactivity levels were detected in the liver and kidneys of the dams at the first sampling time 1 hour after administration. These values were 31% to 49% 24 hours later and 15% to 26% 48 hours after administration. The radioactivity in the foetuses was relatively constant up to 24 hours after administration and then decreased. The radioactivity in the placenta, foetuses and amniotic fluid was 0.35%, 0.35% and 0.09% of the administered dose, respectively. During the first 48 hours after administration, the concentrations in the plasma of the dams were about 2 to 4 times as high as in the plasma of the foetuses. The concentrations in the placenta were always higher than in the foetuses; transfer to the foetuses via the placenta was thus limited (Saillenfait et al. 1999).

Fluxes of 444, 8.6 and 3.9 μg/cm² and hour were calculated for a saturated aqueous solution of **1,2-diethylbenzene** using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), respectively. Assuming the exposure of a 2000 cm² surface area of skin for 1 hour, this would correspond to absorbed amounts of 888, 17.2 and 7.8 mg, respectively.

The corresponding values were 2124, 23.2 and 5.5 mg for **1,3-diethylbenzene** and 2246, 24.3 and 5.7 mg for **1,4-diethylbenzene**.

3.2 Metabolism

Qualitative evidence of the metabolism of 1,2-diethylbenzene to 1,2-diacetylbenzene was found after male Sprague Dawley rats were given an oral 1,2-diethylbenzene dose of 165 mg/kg body weight and day on 4 days a week (Gagnaire et al. 1991).

1-(2′-Ethylphenyl)ethanol, 2,3-diethylphenol and 3,4-diethylphenol are other metabolites of 1,2-diethylbenzene (Payan et al. 1999).

After oral administration of radioactively labelled 1,2-diethylbenzene to male Sprague Dawley rats, the two glucuronides of the stereo-isomeric 1-(2′-ethylphenyl)ethanol were identified as the main metabolites in the urine and bile (about 57% and 79% of the radioactivity in both media; about 40% of the administered dose in the urine). Less than 10% of the radioactivity in the urine and bile was present in the form of neutral metabolites, and only small amounts of the parent substance were recovered. Only traces of 1,2-diacetylbenzene were detected in the urine, faeces and bile; the authors attributed this to the reaction with amino groups in the body (Payan et al. 1999). As 2% to 3% of the dose was found in the body, metabolism to 1,2-diacetylbenzene may have accounted for at least part of this amount. To what extent 1,2-diethylbenzene is metabolized to 1,2-diacetylbenzene is not known, but may be below 3%.

In vitro, the (R)-enantiomer of 1-(2′-ethylphenyl)ethanol formed more rapidly from 1,2-diethylbenzene than the (S)-enantiomer in the presence of S9 mix from rat liver or microsomes, and the ratio between (R)-enantiomer and (S)-enantiomer formation was 1.2:1 in rats in vivo. In contrast, the (S)-enantiomer underwent glucuronidation in the plasma 4 times more rapidly than the (R)-enantiomer both in vitro and in vivo and was eliminated primarily via the kidneys (Payan et al. 2001).

4 Effects in Humans

There are no data available. Likewise, there are no clinical findings available for the sensitizing effects of the diethylbenzenes on the skin or respiratory tract.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

The 4-hour LC_{50} of **1,4-diethylbenzene** was higher than the limit test concentration of 5000 mg/m^3 for Crj:CD(SD) rats. The substance was administered via the nose as an aerosol. No animals died; dyspnoea, ataxia and tremor were observed. Gross-pathological examination did not yield any unusual findings (ECHA 2016 a).

The 7-hour LC_{50} was > 2100 ml/m^3 for a **diethylbenzene isomer mixture** (25% 1,2-diethylbenzene, 40% 1,3-diethylbenzene, 35% 1,4-diethylbenzene). Nasal irritation, dizziness and body weight loss were observed. Necropsy revealed blue discoloration of all tissues and secretions, moderate kidney findings and very mild liver findings (no other details) (ECHA 2016 b).

5.1.2 Oral administration

The oral LD_{50} of **1,4-diethylbenzene** was higher than the limit test dose of 2000 mg/kg body weight for Crj:CD(SD) rats. No animals died, the spontaneous motor activity was reduced and gross-pathological examination did not yield any unusual findings (ECHA 2016 a).

An oral LD_{50} of 2050 mg/kg body weight was determined in Sprague Dawley rats for a **diethylbenzene isomer mixture** (composition not specified). Other studies that investigated another diethylbenzene isomer mixture (composition not specified) reported an LD_{50} in a range from 2520 to 5000 mg/kg body weight for female Sprague Dawley rats and an LD_{50} > 2000 mg/kg body weight for male F344 rats (ECHA 2016 b).

An oral LD_{50} of 1200 mg/kg body weight was established for a diethylbenzene isomer mixture (25% 1,2-diethylbenzene, 40% 1,3-diethylbenzene, 35% 1,4-diethylbenzene) in female white rats (Wolf et al. 1956).

5.1.3 Dermal application

The dermal LD_{50} determined in a study carried out in 2 New Zealand White rabbits according to OECD Test Guideline 402 with a diethylbenzene isomer mixture (composition not specified) was greater than 2000 mg/kg body weight. Effects on the skin were not reported (ECHA 2016 b).

After occlusive application of a diethylbenzene isomer mixture (composition not specified) to the skin of 10 New Zealand White rabbits for 24 hours, the dermal LD_{50} was higher than 5000 mg/kg body weight. Fissures on the exposed skin site were observed in 1 animal, and reduced feed consumption and body weight loss or stagnating weights were determined in all animals on day 7 after exposure (ECHA 2016 b).

5.1.4 Intraperitoneal injection

In rats, an intraperitoneal **1,2-diethylbenzene** dose of 200 mg/kg body weight induced necrosis in the olfactory epithelium and Bowman's glands that increased with the exposure period. Most lesions were reversible 1 month after administration. The same effects were observed after treatment with a **1,2-diacetylbenzene** dose of 40 mg/kg body weight, but no effects were caused by the injection of **1,3-diethylbenzene** or **1,4-diethylbenzene**. The effects were inhibited by the pre-treatment of rats with 5-phenyl-1-pentyne, which is an inhibitor of CYP2F2 and CYP2E1. These lesions were presumably caused by the oxidative metabolism of 1,2-diethylbenzene to 1,2-diacetylbenzene in the olfactory epithelium and were thus systemic effects. The authors pointed out that in the study of Payan et al. (2008) the radioactivity concentrations found in the nasal cavity were 10 times as high as in the plasma or liver after intravenous injection of radioactively labelled 1,2-diethylbenzene (Gagnaire and Boucard 2014). This type of nasal lesion was not found in the only inhalation study that investigated the nose after repeated exposure to a **diethylbenzene isomer mixture** containing 1,2-diethylbenzene (Monsanto 1993; see Section 5.2.1). The intraperitoneal dose of 200 mg/kg body weight would correspond to a 1,2-diethylbenzene concentration of 1160 mg/m^3 after exposure by inhalation for 6 hours at a

respiratory volume of 0.8 l/min/kg body weight and assuming 100% intraperitoneal absorption and 60% absorption by inhalation. The 1,2-diethylbenzene concentration in the inhalation study, however, was only 25 ml/m³ (140 mg/m³); therefore, the concentration might have been too low to produce these nasal lesions.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In an unpublished study from 1958, different species were exposed 127 to 130 times to a **diethylbenzene isomer mixture** (25% 1,2-diethylbenzene, 40% 1,3-diethylbenzene, 35% 1,4-diethylbenzene) at a concentration of 100 ml/m³ for 7 hours a day over a period of 183 days. Groups of 20 male and 20 female rats, 10 male and female guinea pigs, 3 male and female rabbits and 2 female monkeys were used. Control groups with the same number of animals were sham-exposed to air or were not exposed at all. Liver and kidney findings were obtained in the female rats. The tissues and the urine of the exposed animals were discoloured blue and green, respectively. The haematological examination of the rats and of 1 monkey did not yield any adverse findings. In another study, 5 male and 5 female rats, 1 rabbit and 2 guinea pigs were exposed 4 times to 600 ml/m³ within 7 days for 7 hours a day. Another 5 male and 5 female rats were exposed 3 times to 200 ml/m³ within 6 days. The body weight gains of these animals were reduced and the animals were in a poor general state (no other details; ECHA 2016 b). Definite conclusions cannot be drawn for the animals exposed to the higher concentrations because of the short exposure period. As only limited histopathological examinations were carried out, this also applies to the derivation of a NOAEC (no observed adverse effect concentration) for this end point in the animals in the long-term exposure groups. It can however at least be said that clinical signs of neurotoxicity were not observed in several species at a **1,2-diethylbenzene** concentration of 25 ml/m³.

