



**Ministry of Environment  
and Food of Denmark**

Environmental  
Protection Agency

# Toluene

Evaluation of health hazards and proposal  
of health based quality criteria for  
drinking water and soil

Environmental Project No. 1874, 2016

**Title:**

Toluene

**Authors:**

Poul Bo Larsen  
Beata Farkas  
Helle Buchardt Boyd

**Published by:**

The Danish Environmental Protection Agency  
Strandgade 29  
DK-1401 Copenhagen K  
[www.mst.dk](http://www.mst.dk)

**Year:**

2016

**ISBN no.**

978-87-93435-93-3

**Disclaimer:**

The Danish Environmental Protection Agency publishes reports and papers about research and development projects within the environmental sector, financed by the Agency. The contents of this publication do not necessarily represent the official views of the Danish Environmental Protection Agency. By publishing this report, the Danish Environmental Protection Agency expresses that the content represents an important contribution to the related discourse on Danish environmental policy.

Sources must be acknowledged.

# Contents

<b>Contents.....</b>	<b>3</b>
<b>Preface .....</b>	<b>6</b>
<b>1. General description.....</b>	<b>7</b>
1.1 Identity and physical-chemical properties .....	7
1.2 Physical and chemical properties .....	7
1.3 Production and use .....	8
1.4 Environmental occurrence and environmental fate.....	9
1.4.1 Air .....	9
1.4.2 Soil .....	9
1.4.3 Water .....	10
1.4.4 Foodstuffs.....	10
1.4.5 Biodegradation and bioaccumulation .....	10
1.5 Human Exposure .....	10
<b>2. Toxicokinetics .....</b>	<b>12</b>
2.1 Absorption.....	12
2.1.1 Inhalation exposure .....	12
2.1.2 Oral exposure .....	12
2.1.3 Dermal exposure .....	12
2.2 Distribution .....	13
2.3 Metabolism and elimination .....	13
2.4 Mode of action.....	14
<b>3. Human toxicity.....</b>	<b>16</b>
3.1 Single dose toxicity .....	16
3.1.1 Inhalation exposure .....	16
3.1.2 Oral exposure .....	16
3.1.3 Dermal exposure .....	17
3.2 Irritation and sensitisation .....	17
3.2.1 Irritation.....	17
3.2.2 Sensitisation .....	17
3.3 Repeated dose toxicity .....	17
3.3.1 Inhalation .....	17
3.3.2 Oral exposure .....	20
3.3.3 Dermal exposure .....	20

3.4	Toxicity to reproduction .....	20
3.4.1	Inhalation .....	20
3.4.2	Oral exposure .....	20
3.5	Mutagenicity .....	20
3.6	Carcinogenicity .....	21
<b>4.</b>	<b>Animal toxicity .....</b>	<b>22</b>
4.1	Single dose toxicity .....	22
4.1.1	Inhalation exposure .....	22
4.1.2	Oral exposure .....	22
4.1.3	Dermal exposure .....	22
4.2	Irritation and sensitisation .....	22
4.2.1	Irritation .....	22
4.2.2	Sensitisation .....	23
4.3	Repeated dose toxicity .....	23
4.3.1	Inhalation .....	23
4.3.2	Oral exposure .....	23
4.3.3	Dermal exposure .....	26
4.4	Toxicity to reproduction .....	26
4.4.1	Fertility .....	26
	<i>Inhalation</i> .....	26
4.4.2	Development .....	27
4.4.3	Oral exposure .....	29
4.5	Mutagenicity .....	29
4.5.1	<i>In vitro</i> studies .....	29
4.5.2	<i>In vivo</i> studies .....	30
4.6	Carcinogenicity .....	30
<b>5.</b>	<b>Regulations .....</b>	<b>32</b>
5.1	Ambient air .....	32
5.2	Drinking water/ groundwater .....	32
5.3	Soil .....	32
5.4	Occupational Exposure limits .....	33
5.5	Classification .....	33
5.6	IARC .....	33
5.7	Tolerable daily intake .....	33
5.7.1	US EPA (2005), reference dose/concentration .....	33
5.7.2	Health Canada (2014) TDI .....	34
5.7.3	WHO (2004), TDI .....	34
<b>6.</b>	<b>Summary and evaluation .....</b>	<b>35</b>
6.1	Description .....	35

6.2	Environment .....	35
6.3	Human exposure.....	35
6.4	Toxicokinetics .....	36
6.4.1	Single dose toxicity .....	36
6.4.2	Irritation and sensitisation .....	36
6.4.3	Repeated dose toxicity .....	36
6.4.4	Toxicity to reproduction .....	37
6.4.5	Mutagenicity .....	37
6.4.6	Carcinogenicity .....	38
6.5	Animal toxicity .....	38
6.5.1	Single dose toxicity .....	38
6.5.2	Irritation and sensitisation .....	38
6.5.3	Repeated dose toxicity .....	38
6.5.4	Toxicity to reproduction .....	39
6.5.5	Mutagenicity .....	39
6.5.6	Carcinogenicity .....	40
6.6	Evaluation, critical effects NOAELs/LOAELs .....	40
<b>7.</b>	<b>TDI and quality criteria .....</b>	<b>41</b>
7.1	TDI.....	41
7.2	Allocation .....	41
7.3	Quality criterion in drinking water .....	42
7.4	Quality criterion in soil .....	42
7.5	Conclusion, quality criteria .....	43
<b>8.</b>	<b>References .....</b>	<b>44</b>
<b>9.</b>	<b>Appendix 1.....</b>	<b>47</b>

# Preface

This report has been prepared by Poul Bo Larsen, Beata Farkas and Helle Buchardt Boyd, DHI.

The Danish EPA has requested a documentation document for health-based quality criteria for toluene in drinking water and in soil, as the current legislation on drinking water requires testing for toluene if the soil in the water capture zones is polluted with aromates.

The report has been elaborated based on existing expert assessments of toluene, and has been elaborated according to the general practice laid down in the Danish EPA guidance document for the setting of health-based quality criteria for chemical substances in relation to soil, ambient air and drinking water (Vejledning fra Miljøstyrelsen 5/2006).

The report has been subjected to review and written commenting by a steering committee with representatives from the following Danish authorities / institutions:

Danish Health Authority

The Danish Nature Agency

The Danish Veterinary and Food Administration

Danish Regions

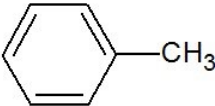
Danish Environmental Protection Agency

# 1. General description

## 1.1 Identity and physical-chemical properties

The name and other identifiers of toluene are given below, Table 1.

**TABLE 1**  
NAME AND OTHER IDENTIFIERS OF TOLUENE (DEPA 2014)

Substance name	Toluene
EC number	203-625-9
CAS number	108-88-3
Synonyms	-
Molecular formula	C <sub>7</sub> H <sub>8</sub>
Structure	
Molecular weight (g/mole)	94.12

## 1.2 Physical and chemical properties

The physical and chemical properties of toluene are shown in Table 2. The listed properties mainly refer to the registration dossiers available at ECHA's website. The registration dossiers may include different values for the same parameter; in this case a range is indicated.

**TABLE 2**  
**PHYSICAL AND CHEMICAL PROPERTIES OF TOLUENE [DEPA 2014; EU-RAR 2003]**

Property	Toluene
Physical state	Colourless liquid
Melting point at 1013 hPa (°C)	-95
Freezing point (°C)	-
Boiling point at 1013 hPa (°C)	110.6
Property	Toluene
Relative density at 20°C (g/cm <sup>3</sup> )	0.87
Vapour pressure	3000 Pa at 20 °C
Surface tension at 25 °C (mN/m)	27.73
Water solubility at 25 °C and pH 7 (mg/L)	573 - 580
Log P (octanol/water) at 20 °C	2.73
Concentration in air	1 ppm $\cong$ 3.83 mg/m <sup>3</sup> at 20 °C and 1013 hPa

Toluene has a pungent odour with an odour threshold in air of 0.64 to 139 mg/m<sup>3</sup>. The odour threshold in water was found to be in the range of 0.024–0.17 mg/l, and the reported taste threshold ranged from 0.04 to 0.12 mg/l (WHO 2004).

### 1.3 Production and use

Toluene is both a constituent of crude oil and a component of the condensate from natural gas production. Thus, it is synthesised together with many other substances in petroleum refinery and chemical plant processes, primarily by catalytic reforming, steam cracking, and dealkylation. Toluene is also recovered during the production of coal-derived chemicals, primarily from coke oven by-products. Part of the toluene recovered during production of coal-derived chemicals is purified for production of commercial grade toluene (DEPA 2014).

The main applications of commercial toluene are as raw materials and auxiliaries in the chemical industry and as solvents in many applications including paints, textile coatings, printing industry, etc.

In Denmark, the use as a solvent is the most significant use of toluene. The consumption of toluene in Denmark accounted about 3 300 tons (SPIN database 2013). Furthermore, toluene is registered under REACH in the tonnage band 1-10 million t/year (DEPA 2014).

Toluene is a constituent of the various fuel streams from petrochemical refining and is a significant constituent in petrol with an average content in EU of 11 % (UKEA 2009b).



## 1.4 Environmental occurrence and environmental fate

### 1.4.1 Air

In ambient air in Denmark, measurements reported in 2011/12 indicate average levels of 3.4-3.6  $\mu\text{g}/\text{m}^3$  of toluene at busy roads in Copenhagen, while the urban background levels were in the range of 1.3-1.6  $\mu\text{g}/\text{m}^3$  (DEPA 2014).

In ambient air, photo-oxidation of toluene takes place rather rapidly, primarily due to reaction with hydroxyl radicals. An experimental half-life of 1.3 days is reported while the half-life calculated with the AOPWIN model was approx. 2 days (EU-RAR 2003/ DEPA 2014).

Geiss et al. (2011) in the European AIRMEX project measured 14 VOC substances including toluene in outdoor and indoor environment in 11 European cities. The measurements were performed outdoor, in schools and public buildings and in homes. Furthermore, personal borne measurements were made. The measured levels of toluene are given in Table 3.

**TABLE 3**  
**MEASURED TOLUENE LEVELS IN OUTDOOR AND INDOOR AIR. DATA FROM THE AIRMEX STUDY GEISS ET AL. (2011)**

	Outdoor $\mu\text{g}/\text{m}^3$	Schools & Publ. Buildings $\mu\text{g}/\text{m}^3$	Homes $\mu\text{g}/\text{m}^3$	Person borne measurement $\mu\text{g}/\text{m}^3$
<b>Toluene, Median level</b>	4.8	7.1	6.5	11.7
<b>Toluene, 95- percentile</b>	33.2	47.6	28.4	55.3

Geiss et al. (2011) noted that especially measurements from the southern parts of Europe influenced the figures as the values from the northern parts of Europe showed considerably lower levels compared to the levels in the southern parts of Europe.

Inside cars, toluene levels of 12 – 101  $\mu\text{g}/\text{m}^3$  were measured depending on driving conditions and temperature (Fedoruk & Kerger 2003).

Inside new cars, a toluene level of 55  $\mu\text{g}/\text{m}^3$  has been measured as a mean level for 5 cars before use (Faber et al. 2014).

### 1.4.2 Soil

The sources for soil and ground water pollution with toluene are usually leakage of fuel from tanks buried in the soil or from other types of hot spot pollutions with fuels or other petrochemical solvents or in connection with old gas plants.

In soil, experimental half-lives for degradation of toluene in the range 83-92 days have been found, but values of a few days are also reported. The EU-RAR (2003) conservatively uses a half-life in soil of 90 days in the risk assessment. No anaerobic degradation half-life has been determined for toluene in sediments, but 34-49 % anaerobic degradation in 2 weeks has been observed at high concentrations of the substance (EU-RAR 2003; DEPA 2014).

An estimated  $K_{oc}$  in soil of 177, based on the Log  $P_{ow}$  value, indicates that toluene has a relatively high mobility in soil (EU-RAR 2003; DEPA 2014).

### 1.4.3 Water

In rain water in Denmark an average toluene level of 0.12 µg/l has been measured (DEPA 2014).

Toluene is often detected in groundwater within the Danish GRUMO survey. In 184 of 2637 samples, toluene was found in the groundwater with a median level of 0.1 µg/l and a maximum level of 2.4 µg/l (Juhler & Felding 2001).

In 2013, GEUS reported that 209 of 908 samples of groundwater contained toluene of which only 1 sample exceeded the drinking water criterion (GEUS 2013). (The values were not indicated, but presumably the drinking water criterion referred to is the ground water criterion for toluene of 5 µg/l).

The volatilisation of toluene from water, as well as from soil surfaces, takes place fast. From surface water the half-life is typically in the order of hours (but depends on water depth, mixing and temperature); a half-life of 4.9 hours has been reported from the surface of a sandy soil with low organic carbon content (EU-RAR 2003/ DEPA 2014)).

Hydrolysis of toluene in water does not take place as the substance does not possess hydrolysable groups. Photolysis in water is regarded as a marginal fate process for toluene as only 8.4 % degradation was found after 17 hours of irradiation at >290 nm (EU-RAR 2003; DEPA 2014)).

### 1.4.4 Foodstuffs

WHO (2004) indicated that levels of toluene in food are very low (without indicating any figures) and the exposure from food is negligible compared to other exposure sources for the substance.

### 1.4.5 Biodegradation and bioaccumulation

Toluene has demonstrated to be readily biodegradable in standard tests. However, the rate of degradation becomes significantly lower at lower concentrations relevant for the environment and if other carbon sources are not available. Thus, slow degradation of toluene in water was observed at concentrations below 31 µg/l if no other carbon sources were present, while 0.9 µg/l degraded to below 0.002 µg/l in 8 days when other such sources were present (EU-RAR 2003). In the EU-RAR (2003), a half-life of 30 days for the aquatic risk assessment is used (DEPA 2014).

