

Toxicological information

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Administrative data

Workers - Hazard via inhalation route

Systemic effects

Long term exposure

Hazard assessment conclusion DNEL (Derived No Effect Level)

Value 77 mg/m³

Most sensitive endpoint repeated dose toxicity

DNEL related information

Overall assessment factor (AF) 3

Modified dose descriptor starting point NOAEC

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion low hazard (no threshold derived)

DNEL related information

DNEL extrapolated from long term DNEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Local effects

Long term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion DNEL (Derived No Effect Level)

Value 293 mg/m³

Most sensitive endpoint irritation (respiratory tract)

DNEL related information

Overall assessment factor (AF) 3

DNEL extrapolated from long term DNEL

Dose descriptor starting point NOAEC

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Workers - Hazard via dermal route

Systemic effects

Long term exposure

Hazard assessment conclusion DNEL (Derived No Effect Level)

Value 180 mg/kg bw/day

Most sensitive endpoint repeated dose toxicity

DNEL related information

Overall assessment factor (AF) 12

Modified dose descriptor starting point NOAEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

DNEL extrapolated from
long term DNEL



Explanation for hazard conclusion

Explanation for hazard
conclusion



[Not publishable]

Local effects

Long term exposure

Hazard assessment
conclusion

no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard
conclusion



[Not publishable]

Acute/short term exposure

Hazard assessment
conclusion

no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard
conclusion



[Not publishable]

Workers - Hazard for the eyes

Local effects

Hazard assessment conclusion low hazard (no threshold derived)

Explanation for hazard conclusion  [Not publishable]

Additional information - workers

B.5.11.3 Worker-DNEL short-term inhalation route

Peak airborne exposure to ethylbenzene vapour may cause local respiratory irritation.

B.5.11.3.1 DNEL based on local respiratory irritation

The dose descriptor is obtained from a study on 12 normal human volunteers exposed for 4 h to fluctuating concentrations of ethylbenzene. The exposure consisted of 8 peaks of 200 ppm separated by troughs of 10 ppm with an integrated mean of 98 ppm. No significant difference was found when comparing the reporting of subjective nasal irritation under these conditions with that at a constant exposure to 10 ppm. Similarly, inflammatory biomarkers in nasal secretion were not affected under these exposure conditions. In addition 12 volunteers with self-reported Multiple Chemical Sensitivity (sMCS) were investigated. Nasal irritation reported by the sMCS subjects was again not significantly increased when comparing the fluctuating exposure condition against constant exposure at 10 ppm. But reported irritation was significantly higher in sMCS subjects in comparison to the normal subjects under the high exposure conditions (van Thriel et al., 2003).

Thus the dose descriptor is the NOAEC of 98 ppm for 4 h of exposure and 200 ppm for short term exposures of 15 min. Irritation is a concentration specific effect, it is therefore not necessary to modify the dose descriptor to take account of differences in breathing rates between volunteers at rest and active workers. The starting point is therefore 200 ppm for short term exposures and 98 ppm of a prolonged duration up to several hours for the normal worker population. It is pointed out that this starting point will lead to a very conservative DNEL since the NOAEC is the highest concentration investigated. The "true" NOAEC is not known but certainly is higher.

There is a further aspect strongly indicating that the NOAEC of 98 ppm for local respiratory irritation is highly conservative: after short term exposure of volunteers (one sniff from a squeeze bottle) the odour detection threshold was in the range of 20 ppm with a broad individual variation. In contrast, anosmics that will only react to (true) trigeminal irritation reported a nasal pungency threshold of 10100 ppm (Cometto-Muniz, 1994). As in the study of van Thriel et al. (2003) subjective reporting was the endpoint recorded, this subjective reporting may not represent true sensory irritation but may be confounded by olfaction. (Problems associated with defining the threshold for olfaction are demonstrated by the recent study of Cometto-Muniz and Abraham (2009). With a more sensitive

approach as compared to the 1994-study, the odour threshold for alkylbenzenes decreased by several orders of magnitude being 6 ppb for ethylbenzene.)

Table B.1: Assessment factors and DNEL calculation for worker-DNEL short-term inhalation local effects