Groups of 10 male and 10 female Sprague Dawley rats were exposed whole-body to a **diethylbenzene isomer mixture** (10.0% 1,2-diethylbenzene, 57.9% 1,3-diethylbenzene, 29.4% 1,4-diethylbenzene, 0.6% *sec*-butylbenzene, < 0.1% isopropylbenzene) at concentrations of 0, 190, 610 or 1400 mg/m³ (34, 110 and 252 ml/m³) for 6 hours a day, on 5 days a week, for 13 weeks. The exposure conditions for the two high concentrations were achieved by spraying the substance into the inhalation chambers. The concentrations in the chambers were determined by passing the air through acetonitrile and subsequent HPLC analysis; they were thus calculated as the sum of vapour and aerosol. A separate analysis was not carried out to quantify the possible aerosol fraction. The study was carried out according to EPA Test Guideline T26-16 and involved haematological, clinico-chemical and ophthalmological examinations, determinations of the weights of the adrenal glands, brain, heart, kidneys, liver, spleen and testes, and histopathological examinations of 37 organs/tissues including the nasal turbinates, spinal cord and sciatic nerve in the control animals and the animals of the high concentration group. The lungs, liver and kidneys were examined in all animals. At 190 mg/m³ and above, the relative liver weights of the males were slightly increased, but independent of the concentration; therefore, this was not considered to be induced by the substance. At 610 mg/m³ and above, the leukocyte

counts were decreased in a concentration-related manner by about 26% to 29% and the lymphocyte counts by 20% to 30% in the male animals. The phosphorus level was increased in the females. The brains and bladders of the males and females were bluish-grey. At 1400 mg/m³ and above, body weight gains were reduced in both sexes and most tissues, including the serum, were a bluish-grey colour. The alanine and aspartate aminotransferase and the creatinine kinase activities were reduced in the females and the potassium and phosphorus levels were increased in the males. The relative kidney weights of the males were slightly increased. Neurotoxicity or adverse histopathological findings were not observed. A NOAEC of 34 ml/m³ (190 mg/m³) was determined for the **diethylbenzene isomer mixture**. The lymphocyte and leukocyte counts were reduced at the LOAEC of 110 ml/m³ (Monsanto 1993). As the diethylbenzene isomer mixture did not cause any neurotoxic effects, even at the highest concentration tested, the NOAEC for clinical neurotoxicity induced by **1,2-diethylbenzene** was 25 ml/m³ (10% of 252 ml/m³) also in this study.

Groups of 12 male, 9-week-old Sprague Dawley rats were exposed whole-body to a **diethylbenzene isomer mixture** (6% 1,2-diethylbenzene, 66% 1,3-diethylbenzene and 28% 1,4-diethylbenzene) at concentrations of 0, 496, 680 or 869 ml/m³ for 6 hours a day, on 5 days a week, for 18 weeks, and the animals were subsequently observed for a period of 6 weeks. Ten animals were used as controls. Nerve conduction velocities and action potentials were determined in these animals. In a second experiment, groups of 15 male, 19-week-old Sprague Dawley rats were exposed whole-body to 0, 646 or 834 ml/m³ for 6 hours a day, on 5 days a week, for 18 weeks and were then observed for 7 weeks. In these animals, the latencies of brainstem auditory evoked potentials (BAEP) were recorded by means of implanted electrodes. The body weight gains were reduced in all animals exposed to diethylbenzene and the skin was bluish-grey. No signs of neurotoxicity were observed in the animals of the first experiment. The motor and sensory conduction velocities of the tail nerve and the amplitudes of the sensory action potential were reduced in all exposed animals in a time and concentration-dependent manner, and these findings were not completely reversible. As was expected, the electrophysiological effects were observed at lower concentrations than the adverse clinical effects. Unsteady gait was observed in some animals of the second experiment and paralysis of the hind legs was found in 1 animal of the group exposed to 834 ml/m³. Two animals of this group died. The 19-week-old rats were thus more susceptible than the 9-week-old rats. At 646 and 834 ml/m³, the BAEP latencies of the 5 waveforms and the interpeak differences (I–V) were significantly prolonged. At 834 ml/m³, the amplitude of the N2P2 component was significantly reduced (Gagnaire et al. 1992 a). Therefore, a LOAEC of 30 ml/m³ (6% of 496 ml/m³) for nerve conduction velocities and action potential amplitudes and a LOAEC of 39 ml/m³ (6% of 646 ml/m³) for clinical neurotoxicity were obtained for **1,2-diethylbenzene**. With a NOAEC of 52 ml/m³ (6% of 869 ml/m³), younger animals were less susceptible to clinical neurotoxicity.

The most sensitive end point was the effect on the amplitudes of the sensory action potentials (ASAP; a decrease of 16%, 13% and 26% compared with the control values at 496, 680 and 869 ml/m³, respectively) and on the latencies of the 5th component of the BAEP (an increase of 2.9% and 9.4% compared with the control values at 646 and 834 ml/m³, respectively). If the NAEC (no adverse effect concentration) is estimated to be equal to a third of the LOAEC of 496 ml/m³ (= 165 ml/m³), this

would correspond to a 4.4% decrease in the ASAP after linear extrapolation of the above concentration – effect relationship. As the relative standard deviation for the mean value of the ASAP of the control animals (208 ± 25 µV) of 12% is higher than the estimated decrease, the action potential determined at the NAEC is within the standard deviation of the controls. After linear extrapolation of the above concentration – effect relationship, the latency of the 5th component of the BAEP would increase by 1.4% at the estimated NAEC of 165 ml/m³. Whether this increase is still within the standard deviation of the control values cannot be calculated on the basis of the published data because only the standard error was reported. Based on the content of 1,2-diethylbenzene, a NAEC of 10 ml **1,2-diethylbenzene**/m³ (6% of 165 ml/m³) can thus be assumed.

Summary: After exposure by inhalation, the most sensitive end points of the effects of the **diethylbenzene isomer mixture** are decreased numbers of leukocytes and lymphocytes at 110 ml/m³ with a NOAEC of 34 ml/m³. For the neurotoxic **1,2-diethylbenzene**, a LOAEC of 30 ml/m³ for effects on the action potentials of peripheral nerves was derived with an estimated NAEC of 10 ml/m³.