An experimental bioconcentration factor (BCF) for fish (golden ide, *Leuciscus idus*) of 90 days has been determined experimentally together with an elimination half-life of less than 2 days, while a BCF = 36 has been calculated based on the Log Pow of 2.7 (ECB, 2003). These values, of which the former is used in the EU risk assessment, indicate a rather low bioaccumulation potential of toluene (DEPA 2014).

## 1.5 Human Exposure

In general, indoor environment and transport in cars can be considered the major sources of toluene exposure for the general population.

Using data from person borne measurements, a 24h average exposure to 11.7 µg/m<sup>3</sup> (median level from 11 European cities) would result in a daily exposure of 234 µg toluene (or 3.3 µg/kg/d) for an adult person (70 kg bw) inhaling 20 m<sup>3</sup> of air per day.

In addition to this “background” exposure level of toluene, exposure may occur in connection with the use of various chemical products containing toluene, e.g. paints/varnishes, stain removers, surface coating, glues, dyes, nail polish etc. The exposure levels connected to these uses will vary

much depend on amount used, toluene concentration in the product, conditions of use (e.g. spray application, room size, ventilation etc.).

## 2. Toxicokinetics

### 2.1 Absorption

#### 2.1.1 Inhalation exposure

The major uptake of toluene vapour is through the respiratory system. A number of investigations in humans (EU-RAR, 2003) have shown that at rest, a three-hour exposure to toluene vapour will result in a systemic absorption amounting to approximately 50 % of the inhaled toluene.

In rats, toluene absorption after inhalation is rapid. During a three-hour exposure to 2.155 mg/m<sup>3</sup>, blood and brain toluene levels reached maximum levels in 53 and 58 minutes, respectively. In dogs exposed to 370-820 mg/m<sup>3</sup> (100-220 ppm) toluene via inhalation for 1-2 minutes, an uptake of approximately 90 % could be determined. The absorption of toluene was similar in the upper and lower respiratory tract (EU-RAR, 2003).

In conclusion, toluene is absorbed rapidly via inhalation and an absorption of about 50 % takes place.

#### 2.1.2 Oral exposure

Case reports of accidents and attempted suicides, and old clinical trials involving toluene administration to humans show that toluene is absorbed via the gastrointestinal tract.

In rats, absorption of toluene via the alimentary tract is slower than the respiratory absorption. Toluene concentration in blood reached maximum values two hours after an oral dose. About 76 % of the dosed toluene was recovered as hippuric acid in the urine, and approximately 18 % was excreted as toluene vapour through the respiratory system. Absorption appears to be nearly 100 % (EU-RAR, 2003).

Thus, toluene is absorbed almost completely from the gastrointestinal channel.

#### 2.1.3 Dermal exposure

The rate of absorption of toluene through human skin has been reported to range from 14 to 23 mg/cm<sup>2</sup> per hour (forearm skin). It has been calculated that bathing in water containing a toluene concentration of 5–500 µg/L (15 minutes/day) would result in an absorbed dermal dose ranging from 0.2 to 20 µg/kg body weight (bw) per day for a 70 kg adult and from 0.4 to 40 µg/kg bw per day for a 10.5 kg infant (Health Canada 2014).

Soaking the skin in a solvent containing 65 % toluene for 5 minutes produced a maximum concentration of toluene in blood of 5.4 µmol/L. This latter experiment, conducted with two volunteers, revealed individual differences in absorption, which is consistent with the high variability reported in a study with six rotogravure printing workers who washed their hands with toluene for 5 minutes; the next morning, toluene levels in alveolar air ranged between 0.5 and 10 mg/m<sup>3</sup> (Health Canada 2014).

In rats, dermal absorption of toluene in aqueous solution was significant, even though only 1 % of the body surface was exposed. For neat toluene exposure, a peak blood concentration of 9.5 µg/mL was reached in connection with hours of exposure (Health Canada 2014).

Thus, dermal exposure should not be disregarded as a potential route for systemic exposure.

## **2.2 Distribution**

Toluene that is absorbed into the blood is distributed throughout the body. A 51-year old man who died from an accidental oral overdose was reported with the highest toluene concentrations per gram tissue in the liver, pancreas, brain, heart, blood, fat and cerebrospinal fluid. A 16-year old man who was found dead from toluene intoxication had higher concentration in the brain than in the liver. Similar findings have been reported in the case of a 20-year old male painter.

Available data from human exposure suggest that more toluene accumulates in the brain than in the liver following inhalation exposure, whereas following oral exposure, the liver contains the greatest concentrations of toluene (US EPA 2005). This agrees well with the general knowledge of first pass metabolism after oral exposure going directly to the liver.

The distribution of toluene in the body is among other factors dependent on the tissue/blood partition coefficients and the metabolism. Toluene can be distributed to various tissues, the amount depending on the tissue/blood partition coefficient, the duration and level of exposure, and the rate of elimination. Additionally, adipose tissue may be a reservoir for toluene. Toluene easily passes the placenta and was found in fetuses in concentrations of about 75 % of that found in the maternal blood. Also, toluene is secreted into breast milk (EU-RAR 2003).

## **2.3 Metabolism and elimination**

The liver is the primary site of toluene metabolism. Toluene is metabolised by sequential hydroxylation and oxidation to benzoic acid. The conjugation of glycine with benzoic acid to form hippuric acid constitutes the major route of toluene detoxification and elimination. The initial step in toluene metabolism is transformation by cytochrome P-450 (CYP) enzymes, which are found mainly in the liver. The most prominent of these transformations is hydroxylation of the methyl group forming benzyl alcohol. Benzyl alcohol is primarily oxidised to benzoic acid, then conjugated with glycine to form hippuric acid (US EPA 2005).

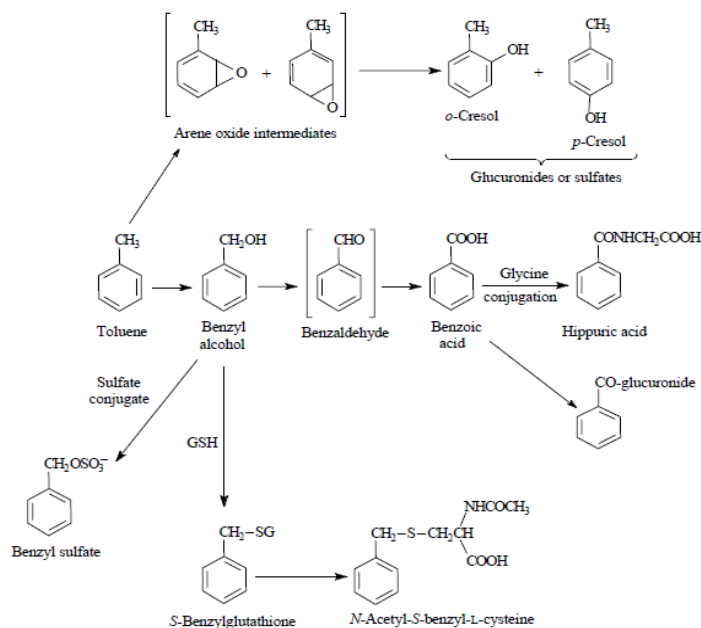


Figure 1. Metabolic pathways for toluene (from IARC 1999)

Toluene or its metabolites may be eliminated via the lungs, the kidneys, or the liver. It was concluded from various studies that around 20 % of the absorbed toluene is eliminated un-metabolised in the expired air. The remaining 80 % of the absorbed toluene is metabolised and excreted in the urine (EU-RAR 2005).

## 2.4 Mode of action

US EPA (2005) discussed issues regarding mode of action:

### *Toxicological mechanisms*

Understanding of the mechanisms by which toluene may exert its toxic effects is limited. However, the parent compound, rather than a metabolite, is believed to be responsible for the observed toxicity.

On a molecular scale, little is known about the mechanisms by which toluene produces acute or residual central nervous system (CNS) effects, but it is reasonable to assume that its toxic effects are due, at least in part, to its general characteristics as a solvent. The Meyer-Overton theory of partitioning of a compound into membrane lipids has been widely accepted for a century. However, other mechanisms have been postulated as well such as effects on GABA receptor functions and alteration of the dopaminergic system or molecular damage caused by toluene induced free radical oxidations.

In the case of chronic toluene exposure, it is not clear that the peak tissue concentration is the appropriate measure of internal dose to use in estimating the continuous exposure concentration that is associated with the observed neurotoxicity. The default duration and dosimetric adjustment method is based on the premise that the total amount of exposure, rather than the momentary tissue concentration, is the appropriate predictor of chronic toxic effects.

### *Susceptibility*

Only limited data exist that examine the potential differences in susceptibility to toluene between children and adults. Children have been shown to have differences in levels of CYP enzymes and several phase II detoxification enzymes (e.g., N-acetyl transferases, UDPglucuronyl transferases,

and sulfotransferases) relative to adults as well as other physiological differences (e.g., children have higher brain mass per unit of body weight, higher cerebral blood flow per unit of brain weight, and higher breathing rates per unit of body weight). However, data on the possible contributions of these differences to potential age-related differences with respect to toluene are lacking.

#### *Exposure route dependence*

Theoretically, the available toluene PBPK models could be used to extrapolate the risks of neurotoxic outcomes from inhalation exposure to oral exposure. However, in the case of toluene, unpublished data suggest that behavioural deficits observed in rats exposed to toluene by inhalation exposure are not observed in rats given toluene by oral gavage at doses expected to produce the same concentrations of toluene in the brain. The mechanism for this apparent difference in the effect of toluene by the oral and inhalation routes is not understood at this time.

# 3. Human toxicity

The description in this chapter is based mainly on the following expert assessments: IARC (1999); EU-RAR (2003), US EPA (2005), and Health Canada (2014).

## 3.1 Single dose toxicity

### 3.1.1 Inhalation exposure

From a series of studies with toluene exposure to human volunteers, the EU-RAR (2003) concluded the following regarding the occurrence of subjective symptoms and neurobehavioural findings in relation to short-term inhalation of toluene vapours.

Headache, dizziness, feeling of intoxication, irritation and sleepiness were recorded to occur with significantly increased frequency at exposure levels from 562 mg/m<sup>3</sup> (150 ppm) down to 281 mg/m<sup>3</sup> (75 ppm). At 150 mg/m<sup>3</sup> (40 ppm) and below the effects did not occur with increased frequency. For these subjective symptoms, a lowest observed adverse effect concentration (LOAEC) of 281 mg/m<sup>3</sup> (75 ppm) and a no observed adverse effect concentration (NOAEC) of 150 mg/m<sup>3</sup> (40 ppm) can be established.

With respect to function in performance tests, inhalations of 281 mg/m<sup>3</sup> (75 ppm) and 562 mg/m<sup>3</sup> (150 ppm) for 7 hours have resulted in significantly worse results in a number of performance tests, indicating a LOAEC of 281 mg/m<sup>3</sup> (75 ppm) for function in performance tests while a NOAEC could not be established.

### 3.1.2 Oral exposure

A number of acute studies and case reports following toluene exposure are available in the literature. Accidental ingestion of toluene was shown to cause severe acute toxicity, including oropharyngeal and gastric irritation with vomiting and hematemesis. Abdominal pain, hemorrhagic gastritis and central nervous system depression were observed following ingestion of approximately 1 L of paint thinner known to contain toluene, as well as death was reported to occur within 30 minutes of ingestion of approximately 60 mL (625 mg/kg bw) of toluene in one individual (Health Canada 2014)

Also, US EPA (2005) reported cases of acute intoxications with toluene. Accidental oral ingestion had been the cause of 15 deaths by paint thinner containing toluene over the period from 1977 to 1986. A 51-year-old man died approximately 30 minutes after he had ingested a large quantity of toluene and the probable cause of death was severe central nervous system depression.

A 46-year-old man ingested approximately one quart of paint thinner containing toluene, which resulted in severe central nervous system depression, severe abdominal pain, diarrhea, and hemorrhagic gastritis. However, the patient recovered after 36 hours of supportive care (US EPA 2005).



### **3.1.3 Dermal exposure**

No data have been reported regarding acute toxic effects in humans following dermal exposure.

## **3.2 Irritation and sensitisation**

### **3.2.1 Irritation**

No data have been reported about toluene causing skin irritation in humans.

With respect to eye irritation, the EU-RAR (2003) referred to two studies using human volunteers from which it was concluded that eye irritation starts somewhere between a toluene vapour concentration of 150 mg/m<sup>3</sup> and 375 mg/m<sup>3</sup>. These values were considered NOAEC and LOAEC-values, respectively (EU-RAR, 2003).

### **3.2.2 Sensitisation**

No human data available (EU-RAR, 2003).

## **3.3 Repeated dose toxicity**

### **3.3.1 Inhalation**

From a large number of case reports and clinical examinations, a series of adverse neurotoxic effects from toluene sniffing have been described in relation to repeated or chronic exposure.

Toluene abusers who have been exposed for long periods of time exhibit a variety of neurologic manifestations, including ataxia, tremor, anosmia, sensorineural hearing loss, dementia, corticospinal tract dysfunction, abnormal brainstem auditory-evoked potentials, and epileptic seizures. Abnormal magnetic resonance imaging findings in toluene abusers include generalised cerebral, cerebellar, and brainstem atrophy; atrophy of the corpus callosum; loss of grey-white matter discrimination; multifocal high signal intensity in the cerebral white matter; and hypointensity of the thalami. Further, optic neuropathies with dyschromatopsia, blindness, and changes in pattern visual-evoked potentials, pendular nystagmus, ocular flutter, opsoclonus (irregular rapid eye movement), bilateral internuclear ophthalmoplegia, and retinal impairment have been reported in participants who chronically sniffed toluene or toluene-based glue (US EPA 2005).