Uncertainties	AF	Justification
Interspecies differences	-	The starting point is obtained from human data so it is not necessary to apply a factor to take account of interspecies differences.
Intraspecies differences	3	There are no data to quantify variability in susceptibility to the irritant effects of ethylbenzene in the human population. Since irritant effects relate to the concentration at the target site it is not necessary to apply a factor to take account of toxicokinetic differences. For workers REACH (2008) proposes an assessment factor of 5. However, following a review of data on respiratory irritants in human volunteers, ECETOC (2003, 2010) concluded that variations between individuals were small. In addition, the population exposed in the workplace is highly homogeneous and the health of the work force is typically good (healthy worker effect). The analysis of assessment factors conducted by ECETOC also showed that estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states supports use of an assessment factor of 3 to account for intraspecies variability within workers. The factor of 3 proposed by ECETOC will be used for this study in humans.
Differences in duration of exposure	1	It is not necessary to apply a factor to take account of duration of exposure.
Dose response and endpoint specific/severity issues	1	The starting point is a NOAEC. In this study, normal volunteers exposed to 200 ppm for short term exposure peaks and to 98 ppm for 4 h did not report irritation. In contrast, slight irritation was reported by a selected group of sMCS persons with a high subjective sensitivity.
Quality of database	1	The quality of the database for this endpoint is adequate and the study was conducted to modern standards. It is therefore not necessary to apply a factor to take account of deficiencies in the quality of the data.
Overall assessment factor: 3		
Endpoint specific DNEL: $200/3 = 67$ ppm for short term exposure peaks (15 min) Or $98/3 = 33$ ppm for single exposures over several hours		

B.5.11.3.2 Selection of worker-DNEL short-term inhalation

A DNEL of 67 ppm was calculated for respiratory tract irritation for short-term exposures of about 15 min. For single exposures of a few hours a DNEL of 33 ppm was derived.

B.5.11.4 Worker-DNEL long-term inhalation route

For long-term repeated exposure the potential of ethylbenzene to induce ototoxicity and developmental toxicity should be discussed. For these endpoints the available human data do not provide sufficient information to allow a human dose descriptor to be identified. A dose descriptor for these endpoints can be identified from animal data. Since the dose-response relationship and evidence base for each endpoint is different it is not clear which is the critical endpoint for risk assessment of long-term repeated exposure. It will therefore be necessary to calculate separate endpoint specific DNELs for each effect to identify the critical long-term DNEL.

B.5.11.4.1 Endpoint specific DNEL for ototoxicity

Repeated inhalation exposure to ethylbenzene vapor was irreversibly ototoxic in rats (Gagnaire et al., 2007). Auditory dysfunction characterised by an elevation of hearing thresholds was localised in the mid frequencies and corresponded to the loss of cochlear outer hair cells, the sensory cells in the inner ear. Hearing loss and cell damage increased with exposure concentrations. In this 90 day rat inhalation study (6 hours/day, 6 day/week) the NOAEC for ototoxicity was extrapolated to be 114 ppm (500 mg/m³) based on destruction of outer hair cells in row 3 occurring already at 200 ppm. In contrast, no effect on hearing ability as indicated by audiometric thresholds was observed at 200 ppm. According to several case reports where hearing deficits in humans occupationally exposed to organic solvents or from people after solvent abuse are described (for review cf Risk Assessment Reports on toluene and styrene), these rat data are taken to be relevant for humans.

Thus, this extrapolated NOAEC of 114 ppm (500 mg/m³) is taken as dose descriptor for DNEL derivation of repeated dose toxicity.

The extrapolated NOAEC of 114 ppm (500 mg/m³) from the rat is (1) multiplied with a factor of 0.45 (for rat absorption percentage of 45%), divided by a divisor of 0.65 (for human absorption percentage after inhalation of 65%) and (2) multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. Further differences regarding the experimental inhalation duration (6 hours/day, 6 days/week) and the working conditions (8 hours/day, 5 days/week) are not considered, because they roughly balance each other.

The calculation results in an adjusted inhalation starting point of 232 mg/m³ (500 x 0.45/0.65 x 6.7/10).

In the overall assessment it has also be taken into consideration that the starting point to define ototoxicity is not auditory dysfunction but histopathological effects on the outer hair cells of the cochlea. Numerous investigations have shown that audiometric hearing deficits occur at higher exposure concentrations than (small) losses of hair cells in the outer row 3 of the cochlea. While the

audiometric effects define the impact of ototoxicity for workers, the use of histopathological effects for the starting point implies a further conservative factor.

Hearing loss is not a specific effect for ethylbenzene but has also been found with other aromatic solvents like styrene, toluene, or xylenes. Detailed mechanistic studies, especially with styrene, have demonstrated that ototoxicity is exerted by the unmetabolized parent chemical. Studies in rats with styrene and toluene showed that hearing loss occurs within a few days of the start of exposure and although it does not increase in severity with prolongation of exposure, the initial effect is irreversible. According to Gangnaire et al. (2007) ethylbenzene led to maximal hearing impairment after about 4 weeks without further deterioration by prolongation of exposure.