5.2.2 Oral administration

Groups of 15 male Sprague Dawley rats were given gavage doses of **1,2-diethylbenzene** of 0, 75 or 100 mg/kg body weight on 4 days a week for 8 weeks, and the animals were subsequently observed for a period of 8 weeks. Body weight loss, blue discoloration of the skin and urine and unsteady gait up to paralysis of the hind legs were observed in the animals. Exposure to 100 mg/kg body weight was lethal to two animals. The latencies of the 5 waveforms of the BAEP were significantly prolonged and the amplitudes were reduced; these changes remained even after the 8-week recovery period. Likewise, the interpeak latency (I–V) increased dose and time-dependently. The results were more conclusive after intraperitoneal injection of **1,2-diacetylbenzene** doses of 10 or 15 mg/kg body weight and day that were given according to the same pattern. By increasing the BAEP latencies following acoustic stimulation, 1,2-diethylbenzene or its metabolite 1,2-diacetylbenzene leads to central nervous effects on hearing; however, peripheral cochlear dysfunction may also be involved in the induction of these effects (Gagnaire et al. 1992 b).

In a test carried out according to OECD Test Guideline 407, which additionally investigated endocrine effects, groups of 5 male and 5 female 8-week-old Crl:CD (SD) rats were given **1,3-diethylbenzene** doses of 0, 25, 150 or 1000 mg/kg body weight and day for 28 days. Another 5 male and 5 female control animals and high dose group animals were observed for 14 days. At 25 mg/kg body weight and day and above, the reticulocytes of the males were increased, but not in a dose-dependent way. The T4 concentrations were increased in a dose-dependent manner. In the females, total cholesterol was increased by 28% at 150 mg/kg body weight and day and above. In male rats, alpha2u-protein deposits were observed in the renal epithelial cells, but this finding is not relevant for humans. Haemosiderin deposits in the spleen were observed in 1 female at 150 mg/kg body weight and day, in 2 female rats at 1000 mg/kg body weight and day and in 4 female rats at the end of the observation period. The body weights of the males were significantly reduced by 4% at 1000 mg/kg body weight and day. The relative liver weights were increased

by 38%, and the relative kidney weights were increased by 10%. In the females, the relative liver weights were increased by 35% and the weights of the thyroid gland were increased by 36%. All effects were reversible after the 14-day recovery period. At 1000 mg/kg body weight and day, the reticulocyte levels were increased in the females. Likewise, the concentrations of total protein, albumin and thyroid-stimulating hormone (TSH) in the blood were increased and the cholinesterase activity and T3 values were decreased. Hypertrophy of the centrilobular hepatocytes was detected in 5 males and 4 females and extramedullary haematopoiesis was increased in 1 male and 1 female. Hyperplasia of the follicular epithelium of the thyroid gland was observed in 1 male animal. The authors reported a NOAEL (no observed adverse effect level) of 150 mg/kg body weight and day for 1,3-diethylbenzene (Yamasaki et al. 2012).

The increased reticulocyte counts in the males and the increased T4 levels were the most sensitive effects at 25 mg/kg body weight and day and above. However, it is questionable whether the increased reticulocyte counts were substance-induced because there was no dose-dependence. The effects on the thyroid gland are plausible because the liver weights were greatly increased and centrilobular hypertrophy was observed in the animals of the high dose group. This suggests enzyme induction, which then leads to the degradation of thyroid hormones due to an increase in glucuronidation. However, this liver cell hypertrophy was observed only at 1000 mg/kg body weight and day. In this group, the TSH levels were increased in both males and females, but the increase was statistically significant only in the females. The increased T4 levels observed at the lower doses were not plausible. The same publication described a study that investigated a second substance with control animals of the same age. In this control group, the T4 level was 25% higher and was thus just as high as the T4 level after exposure to 1,3-diethylbenzene at 25 mg/kg body weight and day. Therefore, the control value in the study with 1,3-diethylbenzene was presumably too low. Haemosiderin deposits in the spleen and increased total cholesterol were observed in the female rats at 150 mg/kg body weight and day and above. The authors did not discuss these findings. These effects were not observed in the male animals up to 1000 mg/kg body weight and day. The dose of 1000 mg/kg body weight and day was a clear LOAEL (lowest observed adverse effect level). The oral dose of 150 mg/kg body weight and day would correspond to about 780 mg/m^3 (140 ml/m^3) after inhalation exposure for 6 hours (respiratory volume: 0.8 l/min/kg body weight, oral absorption: 90% and absorption by inhalation: 60%). However, no effects on the spleen or cholesterol were observed in the 13-week inhalation study with a **diethylbenzene isomer mixture** corresponding to a 1,3-diethylbenzene concentration of 146 ml/m^3 (58% of 252 ml/m^3). Therefore, a 1,3-diethylbenzene dose of 150 mg/kg body weight and day is regarded as the NOAEL.

In a screening test similar to OECD Test Guideline 422 carried out in Japan (summary and tables in English) in 1993, groups of 12 Sprague Dawley rats per sex and dose were given daily gavage doses of **1,4-diethylbenzene** of 0, 30, 150 or 750 mg/kg body weight. The male animals were exposed for 44 days including 14 days before mating and the females were exposed for up to 52 days including 14 days before mating and the period up to day 3 of lactation. Clinico-chemical and haematological examinations were carried out only in the male animals. At 150 mg/kg body weight and day, the blood urea nitrogen levels (0, 30, 150, 750 mg/kg body weight: 120, 125, 136,

138 mg/l; possibly a sign of impaired renal function), alanine aminotransferase activity (17, 18, 23, 25 U/l; adverse nature not known because effects were slight) and relative kidney weights (0.641%, 0.678%, 0.715%, 0.737%; alpha2u deposits in the kidneys were not examined) were significantly increased in the males. The gamma-glutamyl transpeptidase activity was reduced (2.9, 3.0, 2.2, 2.1 U/l; relevance not known). In the male animals treated with 750 mg/kg body weight and day, total protein, albumin, creatinine and total bilirubin were increased in the blood and feed consumption was increased on day 28 and thereafter. The glucose concentration in the blood was reduced. The liver was brown and enlarged and the liver cells were swollen. At this dose level, body weight gains were decreased (♂: –10%; ♀: –16% during gestation) and the liver weights were increased (♂: 18% absolute weights; 24% relative weights; ♀: 18% absolute weights; 21% relative weights). The haematological parameters of the males differed significantly from those of the control animals, in some cases even at the low dose, but there was no evidence of dose dependence or of an adverse nature. A NOAEL of 30 mg/kg body weight and day was determined for the male rats on the basis of the changes in the clinico-chemical parameters, and a NOAEL of 150 mg/kg body weight and day was established for the female rats on the basis of the reduced body weight gains (ECHA 2016 a; MHW 1993 a).

Groups of 12 male Sprague Dawley rats were given gavage doses of a **diethylbenzene isomer mixture** (7% 1,2-diethylbenzene, 58% 1,3-diethylbenzene, 35% 1,4-diethylbenzene) and separate gavage doses of 1,2-diethylbenzene, 1,3-diethylbenzene or 1,4-diethylbenzene. Doses of 0, 500 or 750 mg/kg body weight and day of the mixture were given on 5 days a week for 10 weeks. A **1,2-diethylbenzene** dose of 100 mg/kg body weight and day was administered on 4 days a week for 8 weeks, and **1,3-diethylbenzene** and **1,4-diethylbenzene** doses of 500 mg/kg body weight and day were administered on 5 days a week for 8 weeks. Groups of 10 control animals were given the olive oil vehicle. On day 3 and thereafter, blue discoloration of the skin and urine was found in all animals that were treated with the isomer mixture. In both dose groups, 2 animals died, and body weight gains were reduced in the high dose group. Weakness of the hind legs and unsteady gait were observed in all treated rats and complete paralysis of the hind legs was found in some animals. In week 4 and thereafter, the motor and sensory nerve conduction velocities of the tail nerve and the amplitudes of the sensory action potential were reduced in a dose and time-dependent manner. The electrophysiological examination of the rats that were given 1,2-diethylbenzene yielded the same clinical signs and impairments. These worsened in weeks 1 or 2 and thereafter, but improved during the 8-week observation period. As the action potentials were reduced to a greater extent than the nerve conduction velocities, the authors concluded that these effects were indicators of axonopathy rather than myelinopathy. The 1,3-isomer and 1,4-isomer did not cause any neurotoxic effects or systemic toxicity with the exception of a transient decrease in body weight gains caused by 1,4-diethylbenzene. The authors noted that the treatment of guinea pigs with 1,2-diethylbenzene did not lead to blue discoloration of tissues or urine, nor did it cause neurotoxicity (no other details). Therefore, they assumed that 1,2-diethylbenzene itself did not induce neurotoxicity, but that a metabolite was responsible for the blue discoloration and neurotoxicity (Gagnaire et al. 1990).

