

# Air Toxics Hot Spots Program

## Toluene Reference Exposure Levels

Technical Support Document for the  
Derivation of Noncancer Reference  
Exposure Levels

Appendix D1

August 2020

Air and Site Assessment and Climate Indicator Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency



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Noncancer Reference Exposure Levels

### Appendix D1

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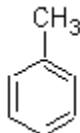
### List of Acronyms

ACGIH	American Conference of Governmental Industrial Hygienists	GTP	glutamyl transpeptidase
AF <sub>HK</sub>	human toxicokinetic variability adjustment factors	HSDB	Hazardous Substances Data Bank
AIC	Akaike Information Criterion	IPL	interpeak latency
ANOVA	analysis of variance	LH	luteinizing hormone
API	American Petroleum Institute	LOAEL	Lowest observed adverse effect level
ATSDR	Agency for Toxic Substances and Disease Registry	LWAE	lifetime weighted average exposure
AUC	area under the curve	MMEF	peak expiratory flow rate at 50% of FVC
BAEP	brainstem auditory evoked potential	MRI	magnetic resonance imaging
BAER	brainstem auditory evoked response	MRL	Minimal risk level
BAL	bronchoalveolar lavage	NES	Neurobehavioral evaluation system
BMC	Benchmark concentration	NHANES	National Health and Nutrition Examination Survey
BMC <sub>05</sub>	Benchmark concentration producing a 5% response rate	NOAEL	No observed adverse effect level
BMCL <sub>05</sub>	the 95% lower confidence limit of the dose producing a 5% response rate	NTP	National Toxicology Program
BMD	Benchmark dose	OECD	Organization for Economic Cooperation and Development
BMDL	estimation of the BMD 95% lower bound confidence limit	PBPK	Physiologically based pharmacokinetics
CAR	Conditioned Avoidance Response	PEFR	peak expiratory flow rate
CCI	color confusion index	ppb	parts per billion
CNS	central nervous system	ppm	parts per million
CTI	California Toxics Inventory	REL	Reference exposure level
CVD	cardiovascular disease	RfC	Reference concentration
ELISA	Enzyme-linked immunosorbent assay	SDT	signal detection task
FAS	fetal alcohol syndrome	SMCBs	small- and medium-sized commercial buildings
FEF <sub>25-75%</sub>	Forced respiratory flow (25-75% of forced vital capacity)	TAC	Toxic air contaminant
FEV1	Forced expiratory volume in 1 second	ToIU	unmetabolized toluene in urine
FSH	follicle stimulating hormone	TOTCI	total confusion index
FVC	Forced vital capacity	TSD	Technical Support Document
GC	gas chromatography	TWA	Time-weighted average
GPT	glutamic-pyruvic transaminase	UDP	Uridine diphosphate glucose
		UF	Uncertainty factor
		VEP	visual evoked potential
		VER	visual evoked response
		VOC	Volatile organic compound

# Toluene Reference Exposure Levels

(Methyl benzene; methyl benzol; phenyl methane; toluol)

CAS Registry Number 108-88-3



## 1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b) (2)). In response to this statutory requirement OEHHA developed a Technical Support Document (TSD) that describes acute, 8-hour, and chronic Reference Exposure Levels (RELs). The TSD was adopted in December 2008 (OEHHA, 2008) and presents methodology for deriving RELs, and in particular explicitly includes consideration of possible differential effects on the health of infants, children, and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*).

Toluene was chosen for a re-evaluation of its previously established RELs because it is one of the prioritized toxic air contaminants selected for focused literature review in the Prioritization of Toxic Air Contaminants Under the Children's Environmental Health Protection Act document (OEHHA, 2001; Table 1A). The noncancer REL TSD guidelines (OEHHA, 2008) have been used to develop the RELs for toluene presented in this document; this document will be added to Appendix D of the TSD.

Toluene is a solvent that has been shown to cause sensory irritation (i.e., eye and upper respiratory irritation) and central nervous system depression in humans at high acute airborne exposures. Prolonged or repeated exposures have been associated with neurophysiological decrements and acquired color vision impairment (dyschromatopsia). The non-cancer adverse health effects of toluene also include severe disabilities of children if the substance is abused by deliberate inhalation during pregnancy for its narcotic effect. Infants and children may be more susceptible to the effects of toluene because their nervous systems are still developing, and because metabolism of toluene in the first years of life is slower compared to that of adults.

This review includes relevant material published through October 2018 and is a technical review of those studies specifically applicable to developing non-cancer acute, 8-hour, and chronic inhalation RELs for toluene.

**Toluene Acute REL**

<i>Reference exposure level</i>	<b>5,000 µg/m<sup>3</sup> (5 mg/m<sup>3</sup>; 1,300 ppb; 1.3 ppm)</b>
<i>Critical effect(s)</i>	Headache, dizziness, slight eye and nose irritation.
<i>Hazard index target(s)</i>	Nervous system; eyes; respiratory system.

**Toluene 8-hour REL**

<i>Reference exposure level</i>	<b>830 µg/m<sup>3</sup> (0.83 mg/m<sup>3</sup>; 220 ppb; 0.22 ppm)</b>
<i>Critical effect(s)</i>	Acquired color vision impairment (dyschromatopsia)
<i>Hazard index target(s)</i>	Eyes

**Toluene Chronic REL**

<i>Reference exposure level</i>	<b>420 µg/m<sup>3</sup> (0.42 mg/m<sup>3</sup>; 110 ppb; 0.11 ppm)</b>
<i>Critical effect(s)</i>	Acquired color vision impairment (dyschromatopsia)
<i>Hazard index target(s)</i>	Eyes

**2. Physical & Chemical Properties** (HSDB (2006) except as noted)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C <sub>7</sub> H <sub>8</sub>
<i>Molecular weight</i>	92.14 g/mol
<i>Density</i>	0.8636 g/cm <sup>3</sup>
<i>Boiling point</i>	110.6 °C
<i>Melting point</i>	-94.9 °C
<i>Vapor pressure</i>	28.4 mm Hg at 25°C
<i>Odor threshold</i>	11 mg/m <sup>3</sup> (2.9 ppm) (Amoore and Hautala, 1983) sweet, pungent, benzene-like odor
<i>Solubility</i>	Soluble in most common organic solvents, considered insoluble in water (0.0526 g/100ml at 25 °C).
<i>Conversion factor</i>	3.76 mg/m <sup>3</sup> = 1 ppm at 25° C

### 3. Major Uses and Sources

Toluene occurs naturally as a component of crude oil and is produced in petroleum refining and coke oven operations (HSDB, 2006). As a result, automobile emissions are the principal source of toluene to the ambient air. Xylenes, ethylbenzene, and benzene are often found together as airborne co-pollutants with toluene. Toluene has been used as a sentinel chemical for benzene in the context of air and water sample monitoring. Toluene is used as a solvent in paints, coatings, synthetic fragrances, adhesives, inks, and cleaning agents. It has also been applied in the production of polymers used to make nylon, plastic soda bottles, and polyurethanes and for pharmaceuticals, dyes, cosmetic nail products, and the synthesis of organic chemicals (Cosmetic Ingredient Review Panel, 1987).