Table B.2: Assessment factors and DNEL calculation for worker DNEL long-term inhalation for ototoxicity

Uncertainties	AF	Justification
Interspecies differences	1	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an inhalation study and is being used to derive an inhalation DNEL. It is therefore not necessary to apply an allometric scaling factor to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	3	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However the population exposed in the workplace is highly homogeneous and the health of the work force is typically good (healthy worker effect) while metabolic differences due to genetic polymorphisms do not automatically require an increased assessment factor since alternative pathways of elimination are often present (ECETOC, 2003, 2010). The analysis of assessment factors conducted by ECETOC also showed that estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states supports use of an assessment factor of 3 to account for intraspecies variability within workers. The default factor of 3 for workers as proposed by ECETOC will therefore be used to take account of intraspecies variability. In total there is a combined inter- and intraspecies assessment factor of 3 that takes into account that the target tissue (outer hair cells in the cochlea, anatomical structure and blood supply of the cochlea) is identical in rats and humans. Furthermore, not hearing deficits but histopathological alterations of the cochlea were used as starting point. As histopathology is

		more sensitive than the physiological effects this is a conservative starting point per se.
Differences in duration of exposure	1	The dose descriptor was obtained from a 90-day inhalation study. Although the DNEL is to be used to assess long-term repeated exposure there is strong evidence showing that the ototoxic effects of ethylbenzene have already occurred within the first four weeks of initial exposure and did not progress in severity with further exposure. On this basis, the duration of this study is adequate for the endpoint being studied and it is not necessary to apply a factor to take account of differences in duration of exposure.
Dose response and endpoint specific/severity issues	1	As an extrapolated NOAEC for the most sensitive endpoint, i.e. histopathological alterations of cochlear hair cells, was used an additional assessment factor is not warranted. The study encompassed a range of concentrations and durations of exposure and provided reliable information on the dose-response relationship and severity of effect.
Quality of database	1	The findings from the key study are supported by findings from several additional studies with similar aromatic solvents conducted to modern regulatory standards by separate groups of researchers. Therefore it is not necessary to apply an additional factor.
Overall assessment factor: 3		
Endpoint specific DNEL: $232/3 = 77 \text{ mg/m}^3$ (8-hours) corresponding to 17.5 ppm		

B.5.11.4.2 Endpoint specific DNEL for developmental toxicity

For DNEL derivation with regard to developmental toxicity two study types can be taken into consideration: (multi) generation and prenatal toxicity studies.

B.5.11.4.2a DNEL based on (multi) generation studies

For definition of a DNEL two studies are available: A guideline 2-generation study with an indication of slight foetotoxicity (reduced foetal body weight and occasional increases in skeletal variations) in the presence of maternal toxicity with a NOAEC for foetal and maternal toxicity of 500 ppm and a preceding probe 1-generation reproduction toxicity study with a NOAEC of 100 ppm based on increased postnatal mortality and body weight gain depression in the offspring. As a general strategy, the guideline 2-generation study with a large number of experimental animals should have precedent over the probe study with a more limited number of animals. Furthermore, the specific life stages relevant for derivation of a DNEL long-term for workers have to be defined.

While exposure during pregnancy and lactation (via the milk) might occur in female workers under rare circumstances (e.g. if maternity leave is not taken), it is not possible that inhalation exposure at the workplace will occur in humans directly after weaning. Therefore, only effects found in offspring until the date of weaning should be taken into consideration to derive a long-term DNEL for workers, but not effects occurring in the offspring exposed by direct inhalation after weaning. A detailed evaluation of the probe 1-generation study leads to a NOAEC for the offspring until weaning of 500 ppm including

body weight development and postnatal mortality. This corresponds to the NOAEC of the guideline 2-generation study.

The NOAEC for developmental effects relevant for the exposure situation at the workplace is 500 ppm with an exposure schedule of 6h/d, 7 d/week. Transformation to the workplace situation (8h/d; 5d/week) would lead to 525 ppm ($500 \times 6/8 \times 7/5$), corresponding to 2300 mg/m³. This NOAEC from the rat is (1) multiplied with a factor of 0.45 (for rat absorption percentage of 45 %) and divided by 0.65 (for human absorption percentage of 65 %) and (2) multiplied by a factor of 0.67 for activity differences of respiratory volumes in workers.

The adjusted starting point is therefore 1070 mg/m³.

Table B.3: Assessment factors and DNEL calculation for worker DNEL long-term inhalation for developmental toxicity (generation studies)