As this study tested 1,2-diethylbenzene both alone and as a component of a mixture with the two other isomers, the following discusses whether the neurotoxicity of the substance was affected by the two non-neurotoxic isomers. When given as a mixture, the dose of 1,2-diethylbenzene was 7% of 750 mg/kg body weight and day and thus 262.5 mg/kg body weight and week. When given alone, the weekly dose of 1,2-diethylbenzene was 400 mg/kg body weight. The slopes of the regression lines shown in the publication are steeper for the end points of motor and sensory nerve conduction velocities (MCV and SCV) and amplitudes of the sensory action potential (ASAP) after administration of 1,2-diethylbenzene; this confirms the expected result that the 1,2-diethylbenzene dose of 400 mg/kg body weight and week causes severer effects than the 1,2-diethylbenzene dose of 262.5 mg/kg body weight and week in the diethylbenzene mixture. Thus, the neurotoxic effects were not increased by the two other isomers. However, the different intensities of the effects (slopes of the regression lines) are also no proof that the two other diethylbenzene isomers substantially decrease the neurotoxicity of 1,2-diethylbenzene. A LOAEL of 35 mg/kg body weight and day (7% of 500 mg/kg body weight) was calculated for the clinical signs of neurotoxicity and neurotoxic effects detected in the electrophysiological examination on the basis of the fraction of **1,2-diethylbenzene** present in the mixture. A NOAEL was not obtained. In addition, the figures show that the effects increase with an increase in time and that a plateau had not yet been reached after 8 weeks.

Summary: Also after oral administration of a **diethylbenzene isomer mixture**, the clinical signs and the electrophysiological findings of neurotoxicity induced by 1,2-diethylbenzene were the most sensitive end points with a LOAEL for **1,2-diethylbenzene** of 35 mg/kg body weight and day. A NOAEL was not obtained. Therefore, 12 mg/kg body weight and day, which is one third of the LOAEL, is assumed to be the NAEL (no adverse effect level).

Increased liver weights and centrilobular hypertrophy were observed in rats given gavage doses of **1,3-diethylbenzene** of 1000 mg/kg body weight, presumably as a result of the induction of metabolizing enzymes of the liver. This affects the thyroid hormones and leads to increased thyroid weights. A NOAEL of 150 mg/kg body weight and day was found.

In a screening study carried out according to OECD Test Guideline 422, **1,4-diethylbenzene** induced changes in the blood urea nitrogen levels, and in the alanine aminotransferase and gamma-glutamyl transpeptidase activity, and led to increased kidney weights in male rats at 150 mg/kg body weight and day and above. The NOAEL was 30 mg/kg body weight and day.

5.2.3 Dermal application

The carcinogenicity study (Section 5.7) with dermal application of 25 µl of a diethylbenzene isomer mixture to the skin of mice 3 times a week did not reveal changes in survival or the incidence of systemic tumours compared with the findings in control animals.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In an in vitro study with reconstructed human epidermis carried out according to OECD Test Guideline 439, **1,4-diethylbenzene** induced cytotoxicity and reduced the cell viability to 12.2% of the negative control value. The positive control test with 5% sodium dodecyl sulfate yielded a cell viability of 2.3%. Therefore, 1,4-diethylbenzene caused irritation of the skin (ECHA 2016 a).

Likewise, 1,4-diethylbenzene induced irritation of the skin in the local lymph node assay in mice (Section 5.4.1).

A **diethylbenzene isomer mixture** (25% 1,2-diethylbenzene, 40% 1,3-diethylbenzene, 35% 1,4-diethylbenzene) caused moderate irritation of the skin and after repeated application (10 to 20 times within 4 weeks) exfoliation of the skin of rabbits (Wolf et al. 1956).

In a skin irritation test carried out according to the current OECD Test Guideline 404, the semi-occlusive application of 0.5 ml of a diethylbenzene isomer mixture to the skin of 3 New Zealand White rabbits for 4 hours caused severe irritation with a primary skin irritation index of 5.2 of 8 (ECHA 2016 b).

In a skin irritation test carried out according to the current and previous OECD Test Guideline 404, 0.5 ml of a diethylbenzene isomer mixture was applied semi-occlusively to the skin of 6 New Zealand White rabbits for 4 hours and occlusively for 24 hours and caused moderate skin irritation with a primary skin irritation index of 3 of 8 for the 24-hour exposure. Superficial and subepidermal necrosis was observed in 2 animals. Moderate to severe erythema was found in all 6 animals after 4-hour application; the findings were reversible in 5 animals after 14 days (ECHA 2016 b). The reasons why the skin irritation observed in this 4-hour test was less marked than that in the test described above are unknown.

In the REACH registration dossier, the diethylbenzene isomers have been classified as irritating to the skin according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (ECHA 2016 b).

5.3.2 Eyes

The HET-CAM test in hens' eggs yielded an irritation index of 10.0 for **1,4-diethylbenzene**. This value was just as high as that for 1% sodium dodecyl sulfate, which was used as the positive control substance. Thus, this test predicted severe eye irritation for 1,4-diethylbenzene (ECHA 2016 a). However, the OECD has not acknowledged the HET-CAM test as a valid replacement for a study of eye irritation.

The instillation of 2 drops of a **diethylbenzene isomer mixture** (25% 1,2-diethylbenzene, 40% 1,3-diethylbenzene, 35% 1,4-diethylbenzene) into the rabbit eye caused mild irritation of the conjunctivae, but no damage to the cornea (Wolf et al. 1956).

In an eye irritation test carried out according to EPA Test Guideline OPP 81-4, 0.1 ml of a diethylbenzene isomer mixture caused mild and reversible irritation after 24-hour application to the eyes of New Zealand White rabbits. After 24, 48 and 72 hours, the average values in all 6 animals were 1.3 for conjunctival erythema, 0 for iritis and 0 for corneal effects. Therefore, the mixture has not been classified as irritating to the eyes (ECHA 2016 b).

In the REACH registration dossiers, the **diethylbenzene isomers** were not assessed to be irritating to the eyes according to the GHS (ECHA 2016 b), whereas **1,4-diethylbenzene** was classified as "causing serious eye damage" (ECHA 2016 a).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

1,4-Diethylbenzene (purity: 99.3%) was tested in a modified local lymph node assay (integrated model for the differentiation of skin reactions; IMDS) in female NMRI mice in dilutions of 1%, 10%, 25% and 50% in acetone/olive oil (3:1) and as the undiluted substance. Deviating from OECD Test Guideline 429, the lymph node cell count and the lymph node weights, rather than cell proliferation, were determined as a measure of sensitization, and the ear weights and ear thickness were determined as a measure of irritation. The positive control substance hexyl cinnamaldehyde increased the lymph node cell count to more than 1.4 times the control value, which is the cut-off criterion for a sensitizing substance in this test. The 1% and 10% dilutions of 1,4-diethylbenzene did not increase the cell count or lymph node weights or the weights and thickness of the ears. These parameters were significantly increased at the other concentrations. The lymph node count was increased 1.4, 1.94 and 1.91 times and the lymph node weights were increased 1.19, 1.58 and 1.87 times compared with the control values. The ear thickness was not consistent at the 5 test concentrations and was 1.09, 1.02, 1.03, 1.02 and 1.13 times the control values with an increase in the test concentrations. However, as the ear weights at the 3 high concentrations were more than 1.1 times the control values (1.10, 1.12 and 1.19 times), thus fulfilling the criterion for an irritant substance, the increased lymph node cell count resulting from the simultaneous irritation was not regarded as evidence that the substance has sensitizing potential (ECHA 2016 a).