The highest concentrations of toluene usually occur in indoor air from the use of common household products (paints, paint thinners, adhesives, synthetic fragrances and nail polish) and cigarette smoke (Sack et al., 1992).

In 2005, the California statewide mean outdoor monitored concentration of toluene was approximately  $2.19 \mu\text{g}/\text{m}^3$  (0.58 ppb) (CARB, 2017). Estimates for toluene emissions from the Statewide 2010 California Toxics Inventory (CTI) were 9867 tons from point sources, 4119 tons from area-wide sources, 10,784 tons from on-road mobile sources, 6088 tons from other mobile sources, and 46 tons from natural sources (CARB, 2010). Among the U.S. general public, the mean toluene blood concentration was  $1.96 \mu\text{g}/\text{m}^3$  (0.52 ppb) in adults and  $0.53 \mu\text{g}/\text{m}^3$  (0.14 ppb) in children (Ashley et al., 1994; Sexton et al., 2005).

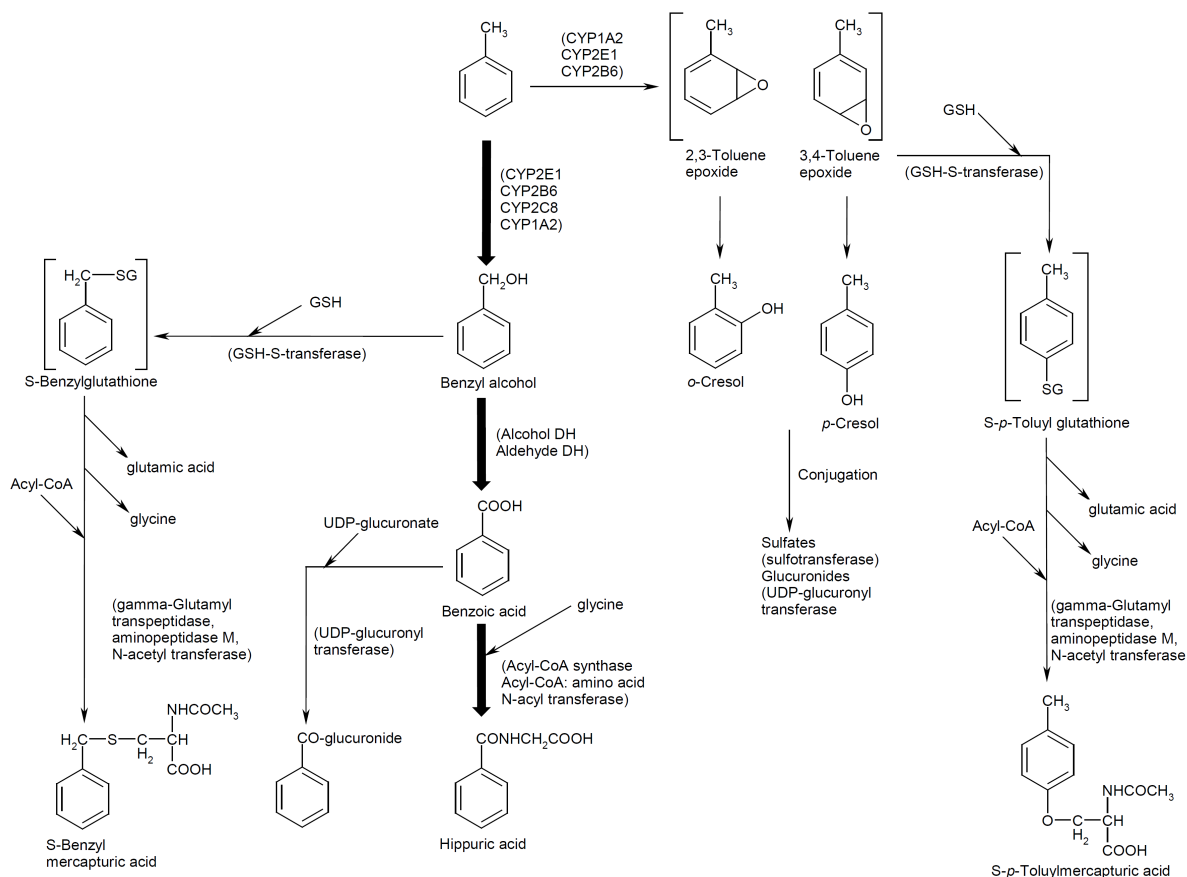
Toluene may also be released to the ambient air during the production, use, and disposal of industrial and consumer products that contain toluene. Levels of toluene measured in rural, urban, and indoor air averaged 1.3, 10.81, and  $31.5 \mu\text{g}/\text{m}^3$  (0.35, 2.88, and 8.4 ppb), respectively (USEPA, 1988). A geometric mean concentration of  $9.8 \mu\text{g}/\text{m}^3$  (2.6 ppb) (range:  $2.6\text{--}16.9 \mu\text{g}/\text{m}^3$ , 0.7–4.5 ppb) toluene was recorded for 12 northern California office buildings in an indoor air quality study (Daisey et al., 1994). A recent study by Wu et al. (2011) identified a geometric mean concentration of  $4.47 \mu\text{g}/\text{m}^3$  (1.19 ppb) (range  $0.44\text{--}200 \mu\text{g}/\text{m}^3$ , 0.12 – 53 ppb) toluene in 37 small- and medium-sized commercial buildings (SMCBs) in California. The main source of toluene in these buildings was from motor vehicle emissions. The highest concentrations of toluene are often found in buildings immediately after construction or renovation (Brown, 2002). Toluene concentrations measured in portable and main building classrooms in California during school hours were found to range between 4.7 and  $21.4 \mu\text{g}/\text{m}^3$  (1.25 – 5.68 ppb) (Shendell et al., 2004).