Uncertainties	AF	Justification
Interspecies differences	1	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an inhalation study and is being used to derive an inhalation DNEL. It is therefore not necessary to apply an allometric scaling factor to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	3	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However the population exposed in the workplace is highly homogeneous and the health of the work force is typically good (healthy worker effect) while metabolic differences due to genetic polymorphisms do not automatically require an increased assessment factor since alternative pathways of elimination are often present (ECETOC, 2003, 2010). The analysis of assessment factors conducted by ECETOC also showed that estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states supports use of an assessment factor of 3 to account for intraspecies variability within workers. The default factor of 3 for workers as proposed by ECETOC will therefore be used to take account of intraspecies variability.
Differences in duration of exposure	1	The NOAEC was derived from the probe 1-generation and the 2-generation study starting from exposure of young adult animals of the F0 generation up to weaning of the F1 generation. This

		represents a worst case scenario for workers being exposed from the start of their working life, during pregnancy up to the end of the breast feeding period without maternity leave. An assessment factor of 1 is therefore appropriate.
Dose response and endpoint specific/severity issues	1	As 500 ppm was a clear NOAEC for developmental effects in F1 offspring before weaning and start of direct inhalation exposure, an assessment factor is not warranted in this respect.
Quality of database	1	The key study was conducted to modern regulatory standards and was adequately reported. On this basis the quality of the database is not considered to contribute uncertainty and it is therefore not necessary to apply an additional factor.

Overall assessment factor: 3

Endpoint specific DNEL: $1070/3 = 357 \text{ mg/m}^3$ (8-hour) corresponding to 81 ppm

B.5.11.4.2b DNEL based on prenatal toxicity studies

The derivation of the DNEL is based on the prenatal toxicity study in rats of Saillenfait et al. (2003) at exposure concentrations of 100, 500, 1000, and 2000 ppm during gestation days 6-20 with a NOAEC of 500 ppm for an exposure regime of 6 h/d throughout gestation days 6-20.

This NOAEC has to be adjusted by a factor of 6/8 to account for different daily exposure durations leading to 375 ppm. The dams were exposed every day during pregnancy and not at 5 d/week, as would have been workers. Due to the short duration of pregnancy in rats this fact is not taken into account by a factor of 7/5, leading to a more conservative DNEL derivation. The NOAEC of 375 ppm from the rat is (1) multiplied with a factor of 0.45 (for rat absorption percentage of 45 %) and divided by 0.65 (for human absorption percentage of 65 %) and (2) multiplied by a factor of 0.67 for activity differences of respiratory volumes in workers.

The adjusted starting point is therefore 174 ppm.

Table B.4: Assessment factors and DNEL calculation for worker DNEL long-term inhalation for developmental toxicity (prenatal toxicity studies)

Uncertainties	AF	Justification
Interspecies differences	1	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an inhalation study and is being used to derive an inhalation DNEL. It is therefore not necessary to apply an allometric scaling factor to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	3	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human

		<p>population. However the population exposed in the workplace is highly homogeneous and the health of the work force is typically good (healthy worker effect) while metabolic differences due to genetic polymorphisms do not automatically require an increased assessment factor since alternative pathways of elimination are often present (ECETOC, 2003, 2010). The analysis of assessment factors conducted by ECETOC also showed that estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states supports use of an assessment factor of 3 to account for intraspecies variability within workers.</p> <p>The default factor of 3 for workers as proposed by ECETOC will therefore be used to take account of intraspecies variability.</p>
Differences in duration of exposure	1	The NOAEC was derived from a prenatal toxicity study with an exposure regime from gestation day 6 up to the end of pregnancy at gestation day 20. An assessment factor of 1 is therefore appropriate.
Dose response and endpoint specific/severity issues	1	As 500 ppm was a clear NOAEC for developmental effects, an assessment factor is not warranted in this respect.
Quality of database	1	The key study was conducted to modern regulatory standards and was adequately reported. On this basis the quality of the database is not considered to contribute uncertainty and it is therefore not necessary to apply an additional factor.
Overall assessment factor: 3		
Endpoint specific DNEL: $174/3 = 58$ ppm (8-hour) corresponding to 255 mg/m ³		

B.5.11.4.3 Selection of worker-DNEL long-term inhalation

The most sensitive endpoint-specific DNEL is that for ototoxicity. This endpoint is relevant for workers. So the endpoint specific DNEL for ototoxicity is identified as the worker-DNEL long-term inhalation.

The worker DNEL long-term inhalation route is 17.5 ppm (8-hr TWA).

B.5.11.5 Worker-DNEL long-term dermal route

No information is available for systemic toxicity after repeated dermal exposure. Therefore the extrapolated NOAEC of 114 ppm (500 mg/m³) based on ototoxicity from the 90 day inhalation rat study is taken for the dermal route, ototoxicity being the critical health effect leading to the lowest worker-DNEL long-term by inhalation.

Expressed as (external) dose the value of 500 mg/m³ corresponds to 190 mg/kg/day (500 mg/m³ x default respiratory volume for the rat for 8 hours of 0.38 m³/kg). With a rat adsorption percentage of 45% after inhalation the internal starting point corresponds to 86 mg/kg/day (190 mg/kg/day x 0.45).