A Bühler test yielded negative findings for the **"polyethylbenzene" mixture** with the CAS number 25340-17-4. A 10% formulation of the mixture in ethanol was used for induction. During the challenge treatment carried out 2 weeks after 3 inductions, none of the 20 female Hartley guinea pigs reacted to a 5% formulation of the mixture in acetone (ECHA 2016 b).

5.4.2 Sensitizing effects on the airways

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a screening test similar to OECD Test Guideline 422 carried out in 1993, groups of 12 Sprague Dawley rats per sex and dose were given daily gavage doses of **1,4-diethylbenzene** of 0, 30, 150 or 750 mg/kg body weight. The male animals were exposed for 44 days including 14 days before mating, and the females were exposed for up to 52 days including 14 days before mating and the period up to day 3 of lactation.

Effects on mating, fertility, the oestrus cycle or parturition were not observed (see Section 5.2.2; ECHA 2016 a; MHW 1993 a).

5.5.2 Developmental toxicity

In a study of the toxic effects on prenatal development carried out according to OECD Test Guideline 414, groups of 22 to 26 Sprague Dawley rats were given daily gavage doses of **1,2-diethylbenzene** of 0, 5, 15, 25 or 35 mg/kg body weight from days 6 to 20 of gestation (vehicle: corn oil). The foetuses were examined on day 21 of gestation. Doses of 15 mg/kg body weight and above led to significant dose-dependent decreases in body weight gains (days 6 to 21 of gestation minus gravid uterus weights: 15, 25, 35 mg/kg body weight: 25.5%, 37.3%, 49,0%), feed consumption of the dams (days 6 to 21 of gestation: 15, 25, 35 mg/kg body weight: 7.9%, 12.4%, 15.5%) and foetal weights per litter (15, 25, 35 mg/kg body weight: 5.6%, 8.5%, 10.6%). Increased mortality or increased incidences of malformations or variations were not found up to the highest dose tested of 35 mg/kg body weight and day. Blue discoloration of the placental tissues (no details whether skin or organs of the foetuses were discoloured) was observed at 15 mg/kg body weight and above. According to the authors, the NOAEL for developmental toxicity and maternal toxicity of 1,2-diethylbenzene was 5 mg/kg body weight (Saillenfait et al. 1999). The reduced foetal weights per litter correlated strongly with the reduced feed consumption and body weight gains of the dams. As no other effects on the foetuses were observed, the Commission considers the dose of 35 mg/kg body weight and day to be the NOAEL for developmental toxicity.

In the screening test with **1,4-diethylbenzene** described under Section 5.5.1, external malformations or effects on the body weights were not observed in the foetuses of the dams treated up to day 4 of lactation with doses up to 750 mg/kg body weight (see Section 5.2.2; ECHA 2016 a; MHW 1993 a).

In a study of the toxic effects on prenatal development similar to OECD Test Guideline 414, groups of 25 Sprague Dawley rats were given daily gavage doses (vehicle: corn oil) of 0, 20, 100 or 200 mg/kg body weight of a **diethylbenzene isomer mixture** (10.0% 1,2-diethylbenzene, 57.9% 1,3-diethylbenzene, 29.4% 1,4-diethylbenzene, 0.6% *sec*-butylbenzene, < 0.1% isopropylbenzene) from days 6 to 15 of gestation. The foetuses were examined on day 20 of gestation. At 100 mg/kg body weight and above, the body weight gains (days 6 to 9 of gestation: controls: 5.0 ± 5.4 g; 100 mg/kg body weight: 0.0 ± 6.9 g; 200 mg/kg body weight: –7 ± 10.4 g) and feed consumption of the dams (days 6 to 9 of gestation: controls: 60.0 ± 7.8 g/kg body weight and day; 100 mg/kg body weight: 52.0 ± 9.0 g/kg body weight and day; 200 mg/kg body weight: 44.0 ± 11.4 g/kg body weight and day) were reduced and greenish-blue discoloration of the amniotic sac was observed. At 200 mg/kg body weight, the average foetal weights were reduced by 6% compared with those of the animals of the control group. Up to the high dose of 200 mg/kg body weight, clinical findings were not observed in the dams, and no increased mortality or increased incidences of malformations or variations were found in the foetuses. The NOAEL for maternal toxicity was 20 mg/kg body weight and day (Monsanto 1992). A NOAEL for developmental toxicity of 200 mg/kg body weight and day was derived from this

study. The only marginally reduced foetal weights are not relevant to the evaluation because maternal feed consumption was reduced and specific effects on development, in particular teratogenicity, were not observed.

The greenish-blue discoloration of the amniotic sac observed in this study after exposure to the diethylbenzene isomer mixture at a dose level of 100 mg/kg body weight and day and above (corresponding to a 1,2-diethylbenzene dose of 10 mg/kg body weight and day) concurs with the blue discoloration of the placental tissues found in the prenatal developmental toxicity study in rats with 1,2-diethylbenzene doses of 15 mg/kg body weight and day and above (Saillenfait et al. 1999). The blue discoloration is caused by the 1,2-diethylbenzene metabolite, 1,2-diacetylbenzene. 1,2-Diacetylbenzene reacts very readily with lysine and glycine to form purplish-blue chromophores (Kim et al. 2002).

In a range-finding study with groups of 6 animals given doses of 0, 20, 50, 100, 150 or 200 mg/kg body weight of a diethylbenzene isomer mixture, the body weight gains of the dams were not impaired up to 50 mg/kg body weight and the foetal weights were reduced at 200 mg/kg body weight. There was no evidence of external malformations (Monsanto 1991).

5.6 Genotoxicity

5.6.1 In vitro

1,4-Diethylbenzene was not found to be mutagenic in pre-incubation tests with the Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 or Escherichia coli WP2uvrA up to the cytotoxic concentration of 78 µg/plate either with or without the addition of metabolic activation. The positive controls induced the expected effects (ECHA 2016 a; MHW 1993 b).

A **diethylbenzene isomer mixture** (composition not specified) was not mutagenic in pre-incubation tests with the Salmonella typhimurium strains TA98, TA100, TA1535 and TA1537 up to the cytotoxic concentration of 50 µg/plate either with or without the addition of metabolic activation. Positive controls were tested, but the results were not reported (ECHA 2016 b).

A **diethylbenzene isomer mixture** (composition not specified) was not mutagenic in plate incorporation tests with the Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 up to the cytotoxic concentration of 50 µg/plate either with or without the addition of metabolic activation. The positive controls induced the expected effects (ECHA 2016 b).

A chromosomal aberration test in Chinese hamster lung cells yielded negative results up to a **1,4-diethylbenzene** concentration of 1.3 mg/ml after 6-hour incubation with or without the addition of metabolic activation. Cytotoxicity was observed only without the addition of metabolic activation. With metabolic activation, the highest concentration corresponded to the limit dose of 10 mM (1.34 mg/ml). Likewise, negative results were obtained up to the cytotoxic 1,4-diethylbenzene concentration of 0.11 mg/ml after 24-hour or 48-hour incubation without the addition of metabolic activation. The positive controls mitomycin C and cyclophosphamide induced the expected effects (ECHA 2016 a; MHW 1993 c).