In relation to occupational inhalation exposure most studies have addressed the neurotoxic potential of toluene exposure. Several cross-sectional studies have been found, in which an exposed group of workers have been compared with a matched control group. Rotogravure printing is an occupation with a relatively pure exposure to toluene. The studies in which the exposure was predominantly to toluene, and where estimated of exposure levels were made, are shown in Table 4 (EU-RAR 2003; Tukes 2013).

TABLE 4. NEUROBEHAVIOURAL EFFECTS FROM OCCUPATIONAL EXPOSURE TO TOLUENE (EU-RAR 2003; TUKES 2013)

Reference	Groups studied	Toluene exposure	Toluene-related effects
<b>Iregren (1982)</b>	34 toluene-exposed rotogravure printers, 34 solvent mixture-exposed subjects, 34 non-exposed controls	150 ppm, reduced to 50 ppm, for an average of 16.3 years. Higher concentrations occurred occasionally.	Increased simple reaction time.
<b>Cherry et al. (1984)</b>	59 toluene exposed workers, 59 non-exposed workers	100-500 ppm for an average of 9.4 years	No effect.
<b>Juntunen et al. (1985)</b>	43 toluene-exposed rotogravure printers, 31 occasionally solvent-exposed controls	117 ppm for approx. 26 years, estimated mean level of exposure during the last year: 78 ppm	No effect.
<b>Larsen &amp; Leira (1988)</b>	22 toluene-exposed rotogravure printers, 19 unexposed controls	50-80 ppm, concentrations exceeding 1000 ppm 5 years previously. No. of years of exposure > 12.	Higher frequency of slight or moderate organic brain syndrome
<b>Lee et al. (1988)</b>	193 toluene-exposed female workers, 65 non-exposed workers	1->150 ppm	Increase in prevalence of subjective symptoms
<b>Ørbæk &amp; Nise (1989)</b>	30 toluene-exposed rotogravure printers, 72 unexposed controls	Mean exposure levels 43 and 157 mg/m <sup>3</sup> (12 and 42 ppm) for a median no. of exposure years of 29 (range 4-43).	Increase in prevalence of subjective symptoms. Impairment in spatial memory
<b>Foo et al. (1990)</b>	30 toluene-exposed workers, 30 low-level toluene exposed controls	88 ppm for an average of 5.7 years in exposed group 13 ppm for an average of 2.5 years in the control group	Impairment in manual dexterity, verbal memory and visual cognitive ability
<b>Muttray et al. (1995)</b>	59 rotogravure workers	Blood conc. of toluene ranging from <0.22 to 7.37 mg/l	No effect on colour vision in 5 tests
<b>Vrca et al. (1995)</b>	49 printing-press workers exposed to toluene, 59 non-exposed controls	40-60 ppm for an average of 21.4 years	Changes in visual-evoked potentials
<b>Boey et al. (1997)</b>	29 toluene-exposed workers, unexposed controls	90.9 ppm in exposed group, 12.2 ppm in control group. Mean blood toluene level 1.25 mg/l vs. 0.16 mg/l in controls	Impairment in psychological test
<b>Freie Universität Berlin (1996)</b>	1324 toluene-exposed rotogravure workers, 154 paper industry workers	80 mg/m <sup>3</sup> , mean blood toluene level 0.3 mg/l	Impairment in short-term memory

Two of these studies specifically addressed the induction of chronic neurotoxic effects from toluene diagnosed as “chronic toxic encephalopathy” or “organic brain syndrome”. The study by Larsen and Leira (1988) showed a higher frequency of organic brain syndrome in subjects exposed to toluene for more than 12 years (50-80 ppm, concentrations exceeding 1,000 ppm 5 years previously). In the

study by Ørbæk and Nise (1989), toluene-exposed workers complained substantially more of neurasthenic symptoms and scored lower in psychometric tests. Mean exposure levels at the time of the investigation were 11 and 42 ppm, while 5 years previously the exposure levels had exceeded 300 mg/m<sup>3</sup>. Both of these studies show an increased prevalence of organic brain syndrome in exposed workers compared with the control group. In both studies the length of employment was high (Larsen and Leira >12 years, Ørbæk and Nise median 29 years), while only recent exposure data were well documented. Exposure during the years preceding the investigation was not well described (EU-RAR 2003).

Overall, the EU-RAR (2014) concluded, due to lack of more precise exposure data, that neither a LOAEC nor a NOAEC could be determined for development of organic brain syndrome.

Hearing-loss was found to be another effect occurring at higher toluene exposure levels. Thus, studies by Morata et al. (1993) and Morata et al. (1997) indicated that occupational exposure to toluene may increase the risk of developing occupational high-frequency hearing loss in noisy environments. In the latter study, this conclusion was based on occupational exposure to toluene in the 0-245 ppm range. However, the studies are not considered appropriate for determining a LOAEC/NOAEC (EU-RAR 2003).

In the US EPA (2005) review on toluene, additional human studies were considered, and the conclusions were very much in concordance with the assessment of the EU-RAR (2003). US EPA (2005) found occupational exposure associated to a variety of adverse neurotoxic effects; the most sensitive endpoints being: impaired colour vision, impaired hearing, and decreased performance in neurobehavioural analysis, changes in motor and sensory nerve conduction velocity, headache and dizziness. As EU-RAR (2003), the US EPA (2005) was not able to identify an individual study from which to derive NOAEL/NOAEL values. Instead, US EPA (2005) collected what they considered the most relevant studies for an overall dose-response analysis of the neurotoxic effects (see Appendix 1).

From these studies covering a range of NOAELs from 20 ppm (77 mg/m<sup>3</sup>) to 48 ppm (184 mg/m<sup>3</sup>), US EPA (2005) estimated an arithmetic mean NOAEL value of 34 ppm (130 mg/m<sup>3</sup>), which was chosen as an overall NOAEL for the neurotoxic effects. It was noted that this NOAEL value was lower than any of the LOAELs identified in the studies.

Health Canada (2014) identified a NOAEL for humans based mainly on two studies conducted by Seeber et al. (2004; 2005) that examined the same population of exposed individuals within 14 rotary printing plants. These studies covered all of the neurological endpoints, including vibration thresholds, colour discrimination, auditory thresholds, attention (symbol-digit substitution, switching attention and simple reaction), memory (digit span forward and backward, immediate and delayed reproduction of pictures) and psychomotor functions (steadiness, line tracing, aiming, tapping, pegboard). Moreover, the neurological effects were investigated in terms of length of exposure, with an average of 21 years as a lifetime-weighted average and an average of 6 years as a current exposure level. The shorter term data were more relevant in the selection of a point of departure, as toluene levels were measured four times over the period of 5 years directly in the breathing environment of workers over full days, whereas long-term data were estimated using a job exposure matrix. In addition to adequate exposure monitoring, the Seeber et al. (2004, 2005) studies had a large sample size, a reference group from the same population as the exposed group, and appropriate controls for age, education and alcohol intake. None of the endpoints investigated within these studies was indicative of an adverse effect following exposure to toluene, and a NOAEL of 26 ppm (100 mg/m<sup>3</sup>) (as an average of highly exposed individuals) was concluded. It should be noted that all effects investigated in other epidemiological studies were observed at concentrations that exceeded 26 ppm. Although the true NOAEL for neurological endpoints may be higher than 26 ppm, Health Canada (2014) considered 26 ppm (100 mg/m<sup>3</sup>) as the most appropriate value.

### **3.3.2 Oral exposure**

No data found.

### **3.3.3 Dermal exposure**

No data found.

## **3.4 Toxicity to reproduction**

### **3.4.1 Inhalation**

Toluene has been shown to cause congenital defects in infants born to mothers who abused toluene during pregnancy. Exposure levels in the available studies, if reported at all, are very high. The clinical and morphometric characteristics of findings in children, where the mothers abused toluene during pregnancy, are identical. Microcephaly, CNS dysfunction, attention deficits and developmental delay had been recorded. Phenotypic similarities included a small mid face, deep-set eyes, micrognathia (smallness of the jaws) and blunting of the fingerprints (EU-RAR 2003).

Studies examining reproductive toxicity of toluene in humans following long-term low-level exposure are less common. Rotogravure printing workers were examined in one study, where 150 male and 90 female were exposed to toluene. Although no quantitative exposure levels were reported, significant association had been identified between toluene exposure and reduced fertility in females. In another study increased spontaneous abortions were found to be associated with exposure to toluene in the workplace at average air concentration levels 88 ppm (range 50-150 ppm) (EU-RAR 2003, Tukes 2013).

These data lend support to the classification as Repr2, H361d (suspected of damaging the unborn child).

### **3.4.2 Oral exposure**

No data found.

## **3.5 Mutagenicity**

Human data on mutagenicity are available from occupationally exposed workers. However, the occupational data do not give any consistent and conclusive answer regarding the genotoxic potential of toluene.

US EPA (2005) found that the majority of studies in toluene-exposed workers reported no differences in chromosomal aberrations between control subjects and toluene-exposed workers. Similarly, humans exposed to toluene have not generally demonstrated increases in SCE, cell cycle delay, or DNA damage as indicated by Comet assay.

However, three studies of exposed workers have found increases in chromosomal breaks, exchanges, and/or gaps relative to controls. In one population of shoe factory workers exposed to solvents (including toluene, gasoline, and acetone), an increase in micronuclei, but not sister chromatid exchanges was found in cultured peripheral lymphocytes. However, the chemical exposure responsible for the increase in micronuclei could not be identified with any certainty. Two other studies have reported genotoxic changes in toluene-exposed workers, but the changes have either been reversible or they could not be directly attributed to toluene exposure due to confounding factors (US EPA 2005).

The EU-RAR (2003) summarised the human data regarding genotoxicity as non-conclusive. Various results have been obtained in a multitude of studies with biological monitoring of various genotoxic effects in peripheral blood lymphocytes from workers exposed to toluene in the occupational environment, but confounding due to co-exposure to ink, other solvents and various

genotoxic substances in the environment could not be excluded. Also, a synergistic effect between toluene exposure and smoking has been demonstrated.

### **3.6 Carcinogenicity**

IARC (1999) evaluated eight epidemiological studies for the discussion of the carcinogenic potential of toluene in humans. Overall, however, that data were too weak for drawing conclusions.

In two of the studies, one concerning shoe manufacturing workers in the United States and one concerning Swedish rotogravure printers, it was believed that toluene was the predominant exposure; in the other studies, there were probably concomitant exposures. Cancers of most sites were not significantly associated with toluene exposure in any study. Stomach cancer mortality was significantly elevated in the Swedish rotogravure printers study, it was slightly, though not significantly, elevated in two other studies, and it was not associated at all in a fourth. Rates of lung cancer were significantly elevated in the cohort of shoe manufacturers and in the Swedish cohort of rotogravure printers, but were not associated at all in two other studies. Colorectal cancer was significantly elevated in the Swedish rotogravure printers study and in a Canadian case-control study, and colon cancer was nonsignificantly elevated in the shoe manufacturer's cohort. While results on leukaemias and lymphomas generally showed no association, these were based on small numbers. Considering the multiple exposure circumstances in most studies and the weak consistency of findings, these results are not strong enough to conclude that there is an association (IARC 1999).

# 4. Animal toxicity

The description in this chapter will be based mainly on the following expert assessments: IARC (1999); EU-RAR (2003), US EPA (2005) and Health Canada (2014).

## 4.1 Single dose toxicity

### 4.1.1 Inhalation exposure

Via inhalation, the 4-hour median lethal concentration (LC<sub>50</sub>) of toluene is 7500 ppm (2828 mg/m<sup>3</sup>) in rats and 5308–7440 ppm (20 011–28 048 mg/m<sup>3</sup>) in mice (Health Canada 2014).

Several acute animal studies have examined the neurological effects of inhaled toluene. In rats exposed to a single inhalation exposure of 500–16 000 ppm toluene, abnormal flash-evoked potentials were reported. Another study demonstrated severe disruption of auditory function and pathological effects in the inner ears (in the cochlea) of rats. This could, however, not be found in guinea pigs where the animals were exposed to 600 ppm toluene for 5 days. Rats exposed to toluene levels up to 3000 ppm for 4 hours prior to behavioural evaluation showed reduced performance in behavioural tests, particularly at the 1780 and 3000 ppm exposure levels.

A biphasic response in mice as well as rats exposed to toluene for 1 hour has been demonstrated, i.e. an increase in activity up to 1000 ppm, and then a decline with the increasing dose (US EPA, 2005).

### 4.1.2 Oral exposure

The acute toxicity of toluene is relatively low. The oral LD<sub>50</sub>-values for toluene in rats ranges from 5300 to 7400 mg/kg bw (Health Canada, 2014).

Neurobehavioural effects were studied in male and female Sprague-Dawley rats that were exposed to single gavage doses of 0, 3, 4.5, or 6 mL toluene/kg (0, 2600, 3900, or 5200 mg/kg, respectively). On days 1 (2–3 hours after exposure), 7, and 14 post-exposure, the animal body weights were recorded, and a functional observation battery (FOB) was conducted to detect neurobehavioural changes. Horizontal motor activities were significantly lower in both sexes at all dose levels on day 1 and the values remained lower in all treated female groups (US EPA 2005).

### 4.1.3 Dermal exposure

From a single reporting, a dermal LD<sub>50</sub> value of 12 400 mg/kg for toluene was described; however, no further details were given (EU-RAR 2003).

## 4.2 Irritation and sensitisation

### 4.2.1 Irritation

Test results from animal studies show that toluene is irritating to the skin in rabbits, mice and guinea pigs. Accordingly, toluene is classified as Skin Irrit 2, H315 (Causes skin irritation).

Although three animal studies show that liquid toluene has some potential to cause eye irritation, data were not considered sufficient to warrant classification for eye irritation.

Toluene vapours may also cause irritation to the respiratory tract in animals, which, however, has only been observed at very high concentrations. The irritative effect of lower toluene concentrations has not been examined (EU-RAR 2003).