It is necessary to convert this internal inhalative dose to an equivalent external dermal dose. There are several studies investigating dermal penetration of ethylbenzene but most of them have severe deficits, either using preparations not relevant for the possible exposure scenarios of ethylbenzene or not allowing for evaporation after dermal contact. The OECD test guideline for skin absorption studies describes a device that should be used for volatile chemicals. Only Susten et al. (1990) used an experimental design similar to that described by OECD. With hairless mice they determined a dermal penetration rate of 3.61%. It is generally recognized that the skin barrier in hairless mice is less efficient than that of humans. Therefore a penetration rate of 4% as used in this calculation is on the conservative side.

With a penetration rate of 4% the internal inhalation dose corresponds to an external dermal dose of $86 \times 100/4 = 2150 \text{ mg/kg/d}$.

Table B.5: Assessment factors and DNEL calculation for worker DNEL long-term dermal for ototoxicity

Uncertainties	AF	Justification
Interspecies differences	4	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The starting point is derived from the internal rat dose in mg/kg/d. Therefore, the allometric scaling factor of 4 is used to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	3	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However the population exposed in the workplace is highly homogeneous and the health of the work force is typically good (healthy worker effect) while metabolic differences due to genetic polymorphisms do not automatically require an increased assessment factor since alternative pathways of elimination are often present (ECETOC, 2003, 2010). The analysis of assessment factors conducted by ECETOC also showed that estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states supports use of an assessment factor of 3 to account for intraspecies variability within workers. The default factor of 3 for workers as proposed by ECETOC will therefore be used to take account of intraspecies variability. In total there is a combined inter- and intraspecies assessment factor of 12 that takes into account that the target tissue (outer hair cells in the cochlea, anatomical structure and blood supply of the cochlea) is identical in rats and humans. Furthermore, not

		hearing deficits but histopathological alterations of the cochlea were used as starting point. As histopathology is more sensitive than the physiological effects this is a conservative starting point per se.
Differences in duration of exposure	1	The dose descriptor was obtained from a 90-day inhalation study. Although the DNEL is to be used to assess long-term repeated exposure there is strong evidence showing that the ototoxic effects of ethylbenzene have already occurred within the first four weeks of initial exposure and did not progress in severity with further exposure. On this basis, the duration of this study is adequate for the endpoint being studied and it is not necessary to apply a factor to take account of differences in duration of exposure.
Dose response and endpoint specific/severity issues	1	As an extrapolated NOAEC for the most sensitive endpoint, i.e. histopathological alterations of cochlear hair cells, was used an additional assessment factor is not warranted. The study encompassed a range of concentrations and durations of exposure and provided reliable information on the dose-response relationship and severity of effect.
Quality of database	1	The findings from the key study are supported by findings from several additional studies with similar aromatic solvents conducted to modern regulatory standards by separate groups of researchers. Therefore it is not necessary to apply an additional factor.
Overall assessment factor: 12		
Endpoint specific DNEL: $2150/12 = 180 \text{ mg/kg/d}$ (8-hours) corresponding to 12500 mg/person/d		

The worker DNEL long-term dermal route for systemic effects is 180 mg/kg/d. As a skin irritation DNEL is not quantifiable from the data this DNEL does not address the potential for local irritation.

General Population - Hazard via inhalation route

Systemic effects

Long term exposure

Hazard assessment
conclusion

DNEL (Derived No Effect Level)

Value 15 mg/m³

Most sensitive endpoint repeated dose toxicity

DNEL related information

Overall assessment factor (AF) 5

Modified dose descriptor starting point NOAEC

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion low hazard (no threshold derived)

DNEL related information

DNEL extrapolated from long term DNEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Local effects

Long term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

DNEL extrapolated from long term DNEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

General Population - Hazard via dermal route

Systemic effects

Long term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

DNEL extrapolated from long term DNEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Local effects

Long term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

General Population - Hazard via oral route

Systemic effects

Long term exposure

Hazard assessment conclusion DNEL (Derived No Effect Level)

Value 1.6 mg/kg bw/day

Most sensitive endpoint repeated dose toxicity

DNEL related information

Overall assessment factor (AF) 40

Modified dose descriptor starting point NOAEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

Acute/short term exposure

Hazard assessment conclusion no hazard identified

DNEL related information

DNEL extrapolated from long term DNEL

Explanation for hazard conclusion

Explanation for hazard conclusion  [Not publishable]

General Population - Hazard for the eyes

Local effects

Hazard assessment conclusion low hazard (no threshold derived)

Explanation for hazard conclusion  [Not publishable]

Additional information - General Population

B.5.11.6 General population DNEL long-term inhalation by exposure via the environment

As discussed in section B.5.11.4 long-term repeated exposure to ethylbenzene has the potential to cause ototoxicity and developmental toxicity.