In a chromosomal aberration test in Chinese hamster ovary (CHO) cells, a **diethylbenzene isomer mixture** (composition not specified) was not found to be clastogenic up to the cytotoxic concentration of 75 µg/plate either with or without the addition of metabolic activation. The positive controls induced the expected effects (ECHA 2016 b).

In an HPRT test carried out according to OECD Test Guideline 476, **1,4-diethylbenzene** was not mutagenic in V79 fibroblasts up to the cytotoxic concentration of 100 µg/ml either with or without the addition of metabolic activation. The positive controls induced the expected effects (ECHA 2016 a).

In an HPRT test similar to OECD Test Guideline 476, a **diethylbenzene isomer mixture** (composition not specified) was not mutagenic in CHO cells up to the cytotoxic concentration of 100 µg/ml either with or without the addition of metabolic activation. The positive controls induced the expected effects (ECHA 2016 b).

5.6.2 In vivo

In a micronucleus test carried out according to OECD Test Guideline 474 in 1991, groups of 18 male and 18 female CD1 mice were given doses of 0, 1000, 2000 or 4000 mg/kg body weight of a **diethylbenzene isomer mixture** (composition not specified) by intraperitoneal injection. Mortality occurred at doses of 2000 mg/kg body weight and above. The bone marrow of the animals was examined 24, 48 and 72 hours after administration, but no increased incidence of micronuclei was found in the 1000 polychromatic erythrocytes that were examined per animal. In the female animals of the highest dose group, the PCE/NCE ratio indicated cytotoxicity after 48 hours. The positive control cyclophosphamide induced the expected effects (ECHA 2016 b).

5.7 Carcinogenicity

When 25 µl of a **diethylbenzene isomer mixture** (composition not specified in the original report; product of Dow Chemical Company; according to ECHA (2016 b): 25% 1,2-diethylbenzene, 40% 1,3-diethylbenzene, 35% 1,4-diethylbenzene) was applied as a 10% solution in acetone to the dorsal skin of 40 male C3H/HeJ mice 3 times a week for the entire lifespan of the animals, 1 squamous cell carcinoma was observed at the application site. In addition, treatment induced epidermal hyperplasia in 7 mice and papillary hyperplasia in 1 mouse. Other skin lesions included hyperkeratosis (23/39), encrustation of the skin surface (5/39), epidermal necrosis (1/39), ulcerative dermatitis (4/39), dermatitis (6/39) and dermal fibrosis (5/39). The control animals were treated with acetone; 1 fibrosarcoma, 1 lymphosarcoma and 4 skin lesions (as above) were found. The survival of the treated animals (502 days) and control animals (499 days) and the systemic tumour incidence did not differ significantly. As squamous cell carcinomas were not found in the historical acetone control group of 604 male C3H/HeJ mice, the squamous cell carcinoma observed in the treated animal was interpreted as substance-induced. In a 10-day range-finding study in which mice were treated daily with 25 µl of a diethylbenzene isomer mixture in undiluted form or as a 50% or 10% solution in acetone, the 10% solution was found to be sufficiently non-irritating and non-toxic for a carcinogenicity study (Dow Chemical

Company 1983). In the REACH registration dossier, attention was drawn to the skin irritation caused by diethylbenzenes, which was considered to be tumourigenic after repeated application; therefore, the tumourigenic effect observed in this study was not regarded as relevant to humans (ECHA 2016 b).

6 Manifesto (MAK value/classification)

Critical effects in Sprague Dawley rats are the decreased number of white blood cells caused by the diethylbenzene isomer mixture, peripheral neurotoxicity caused by 1,2-diethylbenzene, increased liver and thyroid weights and TSH levels caused by 1,3-diethylbenzene, and increased blood urea nitrogen levels, alanine aminotransferase activity and kidney weights and decreased gamma-glutamyl transpeptidase levels caused by 1,4-diethylbenzene.

MAK value.

1. Diethylbenzene isomer mixture. An unpublished 13-week study (Monsanto 1993) in rats with a **diethylbenzene isomer mixture** (10.0% 1,2-diethylbenzene, 57.9% 1,3-diethylbenzene, 29.4% 1,4-diethylbenzene, 0.6% *sec*-butylbenzene, < 0.1% isopropylbenzene) did not report adverse effects on the respiratory tract at a concentration of 252 ml/m^3. A NOAEC of 34 ml/m^3 and a LOAEC of 110 ml/m^3 were obtained for the critical systemic effect, the decrease in white blood cells; systemic effects are therefore the most relevant effects. The blood:air partition coefficient, which was calculated according to Buist et al. (2012), was 80 to 120 for the isomers. Therefore, the fact that the respiratory volume at the workplace is higher than under experimental conditions has to be taken into account (see List of MAK and BAT Values, Sections I b and I c). The NOAEC corresponds to a concentration of **4.25 ml/m^3** for humans at the workplace (Table 1).

Therefore, 5 ml/m^3 has been established as the MAK value for a diethylbenzene isomer mixture. At a concentration of 5 ml/m^3, a diethylbenzene isomer mixture with a 1,2-diethylbenzene fraction of about 10% would contain about 0.5 ml 1,2-diethylbenzene/m^3. The MAK value for 1,2-diethylbenzene of 1 ml/m^3 (see below) should not be exceeded.

2. Individual isomers. Co-exposure to 2-butanone potentiates the neurotoxicity induced by *n*-hexane (Noraberg and Arlien-Søborg 2000), probably by inhibiting the phase II metabolism of 2,5-hexanedione (Yu et al. 2002). After oral administration, the neurotoxic effects of 1,2-diethylbenzene were not substantially inhibited or increased by the two other isomers included in the diethylbenzene mixture (see Section 5.2.2; Gagnaire et al. 1990). As the vapour pressures of the 3 isomers are very similar, it is assumed that the isomer ratio in the air corresponds to that in the liquid, and the oral and inhalation studies with diethylbenzene isomer mixtures can thus be used for the derivation of limit values for the individual substances.

1,2-Diethylbenzene. The severity of the neurotoxicity caused by 1,2-diethylbenzene depends on its metabolism to 1,2-diacetylbenzene or, rather, the further metabolism and elimination of 1,2-diacetylbenzene. It is thus dependent on the body burden. As there are no data for the body burden in either rats or humans, possible