#### **4.2.2 Sensitisation**

In a well conducted guinea pig maximisation study, no evidence of skin sensitisation was found, suggesting that toluene is not a skin sensitizer in humans (EU-RAR 2003)

### **4.3 Repeated dose toxicity**

#### **4.3.1 Inhalation**

Repeated dose inhalation studies with toluene exposure of experimental animals have been conducted abundantly. As the focus of this report mainly is the oral exposure to toluene, the data from inhalational exposure are not described in details. Furthermore, critical dose levels in relation to inhalational exposure to humans have been found from the extensive human data, and thus only a short overview will be given concerning the experimental animal data.

The focus has been on effects on the central nervous system (EU-RAR 2003):

After inhalational exposure of rats to toluene at 1500 ppm (5625 mg/m<sup>3</sup>) for 6 months, a reduced number of neurones in the hippocampus and a reduced hippocampal weight was found. In very young rats exposed to toluene via inhalation on postnatal day 1-28 at 100 and 500 ppm (380 and 1900 mg/m<sup>3</sup>), reduced volume of certain hippocampal structures was detected. Changes in brain neurochemistry in rats have also been described. Effects were found at an exposure level of 80 ppm (300 mg/m<sup>3</sup>) after only 3 days of exposure. Effects on brain neurochemistry was found after long-term exposure at 500 ppm (1900 mg/m<sup>3</sup>) and was still present six months after the last exposure indicating possibly irreversible changes.

The ototoxicity of toluene in the rat is well documented by behavioural, electrophysiological, and morphological techniques. Impaired hearing function was caused by exposure concentration levels of 1000-1400 ppm (3800-5320 mg/m<sup>3</sup>) for 2-8 weeks. In one study, an exposure level of 700 ppm (2660 mg/m<sup>3</sup>) was determined as a NOAEC for auditory toxicity. Effects on morphology of outer hair cells and auditory function have been found already after 5 days of exposure to 1400 ppm of toluene. The effect seems to be irreversible. However, transient auditory system impairment has been revealed at a much lower toluene concentration when using distortion product otoacoustic emission to evaluate auditory function. There are strong indications from several studies in rats of an interaction between toluene and noise with respect to effects on auditory functions.

Overall, the lowest LOAEL value was found for other effects such as nasal toxicity and forestomach ulcers in chronic inhalation studies in which these effects were found down to the lowest dose tested at 600 ppm (2280 mg/m<sup>3</sup>).

A NOAEC of 300 ppm (1125 mg/m<sup>3</sup>) was found from another chronic inhalation study, in which no adverse effects were noted at this dose levels (the highest dose level tested).

#### **4.3.2 Oral exposure**

The data on repeated oral exposure will be presented and discussed in the light of the assessments of EU-RAR (2003); WHO (2004); US EPA 2005, and Health Canada (2014) as some differences in the interpretation of the data exist among these expert assessments.

An overview regarding the oral repeated dose toxicity studies is given in Table 5.

TABLE 5 OVERVIEW OF EXPERIMENTAL STUDIES ON REPEATED DOSE TOXICITY

Species/ strain	Duration/ Dose levels/ Chemical form	Effects (mg toluene/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
<b>B6C3F1 Mice (Four females/ group)</b>	14 days  0, 600 mg/kg/d. Oral gavage	Mean number of leukocytes was 30 % lower in treated animals while the mean number of circulating reticulocytes was almost twice the mean value for the control group.	Not identified	600	Burns et al. (1994)
<b>CD-1 Mice (Five mice/ group)</b>	28 days  0, 17, 80, 405 mg toluene/L Drinking water ad libitum  (0, 5, 22, 105 mg/kg/day)	Increased rel. liver weight Decreased rel. thymus weight. Immune response measured as decrease in antibody formation towards injection of sheep red blood cells.	22	105 (organ weight and immune response)	Hsieh et al. (1989)
<b>CD-1 Mice (Five mice/ group)</b>	28 days  0, 80, 325 mg toluene/L. Drinking water ad libitum (0, 22, 85 mg/kg/day)	22 Significant increase in Immune suppression (mitomycin C- blocked YAC-1 cells as stimulators).	Not identified.	22	Hsieh et al. (1990b)
<b>CD-1 Mice (Five mice/ group)</b>	28 days  0, 20, 100, 500 mg toluene/L. Drinking water ad libitum.  (0, 5, 22, 105 mg/kg/day)	105 Decreased production of IL-2 by splenocytes.	22	105	Hsieh et al. (1991)
<b>CD-1 Mice</b>	28 days  0, 17, 80, 405 mg toluene/L. Drinking water ad libitum.  (0, 5, 22, 105 mg/kg/day)	Significant increase in norepinephrine and its metabolite as well as in serotonin in all dose groups.	Not identified.	Not identified.	Hsieh et al. (1990a)
<b>B6C3F1 Mice 10 mice/sex /group</b>	13 weeks  0, 312, 625, 1250, 2500 or 5000 mg/kg were administered 5 days per	312 Abs. and rel. liver weight increase	Not identified.	312 (organ weight)	NTP, 1990



Species/ strain	Duration/ Dose levels/ Chemical form	Effects (mg toluene/kg bw/day)	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Reference
	week. Toluene in corn oil by gavage.  (average daily doses during a week 0, 223, 446, 893, 1786 and 3571 mg/kg)				
<b>F-344 Rats</b>  <b>10</b> <b>rats/sex/group</b>	13 weeks  0, 312, 625, 1250, 2500 or 5000 mg/kg were administered 5 days per week. Toluene in corn oil by gavage.  (average daily doses during a week 0, 223, 446, 893, 1786 and 3571 mg/kg)	625 Liver and kidney weight increase in male rats. (p<0.05)  1250 Necrosis in hippocampus in males and females	312	625 (organ weight)	NTP, 1990

As indicated below, the interpretation of the data from the NTP (1990) studies in rats and mice differs somewhat between the various expert groups.

The assessment of EU-RAR (2003) acknowledged the increase in absolute and relative liver and kidney weights at the lower dose levels in the 90 days NTP studies; however, these effects were interpreted as toxicologically non-significant. Consequently, the dose level 625 mg/kg was considered as a NOAEL as neuron necrosis in the brain was found at doses of 1250 mg/kg and above. This was seen as a clearly adverse effect and therefore considered as a LOAEL.

WHO (2004) also used the NTP studies as the most valid studies for N(L)OAEL derivation. The NOEL in this rat study was indicated to 312 mg/kg body weight per day and the NOAEL was 625 mg/kg body weight per day, based on increased absolute and relative kidney weights (without histopathology). (The distinction between the NOEL and the NOAEL values was not further described by WHO (2004)). In mice, an increased relative liver weight was the most sensitive effect, being present in females at the lowest dose tested, 312 mg/kg body weight per day; in the absence of histopathology, this was likely to reflect adaptive change. High-dose animals showed clinical signs of neurotoxicity, and myocardial degeneration was detected in several mice.

US EPA (2005), however, considered that the most critical and consistent effects were on the kidneys found in an NTP study with rats.

In the (NTP 1990) studies both sexes of F-344 rats and both sexes of B6C3F1 mice were exposed to toluene by gavage for 13 weeks at dose levels of 0, 312, 625, 1250, 2500 or 5000 mg/kg administered 5 days per week. (When adjusted to an average daily dose over a week the dose levels were 0, 223, 446, 893, 1786 or 3571 mg/kg/day). In male rats, absolute and relative weights of both the liver and kidney were significantly increased (p<0.05) at doses greater than or equal to 446 mg/kg/day. Absolute kidney weights were 100, 107, 112, 119, and 113 % of controls; relative kidney weights were 100, 100, 106, 114, and 146 % of controls for the 0, 312, 625, 1250, 2500 mg/kg/day dose levels. Histopathologic lesions in the liver consisted of hepatocellular hypertrophy, occurring at doses greater than 2500 mg/kg/day. In the brain, mineralised foci and necrosis of neuronal cells

were observed in males and females at 2500 mg/kg/day. The study in rats established a NOAEL of 312 mg/kg/day based on increases in liver and kidney weights of male rats at 625 mg/kg/day (LOAEL). It should be noted that no increase in kidney weight was seen in the parallel study in B6C3F1 mice, indicating a species difference in the response.

In female mice, absolute liver weights were increased in the 312 and 2500 mg/kg/day groups, but not in the other treated groups; relative liver weights were increased in all treated groups. No other changes in organ weights were seen in female mice. Several small but statistically significant changes occurred in hematologic parameters, but did not appear to be related to toluene exposure as no dose-response was observed. No histologic changes in the liver, brain, kidneys, or bladder of any group were reported (US EPA 2005).

Health Canada (2014) concluded the adverse neurological effects as the most critical effects from oral exposure to animals and considered these data supported by the findings from inhalation data on experimental animals as well as on humans. It was noted that one study found that oral exposure through drinking water at toluene concentrations as low as 17 mg/L over 28 days (corresponding to a daily intake of 5 mg/kg bw) increased norepinephrine, dopamine and serotonin levels in the hypothalamus of male CD-1 mice as well as in other regions of the brain. Another study by oral gavage indicated neuronal necrosis in the dentate gyrus and Ammon's horn of the hippocampus in male and female rats at doses as low as 1250 mg/kg bw (Health Canada 2014).

However, also immunosuppressive responses from toluene exposure were acknowledged. One study of mice exposed via drinking water showed a decrease in thymus weight, splenocyte lymphoproliferation in response to alloantigens, antibody plaque-forming cell responses and interleukin-2 production, but only at a high dose of 405 mg/L. These findings were supported in another study by the same group using the same doses. An additional study in which the highest dose was 325 mg/L showed no obvious immunotoxic effects (Health Canada 2014).

In summary, effects on liver and kidneys should be considered the most critical effects from subchronic exposure to toluene. Effects on neurotransmitter level and immune response observed in mice after 28 days of oral exposure are considered very uncertain endpoints in relation to risk assessment as the implication/ relevance of these findings are uncertain. Although WHO (2004) used a dose level of 312 mg/kg/d as a LOAEL in relation to increased liver weight in mice, the significance of this finding seems debatable, as the effect did not occur consistently at higher dose levels.

Thus the NOAEL of 312 mg/kg/day established by US EPA (2005) based on increased kidney weights, which was consistently found in male rats at all of the higher dose levels, is considered as the best documented NOAEL value.

#### **4.3.3 Dermal exposure**

No data available.

### **4.4 Toxicity to reproduction**

#### **4.4.1 Fertility**

##### ***Inhalation***

In a combined two-generation fertility and teratogenicity inhalation study, groups of at least 10 male and 20 female Charles River CD rats were exposed to either 0, 375, 1875, or 7500 mg/m<sup>3</sup> (0, 100, 500, or 2000 ppm) toluene 6 hours/day, 7 days/week during an 80-day premating period and a 15-day mating period. Females were further exposed on days 1-20 of gestation and during day 5-21 of lactation. In the P generation, a slight inhibition of body weight gain was observed in males at

500 and 2000 ppm, and minor reductions in maternal body weight were reported during gestation and lactation in the group of females exposed to 2000 ppm. Toluene did not affect fertility in this study (EU-RAR 2003).

Groups of 15 Sprague-Dawley rats were exposed to air, 600 or 2000 ppm of toluene vapour, 6 hours/day (Ono et al., 1996). Male rats 7 weeks of age were exposed for 90 days, starting 60 days before mating. Female 10-week old rats were exposed from 14 days before mating until day 7 of gestation. Female rats were paired on a 1:1 basis with male rats of the same dose group. Except for one rat pair in the 600 ppm group, all pairs copulated. Only one female rat, in the 2000 ppm group, did not become pregnant. Pregnant females were sacrificed on day 20 of gestation and the uterus was removed. No statistically significant differences were observed between exposed and unexposed dams with respect to number of corpora lutea, implantations, live fetuses, sex ratio, malformations (0 in all groups), foetal weight, or foetal deaths. Eight males from each group were sacrificed the day after the last exposure. Quantitative morphometry of the spermatogenic cycle stages was carried out. The remaining males were sacrificed on the second day after the last exposure, and examined for spermatozoa and elemental analysis. In males exposed to 2000 ppm, kidney weight increase accompanied by basophilic changes and tubular necrosis, and thymus weight decrease indicated toxic effect of toluene. Relative and absolute epididymides weights were decreased at 2000 ppm. No abnormalities of testes and epididymides were detected on histopathological examination. The number of spermatogenic cells counted at 3 stages was not affected by toluene exposure. The sperm count was significantly decreased (approximately 20-25 %) at 2000 ppm. Also at 600 ppm, a decreased sperm count was found (approximately by 10 %), this was not statistically significant. Sperm motility was not affected.

This study indicates that toluene causes a reduction in epididymal weight and sperm count in male rats at 2000 ppm. That fertility was not affected is not surprising, as this parameter is relatively insensitive in the rat (EU-RAR 2003).

Further, Health Canada (2014) referred to a study in which female Wistar rats (P generation) were exposed to 0 (n=38), 1125, 2250, 3750, or 4500 mg/m<sup>3</sup> (0, 300, 600, 1000 or 1200 ppm) (n=23 to 29 in exposed groups) toluene 6 hours/day on day 9 to 21 of pregnancy. The adult F1-generation was mated and the fertility was determined. Mating and pregnancy indexes were unaffected. The fertility index of F1 rats prenatally exposed to 600 ppm was significantly increased compared with the control group, but since no concentration relation was present, it was concluded that the difference had occurred by chance.

#### **4.4.2 Development**

Tukes (2013) made an overview of the most important developmental toxicity studies, see Table 7.