### 4. Metabolism

Studies in human subjects and laboratory animals indicate that toluene is readily absorbed from the respiratory and gastrointestinal tracts, but is only slowly absorbed through skin when applied topically (ACGIH, 2010). Thus, toluene was not assigned a skin notation by ACGIH for worker incidental contact with the liquid. Toluene is rapidly taken up into the bloodstream (Carlsson 1982) and distributed to various brain regions (Gerasimov et al. 2002). The metabolism of toluene is depicted in Figure 1. The initial step in toluene metabolism is transformation by cytochrome P-450 (CYP) enzymes, which occurs mainly in the liver. The most prominent of



these transformations is hydroxylation of the methyl group to form benzyl alcohol followed by oxidation to benzoic acid (Tassaneeyakul et al., 1996; Nakajima et al., 1997). Most of the benzoic acid is then conjugated with glycine to form hippuric acid, but a small portion can be conjugated with UDP-glucuronate to form the acyl-glucuronide. A minor CYP-related pathway involves a transient epoxidation of the aromatic ring to form either *o*- or *p*-cresol. The cresols may undergo a variety of conjugation reactions, forming mainly sulfates and glucuronides. Glutathione conjugation may also occur resulting in S-benzylglutathione and S-benzylmercapturic acid (conjugation to benzyl alcohol), or S-*p*-toluyl glutathione and S-*p*-toluylmercapturic acid (conjugation to the epoxidated ring). Much of the remaining toluene is exhaled unchanged. The urinary excretion of toluene and its metabolites is rapid, with the major portion excreted within 12 hours of exposure (Baelum et al., 1993).



**Figure 1. Proposed pathways for toluene metabolism.** Proposed enzymes are noted in parentheses. CoA = coenzyme A; CYP = cytochrome P-450; DH = dehydrogenase; GSH = glutathione; UDP = uridine 5'-diphosphate. Sources: US EPA (2005), Angerer et al. (1998), IARC (1999), Nakajima and Wang (1994), Nakajima et al. (1997), and Tassaneeyakul et al. (1996).

CYP2E1 is considered to be the most active hepatic isozyme in metabolizing toluene to benzyl alcohol, particularly at low exposure concentrations (Nakajima and Wang, 1994; Nakajima et al., 1997). Other isozymes, including CYP2B6, CYP2C8, CYP1A2, and CYP1A1 are also active in forming benzyl alcohol. CYP1A2 is thought to be the main isozyme in forming *o*-cresol and other

minor metabolites. CYP2E1 is undetectable in the fetal liver, but develops rapidly within hours after birth (Cresteil, 1998; Vieira et al., 1996). CYP1A2 and other hepatic CYPs begin to develop by about one month of age. A majority of the CYPs, including CYP2E1, are near adult concentrations by one year of age. Phase II enzymes, such as some glutathione-S-transferases isozymes, are present in the fetal liver (Cresteil, 1998). Other isoforms that are present at very low levels in the fetal liver increase soon after birth.

The abundance of CYPs in the human lung is only about 10% of the specific content found in the liver (Zhang et al., 2006). However, the lung may represent as important a site for toluene metabolism by the inhalation route. Many of the same isozymes involved in hepatic toluene metabolism are also present in the lung, including CYP2E1, CYP1A1, CYP1A2, CYP2B6 (Bernauer et al., 2006; Hukkanen et al., 2002; Zhang et al., 2006). In addition, CYP2A13 is expressed predominantly in the pulmonary tract and showed a high activity for benzyl alcohol formation from toluene (Fukami et al., 2008). Similar to the liver, many of the pulmonary CYP isozymes are not expressed or are expressed at very low levels at birth, but begin to develop soon thereafter (Day et al., 2006; Fanucchi, 2006). The increase in CYP expression occurs in parallel with the differentiation of the epithelium in growing children, which indicates that enzymatic activity of many CYP isozymes are age-dependent. However, lung development continues for approximately 8-12 years. Phase II enzymes appear to be expressed earlier in development compared to CYP monooxygenases, suggesting a role in protecting the lung during the period of rapid cellular differentiation and cell division (Fanucchi, 2006).

To conclude, the available evidence indicates that hepatic metabolism of toluene by infants may not reach adult levels until about one year of age. However, metabolism of toluene by pulmonary CYP isozymes in children may not reach adult levels until considerably later. Thus, infants may be more susceptible to toxic effects of toluene due to slower metabolism during the early years of life.

The toluene urinary metabolite hippuric acid is frequently used as a biological marker for monitoring toluene exposure in the workplace (Morata et al., 1997; Thetkathuek et al. 2015). However, it is a common urinary constituent from food sources, which may result in high background levels of the metabolite in urine. Co-exposure to other compounds, including styrene, ethyl benzene and benzoic acid, can result in interference since hippuric acid is also a common metabolite of these compounds. Because the background level of hippuric acid will mask occupational exposure at the TLV value of 20 ppm, the ACGIH (2010) has recommended toluene in blood, unchanged toluene in urine, or the urinary metabolite *o*-cresol to be used as markers of toluene exposure. *O*-cresol is a more sensitive indicator of exposure than hippuric acid and can be used to assess low exposures to toluene.

With exposure to the same concentration of toluene, men were found to have a greater rate of excretion (in  $\mu\text{mol}/\text{min}$ ) of hippuric acid and *o*-cresol compared to women (Baelum et al., 1987). The difference in excretion rate was correlated to body weight and to work level. However, when corrected for urinary creatinine, the excretion rate of hippuric acid and *o*-cresol was greater in women. The authors also noted there was a large inter-individual variation in urinary metabolite levels among the test subjects. Under similar exposure conditions, the alveolar toluene concentration tended to be higher in females than in males both at rest and during exercise (Baelum et al., 1990). The excretion of the metabolites hippuric acid and *o*-cresol were found to be correlated to the alveolar toluene concentration at rest but not during work. The tendency

towards a higher alveolar toluene concentration in females than in males could not be explained by correlations to body size, nutritional status, or to lung volumes. The authors speculated that this gender difference may be caused by the different fat distribution or by altered circulation.

Tardif et al. (1995) used a physiologically-based pharmacokinetic (PBPK) model, developed and validated in the rat, to predict the uptake and disposition kinetics of a toluene and xylene mixture in humans. They substituted the rat physiological parameters and the blood:air partition coefficient with those of humans, and kept all other model parameters species-invariant. The human toluene and xylene mixture PBPK model, developed based on the competitive metabolic inhibition mechanism of interaction elucidated in the rat, simulated adequately the kinetics of toluene and xylene during combined exposures in humans. The simulations with this PBPK model indicate that an eight hour co-exposure to concentrations that remain below the current of the American Conference of Governmental Industrial Hygienists Threshold Limit Value for toluene (50 ppm or 190 mg/m<sup>3</sup>) and xylene (100 ppm) would not result in significant pharmacokinetic interferences. This suggests that data from biological monitoring of worker exposure to these solvents would be unaffected by co-exposures.