Table 1 Studies in rats relevant to the derivation of the MAK value

Substance Reference	Duration, concentration/dose	LOAEC/L	NOAEC/L	Corresponding concentration in the air at the workplace (ml/m³)[a)]
DEB mixture 25% 1,2-DEB 40% 1,3-DEB 35% 1,4-DEB ECHA 2016 b	183 days, 7 hours/day, 5 days/week, 100 ml/m³	–	> 100 ml/m³ (clinical neurotoxicity) = 25 ml 1,2-DEB/m³	
DEB mixture 10% 1,2-DEB 58% 1,3-DEB 29% 1,4-DEB Monsanto 1993	13 weeks, 6 hours/day, 5 days/week, 34, 110, 252 ml/m³	110 ml/m³ (leukocyte count)	**34 ml/m³**	4.25[b)]
		–	> 252 ml/m³ (clinical neurotoxicity) = **25 ml 1,2-DEB/m³**	3.1[b)] for 1,2-DEB
DEB mixture 6% 1,2-DEB 66% 1,3-DEB 28% 1,4-DEB Gagnaire et al. 1992 a	18 weeks, 6 hours/day, 5 days/week, 496, 680, 869 ml/m³ **9 weeks old**	496 ml/m³ (nerve conduction velocity, action potential) = 30 ml 1,2-DEB/m³	– (electrophysiological neurotoxicity) NAEC = **10 ml 1,2-DEB/m³**	1.25[b)] for 1,2-DEB
		–	> 869 ml/m³ (clinical neurotoxicity) = 52 ml 1,2-DEB/m³	
	646, 834 ml/m³ **19 weeks old**	646 ml/m³ (BAEP, clinical neurotoxicity) = 39 ml 1,2-DEB/m³	– (electrophysiological and clinical neurotoxicity)	
DEB mixture 7% 1,2-DEB 58% 1,3-DEB 35% 1,4-DEB Gagnaire et al. 1990	10 weeks, 5 days/week, 500, 750 mg/kg body weight	500 mg/kg body weight (nerve conduction velocity, action potential, clinical neurotoxicity) = 35 mg 1,2-DEB/kg body weight	– (electrophysiological and clinical neurotoxicity) NAEC = **12 mg 1,2-DEB/kg body weight**	1.4[c)] for 1,2-DEB

Table 1 (continued)

Substance Reference	Duration, concentration/dose	LOAEC/L	NOAEC/L	Corresponding concentration in the air at the workplace (ml/m³)[a)]
1,3-DEB Yamasaki et al. 2012	28 days, 7 days/week, 25, 150, 1000 mg/kg body weight	1000 mg/kg body weight (liver weights, liver hypertrophy, TSH, relative thyroid weights)	**150 mg/kg body weight**	8.3[d)]
1,4-DEB MHW 1993 a	♂: 44 days, ♀: 52 days, 7 days/week, 30, 150, 750 mg/kg body weight	150 mg/kg body weight (♂: BUN, ALAT, kidney weights, GGT)	**30 mg/kg body weight**	2.5[e)] (from NOAEL) 4.2[e)] (from LOAEL/3)

[a)]: including time extrapolation (1:2) and extrapolation of the data from animal studies to humans (1:2)
[b)]: taking into account the increased respiratory volume (1:2)
[c)]: 1:4 (kinetic difference between rats and humans) × 70 kg/10 m^3 × 0.9 (oral absorption in animals)/0.6 (absorption by inhalation in humans)
[d)]: 1:4 × 70 kg/10 m^3 × 0.9 (oral absorption in animals)/0.6 (absorption by inhalation in humans) × 7 (7 days/week in animals)/5 (5 days/week in humans) × 1:3 (additional time extrapolation because of subacute exposure)
[e)]: NOAEL or LOAEL/3 × 1:4 × 70 kg/10 m^3 × 0.9 (oral absorption in animals)/0.6 (absorption by inhalation in humans) × 7 (7 days/week in animals)/5 (5 days/week in humans) × 1:2 (additional time extrapolation because exposure duration is between subacute and subchronic)

differences in species sensitivity cannot be assessed quantitatively. Clinical signs of neurotoxicity were not observed after the long-term exposure of several species to a concentration of 100 ml/m³ of a **diethylbenzene isomer mixture** (1,2-diethylbenzene concentration of 25 ml/m³) (ECHA 2016 b). There was evidence that 1,2-diethylbenzene does not cause neurotoxic effects in guinea pigs (Gagnaire et al. 1990) and that rats react more sensitively to 1,2-diacetylbenzene than mice (Tshala-Katumbay et al. 2005). However, humans are generally sensitive to the neurotoxicity caused by gamma-diketones, as was demonstrated for *n*-hexane. Therefore, the experimental data obtained in rats are extrapolated to humans according to the standard procedure (1:2).

A decrease in the NOAEC for clinical neurotoxicity induced by 1,2-diethylbenzene cannot be observed when the 13-week study (25 ml/m³; highest concentration) is compared with the chronic study (25 ml/m³). The LOAEC for clinical neurotoxicity after 18 weeks was 39 ml/m³. However, because of the poor reversibility of the findings, the effects may accumulate with an increase in the exposure duration. Even for *n*-hexane, which was investigated more thoroughly, none of the available studies which included electrophysiological determinations were carried out for more than 6 months. In rats, the NOAEC was 200 ml/m³ after 8 weeks and 100 ml/m³ after 16 to 24 weeks (supplement "n-Hexane" 2000). There is thus evidence of a decrease in the NOAEC with an increase in the exposure duration, and this is also assumed for 1,2-diethylbenzene.

The 13-week inhalation study with a **diethylbenzene isomer mixture** reported a NOAEC of about 25 ml 1,2-diethylbenzene/m³ (highest concentration tested) for the clinical neurotoxicity of 1,2-diethylbenzene (Monsanto 1993). The 1,2-diethylbenzene concentration for the workplace calculated from the NOAEC is **3.1 ml/m³** (Table 1).

In the 18-week inhalation study with a **diethylbenzene isomer mixture**, the LOAEC for 1,2-diethylbenzene was 30 ml/m³ for effects on the nerve conduction velocity and action potential amplitude (Gagnaire et al. 1992 a). The NAEC is estimated to be one third of the LOAEC (see Section 5.2.1). A workplace concentration of **1.25 ml/m³** is calculated from the NAEC (Table 1).

In the 10-week oral study with a **diethylbenzene isomer mixture**, the LOAEL for 1,2-diethylbenzene was 35 mg/kg body weight for clinical neurotoxicity, effects on the nerve conduction velocity and action potential amplitude (Gagnaire et al. 1990). The NAEL is estimated to be one third of the LOAEL. A concentration at the workplace of **1.4 ml/m³** is calculated from the NAEL (Table 1).

Very similar limit values were thus obtained from all three studies. Therefore, a MAK value for 1,2-diethylbenzene of 1 ml/m³ has been established. It should provide protection also from subclinical effects on nerve conduction velocities and action potentials.

1,3-Diethylbenzene and 1,4-diethylbenzene. The 4-week study with **1,3-diethylbenzene** yielded a LOAEL of 1000 mg/kg body weight for increased relative liver weights, relative thyroid weights and TSH with a NOAEL of 150 mg/kg body weight (Yamasaki et al. 2012). This is used to calculate a workplace concentration of **8.3 ml/m³** (Table 1).

The screening study with **1,4-diethylbenzene** reported a LOAEL of 150 mg/kg body weight (clinico-chemical findings in males that were exposed for 44 days,

therefore time extrapolation 1:4) and a NOAEL of 30 mg/kg body weight and day (ECHA 2016 a; MHW 1993 a). This is used to calculate a workplace concentration of **2.5 ml/m³** (Table 1).

As there is a wide margin between the NOAEL and the LOAEL of 150 mg/kg body weight, the actual NAEL of this substance may be even higher. The same calculation with a NAEL of 50 mg/kg body weight (LOAEL/3) yields a workplace concentration of **4.2 ml/m³** (Table 1).

The data do not show a significant difference in potency between 1,3-diethylbenzene and 1,4-diethylbenzene. As the 13-week inhalation study with the diethylbenzene isomer mixture had a longer exposure period and inhalation is more similar to exposure at the workplace than gavage administration, the study was used to derive a MAK value of 5 ml/m³ also for the two isomers 1,3-diethylbenzene and 1,4-diethylbenzene,

As systemic effects are clearly the critical effects (Monsanto 1993), the individual isomers are not expected to induce sensory irritation up to 5 ml/m³.

Peak limitation.

All diethylene benzene isomers have been classified in Peak Limitation Category II because of the critical systemic effects.

1,2-Diethylbenzene. An excursion factor of 8 has been established for 1,2-diethylbenzene because, as in the case of *n*-hexane (excursion factor of 8), the peripheral neurotoxicity is cumulative and does not depend on exposure peaks.