TABLE 7 OVERVIEW OF EXPERIMENTAL STUDIES ON DEVELOPMENTAL TOXICITY (TUKES 2013)

Species/ strain	Duration/ Dose levels	Effects (mg Toluene/m <sup>3</sup> or ppm)  NOAEL (mg Toluene/ m <sup>3</sup> or ppm)	Reference
<b>Rat (Wistar (Bor: Wisw/spf, TNO))</b>	0, 1131, 2261, 3768, 4522 mg/m <sup>3</sup> Inhalation exposure 6 h/day (day 9-21 of pregnancy)	NOAEC (offspring behaviour): 4522 mg/m <sup>3</sup> NOAEC (maternal toxicity): 2261 mg/m <sup>3</sup> air (lower maternal bodyweight gain at 3768 and 4522 mg/m <sup>3</sup> ) NOAEC (developmental toxicity): 2261 mg/m <sup>3</sup>	Thiel R and Chahoud I (1997)
<b>Rat (CrI: CD (SD) BR VAF/Plus)</b>	0, 938, 2812, 5625 or 11250 mg/m <sup>3</sup> Inhalation exposure: 6 h/day (gestation day 6-15)	NOAEC (maternal toxicity): 2812 mg/m <sup>3</sup> NOAEC (developmental toxicity, lower foetal weight): 2812 mg/m <sup>3</sup>	Publication 2 (see annex: confidential information)
<b>Rat (Sprague- Dawley)</b>	0, 1880 or 5650 mg/m <sup>3</sup>  Inhalation exposure: 6 h/day (day 6-20 of pregnancy)	NOAEC (maternal toxicity): 1880 mg/m <sup>3</sup> NOAEC (teratogenicity): 1880 mg/m <sup>3</sup> NOAEC (developmental toxicity , lower foetal weight): 1880 mg/m <sup>3</sup>	Saillenfait, A-M, Gallissot, F, Sabate, J-P, Bourges- Abella, N and (2007)
<b>Rabbit (Himalayan)</b>	0, 113, 377, 1131, 1880 mg/m <sup>3</sup>  Inhalation exposure: 6 h/day (days 6-18 of gestation)	NOAEC (maternal toxicity): 1880 mg/m <sup>3</sup> NOAEC (teratogenicity /malformation diaphragmatic hernia): 1880 mg/m <sup>3</sup> NOAEC (developmental toxicity): 1880 mg/m <sup>3</sup>	Klimisch H-J, Hellwig J, Hofmann A (1992)
<b>Rat (Mol:WIST)</b>	4522 mg/m <sup>3</sup> Inhalation exposure: 6 h/day (day 7 of gestation to day 18 lactation)	LOAEC (developmental toxicity, lower birth weight, behavioural effects): 4522 mg/m <sup>3</sup>	Hass U, Lund SP, Hougaard KS and Simonsen L (1999)

Based on the data, it was concluded that effects indicating developmental neurotoxicity occurred at exposure levels of 4522 mg/m<sup>3</sup> and higher. As the effect has not been examined at a lower exposure levels a NOAEC for other developmental effects is set to 2261 mg/m<sup>3</sup> (Tukes 2013).

EU-RAR (2003) concluded that toluene causes developmental toxicity in rats in the absence of maternal toxicity. In offspring, behavioural effects from prenatal exposure include increased spontaneous activity and impairments of cognitive functions which was seen at 4522 mg/m<sup>3</sup> (Hougaard et al., 1999, Hass et al., 1999). Thus a NOAEC for effects on birth weight and postnatal development of 2250 mg/m<sup>3</sup> was concluded by the EU-RAR (2013) based on the study by Thiel and Chahoud (1997).

#### 4.4.3 Oral exposure

There are far less studies available that investigated the reproductive effects of oral toluene exposure. Mice exposed to a high dose of 2350 mg/kg bw on gestation days 7 through 14 showed no effects on litter variability.

There is one study available where forty-eight female Nya:NYLAR mice had been exposed pre and postnatally to toluene in the drinking water. The concentration of toluene had ranged from 0, 16, 80 or 400 ppm (estimated 0, 7.2, 14.4 and 72 mg/kg/day). Exposure began in the first hour of the 60-hour mating period and continued throughout pregnancy and lactation. The offspring was maintained on the same drinking water from weaning at 21 days of age through behavioural testing. Effect was noted in all dose groups on motor coordination with an inverse dose-response relationship. No effects of toluene exposure were seen on maternal fluid consumption, offspring mortality rate, development of eye or ear openings, or surface-righting response.

Moreover, the National Institute of Occupational Safety and Health (NIOSH) conducted a study to determine the Maximum Tolerable Dose (MTD) for toluene in adult female CD-1 mice and then use the MTD to determine adverse reproductive effects in timed-pregnant (5-day) mice. Doses of 0, 735, 1470, 2945, 5890 and 8700 mg/kg toluene were administered by gavage to groups of ten female mice for eight consecutive days. There were no statistically significant differences between test and control groups in any of the categories of reproductive toxicity.

A series of studies were also conducted examining the effects of oral prenatal toluene exposure on the development of rats. 520 mg/kg toluene in corn oil gavaged on gestational days 6-19 resulted significant decrease in weight gain (24 % and a 12 % reduction in food consumption). Foetal body weights, organ weights and placenta weights were significantly decreased in toluene-exposed animals. No gross foetal malformations were reported. In another study, where pregnant rats received 650 mg toluene/kg in corn oil on gestation days 6-9, significantly decreased foetal weights, decreased organ weights (brain, liver, heart, kidney) and a delay in skeletal ossification were reported. Histologic analysis of the brain revealed decreased neuronal packaging and alterations in the pattern of staining with bromodeoxyuridine (US EPA, 2005).

However, the EU-RAR (2003) noted that foetal effects were only seen at levels with clear maternal toxicity, so no conclusions regarding developmental toxicity could be made from the oral data.

#### 4.5 Mutagenicity

Studies of toluene in cultured cells and experimental animals provided very little evidence of genotoxic activity (EU-RAR, 2003; Health Canada 2014).

##### 4.5.1 *In vitro* studies

There are extensive data available on the lack of mutagenicity of toluene to the standard *Salmonella typhimurium* test strains (TA1535, TA1537, TA1538, TA98 and TA100) and other *S. typhimurium* test strains in the plate incorporation assay. Toluene has a boiling point of 110.6 °C, and the standard plate assay is not considered to be able to accommodate volatile substances without modifications, for example, taping of the plates or use of a desiccator. In addition, toluene has, however, been found negative in a pre-incubation test with the standard *Salmonella typhimurium* test strains, which may be considered to be adequate for the test of compounds with boiling points from 107 °C to 132 °C (EU-RAR 2003).

The genotoxicity of toluene *in vitro* has been evaluated in several types of mammalian cells, including cell lines with mouse lymphomas or Syrian hamster embryo cells, primary rat hepatocytes and human lymphocytes. At non-cytotoxic doses, toluene does not appear to induce biologically significant increases in forward mutations, sister chromatid exchanges, micronuclei or DNA damage *in vitro*. Significant levels of cytotoxicity have been reached in most studies (EU-RAR 2003).

#### 4.5.2 *In vivo* studies

The EU-RAR (2003) found that toluene did not induce biologically significant increases in micronuclei and chromosomal aberrations in the bone marrow of mice and rats or DNA damage in peripheral blood cells, bone marrow, and liver of mice (see overview in Table 8). In a dominant lethal assay, toluene was not mutagenic to the sperm of mice in the doses tested, as it did not cause increases in pre or postimplantation loss of embryos (EU-RAR, 2003).

TABLE 8 GENOTOXICITY OF TOLUENE IN VIVO. OVERVIEW FROM EU-RAR (2003)

Test object	Protocol	Results	Reference
<b>Rats, strain not specified</b>	Bone marrow clastogenicity 22-215 mg/kg, i.p.	Negative	Jagannath et al. (1978)
<b>CD1 mice</b>	Dominant lethal assay 100 and 400 ppm, inhalation	Negative	Brusick and Mazursky (1981)
<b>CD1 mice</b>	Bone marrow clastogenicity 860 and 1720 mg/kg, oral gavage	Negative	Gad-El-Karim et al. (1984)
<b>NMRI and B6C3F1 mice</b>	Bone marrow micronucleus tests 104-435 mg/kg, i.p.	Negative	Mohtashamipur et al. (1985; 1987)
<b>Sprague Dawley rats</b>	Bone marrow micronucleus and clastogenicity test 108.75-440 mg/kg, i.p	Negative	Roh et al., (1987)
<b>Human volunteers</b>	SCE's in blood lymphocytes 50 ppm, 7h, 3 days	Negative	Richer et al. (1993)
<b>BDF1 mice</b>	Single cell gel assay (DNA damage) 500 ppm, inhalation	Negative	Plappert et al. (1994)

IARC (1989) noted that positive results have been obtained in three cytogenetic studies performed in the former USSR in the 1970's. In two of the studies, rats were receiving up to 1000 mg/kg bw of toluene by subcutaneous injections, and in one study rats were exposed to atmospheres containing 610 mg/m<sup>3</sup> of toluene. However, these significant cytogenetic responses might have been due to contamination with benzene.

#### 4.6 Carcinogenicity

IARC (1999) put emphasis on two long-term animal carcinogenicity studies using inhalational exposure:

In one study, groups of 60 male and 60 female B6C3F1 mice, 9–10 weeks of age, were exposed to toluene (purity, > 99 %) by whole-body inhalation at concentrations of 0 (controls), 120, 600 or 1200 ppm (0, 450, 2260 or 4520 mg/m<sup>3</sup>) for 6.5 h per day on five days per week for 104 weeks. In another study, groups of 60 male and 60 female Fischer 344 rats, six to seven weeks of age, were exposed to toluene (purity, > 99 %) by whole-body inhalation at concentrations of 0 (controls), 600 or 1200 ppm (0, 2260 or 4520 mg/m<sup>3</sup>) for 6.5 h per day on five days per week for 103 weeks.

No significant increase in the tumour incidences was observed in these studies.

It was noted that toluene was tested for carcinogenicity in one strain of rats by gavage at one dose level and in a further study in rats by inhalation. However, these studies were considered inadequate for evaluation. Also, toluene was used as a vehicle control in a number of skin-painting studies. Some of these studies were inadequate for evaluation. In others, repeated application of toluene to the skin of mice did not result in an increased incidence of skin tumours.

Overall, IARC (1999) concluded that “there is evidence suggesting lack of carcinogenicity of toluene in experimental animals”.

This conclusion is consistent with the conclusions in the other expert assessments made by EU-RAR (2003); WHO (2004); US EPA (2005) and Health Canada (2014).

# 5. Regulations

## 5.1 Ambient air

C-value for emission to ambient air, DK (DEPA 2008a): 0.4 mg/m<sup>3</sup>

## 5.2 Drinking water/ groundwater

Limit value in groundwater, DK (DEPA 2015): 5 µg/L

Limit value drinking water, DK: No limit value has been established in Denmark.

Guideline value, (WHO 2004): 700 µg/L

WHO (2004) based the guideline value on a TDI level of 223 µg/kg bw/d; allocation of 10 % of the TDI to drinking water, a body weight of 60 kg and a daily ingestion of drinking water of 2 l.

Health based drinking water value, Health Canada (2014): 60 µg/L (draft proposal)

An aesthetic objective, Health Canada (2014): 24 µg/L (draft proposal)

Health Canada (2014) based the health based guideline value on a TDI level of 9.7 µg/kg bw/d; allocation of 20 % of the TDI to drinking water, a body weight of 70 kg and a daily ingestion of drinking water of 2.13 l.

The proposal for an aesthetic guideline value was based on a study by Alexander et al. (1982). In this study, the aqueous odour and taste thresholds for various chemicals including toluene were determined. The *odour threshold* values were reported as milligrams of compound per litre of odour-free water at 60 °C. The *taste threshold* values were reported as milligrams of toluene per litre of odour-free water at 40 °C. For toluene in water, two odour threshold measurements of 0.024 mg/L were reported. Also for toluene in water, two taste threshold measurements of 0.12 and 0.16 mg/L (average value 0.14 mg/L) were reported.

Ambient water quality criteria, US EPA 2014: 300 µg/L (draft proposal)

The quality criteria is considering the human health concern as US EPA (2014) based the guideline value on 300 µg/L on an oral reference dose, RfD of 80 µg/kg bw/d; allocation of 20 % of the RfD to drinking water; a body weight of 80 kg and a daily water consumption rate of 3 l. Also, contribution of toluene from ingestion of fish living in the water was considered in derivation of the value.

## 5.3 Soil

No specific limit value for toluene in soil has been established in Denmark. However, a quality criterion of 0.4 mg/m<sup>3</sup> in indoor and ambient air (evaporation criterion) applies for toluene in relation to evaporation from soil.

UK, soil (UK-EA 2009): 610 mg/kg (residential)  
120 mg/kg (allotment)

UK-EA (2009) based the guideline value on a TDI value of 223 µg/kg bw/d. The TDI value was divided on exposure from direct ingestion of soil (1 %); consumption of home-grown produce (36



%) inhalation of vapours entering into the house (56 %) in addition to normal background exposure (7 %). This distribution of the toluene exposure and the guideline value in drinking water were estimated based on models for distribution using the physical-chemical characteristics of toluene. For allotments, 99.4 % of the toluene exposure was considered to be in relation to ingestion of home grown produce.

<i>Canada, soil (CCME 2004):</i>	0.37 mg/kg for coarse fraction in surface soil
	0.08 mg/kg for fine fraction in surface soil

The Canadian values were calculated in order to prevent toluene to leak from soil into the ground water and reach unacceptable concentrations for use as drinking water. When considering children's soil ingestion, the guideline value was calculated to 22 000 mg/kg (CCME 2004).