Pelekis et al. (2001) used validated PBPK models and simplified physiological-model-based algebraic equations to translate ambient exposure concentration (1 ppm of a VOC for 30 days) to tissue dose in adults and children for selected VOCs, including toluene. This approach derived a pharmacokinetic (PK) Uncertainty Factor (UF) for human adult heterogeneity within a human population and an adult-to-child PK UF based on the range of human physiological parameters used in PBPK models. The adult-to-child UF assumed a child 10 kg in weight, equivalent to an age between 1 and 2 years. The results indicated that there was no significant difference between the UF for human adult PK variability and the PK variability for the adult-to-child UF. Therefore, the authors concluded that the standard human adult PK default of 3.16 is sufficient to protect children's health as well. The lack of a significant difference between the two PK UFs was primarily due to the hepatic clearance for highly metabolized VOCs, such as toluene, being nearly identical for both adults and children. This model is supported by the findings of Nong et al. (2006) below, which calculated an adult-child PK variability factor of 1.5 (i.e., the adult-child variability factor, calculated as the ratio of the 95<sup>th</sup> percentile value over the 50<sup>th</sup> percentile value for the adult and children 1-11 years of age).

The simulations run by Nong et al. (2006) indicated that neonates with higher levels of CYP2E1 (4.33 to 55.93 pmol/mg protein) as well as older children would have a lower AUC (0.16 to 0.43 µg/ml × hr). The latter values were closer to those simulated for adults. Similar results were also obtained for 7 hr exposure to 17 ppm (64 mg/m<sup>3</sup>) toluene, a scenario previously evaluated in human volunteers. The authors did not explicitly address whether CYP metabolism of toluene is expected to be similar in adult men and women. The adult-child metabolism variability factor, calculated as the ratio of the 95<sup>th</sup> percentile value for the following age groups over the 50<sup>th</sup> percentile value for the adult, was 3.9 for neonate low metabolizers, 1.6 for infants (1 month–1 year), 1.5 for children (1–11 years), and 1.4 for adolescents (12–17 years). The lack of large variability in the adult-child PK factor was explained by Nong et al. (2006) on the basis of CYP2E1 levels in neonates, children and adults. CYP2E1 maturation occurs rapidly after birth, and the enzyme content is a more sensitive parameter than hepatic blood flow rate in the neonates whereas metabolism is more sensitive to blood flow in all other age groups. The outcome is that hepatic metabolism of toluene would appear to be limited by enzyme content at

birth and evolve gradually to a flow-limited condition with increasing age. Not surprisingly, the rate of metabolism and AUC of toluene in high metabolizing neonates and older children is comparable to adults.

Mörk et al. (2014) derived human toxicokinetic variability adjustment factors ( $AF_{HK}$ ) for toluene using previously validated PBPK models, based on toluene surrogate concentrations in blood for six different age groups: 3 month-old and 1 year-old infants, 5, 10, and 15 year-old children, and adults. The metabolism of toluene was modeled in both liver and lungs, was saturable, and obeyed Michaelis-Menten kinetics. The resulting PBPK model was used to simulate the blood toluene concentration profiles in each age group, assumed to be exposed to 1.3 ppm (4.9 mg/m<sup>3</sup>) toluene in the air through inhalation over 24 hours. The  $AF_{HK}$  values were calculated as the ratio between 95<sup>th</sup> percentile of the surrogate dose in each respective age group and the median (50<sup>th</sup>) surrogate dose in the whole population. The ratios were 1.3 for 3 month-olds and 1.5 or 1.6 for all other age groups. In this model, only slight differences between age groups were seen, similar to what was observed by Nong et al. (2006) above, except for < 1 month neonates in Nong. The authors did not discuss what caused the difference in modeling results between the two studies. However, Nong et al. used the median value for adults in the denominator, rather than the population as a whole as Mörk et al. did. Gender differences in the toluene  $AF_{HK}$  were small. The  $AF_{HK}$  for adult males and females were 1.5 and 1.6, respectively.

## 5. Acute Toxicity of Toluene

### Acute Toxicity to Adult Humans

Acute toxic effects to the central nervous system (CNS), cardiovascular, hematopoietic, reproductive, and respiratory systems, as well as to the liver, kidneys, skin, and sensory organs have been reported for toluene (Fishbein, 1988). The CNS is a primary target organ for toluene toxicity in both humans and animals for acute and chronic exposures. CNS dysfunction (which is often reversible) and narcosis have been frequently observed in humans acutely exposed to low or moderate levels of toluene by inhalation; symptoms include fatigue, sleepiness, headaches, and nausea.

Toluene is one of the most widely abused chemical substances due to its sweet, pungent smell and its narcotic effect, especially among teenagers and young adults because it is inexpensive and easy to obtain (Lubman et al. 2008; Howard et al. 2011). Death has occurred at very high levels of exposure from abuse of toluene-containing solvents (Paterson and Sarvesvaran, 1983; Takeichi et al., 1986; Ameno et al., 1992; Shibata et al., 1994; Kamijo et al., 1998; Argo et al., 2010; Tang et al., 2005). High concentrations of toluene have been found in the victims' blood, brain, lung, kidney, and liver. Autopsy findings include cerebral edema, congestion of cerebral veins, pulmonary edema, and pancreatic and renal congestion. Acute abusers of toluene solvent have also developed severe muscle paralysis, hypokalemia, renal tubular acidosis, and hyperchloremic metabolic acidosis.

### *Case Reports*

Two separate workplace incidents involving acute inhalation exposure to toluene in several workers resulted in euphoria, drunkenness, dizziness, nausea, confusion, incoordination, drowsiness, and loss of consciousness (Longley et al., 1967). The toluene concentrations were estimated at 10,000 to 30,000 ppm (40,000 to 110,000 mg/m<sup>3</sup>) although no actual measurements were made. No long-term follow-up of the exposed workers was conducted. Cardiac arrhythmia has also been reported in humans acutely exposed to toluene (Dinwiddie, 1994). Following inhalation of a very high, but unknown level of toluene, an individual died from severe CNS depression (Kamijo et al., 1998). Constriction and necrosis of myocardial fibers, swollen liver, congestion and hemorrhage of the lungs, and tubular kidney necrosis were also reported.