For ototoxicity:

Repeated inhalation exposure to ethylbenzene vapor was irreversibly ototoxic in rats (Gagnaire et al., 2007). Auditory dysfunction characterised by an elevation of hearing thresholds was localised in the mid frequencies and corresponded to the loss of cochlear outer hair cells, the sensory cells in the inner ear. Hearing loss and cell damage increased with exposure concentrations. In this 90 day rat inhalation study (6 hours/day, 6 day/week) the NOAEC for ototoxicity was extrapolated to be 114 ppm (500 mg/m³) based on destruction of outer hair cells in row 3 occurring already at 200 ppm. In contrast, no effect on hearing ability as indicated by audiometric thresholds was observed at 200 ppm. According to several case reports where hearing deficits in humans occupational exposed to organic solvents or from people after solvent abuse are described (for review cf Risk Assessment Reports on toluene and styrene), this rat data are taken to be relevant for humans.

Thus, this extrapolated NOAEC of 114 ppm (500 mg/m³) is taken as dose descriptor for DNEL derivation of repeated dose toxicity for general population (consumer).

The extrapolated NOAEC of 114 ppm (500 mg/m³) from the rat is (1) multiplied with a factor of 0.45 (for rat absorption percentage of 45%), divided by a divisor of 0.65 (for human absorption percentage after inhalation of 65%). A factor for activity-driven differences of respiratory volumes is not necessary for the general population (via environment). Further differences regarding the experimental inhalation duration (6 hours/day, 6 days/week) and the environmental exposure conditions (24 hours/day, 7 days/week) have to be taken into consideration by factors of 6/24 and 6/7.

The calculation results in an adjusted inhalation starting point of
 $114 \times 0.45 : 0.65 \times 6/24 \times 6/7 = 17\text{ppm}$

In the overall assessment it has also be taken into consideration that the starting point to define ototoxicity is not auditory dysfunction but histopathological effects on the outer hair cells of the cochlea. Numerous investigations have shown that audiometric hearing deficits occur at higher exposure concentrations than (small) losses of hair cells in the outer row 3 of the cochlea. While the

audiometric effects define the impact of ototoxicity for humans, the use of histopathological effects for the starting point implies a further conservative factor.

Hearing loss is not a specific effect for ethylbenzene but has also been found with other aromatic solvents like styrene, toluene, or xylenes. Detailed mechanistic studies, especially with styrene, have demonstrated that ototoxicity is exerted by the unmetabolized parent chemical. Studies in rats with styrene and toluene showed that hearing loss occurs within a few days of the start of exposure and although it does not increase in severity with prolongation of exposure, the initial effect is irreversible. According to Gagnaire et al. (2007) ethylbenzene led to maximal hearing impairment after about 4 weeks without further deterioration by prolongation of exposure.

Table B.6: Assessment factors and DNEL calculation for general population (consumer) (DIY) DNEL long-term inhalation for ototoxicity

Uncertainties	AF	Justification
Interspecies differences	1	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an inhalation study and is being used to derive an inhalation DNEL. It is therefore not necessary to apply an allometric scaling factor to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However an analysis of assessment factors conducted by ECETOC (2003, 2010) showed that metabolic differences due to genetic polymorphisms do not to automatically require an increased assessment factor since alternative pathways of elimination are often present. This consideration, together with estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states, supports use of an assessment factor of 5 to account for intraspecies variability within the general population. The default factor of 5 for the general population as proposed by ECETOC (2003, 2010) will therefore be used to take account of intraspecies variability. In total there is a combined inter- and intraspecies assessment factor of 5 that takes into account that the target tissue (outer hair cells in the cochlea, anatomical structure and blood supply of the cochlea) is identical in rats and humans. Furthermore, not hearing deficits but histopatological alterations of the cochlea were used as starting point. As histopathology is more sensitive

		than the physiological effects this is a conservative starting point per se.
Differences in duration of exposure	1	The dose descriptor was obtained from a 90-day inhalation study. Although the DNEL is to be used to assess long-term repeated exposure there is strong evidence showing that the ototoxic effects of ethylbenzene have already occurred within the first four weeks of initial exposure and did not progress in severity with further exposure. On this basis, the duration of this study is adequate for the endpoint being studied and it is not necessary to apply a factor to take account of differences in duration of exposure.
Dose response and endpoint specific/severity issues	1	As an extrapolated NOAEC for the most sensitive endpoint, i.e. histopathological alterations of cochlear hair cells, was used an additional assessment factor is not warranted. The study encompassed a range of concentrations and durations of exposure and provided reliable information on the dose-response relationship and severity of effect.
Quality of database	1	The findings from the key study are supported by findings from several additional studies with similar aromatic solvents conducted to modern regulatory standards by separate groups of researchers. Therefore it is not necessary to apply an additional factor.
Overall assessment factor: 5		
Endpoint specific DNEL: $17/5 = 3.4$ ppm (24-hours) corresponding to 15 mg/m ³ .		