## 5.4 Occupational Exposure limits

<i>OEL-8h, DK (DME 2012):</i>	25 ppm (94 mg/m <sup>3</sup> )
-------------------------------	--------------------------------

<i>OEL-8h, EU (Com Dir 2006):</i>	50 ppm (192 mg/m <sup>3</sup> )
-----------------------------------	---------------------------------

## 5.5 Classification

Toluene has an EU-harmonised classification as:

<i>Flam Liq2, H225:</i>	<i>Highly flammable liquid and vapour;</i>
<i>Asp Tox1, H304:</i>	<i>May be fatal if swallowed and enters airways</i>
<i>Skin Irrit2, H315:</i>	<i>Causes skin irritation</i>
<i>STOT SE3, H336:</i>	<i>May cause drowsiness or dizziness</i>
<i>Repr2, H361d:</i>	<i>Suspected of damaging the unborn child</i>
<i>STOT RE2, H373:</i>	<i>May cause damage to organs through prolonged or repeated exposure</i>

## 5.6 IARC

IARC (1999) placed toluene in IARC Group 3 (not classifiable as to its carcinogenicity to humans) based on

- inadequate evidence in humans for the carcinogenicity of toluene
- evidence suggesting lack of carcinogenicity of toluene in experimental animals

## 5.7 Tolerable daily intake

### 5.7.1 US EPA (2005), reference dose/concentration

*RfD, oral (US EPA 2005): 0.08 mg/kg/day*

US EPA (2005) derived the oral reference dose from a Benchmark Dose Level (BMDL) of 238 mg/kg/d as a starting point. This value was calculated from the data on increased kidney weight in male rats in the NTP (1990) study. This BMDL corresponded to the estimated lower 95-percentile dose level associated with a 10 % increase in individuals having a kidney weight higher than the 98th percentile of kidney weights in the control group.

For further derivation of the reference dose, a total uncertainty factor (UF) of 3000 was applied: A factor 10 for extrapolation for interspecies differences; a factor 10 for consideration of intraspecies variation; a factor 10 for use of a subchronic study to estimate chronic effects; and a further factor of 3 for insufficiencies in the database to account for the lack of adequate data on endpoints of potential concern for toluene, including neurotoxicity, two-generation reproductive toxicity, and immunotoxicity.

*RfC, inhalation (US EPA 2005): 5 mg/m<sup>3</sup>*

US EPA (2005) derived the inhalation reference concentration from the overall NOAEL of 34 ppm (128 mg/m<sup>3</sup>) derived from the occupational studies in relation to the neurotoxicity of the substance. This level from the occupational environment was further adjusted to continuous population exposure (128 mg/m<sup>3</sup> x 10m<sup>3</sup>/20m<sup>3</sup> x 5 days/7 days = 46 mg/m<sup>3</sup>) and further, an uncertainty factor of 10 was used to consider intraspecies variability. From this, an RfC of 5 mg/m<sup>3</sup> was calculated.

#### **5.7.2 Health Canada (2014) TDI**

*TDI: 9.7 µg/kg bw/d*

The basis for this value was a NOAEC value of 26 ppm (98 mg/m<sup>3</sup>) with respect to neurotoxicity observed in two studies on occupational exposure (Seeber et al., 2004 and Seeber et al., 2005). Using PBPK modelling, this value was converted to a daily human oral dose of 0.097 mg/kg bw/d. Using a interspecies uncertainty factor of 10 a TDI level of 9.7 µg/kg bw/d was obtained.

#### *DHI Comment:*

According to Health Canada (2014), an inhalational NOAEC of 26 ppm (98 mg/m<sup>3</sup>) from occupational exposure was by PBPK modelling estimated to result in a blood concentration of 0.0075 mg/L. For obtaining this blood level, the human oral dose was modelled to 0.097 mg/kg bw per day.

However, this oral dose level seems very low, as for an adult person (70 kg) this equals 6.79 mg toluene/d as an external oral dose. This daily oral dose should then be equivalent to inhalation NOAEC of 98 mg/m<sup>3</sup> during an 8-hour working day. For an inhalation volume of 10 m<sup>3</sup> air per working day this would correspond to inhalation of 980 mg/d. Considering a retention of 50 % by inhalation, this would result in an internal dose of 490 mg/d. This is far above an internal calculated exposure of 6.79 mg/d. Thus, for the moment, and as no further details concerning the PBPK modelling was given, it seems premature and rather uncertain to consider this oral dose level as a starting point for a risk assessment.

#### **5.7.3 WHO (2004), TDI**

*TDI: 223 µg/kg bw/d*

This value was obtained from a LOAEL of 312 mg/kg for marginal hepatotoxicity observed in mice in the 90-day repeated oral study by NTP (1990). This level corresponds to 223 mg/kg body weight per day for 7 days per week dosing. Further, an uncertainty factor of 100 was applied for considering inter and intraspecies variation, and a further factor of 10 was applied to account for the short duration of the study and use of a LOAEL instead of a NOAEL.

# 6. Summary and evaluation

## 6.1 Description

Toluene is an organic solvent and is a constituent of various fuel streams from petrochemical refining. In EU the average content of toluene in petrol is about 11 %. Toluene has a pungent odour, a boiling point of 110.5 °C, and a vapour pressure of 3 kPa at 20 °C. The water solubility is about 580 mg/L.

The odour threshold in water was found to be in the range of 0.024–0.17 mg/l, and the reported taste threshold ranged from 0.04 to 0.12 mg/l (data from Alexander et al. 1982 that was quoted and taken into account by WHO (2002) and Health Canada (2014)). It should be noted that these low levels may be of importance in cases where a limit value for drinking water should also protect against odour and taste from the drinking water.

The main applications of commercial toluene are as raw materials and auxiliaries in the chemical industry and as solvents in many applications including paints, textile coatings, printing industry, etc.

## 6.2 Environment

In ambient air in Denmark, measurements indicate average levels of 3.4-3.6 µg/m<sup>3</sup> of toluene at busy roads in Copenhagen, while the urban background levels are in the range of 1.3-1.6 µg/m<sup>3</sup>.

In ambient air, photo oxidation of toluene takes place rather rapidly, primarily due to reaction with hydroxyl radicals with an experimental half-life of 1.3 days.

In indoor air, typical levels are below 10 µg/m<sup>3</sup>, but the concentration may vary depending on indoor sources for toluene emissions. Inside cars, toluene levels of 12 – 101 µg/m<sup>3</sup> were measured depending on driving conditions and temperature.

In rainwater in Denmark, an average toluene level of 0.12 µg/l has been measured and in groundwater, a median and maximum level of 0.1 µg/l and 2.4 µg/l, respectively, were measured.

The volatilisation of toluene from water, as well as from soil surfaces, takes place fast. From surface water, the half-life is typically in the order of hours, and a half-life of 4.9 hours has been reported from the surface of a sandy soil with low organic carbon content.

In soil, experimental half-lives for degradation of toluene in the range of 83-92 days have been found, but values of a few days are also reported.

Data from standard testing indicate toluene to be readily biodegradable with a half-life of about 30 days in the aquatic environment.

## 6.3 Human exposure

In general, indoor environment and transport in cars can be considered the major sources of toluene exposure for the general population. Using data from person borne measurements, a 24h average exposure to 11.7 µg/m<sup>3</sup> (median level from 11 European cities) would result in a daily exposure of 234 µg toluene for an adult person inhaling 20 m<sup>3</sup> of air per day.

## 6.4 Toxicokinetics

Toluene is absorbed almost completely from the gastrointestinal channel in animals after oral exposure.

By the inhalation route, approximately 50 % of inhaled toluene is taken up, depending on pulmonary ventilation.

Dermal uptake after skin exposure to liquid toluene occurs to a limited degree. It has been calculated that bathing in water containing a toluene concentration of 5–500 µg/L (15 minutes/day) would result in an absorbed dermal dose ranging from 0.2 to 20 µg/kg body weight (bw) per day for a 70 kg adult and from 0.4 to 40 µg/kg bw per day for a 10.5 kg infant.

Dermal exposure to toluene vapours is not likely to be an important route.

Toluene is distributed to various tissues, and higher concentrations in the brain than in the blood are obtained. Adipose tissue may be a reservoir for toluene. Toluene easily passes the placenta and is found in the foetus in concentrations of about 75 % of that found in the maternal blood. Toluene is secreted into maternal milk.

The half-life in human tissue may be up to three days, whereas in blood toluene rapidly declines after cessation of exposure.

A proportion (around 20 %) of the absorbed toluene is eliminated in the expired air. The remaining 80 % of the absorbed toluene is metabolised in the liver by the P450 system into benzyl alcohol and to benzoic acid, which are conjugated with either glycine or glucuronic acid and excreted in the urine as hippuric acid or benzoyl glucuronide. Toluene is also metabolised to a small extent to o-cresol and p-cresol.

### 6.4.1 Single dose toxicity

Accidental ingestion of toluene has caused severe acute toxicity, including nervous system depression; oropharyngeal and gastric irritation with vomiting and hematemesis.

With respect to inhalation, headache, dizziness, feeling of intoxication, irritation and sleepiness were found to occur with significantly increased frequency at exposure levels from 562 mg/m<sup>3</sup> (150 ppm) down to 281 mg/m<sup>3</sup> (75 ppm).

### 6.4.2 Irritation and sensitisation

No reporting was found regarding dermal irritation and sensitisation.

Toluene vapours causes eye irritation in humans starting at concentrations of 150 mg/m<sup>3</sup> - 375 mg/m<sup>3</sup>.

### 6.4.3 Repeated dose toxicity

Toluene abusers who have been exposed through sniffing for long periods of time exhibit a variety of neurologic manifestations, including ataxia, tremor, anosmia, sensorineural hearing loss, dementia, corticospinal tract dysfunction, abnormal brainstem auditory-evoked potentials, and epileptic seizures. Abnormal magnetic resonance imaging findings in toluene abusers include generalised cerebral, cerebellar, and brainstem atrophy; atrophy of the corpus callosum; and loss of grey-white matter discrimination. Further, optic neuropathies with dyschromatopsia, blindness, and changes in pattern visual-evoked potentials, pendular nystagmus, ocular flutter, opsoclonus (irregular rapid eye movement), bilateral internuclear ophthalmoplegia, and retinal impairment have been reported.

In relation to occupational inhalation exposure to toluene, most studies have addressed the neurotoxic potential of toluene exposure. Studied endpoints affected by toluene exposure were hearing loss; impaired colour vision; impaired performance in neurobehavioural testing, and

subjective CNS symptoms. Several cross-sectional studies have been found, in which an exposed group of workers have been compared with a matched control group. Rotogravure printing is an occupation with a relatively pure exposure to toluene.

Neither the assessments of EU-RAR (2003) nor US EPA (2005) were able to identify robust individual studies from which to make clear conclusions regarding NOAEL/LOAEL values. Instead, US EPA (2005) collected what they considered the most relevant studies for an overall dose-response analysis of the neurotoxic effects (see Appendix 1).

From these studies covering a range of NOAELs from 20 to 48 ppm, US EPA (2005) estimated an arithmetic mean NOAEL value of 34 ppm. This was chosen as an overall NOAEL for the neurotoxic effects and it was noted that this NOAEL value was lower than any of the LOAELs identified in the studies.

Health Canada (2014) identified a NOAEL for humans based mainly on two studies conducted by Seeber et al. (2004; 2005) that examined the same population of exposed individuals within 14 rotary printing plants. These studies covered neurological endpoints such as vibration thresholds, colour discrimination, auditory thresholds, attention (symbol–digit substitution, switching attention and simple reaction), memory (digit span forward and backward, immediate and delayed reproduction of pictures) and psychomotor functions (steadiness, line tracing, aiming, tapping, pegboard). None of the endpoints investigated within these studies was indicative of an adverse effect following exposure to toluene and a NOAEL of 26 ppm or 98 mg/m<sup>3</sup> (as an average of highly exposed individuals) was concluded. It should be noted that all effects investigated in other epidemiological studies were observed at concentrations that exceeded 26 ppm. Although the true NOAEL for neurological endpoints may be higher than 26 ppm, Health Canada (2014) considered 26 ppm the most appropriate value.

Thus, from the evaluations of US EPA (2005) and Health Canada (2014) a NOAEL towards neurological effects from long term occupational exposure in the range of 26 ppm – 34 ppm (100 mg/m<sup>3</sup> – 130 mg/m<sup>3</sup>) can be identified.

#### **6.4.4 Toxicity to reproduction**

Toluene has been shown to cause a series of congenital defects in infants born to mothers who abused toluene (sniffing) during pregnancy. Thus, toluene abuse has been related to a syndrome in human foetuses characterised by physical and neurological abnormalities, resembling the foetal alcohol syndrome.

Studies examining reproductive toxicity of toluene in humans following long-term low-level exposure are less common. Rotogravure printing workers were examined in one study, where 150 male and 90 female were exposed to toluene. Although no quantitative exposure levels were reported, significant association had been identified between toluene exposure and reduced fertility in females. In another study, increased spontaneous abortions were found to be associated with exposure to toluene in the workplace at average air concentration levels 88 ppm (337 mg/m<sup>3</sup>) (range 50–150 ppm or 192–575 mg/m<sup>3</sup>).

These data lend support to the classification as Repr2, H361d (suspected of damaging the unborn child).

#### **6.4.5 Mutagenicity**

Human data on mutagenicity are available from occupationally exposed workers. However, the occupational data do not give any consistent and conclusive answer regarding the genotoxic potential of toluene.

#### **6.4.6 Carcinogenicity**

IARC (1999) evaluated eight epidemiological studies for the discussion of the carcinogenic potential of toluene in humans. Overall, the data were too weak for drawing conclusions with respect to carcinogenicity.

### **6.5 Animal toxicity**

#### **6.5.1 Single dose toxicity**

Toluene has low acute toxicity via inhalation and the oral route. In rats, an LC<sub>50</sub> value of 28.1 mg/l/4h and an oral LD<sub>50</sub> value of 5.58 g/kg have been reported. A dermal LD<sub>50</sub> of 12.4 g/kg has been determined in the rabbit.