Kao et al. (2014) reported leukoencephalopathy (structure alteration of the brain white matter) with magnetic resonance imaging (MRI) features atypical from those of chronic solvent intoxication after a 49-year-old man exposed to probable very high levels of toluene during a lacquer thinner explosion. The man ignited a fire while stripping a floor with lacquer thinner, and was sent to the emergency department with second and third degree burns of the face and four extremities, but he was alert and cooperative. In the following month of burn intensive care, in spite of the aggressive treatments, the patient's consciousness rapidly deteriorated to coma. MRI of the brain at 35 days revealed multiple white matter lesions with perifocal edema. Brain biopsy of the largest lesion at 3 months revealed diffuse white matter necrosis without evidence of microorganism or significant inflammatory infiltration. The patient died of multiple organ failure in the fourth month. Since the patient's MRI features were different from those typical for chronic (min. 3 years) paint thinner abusers, the authors hypothesized that the lacquer thinner explosion exposed the patient to an extraordinarily high level of toluene, resulting in severe neurologic deterioration, white matter necrosis and disruption of the blood-brain barrier. However, the authors assumed toluene as the main component responsible for neurotoxicity without a blood or urine sample.

Camara-Lemarroy et al. (2015) assessed 20 patients admitted to an emergency department due to acute toluene intoxication by recent inhalation of toluene (paint thinner), among whom three young females (average age 25.3 yrs) died of cardiac rhythm abnormalities, with altered mental status, severe acidosis, hypokalemia and acute oliguric renal failure. In all the 20 patients, the potential exposure levels were not estimated, while the most common symptoms were muscular weakness or paralysis, altered mental status, nausea, vomiting and abdominal pain. Proteinuria (renal glomerular injury), liver injury and rhabdomyolysis were also common. The authors concluded that the hallmarks of acute toluene intoxication are hypokalemic paralysis and metabolic acidosis.

Lin and Liu (2015) reported two cases of occupational toluene-poisoning and described their brain MRI characteristics. Case 1 was a 31-year male factory worker who was admitted to the emergency department (ED) due to refractory convulsions, lack of vitality, and general soreness from painting work days and nights for one week in a room with no air conditioning. The patient's urine sample confirmed toluene intoxication, and cerebral MRI showed symmetric brain lesions, common for neurological disorders. Case 2 was of a 61-year-old material-processing factory worker who was admitted to the ED for deteriorating mental status and unsteady gait for two months, due to prior painting work for long hours over 5 days in a poorly air-conditioned room. The patient's electroencephalography disclosed diffuse cortical dysfunction, and cerebral

MRI showed bilateral periventricular white matter change with corpus callosum involvement. The patient's urine sample confirmed the toluene intoxication diagnosis. In both cases, increased signal intensity over the corpus callosum was observed in the initial brain MRI. This finding was thought to be related to increased iron deposition and cytotoxic edema. The abnormalities disappeared in the follow-up MRI scans. The authors suggested that the two patients experienced acute or subacute brain injury due to toluene exposure, because with long term toluene abusers, the cerebral atrophy is irreversible.

Djurendic-Brenesel et al. (2016) reported two cases of fatal intoxication with toluene due to glue sniffing. Case 1 was of an 18-year-old male with a history of glue sniffing brought to an emergency center for detoxification but who did not survive. Case 2 was of a 29-year-old male found lying dead at a river bank. Using the gas chromatography/mass spectrometry (GC/MS) method, the presence of toluene in biological samples were confirmed in both cases. Using the GC/flame ionization detector (FID) method, the quantitative analysis of gastric content, femoral blood, kidney, bile, liver and brain samples from both cases revealed toluene concentrations ranging from 3.81 to 20.97 mg/kg.

Yasar et al. (2016) reported a 21-year-old man arrived at a military medical academy emergency department with dyspnea and confusion. The authors reported the cause to be intentional acute toluene inhalation. The authors could not obtain the history of toluene abuse, frequency and duration of toluene exposure from the patient. The patient was diagnosed with cardiomyopathy. After potassium replacement, and metoprolol and ramipril administration, the patient had a dramatic clinical recovery and was discharged eight days after his hospitalization. The authors stated that no relapse of cardiomyopathy was observed during the follow-up period (timepoints unspecified).

Dharmarajan and Ammar (2017) reported a 31-year-old woman presented to the hospital with generalized weakness and lower back and abdominal pain. Laboratory tests revealed symptoms of toluene intoxication and elevated blood toluene level. The patient denied paint or glue sniffing, but said that she had been exposed to paint while working on a friend's house. After potassium administration over 2 days, the symptoms resolved and the patient was discharged.

### *Controlled Chamber Studies*

#### *Neurological effects (CNS, sensory irritation, neurobehavioral and psychometric tests)*

In a subacute chamber study, three volunteers were exposed to increasing concentrations of toluene ranging from 50 to 800 ppm (190 – 3000 mg/m<sup>3</sup>) for up to 8 hours per day, 2 times per week over 8 weeks (Von Oettingen et al., 1942). Only two exposures per week were conducted to allow for sufficient recovery time between exposures. Concentrations of 50 and 100 ppm (190 and 380 mg/m<sup>3</sup>) resulted in minor symptoms of fatigue, drowsiness, and headache toward the end of the 8-hour exposures in some of the volunteers. At exposures of 200 to 400 ppm (750 to 1,500 mg/m<sup>3</sup>), symptoms of muscular weakness, confusion, impaired coordination, paresthesia (numbness of the skin), and nausea were also reported with some symptoms lasting for several hours after exposure. Insomnia was also reported in all subjects. Exposure to 600 and 800 ppm (2300 and 3000 mg/m<sup>3</sup>) resulted in increased severity of symptoms with

considerable aftereffects in all 3 subjects (severe nervousness, muscular fatigue, and insomnia) lasting up to several days. The authors concluded that 8-hour exposure to 200 ppm (750 mg/m<sup>3</sup>) toluene produces definite impairment of coordination and reaction time.

Reaction time and perceptual speed were studied using psychophysiological tests in 12 young male subjects exposed by inhalation to toluene concentrations of 100, 300, 500, and 700 ppm (380, 1100, 1900, and 2600 mg/m<sup>3</sup>) in a successively increasing manner, each for a 20-minute interval with a pause of 5 min between the second and third 20-min intervals (Gamberale and Hultengren, 1972). Menthol was used to mask the odor of toluene. Statistically significant impaired simple reaction time was apparent following exposure to 300 ppm (1100 mg/m<sup>3</sup>) toluene. A statistically significant impairment in perceptual speed was observed at 700 ppm (2600 mg/m<sup>3</sup>) toluene. No effects were observed at 100 ppm (380 mg/m<sup>3</sup>).