Diethylbenzene isomer mixture, 1,3-diethylbenzene and 1,4-diethylbenzene. The basic excursion factor of 2 has been established for these isomers because half-lives for 1,3-diethylbenzene or 1,4-diethylbenzene are not available.

As adverse effects on the respiratory tract of rats were not observed at 252 ml/m³ (Monsanto 1993), irritation is not expected to occur at the permitted short-term concentrations of 8 or 10 ml/m³.

Prenatal toxicity.

1,2-Diethylbenzene. A prenatal developmental toxicity study carried out with **1,2-diethylbenzene** in rats according to OECD Test Guideline 414 revealed dose-dependent significant decreases in the body weight gains and feed consumption of the dams and in the foetal weights at 15 mg/kg body weight and above (Saillenfait et al. 1999). The NOAEL for developmental toxicity was 35 mg/kg body weight and day because no other effects on the foetuses were observed apart from the reduced foetal weights that correlated with the reduced maternal feed consumption. The following toxicokinetic data are used to extrapolate this NOAEL to a concentration in workplace air: the corresponding species-specific correction value for the rat (1:4), the oral absorption of 90%, the body weight (70 kg) and the respiratory volume (10 m³) of the person, and the 60% absorption by inhalation. This results in a concentration of 92 mg/m³ (17 ml/m³), which is 17 times as high as the MAK value for 1,2-diethylbenzene of 1 ml/m³.

This would justify classification in Pregnancy Risk Group C.

As peripheral neurotoxicity is the critical effect for the derivation of a MAK value for 1,2-diethylbenzene, the developmental neurotoxicity has to be assessed.

There are no data available for 1,2-diethylbenzene that can be used to compare the sensitivity for peripheral neurotoxicity of animals exposed in utero with that of animals exposed in adulthood. Therefore, acrylamide is used as a reference substance. Common to both substances is that they induce peripheral axonopathy, the axon is the site of action, and binding to sulfhydryl groups of neurofilaments is the assumed mechanism. Acrylamide additionally causes damage to the central nervous system and is therefore regarded as a worst-case read-across substance. A 2-generation study in rats with exposure to acrylamide in drinking water (Tyl et al. 2000) yielded a LOAEL for the end points head tilt and foot splay of 5 mg/kg body weight and day (NOAEL: 0.5 mg/kg body weight and day) for the male offspring and a corresponding LOAEL of 0.5 mg/kg body weight and day (no NOAEL because 0.5 mg/kg body weight and day was the lowest dose tested) for the male adult animals. The comparison of the effect doses showed that the offspring were 10 times less sensitive than adult animals for the clinical signs of peripheral neurotoxicity. A comparison of the histopathological changes of the peripheral nerves of the offspring from the 2-generation study and those of adult rats after administration by drinking water revealed the following: The 2-generation study reported minimal to mild fragmentation or swelling of axons of peripheral nerves in 6/6 male and 0/3 female offspring at 5 mg/kg body weight and day (Tyl et al. 2000). Following 93-day exposure in adulthood, questionable to very mild degeneration of the peripheral nerves was observed in 9/10 males and 6/10 females at 5 mg/kg body weight and day, and no unusual findings were detected at 1 mg/kg body weight and day (Burek et al. 1980). Likewise, with regard to the histopathological changes of peripheral nerves, the offspring did not react with greater sensitivity than adult rats.

The data available for acrylamide showed that rats exposed in utero were not more sensitive for peripheral neurotoxic effects than adult animals. This is also assumed for 1,2-diethylbenzene based on the worst-case read-across substance acrylamide. As peripheral neurotoxic effects are not expected to occur in animals exposed in utero and as the 17-fold margin between the NOAEC for developmental toxicity and the MAK value of 1 ml/m^3 is sufficiently large, 1,2-diethylbenzene has been classified in Pregnancy Risk Group C.

Diethylbenzene isomer mixture. In a prenatal developmental toxicity study with a diethylbenzene isomer mixture (10.0% 1,2-diethylbenzene, 57.9% 1,3-diethylbenzene, 29.4% 1,4-diethylbenzene) in rats, the body weight gains and feed consumption of the dams were reduced at 100 mg/kg body weight (Monsanto 1992). At 200 mg/kg body weight, the average body weights of the foetuses were reduced only marginally compared with those of the control animals. Specific effects on development, in particular teratogenicity, were not observed up to the highest dose of 200 mg/kg body weight. After toxicokinetic extrapolation (see above), the NOAEL for developmental toxicity of 200 mg/kg body weight corresponds to a concentration of 526 mg/m^3 (94 ml/m^3), which is about 20 times the MAK value of 5 ml/m^3. As this margin is sufficient and neurotoxicity is not the critical end point for the mixture, the mixture is classified in Pregnancy Risk Group C.

1,3-Diethylbenzene and 1,4-diethylbenzene. There are no developmental toxicity studies available for the isomers 1,3-diethylbenzene and 1,4-diethylbenzene. However, the study with the diethylbenzene isomer mixture containing 57.9% 1,3-diethylbenzene and 29.4% 1,4-diethylbenzene did not report specific effects on development up to the highest dose of 200 mg/kg body weight; this dose was maternally toxic. In the case of 1,3-diethylbenzene, there is a sufficient margin between the MAK value and the calculated concentration in air. The calculated concentration of 1,4-diethylbenzene in air (28 ml/m^3) is only 6 times as high as the MAK value, but as only marginal foetotoxicity was observed at this maternally toxic dose, both isomers have been classified in Pregnancy Risk Group C.

Carcinogenicity.

Epicutaneous application of a diethylbenzene isomer mixture to the skin of male mice caused a squamous cell carcinoma in 1 of 40 animals; it was regarded as substance-induced because this type of tumour has rarely been described in negative control animals. Concurrently, skin lesions were observed and the diethylbenzenes cause acute skin irritation. As the diethylbenzenes are not genotoxic, the promotion of spontaneously initiated cells is assumed to be the cause. However, as only one animal was affected and irritant effects on the skin of the animals in the basal membrane and the underlying epidermis may induce indirect carcinogenic effects resulting from the injury, diethylbenzenes have not been classified in any of the categories for carcinogens.

Germ cell mutagenicity.

Diethylbenzenes are not genotoxic in vitro or in vivo. Therefore, diethylbenzenes have not been classified in any of the germ cell mutagen categories.

Absorption through the skin.

As data from in vitro or in vivo studies are not available, the absorption of the substance through the skin is estimated on the basis of the mathematical models. As the results for other alkyl aromatics obtained using the model of Fiserova-Bergerova et al. (1990) coincide better with the in vivo data available for these substances, this model is used to assess this group of substances. On the basis of model calculations (Section 3.1) and assuming exposure of a 2000 cm^2 surface area of skin for 1 hour, dermal absorption of a maximum 888 to 2200 mg is estimated for humans after exposure to a saturated aqueous solution of diethylbenzene isomers.

Therefore, the uptake via the skin as calculated using the model is markedly higher than the 33.6 mg of 1,2-diethylbenzene and 168 mg of 1,3-diethylbenzene and 1,4-diethylbenzene that are absorbed after inhalation exposure at the level of the MAK value at a respiratory volume of 10 m^3 and 60% absorption by inhalation. Therefore, diethylbenzenes have been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization.

There are no clinical findings or clearly positive experimental results available that demonstrate that diethylbenzenes have sensitizing effects on the skin. Likewise, as no data for respiratory tract sensitization are available, diethylbenzenes have not

been designated with “Sh” or “Sa” (for substances which cause sensitization of the skin or airways).

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completed March 22, 2017