#### **6.5.2 Irritation and sensitisation**

In conclusion, toluene is irritating to skin and to eyes in animals. However, the data only warrant classification with respect to skin (EU-RAR, 2003).

#### **6.5.3 Repeated dose toxicity**

##### *Inhalation*

Repeated inhalation exposure to toluene in experimental animals has, depending of duration and concentration levels, caused CNS depression, brain damage, change in brain neurochemistry and hearing loss. Nasal toxicity and effects in forestomach occurred down to the lowest concentrations.

Reduced number of neurones in the hippocampus and a reduced hippocampal weight were found after exposure of rats to toluene at 1500 ppm (5625 mg/m<sup>3</sup>) for 6 months.

Also, in very young rats exposed to toluene via inhalation on postnatal day 1-28 at 100 and 500 ppm (380 and 1900 mg/m<sup>3</sup>), reduced volume of certain hippocampal structures was detected.

Effects on brain neurochemistry were found after long-term exposure at 500 ppm (1900 mg/m<sup>3</sup>) and was still present six months after the last exposure indicating possibly irreversible changes. After only 3 days of exposure, changes in brain neurochemistry were found at an exposure level of 80 ppm (300 mg/m<sup>3</sup>).

The ototoxicity of toluene in the rat is well documented by behavioural, electrophysiological, and morphological techniques. Impaired hearing function was caused by exposure concentration levels of 1000-1400 ppm (3800-5320 mg/m<sup>3</sup>) for 2-8 weeks. In one study, an exposure level of 700 ppm (2660 mg/m<sup>3</sup>) was determined as a NOAEC for auditory toxicity.

However, the lowest LOAEL value associated to chronic exposure was found for nasal toxicity and forestomach ulcers as these effects were found down to the lowest dose tested at 600 ppm ((2280 mg/m<sup>3</sup>).

In another chronic inhalation study a NOAEC of 300 ppm (1125 mg/m<sup>3</sup>) was found as no effects were found at this exposure level.

##### *Oral exposure*

Two 90 days oral NTP studies conducted with F-344 rats and B6C3F1 mice are considered the most important studies for assessing the toxicological potential from oral exposure to toluene.

In these studies toluene was dosed by gavage for 13 weeks at dose levels of 0, 312, 625, 1250, 2500 or 5000 mg/kg during 5 days per week. In male rats, absolute and relative weights of both the liver and kidney were significantly increased ( $p < 0.05$ ) at doses greater than or equal to 625 mg/kg/day. Absolute kidney weights were 100, 107, 112, 119, and 113 % of controls; relative kidney weights were 100, 100, 106, 114, and 146 % of controls for the 0, 312, 625, 1250, 2500 mg/kg/day dose levels. Histopathologic lesions in the liver consisted of hepatocellular hypertrophy, occurring at doses greater than 2500 mg/kg/day. In the brain, mineralised foci and necrosis of neuronal cells were

observed in male and female rats at 2500 mg/kg/day. The study in rats established a NOAEL of 312 mg/kg/day based on increases in liver and kidney weights of male rats at 625 mg/kg/day (LOAEL). In female mice, absolute liver weights were increased in the 312 and 2500 mg/kg/day groups, but not in the other treated groups; relative liver weights were increased in all treated groups. No other changes in organ weights were seen in female mice. No histologic changes in the liver, brain, kidneys, or bladder of any group were reported. It should be noted that no increase in kidney weight was seen in the 1 study in B6C3F1 mice, indicating a species difference in the response.

In summary, effects on liver and kidneys are considered the most critical effects from subchronic exposure to toluene. Thus the NOAEL of 312 mg/kg/day established by US EPA (2005) based on increased kidney weights, which was consistently found in male rats at all of the higher dose levels, is considered as the best documented NOAEL value.

Other effects such as effects on neurotransmitter level and immune response observed in mice after 28 days of oral exposure are considered very uncertain endpoints in relation to risk assessment, as the implication/ relevance of these findings are uncertain. Although WHO (2004) used a dose level of 312 mg/kg/d as a LOAEL in relation to increased liver weight in mice as the most critical finding, the significance of this finding seems debatable, as the effect did not occur consistently at higher dose levels.

US EPA (2005) derived based on data on increased kidney weights in male rats, a BMDL value of 238 mg/kg/day which may be considered the most relevant starting point for risk assessment of repeated oral exposure to toluene.

#### **6.5.4 Toxicity to reproduction**

In a combined two-generation fertility and teratogenicity inhalation study, rats were exposed to either 0, 375, 1875, or 7500 mg/m<sup>3</sup> (0, 100, 500, or 2000 ppm) toluene 6 hours/day, 7 days/week during an 80-day premating period and a 15-day mating period. Females were further exposed on days 1-20 of gestation and during day 5-21 of lactation.

In this study and in further prenatal developmental toxicity studies, no effects on fertility were observed.

In rats, lower foetal and birth weights have been found in offspring of dams exposed to inhalation concentrations around 1000 ppm (3750 mg/m<sup>3</sup>). Long-lasting developmental neurotoxicity (impairment of learning ability) has been demonstrated in offspring exposed prenatally or pre and postnatally to 1200 ppm (4560 mg/m<sup>3</sup>).

Overall, a NOAEC for effects on birth weight and postnatal developmental effects of 2250 mg/m<sup>3</sup> was concluded by the EU-RAR (2013).

Altogether, the experimental animal findings supported by human data indicating increase in spontaneous abortions lead to an EU harmonised classification as Repro. 2, H361d.

#### **6.5.5 Mutagenicity**

Toluene has been extensively tested in *in vitro* assays showing lack of mutagenicity in bacteria as well as genotoxic and mutagenic effects in mammalian cells.

In *in vivo* assays, toluene has not induced biologically significant increases in micronuclei and chromosomal aberrations in the bone marrow of mice and rats or DNA damage in peripheral blood cells, bone marrow, and liver of mice. In a dominant lethal assay, toluene was not considered mutagenic to the sperm of mice in the doses tested, as it did not cause increases in pre or postimplantation loss of embryos.

Overall, toluene is not considered a genotoxic substance.

#### **6.5.6 Carcinogenicity**

Two long-term animal carcinogenicity studies using inhalational exposure were performed.

In one study, groups of 60 male and 60 female B6C3F1 mice, 9–10 weeks of age, were exposed to toluene (purity, > 99 %) by whole-body inhalation at concentrations of 0 (controls), 120, 600 or 1200 ppm (0, 450, 2260 or 4520 mg/m<sup>3</sup>) for 6.5 h per day on five days per week for 104 weeks.

In another study, groups of 60 male and 60 female Fischer 344 rats, six to seven weeks of age, were exposed to toluene (purity, > 99 %) by whole-body inhalation at concentrations of 0 (controls), 600 or 1200 ppm (0, 2260 or 4520 mg/m<sup>3</sup>) for 6.5 h per day on five days per week for 103 weeks.

No significant increases in tumour incidence were observed in these studies.

Less reliable studies using oral and dermal exposure also indicate lack of a carcinogenic potential.

Overall, IARC (1999) has concluded that: “there is evidence suggesting lack of carcinogenicity of toluene in experimental animals”.

#### **6.6 Evaluation, critical effects NOAELs/LOAELs**

As found by others (WHO, 2004; EU-RAR, 2003; US EPA, 2005), the 90 days NTP (1990) oral studies with rats and mice seem to form the most relevant and robust basis for identifying a critical oral dose level.

The EU-RAR (2003) concluded a NOAEL of 625 mg/kg/d from the rat and mouse study, as doses of and above 1250 mg/kg in rats caused neurone necrosis in the brain. The EU-RAR did not - in contrast to WHO (2003) and US EPA (2005) - consider increased liver and kidney weights at the dose level of 625 mg/kg/d adverse effects. While increased liver weight was suggested to be due to metabolic stimulation, no clear justification for waiving increased kidney weight as an adverse effect was given.

While WHO (2003) defined increased liver weight in mice as the critical endpoint (with a LOAEL of 325 mg/kg/d), US EPA (2005) found increased kidney weight in both male and female rats as the most consistent finding with a NOAEL of 325 mg/kg/day for increases in kidney weights in male rats. This was especially considered a relevant endpoint for humans also, as adverse effects in kidneys in humans have been observed from oral intoxications as well as from occupational inhalation. It was noted by US EPA (2005) that the increase in liver weight found in female mice at the lowest dose level of 325 mg/kg/d was not found at higher dose levels of 625 and 1250 mg/kg/d, but first at 25 000 mg/kg/d.

Therefore, overall, adverse effects on kidneys are to be considered the most critical endpoint for oral exposure to toluene. As point of departure for TDI estimation, the BMDL value of 238 mg/kg/d derived by the US EPA (2005) is considered the most adequate. This BMDL corresponded to the lower bound on the dose associated with a 10 % increase in individuals having a kidney weight higher than the 98th percentile of kidney weights in the control level.



# 7. TDI and quality criteria

## 7.1 TDI

According to Danish EPA (2006) guidance for derivation of health based limit values, the TDI value may be calculated from the Benchmark Dose Level (BMDL) as follows:

$$\text{TDI} = \frac{\text{BMDL}}{\text{UF}_I * \text{UF}_H * \text{UF}_L}$$

The BMDL value of 238 mg/kg/d pertains to 5 days of exposure during a week. Thus, corrections should be made according to daily exposure level, i.e.

$$\text{BMDL}(\text{corr}) = 5\text{d}/7\text{d} \times 238 \text{ mg/kg d} = 170 \text{ mg/kg/d}$$

$$\text{TDI} = \frac{\text{BMDL}}{\text{UF}_I * \text{UF}_H * \text{UF}_L} = \frac{170 \text{ mg /kg bw/day}}{10 * 10 * 3 * 2} = 0.28 \text{ mg/kg bw/day}$$

UF<sub>I</sub>: a default interspecies factor of 10 is used to extrapolate from rats to humans

UF<sub>H</sub>: a default intraspecies factor of 10 is used to account for differences in the human population

UF<sub>L</sub>: a factor 3 is used for extrapolation from a BMDL<sub>10</sub> level to a no-effect level and an additional factor of 2 for extrapolating from a subchronic study to chronic lifetime exposure

It is well known that neurotoxic effects are the most critical human endpoint in relation to long term inhalation exposure, and US EPA (2005) and Health Canada (2014) identified a human NOAEL of 34 and 26 ppm for these effects, respectively. In order to compare whether the TDI value above, which is based on the adverse effects on the kidneys, also would protect against neurotoxic long term effects, comparison can be made for the inhalational dose at 30 ppm to the oral TDI value derived above. At an 8 hr dose level of 30 ppm (113 mg/m<sup>3</sup>) a worker would inhale a daily dose of 10 m<sup>3</sup> (inhalation volume during a working day) x 113 mg/m<sup>3</sup> = 1130 mg/d. If a retention rate of 50 % is assumed, this would result in a daily internal dose of 565 mg/d or 8 mg/kg/d (per working day, assuming a body weight of 70 kg). An average dose level over a week would then be 5/7 x 8 mg/kg/d = 5.8 mg/kg/d as a NOAEL exposure.

If deriving a TDI value from this level, an intraspecies factor of 10 would normally be used and a TDI value of 0.58 mg/kg/d would be achieved. As children may be more vulnerable to neurotoxic effects even a higher intraspecies factor might be used. If a higher factor of 20 would be used, it would result in a TDI value of 0.30 mg/kg/d, which is very comparable to the TDI value of 0.28 mg/kg/d calculated based on the adverse effects on kidneys observed in the oral data from experimental animals.

## 7.2 Allocation

Various other sources especially indoor air and emission from vehicles contribute to the daily toluene exposure of the general population. Also, the use of consumer products may be significant

sources. Thus, it is considered prudent only to allocate 10 % of the TDI value to drinking water or soil.

### 7.3 Quality criterion in drinking water

$$QC_{dw} = \frac{TDI * Y}{\text{Ingestion of drink.water}} = \frac{0.28 \text{ mg/kg bw/day} * 0.1}{0.03 \text{ l/kg bw/day}} = 0.93 \text{ mg/l}$$

Y: allocation

Ingestion of drinking water: 0.03 l/kg bw/day (based on a median ingestion rate for 1-10 year-old children, DEPA (2006)).

It should be noted that this value for a health based quality criterion by far exceeds the taste and odour threshold for toluene in water.

Health Canada (2014) recommended an aesthetic limit value of 24 µg/l for protecting against odour in water. This value was based on a threshold level for odour determined by Alexander et al. (1982).

Alexander et al. (1982) determined the odour and taste threshold values in water by using a panel of experienced personnel. A standard odour calibrating substance (1-butanol) was used to select the panellists. The panellists selected were considered to be more sensitive than otherwise reported elsewhere in the literature. Each panellist was subjected to two flasks containing odour and taste-free water and one flask containing a dilution of the test substance. Two panellists were used for each test for odour and taste. The water temperature was 40 °C in tests for the taste threshold and 60 °C in the tests for odour threshold. Thus, the procedure used by Alexander et al. (1982) may be considered a valid approach for determining odour and taste thresholds. However, the use of rather sensitive test panellists compared to the general population may have resulted in rather conservative threshold values. Also the relatively high testing temperatures of 40 °C and 60 °C of the water samples in the flasks may especially for toluene as a volatile compound have resulted in lower threshold values when compared to testing at lower temperatures. So, altogether the threshold values found by Alexander et al. (1982) may be considered conservative and protective values. Furthermore, it is difficult to assess how the data obtained by Alexander et al. (1982) would comply to test results considering the methodology used in odour test standards of today (ASTM E679 - 04(2011) or EN 13725:2003). Thus, until more standardised test data are available on the odour threshold of toluene in water, a drinking water quality criterion for toluene at a rounded figure of 25 µg/l is proposed as a conservative and protective value.