Stewart et al. (1975) exposed groups of male subjects in a chamber to a different concentration of toluene (0, 20, 50 or 100 ppm, 0, 80, 190 or 380 mg/m<sup>3</sup>) each week over four-week period. Three different groups of 2-4 subjects each, consisting of 1-, 3- and 7.5-hour exposure groups, were exposed each day for up to 5 days per week. During the fifth week three groups of male subjects (2-4 subjects per group), divided into 1-, 3- and 7.5-hour exposure groups, were exposed to a fluctuating concentration of toluene between 50 and 150 ppm (190 and 570 mg/m<sup>3</sup>) each day for 5 days. In addition, three groups of female subjects (2 or 4 subjects per group) were divided into 1-, 3- and 7.5-hour exposure groups and exposed to 100 ppm (380 mg/m<sup>3</sup>) toluene each day for 5 days.

All subjects immediately perceived a mild to strong odor for all toluene exposures when entering the chamber, but it was usually not detectable after the first hour of exposure (Stewart et al., 1975). Subjective responses included a 3-fold greater complaint of eye, nose and throat irritation in the 3-hour male and female subjects (a total of 8 subjects) exposed to 100 ppm toluene. However, no increase in sensory irritation was found in the 1- and 7.5-hour toluene-exposed groups. The authors could not explain this discrepancy. The nostrils of both 7.5-hour male subjects (n=2) exposed to a fluctuating concentration of toluene between 50 and 150 ppm (190 and 570 mg/m<sup>3</sup>) were inflamed and infected on the fifth day of exposure, but only one subject complained of sensory irritation. No increase in drowsiness, fatigue, sleepiness or headache was observed among toluene-exposed groups.

Cognitive task testing revealed a decrement in the ability of 7.5-hour exposed females (n = 4) to concentrate on the alertness test. However, no effect for this test was observed in 3-hour exposed females or in exposed male groups. Other cognitive tests performed (time estimation, coordination, arithmetic, and inspection tests) did not show an effect due to toluene exposure. Spontaneous electroencephalograms and visual evoked responses (VER) were recorded in the 7.5-hour (n = 6) and fluctuating concentration subjects (n = 2). One of six male subjects exposed to 100 ppm (380 mg/m<sup>3</sup>) toluene for 7.5 hours showed a significant increase in the amplitude of the VER on the fifth day of exposure. A significant reduction in VER amplitude during exposure to 150 ppm (570 mg/m<sup>3</sup>) toluene was also noted during week 5. The authors suggest these responses are a sign of a pre-narcosis state. The authors concluded that there was suggestive evidence of deleterious effects on subjects exposed to 100 ppm (380 mg/m<sup>3</sup>) toluene.

Andersen et al. (1983) studied nasal mucus flow, lung function, psychometric performance, and subjective responses in 16 young healthy males exposed to toluene concentrations of 10, 40, and 100 ppm (38, 150, and 380 mg/m<sup>3</sup>) for 6 hours. The subjects were divided into four groups and the exposures used a balanced Latin-square design with chamber exposures over a 4-day period. No masking agents were used to disguise the odor of toluene. Data from the psychometric performance tests were examined by analysis of variance with  $p < 0.05$  used as the level of significance. For statistical analysis of subjective tests, evaluations were collected four times each day. The average score during exposure and the control score were ranked for each subject, and nonparametric statistical methods (Friedman's test and van Elteren's test) were applied to evaluate the effect of exposure.

A significant correlation of both increasing odor level and bad air quality was observed with increasing toluene concentration. The odor impression was significantly different from control at all toluene concentrations. Adaptation to the odor of toluene was noted during all exposures. However, in three subjects the odor sensation was strong and felt to be unacceptable at the 100 ppm (380 mg/m<sup>3</sup>) exposure level. Exposures to 10 and 40 ppm (38 and 150 mg/m<sup>3</sup>) toluene were without subjective irritation effects. During the 100 ppm (380 mg/m<sup>3</sup>) exposure, statistically significant ( $p < 0.05$ ), but mild irritation was experienced in the eyes and/or nose in 10 of the subjects; the other six subjects did not report any irritation during exposure to 100 ppm (380 mg/m<sup>3</sup>) toluene. The irritation was felt just after the exposure began and was constant throughout the duration of exposure. No irritation occurred in the throat or the lower airways. About half of the subjects experienced a statistically significant increase in the occurrence of headaches, dizziness and feeling of intoxication at 100 ppm (380 mg/m<sup>3</sup>) that was slight to moderate in intensity. None of the subjects reported nausea or cough.

The subjects in the Andersen et al. study also reported that it became more difficult to participate in the battery of psychometric tests and that their reaction time felt impaired at 100 ppm (380 mg/m<sup>3</sup>). In the eight psychometric performance tests covering visual perception, motor performance, the coordination between visual perception and motor performance, vigilance, and intellectual capacity, no significant objective changes compared to control exposures were observed, although there was a borderline significant correlation ( $0.05 < p < 0.10$ ) for the results of three of the tests – the screw-plate test, Landolt's ring test, and the number of errors in multiplication test. Toluene exposure did not cause changes in the nasal mucus flow, as measured by tagged particle movement from the oropharynx over time, or lung function tests as measured by forced expiratory vital capacity (FVC), forced expiratory volume in one second after maximal expiration (FEV<sub>1</sub>) and in forced expiratory flow during exhalation of the middle part of FVC (FEF<sub>25-75</sub>). In addition, nasal flow resistance, as measured by an oronasal mask with a pneumotachometer, was not affected by toluene exposure. For this study, the authors concluded that pure toluene up to 100 ppm (380 mg/m<sup>3</sup>) is only slightly irritating to the mucous membranes, reduces perceived air quality, is moderately odorous, and causes some minor, nonsignificant changes in performance.

Dick et al. (1984) examined the comparative psychomotor effects of methyl ethyl ketone and toluene individually and in combination. Female and male subjects (age = 18-38 years,  $n$  unstated) from college campuses were tested for their ability to pass an "extensive" physical exam that included neurological screening. Subjects were exposed in a chamber to air, which included a 2-minute 25 ppm (94 mg/m<sup>3</sup>) "charge" of a toluene "placebo" exposure ( $n = 12$ ), or



100 ppm (380 mg/m<sup>3</sup>) toluene (n = 30 or 32) nominally, for 4 hours. The subjects were tested on their performance in three tasks before, during, and/or after (up to 1.2 hours post-exposure) the exposures. There was no true control group breathing plain air in this study. Tasks included Choice Reaction Time, visual-vigilance, and pattern recognition tests of alertness and psychomotor function. Subjects also participated in body burden measures of toluene via breath samples at the following timepoints: 1) the end of the pre-exposure period; 2) 1-2 hours into the exposure period; 3) at the end of the exposure period; and 4) 90 minutes post exposure. While the authors stated that pre-exposure tests provided baseline data for comparison with other periods, it was not apparent that this was done for toluene. Unstated univariate tests and multivariate analysis of covariance (MANCOVA) were performed.