For derivation of a worker-DNEL long-term based on reproductive effects in the a multi-generation study, effects observed for the time between weaning and puberty did not apply. On the other hand, effects occurring during pre- and postnatal phases have to be taken into account for exposures via the environment.

According to section B.5.11.4.2a for definition of a DNEL two studies are available: A guideline 2-generation study with an indication of slight foetotoxicity (reduced foetal body weight and occasional increases in skeletal variations) in the presence of maternal toxicity with a NOAEC for foetal and maternal toxicity of 500 ppm and a preceding probe 1-generation reproduction toxicity study with a NOAEC of 100 ppm based on increased postnatal mortality and body weight gain depression in the offspring. This NOAEC of 100 ppm was governed by effects occurring after weaning when the offspring was directly exposed by inhalation. As a general strategy, the guideline 2-generation study with a large number of experimental animals should have precedence over the probe study with a more limited number of animals. But in order to derive a DNEL as conservative as possible for the general population exposed via the environment, the NOAEC of the probe 1-generation study will be used as starting point.

In this probe reproductive toxicity study rats were exposed for 6 hours per day, 7 days per week. Since animals were exposed for 6 hours per day, whereas the exposure via the environment is continuous, it is necessary to adjust the starting point by a factor of 6/24. This NOAEC of 100 ppm from the rat is further multiplied with a factor of 0.45 (for rat absorption percentage of 45 %) and divided by 0.65 (for human absorption percentage of 65 %). An adjustment for physical activity is not necessary.

The corrected starting point is therefore

$$100 \times 6/24 \times 0.45/0.65 = 17.3 \text{ ppm.}$$

Table B.7: Assessment factors and calculation for general population DNEL long-term inhalation via environment for developmental toxicity based on generation studies

Uncertainties	AF	Justification
Interspecies differences	1	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an inhalation study and is being used to derive an inhalation DNEL. It is therefore not necessary to apply an allometric scaling factor to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However an analysis of assessment factors conducted by ECETOC (2003, 2010) showed that metabolic differences due to genetic polymorphisms do not to automatically require an increased assessment factor since alternative pathways of elimination are often present. This consideration, together with estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states, supports use of an assessment factor of 5 to account for intraspecies variability within the general population. The default factor of 5 for humans via environment as proposed by ECETOC will therefore be used to take account of intraspecies variability.
Differences in duration of exposure	1	The NOAEC was derived from the 2-generation study covering the whole reproductive cycle. An assessment factor of 1 is therefore appropriate.
Dose response and endpoint specific/severity issues	1	The NOAEC of 100 ppm was derived from generation studies covering the whole reproductive cycle. Therefore no additional assessment factor is necessary.
Quality of database	1	The key study was conducted to modern regulatory standards and was adequately reported. On this basis the quality of the database is not considered to contribute uncertainty and it is therefore not necessary to apply an additional factor.
Overall assessment factor: 5		
Endpoint specific DNEL: $17.3/5 = 3.5 \text{ ppm}$		

For prenatal effects the derivation of the DNEL is based on the prenatal toxicity study in rats of Saillenfait et al. (2003) at exposure concentrations of 100, 500, 1000, and 2000 ppm during gestation days 6-20 with a NOAEC of 500 ppm for an exposure regime of 6 h/d throughout gestation days 6-20.

This NOAEC has to be adjusted by a factor of 6/24 to account for different daily exposure durations leading to 125 ppm. The dams were exposed every day during pregnancy. The NOAEC of 125 ppm from the rat is further multiplied with a factor of 0.45 (for rat absorption percentage of 45 %) and divided by 0.65 (for human absorption percentage of 65 %).

The adjusted starting point is therefore 86.5 ppm.

Table B.8: Assessment factors and DNEL calculation for general population (via environment) DNEL long-term inhalation for developmental toxicity (prenatal toxicity studies)

Uncertainties	AF	Justification
Interspecies differences	1	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an inhalation study and is being used to derive an inhalation DNEL. It is therefore not necessary to apply an allometric scaling factor to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However an analysis of assessment factors conducted by ECETOC (2003, 2010) showed that metabolic differences due to genetic polymorphisms do not automatically require an increased assessment factor since alternative pathways of elimination are often present. This consideration, together with estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states, supports use of an assessment factor of 5 to account for intraspecies variability within the general population. The default factor of 5 for the general population as proposed by ECETOC will therefore be used to take account of intraspecies variability.
Differences in duration of exposure	1	The NOAEC was derived from a prenatal toxicity study with an exposure regime from gestation day 6 up to the end of pregnancy at gestation day 20. An assessment factor of 1 is therefore appropriate.
Dose response and endpoint	1	As 500 ppm was a clear NOAEC for developmental effects, an assessment factor is not warranted in this respect.

specific/severity issues		
Quality of database	1	The key study was conducted to modern regulatory standards and was adequately reported. On this basis the quality of the database is not considered to contribute uncertainty and it is therefore not necessary to apply an additional factor.
Overall assessment factor: 5		
Endpoint specific DNEL: $174/5 = 17.3$ ppm (8-hour) corresponding to 76 mg/m^3		

B.5.11.6h. Selection of DNEL long-term inhalation for the general population (via environment)

In conclusion a DNEL long-term for the general population by exposure via the environment of 3.4 ppm is proposed based on ototoxic effects. This DNEL is very similar to that derived from toxic effects observed in generation studies being 3.5 ppm.