### 7.4 Quality criterion in soil

A health based soil quality criterion can be calculated based on children's soil ingestion according to the guideline from DEPA (2006):

$$QC_{soil} = \frac{TDI * Y * BW}{\text{Ingestion soil}} = \frac{0.28 \text{ mg/kg bw/day} * 0.1 * 13 \text{ kg}}{0.0001 \text{ kg}} = 3640 \text{ mg/kg}$$

Y: allocation

Ingestion of soil: 100 mg/d (based on a median ingestion rate for 1-3 year-old child, DEPA (2006))

BW: 13 kg (body weight of a 1-3 year-old child, DEPA (2006)).

#### 7.4.1 Evaporation from soil

It should be noted that the calculated high toluene level in soil on 3640 mg/kg most probably will result in excession of the evaporation criterion for soil due to the high volatility of the substance, and a strong odour from toluene due to the rather low odour threshold level in air, and will

therefore not be considered a relevant soil quality criterion. The best documented odour threshold level of toluene in air, was estimated to 1.1 mg/m<sup>3</sup> by Nagata et al. (2003), who used a triangle odour bag test method during systematic testing for odour threshold for 223 substances. The evaporation criterion which is 0.4 mg/m<sup>3</sup>, based on the current C value (limit value in ambient air), should be considered the most relevant criterion for toluene polluted soil.

## **7.5 Conclusion, quality criteria**

*Drinking water:* 25 µg/l (for protection against odour from toluene)

Odour, and not the toxicological effects, is the limiting factor for toluene content in drinking water.

*Soil:* 0.4 mg/m<sup>3</sup> (evaporation criterion), which is based on the current C value (limit value in ambient air and close to the odour threshold), is the limit value for toluene content in soil.

# 8. References

Alexander, H.C., McCarty, W.M., Bartlett, E.A. and Syverud, A.N. (1982). Aqueous odor and taste threshold values for industrial chemicals. J. Am. Water Works Assoc., 74: 595–599.

ASTM E679 - 04(2011). Standard Practice for Determination of Odor and Taste Thresholds By a Forced-Choice Ascending Concentration Series Method of Limits CCME (2004). Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health. Toluene 2004. Canadian Environmental Quality Guidelines. Canadian Council of Ministers of the Environment,

Com Dir (2006). COMMISSION DIRECTIVE 2006/15/EC of 7 February 2006 establishing a second list of indicative occupational exposure limit values in implementation of Council Directive 98/24/EC and amending Directives 91/322/EEC and 2000/39/EC

DEPA (2006). Metoder til fastsættelse af kvalitetskriterier for kemiske stoffer i jord, luft og drikkevand med henblik på at beskytte sundheden. Vejledning fra Miljøstyrelsen Nr. 5.

DEPA (2008a). Supplement til B-værdivejledningen 2008. Miljøprojekt Nr. 1252. Danish Environmental Protection Agency.

DEPA (2008b). Olie i jord–forslag til analysemetode og justering af jordkvalitetskriterier, samt grundlag for afskæringskriterier. Miljøprojekt Nr. 1225 2008. Teknologiuudviklings-programmet for jord- og grundvandsforurening

DEPA (2014). Survey of toluene. Part of the LOUS review Environmental project No. 1613, 2014. Danish Environmental Protection Agency.

DEPA (2015). Liste over kvalitetskriterier i relation til forurenede jord og kvalitetskriterier for drikkevand. June 2015. Danish Environmental Protection Agency. <http://mst.dk/virksomhed-myndighed/kemikalier/graensevaerdier-og-kvalitetskriterier/graensevaerdier-for-vand/>

DEPA (2016). Kortlægning og risikovurdering af toluen og andre neurotoksiske stoffer i børneværelser. (Survey and risk assessment of toluene and other neurotoxic substances in children's rooms). Environmental Project xxx. Danish Environmental Protection Agency. (Under publication)

DME (2012). Statutory order on changing the statutory order on limit values for substances and materials, No. 986 of 11/10/2012. Danish Ministry of Employment

EN 13725:2003. Air quality. Determination of odour concentration by dynamic olfactometry

EU-RAR (2003). European Union Risk Assessment Report Toluene. Volume: 30. Institute for Health and Consumer Protection, European Chemicals Bureau, European Commission.

Faber J et al., 2014. Comparison of air pollution by VOCs inside cabins of new vehicles. *Env. Nat. Res. Research* 4 (3), 155-165.

Fedoruk MJ and Kerger BD, 2003. Measurement of volatile organic compounds inside automobiles. *J. Exp. Anal. Env. Epidem.* 13, 31-14

Geiss O, Giannopoulos G, Tirendi S, Barrero-Moreno J, Larsen BR, Kotzias D, 2011. The AIRMEX study - VOC measurements in public buildings and schools/kindergartens in eleven European cities: Statistical analysis of the data. *Atmospheric Environment* 45, 3676-3684.

GEUS (2013). Grundvandsovervågning. Grundvand Status og udvikling 1989 – 2012. De Nationale Geologiske Undersøgelser for Danmark og Grønland (GEUS).

Health Canada (2014). Toluene, Ethylbenzene and Xylenes in Drinking Water. Document for Public Comment. Prepared by the Federal-Provincial-Territorial. Committee on Drinking Water. <http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/toluene/index-eng.php#1>.

IARC (1999). Toluene in: Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 71.

Juhler R & Felding G (2001). Organiske mikroforureninger i dansk grundvand. *Dansk Kemi*, 82, nr. 4, 17-21.

Nagata Y, 2003. Measurement of odor threshold by triangle odor bag method. Odor measurement review. Tokyo (Japan): Office of Odor, Noise and Vibration, Environmental Management Bureau, Ministry of Environment, p 118-127.

Seeber, A., Schäper, M., Zupanic, M., Blaszkewicz, M., Demes, P., Kiesswetter, E. and van Thriel, C. (2004). Toluene exposure below 50 ppm and cognitive function: a follow-up study with four repeated measurements in rotogravure printing plants. *Int. Arch. Occup. Environ. Health*, 77: 1–9.

Seeber, A., Demes, P., Kiesswetter, E., Schäper, M., van Thriel, C. and Zupanic, M. (2005). Changes of neurobehavioral and sensory functions due to toluene exposure below 50 ppm? *Environ. Toxicol. Pharmacol.* 19:635–643.

Tukes (2013). SUBSTANCE EVALUATION REPORT Background document for the purpose of substance evaluation under REACH for Toluene. Finnish Safety and Chemical Agency

UK-EA (2009a). Contaminants in soil: updated collation of toxicological data and intake values for humans. Toluene. Better Regulation Science Programme. Science report: SC050021

UK-EA (2009b). Soil Guideline Values for toluene in soil. Science Report SC050021 / toluene SGV. Environment Agency.

US EPA (2005). TOXICOLOGICAL REVIEW OF TOLUENE. In Support of Summary Information on the Integrated Risk Information System (IRIS). September 2005. EPA/635/R-05/004

US EPA (2014). Draft Update of Human Health Ambient Water Quality Criteria: Toluene. EPA 820-D-14-063 May 2014 -

WHO (2004). Toluene in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/03.04/116.

# 9. Appendix 1

**TABLE 1. SELECTED SUBSET OF OCCUPATIONAL STUDIES OF NEUROLOGICAL EFFECTS FROM INHALATION OF TOLUENE (US EPA 2005)**

<b>Study number in Figure 1 and reference</b>	<b>Number of workers and duration of exposure (average years <math>\pm</math> SD)</b>	<b>NOAEL (ppm)</b>	<b>LOAEL (ppm)</b>	<b>Effect/test</b>	<b>Response level at the LOAEL (statistically significant response compared to controls)<sup>a</sup></b>	<b>Noted potential limitations</b>
1. Abbate et al., 1993	Reference (n=40), exposed (n=40)  (12-14 years; no SD reported)	None <sup>b</sup>	97	Brainstem response auditory-evoked potential	28% increase of the latency shift for wave-I during passage from 11 to 90 repetitions.	
2. Boey et al., 1997	Reference (n = 29) exposed (n = 29)  (4.9 $\pm$ 3.5 years; range of 1-13 years)	None	91	Neuropsychological examination; digit span, visual reproduction, Benton visual retention test, trail making test, symbol digit modality test, grooved pegboard test, and finger tapping tests	Increased time to complete the grooved pegboard test 7% and 6% for dominant and non-dominant hands respectively,	Control workers were exposed to 12 ppm toluene

Study number in Figure 1 and reference	Number of workers and duration of exposure (average years $\pm$ SD)	NOAEL (ppm)	LOAEL (ppm)	Effect/test	Response level at the LOAEL (statistically significant response compared to controls) <sup>a</sup>	Noted potential limitations
					increase in time to complete trail-making test parts A&B, 31% & 28%, respectively; 15% decrease in backward digit span test; 12% and 10% decrease in symbol digit modality test for written and oral sections, respectively.	
3. Cavalleri et al., 2000	Reference (n=16), exposed (n=33)  (9.75 years; no SD)	None	42	Color vision impairment (Lanthony D-15)	29% increase in CCI and 49% increase in total confusion index (TOCI) (reported as mean of both	Exposure measured from urinary excretion of toluene: on the basis of previous data, air concentrations estimated to be



Study number in Figure 1 and reference	Number of workers and duration of exposure (average years $\pm$ SD)	NOAEL (ppm)	LOAEL (ppm)	Effect/test	Response level at the LOAEL (statistically significant response compared to controls) <sup>a</sup>	Noted potential limitations
	reported)				eyes).	42 ppm.
4. Eller et al., 1999	Reference (n=19), low exposure (n=30), high exposure (n=49)  low exposure (1-12 years; no SD reported) high exposure (>12 years)	20	>100	Neuropsychological examination (Cognitive Function Scanner); verbal and nonverbal learning and memory, visuomotor function, computerized neurological examination (CATSYS, TREMOR, and SWAY), subjective assessment	13% increase in performance time on Bourdon Wiersma Test but no increase in the number of missed or incorrect detections; 33% of exposed population reported concentration difficulties.	The high exposure classification was based on historical exposures which may have exceeded 100 ppm for up to 27 years.
5. Foo et al., 1990	Reference (n=30), exposed (n=30)  (5.7 $\pm$ 3.2	None	88	Neurobehavioural tests: Benton visual retention test, visual reproduction, trail making, grooved pegboard,	Increased time to complete the trail-making test parts A&B, 51% &	Control workers were exposed to 13 ppm toluene for 2.5 $\pm$ 3.2 years. The education level

<b>Study number in Figure 1 and reference</b>	<b>Number of workers and duration of exposure (average years <math>\pm</math> SD)</b>	<b>NOAEL (ppm)</b>	<b>LOAEL (ppm)</b>	<b>Effect/test</b>	<b>Response level at the LOAEL (statistically significant response compared to controls)<sup>a</sup></b>	<b>Noted potential limitations</b>
	years)			digit span, digit symbol, finger tapping, and simple reaction time	63%, respectively; 25% decrease in digit symbol test performance; 16% decrease in total digit span test scores (both forward and backward).	was lower in the exposed group. As a result, data from the neurobehavioural tests were adjusted for years of education using a generalized linear model.
6. Murata et al., 1993	Reference (n=10), exposed (n=10)  (11 years; range of 1-36 years; no SD reported)	None	83	Electrophysiological analysis of maximal motor and sensory nerve conduction velocity (MCV & SCV)	9% reduction in the MCV in the forearm and 6% reduction in the SCV in the palm.	Exposed workers were matched for age but not alcohol consumption.
7. Nakatsuka et al.,	Reference (n=120), exposed	44-48	None	Color vision impairment (Lanthony's new	No measured effect on color vision.	In lieu of determining exposure

<b>Study number in Figure 1 and reference</b>	<b>Number of workers and duration of exposure (average years <math>\pm</math> SD)</b>	<b>NOAEL (ppm)</b>	<b>LOAEL (ppm)</b>	<b>Effect/test</b>	<b>Response level at the LOAEL (statistically significant response compared to controls)<sup>a</sup></b>	<b>Noted potential limitations</b>
1992	(n=174)			color test and Ishihara's color vision test)		duration, groups were age-matched to control for effects of aging on color vision.
8. Neubert et al., 2001	Ref-ex (n=109), ref-int (n=48), exp gp I (n=316), exp gp II (n=535 ), exp gp III (n=308), exp gp IV (n=65)	39 (exp gp 1)	81 (ex gp IV)	Psychophysiological and psychomotor testing: verbal memory span, visuomotor performance, immediate visual memory, self-rating of feeling, biosensory vigilance, critical flicker fusion frequency test, personality dispositions	5% reduction in ascending flicker fusion frequency.	Exposure was identified as chronic but the duration was not reported.
9. Vrca et al., 1995	Reference (n=59), exposed (n=49)	None	40-60	Visual evoked potentials	The amplitudes of visual evoked brain potentials	Exposure levels were estimated based on urinary levels of metabolites and

Study number in Figure 1 and reference	Number of workers and duration of exposure (average years $\pm$ SD)	NOAEL (ppm)	LOAEL (ppm)	Effect/test	Response level at the LOAEL (statistically significant response compared to controls) <sup>a</sup>	Noted potential limitations
	(21.4 $\pm$ 7.4 years)				were 24, 43, and 55% higher for N75, P100, and N145, respectively.	toluene levels in blood.
10. Zavalic et al., 1998a	Reference (n=90), low exposure (n=46), high exposure (n=37)  low exposure (16.21 $\pm$ 6.1 years) high exposure (18.34 $\pm$ 6.03 years)	32	132	Color vision impairment (Lanthony D-15)	10-14% increase in CCI (both eyes).	The results from this investigation were reported in several publications (Zavalic et al., 1998a,b,c); some reporting discrepancies exist regarding the number of workers in the exposed and control groups and the statistical analyses.

<sup>a</sup> Not all studies examined all neurotoxicity endpoints.

## **Toluene**

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to toluene. This resulted in the present report which includes estimation of a quality criterion in drinking water and soil for toluene.