Results indicated that toluene concentration averaged  $100.1 \pm 2.5$  ppm, range  $96.4 \pm 7.9$  to  $102.1 \pm 0.9$  ppm ( $379 \pm 9.4$  mg/m<sup>3</sup>, range  $363 \pm 30$  to  $384 \pm 3.4$  mg/m<sup>3</sup>) over the 4-hour exposure period. Breath toluene levels averaged  $24 \pm 5.6$  mg/m<sup>3</sup> ( $6.5 \pm 1.5$  ppm) over the 4-hour exposure period, and were  $18 \pm 4.9$ ,  $26 \pm 8.3$ ,  $29 \pm 4.9$ , and  $29 \pm 3.4$  mg/m<sup>3</sup> at timepoints 1-4, respectively. The authors stated that under constant exposure conditions, toluene breath concentrations reach steady state within two hours and remain relatively fixed at 10-20% of the exposure chamber concentration. Psychomotor tests showed that although Visual-vigilance and pattern recognition test scores trended downward in the 100 ppm (380 mg/m<sup>3</sup>) group versus the placebo, with the percentages of correct hits differing most between groups during the first two hours of exposure, only the Visual-vigilance test yielded a statistically significant difference ( $p = 0.01$  via Manacova analysis). Despite this result, the report concluded it was unlikely toluene produces biologically significant cognitive decrements at 100 ppm (380 mg/m<sup>3</sup>). The authors stated that 100-ppm toluene effects in this study were marginal at most.

Two groups of middle-aged workers (43 men per group) were recruited for an acute toluene exposure study. Group 1 were printers with occupational exposure to solvents (mainly ethanol, ethyl acetate, and ethoxyethanol, with small amounts of toluene); Group 2 did not have similar solvent exposures (Baelum et al., 1985). Forty-one subjects (20 from Group 1 and 21 from Group 2) were exposed once to 100 ppm (380 mg/m<sup>3</sup>) of toluene for 6.5 hours while 45 subjects (23 from Group 1 and 22 from Group 2) were exposed to clean air. No masking agent was used to disguise the odor of toluene. The subjects completed questionnaires to grade subjective symptoms before exposure, at onset of exposure, and at the end of exposure. Ten different performance tests were used to measure psychomotor skills, perceptual skills and vigilance. Six tests were on different aspects of visuomotor coordination, while the other four were on perceptual speed and quality, and higher cortical functions. The results were evaluated using an analysis of variance with  $p < 0.05$  used as the level of significance.

Toluene exposure had an effect on only one of the six visuomotor coordination tests; toluene-exposed printers (Group 1) were significantly slower in completing the peg board test compared to printers exposed to clean air. However, no effect was seen between toluene exposed controls and clean air controls (Group 2). Visual perception in the Landolt's ring test was significantly affected in toluene-exposed printers compared to printers exposed to clean air. Again, no effect was seen between toluene exposed controls and clean air controls. In the color discrimination test, printers initially performed better overall due to their professional training and education. However, the control groups had a reduced number of errors in the second test during the day, while the toluene groups did not improve ( $p < 0.01$ ). The authors concluded that toluene caused

decreased psychometric performance, mainly in visual perceptual speed and accuracy, with a tendency toward greater sensitivity in printers.

For the subjective symptoms in the Baelum et al. study, Group 2 workers exposed to toluene experienced statistically significant eye, nose and throat irritation with exposure to toluene, which was immediate and remained almost constant throughout the exposure. In Group 1 workers exposed to toluene, only irritation of the nose was statistically significantly increased with toluene exposure. The subjective data were presented only in graphical form as percent responding; no absolute numbers of subjects responding or values of percent of subjects responding were provided. Among other subjective complaints, poor air quality and a strong odor were experienced immediately among the toluene-exposed workers, but declined towards the end of exposure. Fatigue ratings were increased significantly at the end of exposure in both toluene-exposed groups. Feeling of intoxication increased significantly in both exposed groups throughout exposure.

Olson et al. (1985) exposed 16 healthy men to either 0 or 300 mg/m<sup>3</sup> (80 ppm) toluene in a chamber for four hours to test for nervous function deficits and subjective effects. The performance tests were administered when subjects first entered the chamber, after exposure for 2 hours, and after 4 hours of exposure. Analysis of variance was used to determine statistical significance ( $p < 0.05$ ) of the performance tests. Isoamyl acetate was administered in the chamber during the control exposures to disguise the absence of solvent. However, 12 of 16 subjects were able to identify the control condition. The performance on the tests, including simple reaction time, memory reproduction and choice reaction time, was unaffected by toluene exposure. Subjective ratings for sensory irritation, headache, nausea, tiredness, feeling of stress and ability to concentrate were also unaffected by toluene exposure.

A battery of neurobehavioral and performance tests were conducted among 42 young men and women (21 men and 21 women) exposed to 0, 75, and 150 ppm (0, 280, and 560 mg/m<sup>3</sup>) toluene for 7 hours in a chamber (Echeverria et al., 1989). Menthol was used as a masking agent to disguise the odor of toluene. Fourteen subjects were randomly assigned to each of the three groups and were exposed to one of the above 3 concentrations on each day of the 3 days of testing. A 3 × 3 Latin square study design was employed, so each group of 14 subjects was exposed to a different order of toluene concentrations. A 5-10% decrement in performance was considered significant if it was consistent with a linear trend at  $p < 0.05$ . Tests were conducted daily before, during and after exposure where each subject served as their own control. Scheffe's 95% confidence intervals were used to identify significant differences in scores between the control and exposed groups.

With these criteria, Echeverria et al. found statistically significant linear trends for decrements in verbal short term memory (digit span test, 6.0% decrement), visual pattern memory (number correct, 5.0% decrement), visual perception (pattern recognition latency, 12.1% decrement), psychomotor skills (critical tracking test, 3.0% decrement), and manual dexterity (one hole test, 6.5% decrement), with a statistically significant score difference between 0 and 150 ppm (570 mg/m<sup>3</sup>) exposures. A significant difference was also noted between the 0 and 75 ppm exposure groups for the pattern recognition latency test. No gender-related differences in the neurobehavioral and performance tests were observed.