B.5.11.7 General population (via environment) DNEL long-term oral route

For deriving a DNEL long-term oral route for the general population (via environment) two approaches should be considered:

starting from the lowest DNEL long-term inhalation route (ref section B.5.11.7b) for the general population (via environment)

starting from the rat 90-day oral study (Mellert et al., 2007) thereby obviating a route-to-route extrapolation.

a) starting from the DNEL long-term inhalation route for the general population (via environment)

The lowest general population DNEL long-term for the inhalation route via environment was calculated to be 3.4 ppm, corresponding to 15 mg/m^3 (see section B.5.11.7.b) based on ototoxicity. A respiratory volume of $20 \text{ m}^3/\text{person/d}$ is used for the general population (ECHA, 2008) and an uptake by inhalation of 65%. This leads to an internal body burden of $15 \times 20 \times 0.65 = 195 \text{ mg/person/d}$, corresponding to 2.8 mg/kg/d .

After oral exposure up to 92% of the ethylbenzene dose could be recovered as urinary metabolites in rabbits (El Masry et al., 1952) and 84% in urine and feces of rats (Climie et al., 1983); therefore 100% absorption by oral exposure is taken for humans as default.

Thus, for the general population (via environment) the DNEL long-term oral derived from that by inhalation is 2.8 mg/kg/d .

b) starting from the rat 90-day oral study

An increase in liver and kidney weights of rats and mice without histopathological alterations has been found in several studies. These changes are most probably related to enzyme induction. The NOAEL in a guideline oral 90 day study with rats was 75 mg/kg bw/d (LOAEL 250 mg/kg bw/d) based on indications for a mild regenerative anemia and liver changes indicative of microsomal enzyme induction.

The animals were dosed daily by gavage. A 84% oral absorption is used for rats (Climie et al., 1983) and 100% for humans as conservative default leading to an internal dose of $75 \times 0.84 = 63 \text{ mg/kg/d}$ as starting point.

Table B.9: Assessment factors and DNEL calculation for the general population (via environment) long-term oral (based on rat oral study)

Uncertainties	AF	Justification
Interspecies differences	4	For interspecies variability the assessment factors proposed by ECETOC (2003, 2010) are used. The dose descriptor was obtained from an oral study and is being used to derive an inhalation DNEL. Therefore an allometric scaling factor of 4 is used to take account of differences in basal metabolic rates between animals and humans. According to ECETOC the additional assessment factor of 2.5 to quantify other differences between animals and humans that could affect interspecies extrapolation is scientifically not justified and a factor of 1 is appropriate. On this basis the default factor of 1 to account for other species differences will be applied.
Intraspecies differences	5	There are no data to quantify variability in susceptibility to the effects of long-term exposure to ethylbenzene in the human population. However an analysis of assessment factors conducted by ECETOC (2003, 2010) showed that metabolic differences due to genetic polymorphisms do not to automatically require an increased assessment factor since alternative pathways of elimination are often present. This consideration, together with estimates of the upper 95th percentile of the distribution of variability in toxicokinetic and toxicodynamic parameters for populations of different ages, genders and disease states, supports use of an assessment factor of 5 to account for intraspecies variability within the general population. The default factor of 5 for the general population as proposed by ECETOC will therefore be used to take account of intraspecies variability.
Differences in duration of exposure	2	The NOAEL was obtained from a 90-day oral rat study. For extrapolation to chronic exposure an assessment factor of 2 is therefore appropriate according to REACH (2008) and ECETOC (2003, 2010).
Dose response and endpoint specific/severity issues	1	As 75 mg/kg/d was a clear NOAEL with a dose response relationship for toxicologically relevant effects at higher doses, an assessment factor is not warranted in this respect.
Quality of database	1	The key study was conducted to modern regulatory standards and was adequately reported. On this basis the quality of the database is not considered to contribute uncertainty and it is therefore not necessary to apply an additional factor.
Overall assessment factor: 40		

Endpoint specific DNEL: $63/40 = 1.6$ mg/kg/d

In conclusion, the DNEL long-term for the general population (via environment) by oral exposure is 1.6 mg/kg/d.