Echeverria et al. also noted reports of subjective symptoms of headache (19, 26, and 33% at 0, 75, and 150 ppm, respectively), eye irritation (17, 21, and 48% at 0, 75, and 150 ppm, respectively), and number observed sleeping during exposure (7, 14, and 22% at 0, 75, and 150 ppm, respectively) that increased with increasing dose. The dose-dependent increase ( $p < 0.001$ ) in the number of observations of subjects sleeping during the exposure was concluded by the authors to be the most convincing evidence of toluene affecting the central nervous system. Although statistical analysis between dose levels was not provided for subjective symptoms by the authors, the data suggest a statistically significant increase in headache and eye irritation at 150 ppm (570 mg/m<sup>3</sup>).

Half the subjects correctly guessed their order of exposure despite the use of menthol as a masking agent. However, a comparison of performance between the successfully blinded subjects and the non-blinded subjects showed no significant differences. The authors concluded that toluene had significant but small acute behavioral effects mostly in the range of 2 to 7% performance decrements at 150 ppm (570 mg/m<sup>3</sup>).

Baelum et al. (1990) conducted another chamber study in healthy adults (32 males and 39 females) in which exposures were to a constant concentration of 93 ppm (350 mg/m<sup>3</sup>) toluene, or a fluctuating concentration of toluene between 300 and 50 ppm (TWA of 102 ppm, 380 mg/m<sup>3</sup>) for 7 hours. Specifically, fluctuating exposures consisted of 14 episodes of 30 min with an increasing concentration reaching a peak of 300 ppm (1100 mg/m<sup>3</sup>) after 5 min, then decreasing to 50 ppm (190 mg/m<sup>3</sup>) over 10 min where the concentration remained for another 15 min. The subjects were divided into three groups of 23-24 in which each group had a single exposure to either control, constant toluene concentration, or the varying toluene concentration. Three periods of exercise on an ergometer cycle for 15 min were conducted during each exposure, with workloads of 40 and 60% of maximal aerobic capacity. Subjects exposed to the varying concentration of toluene were exercised during peak concentrations. Subjective ratings and four performance tests (peg board, color test, vigilance clock test, five-choice serial reaction test) were conducted twice during exposure. Standard analysis of variance was used to test each variable using  $p < 0.05$  as the measure for statistical significance.

For subjective findings, bad air quality, odor level, irritation of nose and lower airways, feeling of intoxication and dizziness were greater in the toluene exposed groups compared to the control group (Baelum et al., 1990). There was no difference between the two exposed groups. No differences between any groups were observed for the performance tests, although a tendency ( $0.05 < p < 0.10$ ) towards lower score and more errors in the vigilance test was found in the toluene exposed groups. The only apparent gender difference observed was that females worked faster on the peg board test and performed better on the color test compared to males. The authors concluded that the subjective sensory irritation and light neurotoxic symptoms occurred with toluene exposure in accordance with their previous study, while only a weak indication in performance tests was observed, unlike their previous study.

Echeverria et al. (1991) compared the abilities of toluene and ethanol to produce non-specific depressive CNS effects in human volunteers. Only experiments involving toluene are discussed herein. Paid subjects ( $n = 14$ /group; male and female; age 18-35 years,  $\geq 1$  year college education) were exposed to toluene (0, 282, and 564 mg/m<sup>3</sup>, or 0, 75, and 150 ppm) and menthol (an odor masking agent; 0.29 mg/m<sup>3</sup> or 0.078 ppm) for seven hours/day over three days. The subjects were evaluated on their performance in several behavioral tests that

measured short-term pattern memory (Sternberg, digit span, and Benton tests); perception (pattern recognition test); psychomotor skill (simple reaction time, continuous performance, symbol-digit, eye-hand coordination, finger tapping, and critical tracking tests); manual dexterity (one hold test); mood [profile on mood scales (POMS)]; fatigue (fatigue checklist); and verbal ability. Voluntary subject observations regarding sleep and exposure-related ailments were also noted. Each day, pre- (baseline control) and post-exposure test performance were measured at 08:00 and 16:00, respectively. Toluene exposure concentrations varied for each group on a daily basis such that all subjects experienced every exposure condition by the end of the study (one exposure concentration/day).

According to the study authors, complaints of eye irritation and headaches increased in a dose-dependent manner. However, the authors did not provide a quantitative estimate of these effects. At the highest exposure concentration, notable effects included performance decrements in digit span (-6%), pattern recognition (-12%), pattern memory (-5%), one hole (-5%), and critical tracking (-3%) tests with ANOVA analysis. By grouping males and females together for the study, the authors imply that there were no gender differences in responses to the neurobehavioral tests. However, the authors did not explicitly state this.

Rahill et al. (1996) conducted an assessment of physical and neuropsychological performance during low-level exposure to toluene. Healthy, non-smoking male (n = 2) and female (n = 4) Caucasian volunteers between the ages of 27 and 38 years were exposed to conditioned room air or 380 mg/m<sup>3</sup> (100 ppm) of toluene for 6 hours in an exposure chamber in a double-blind, randomized manner. Each subject served as his/her own control, as the experimental design involved a two-period crossover with at least 14 days separating the two successive exposure periods.

Subjects exercised for 30 minutes on a bicycle ergometer during the exposure period to approximately quadruple their resting ventilation. Respiratory function was measured before and immediately after exposure, and levels of toluene in blood and exhaled breath were measured before, twice during, immediately after, and 1 hour and 2 hours after exposure, respectively. Two computerized neuropsychological tests were performed in three repetitions: a brief standard battery, the Automated Neuropsychological Assessment Metric (ANAM), which lasted 20 minutes and required the subject to respond to 7 screen tests presented individually, and an hour-long multitask performance test using the SYNWORK system that required the subject to respond to 4 simultaneous work windows on the computer screen.

Results showed an increase in absorption of toluene during exercise, with mean blood and exhaled air samples averaging 1.5 µg toluene/g blood and 28 ppm (104 mg/m<sup>3</sup>) of toluene, respectively. No significant changes were observed in lung function following toluene exposure relative to baseline or exposure to conditioned air. Exposure to toluene was found to significantly alter both performance and response time on neuropsychological tests: overall performance (Composite score) in the prolonged multitasking test declined during toluene exposure relative to the room air condition ( $F = 29.2$ ,  $p = 0.005$ ), with a 10% reduction during the 6<sup>th</sup> hour of toluene exposure, and the mean response time indicated by the ANAM battery was significantly longer for toluene exposure than for conditioned air. Further, the Composite score improved with exercise under the room air condition but remained flat throughout the exposure period to toluene. No apparent gender-related differences were observed, although the number of subjects were too small for statistical verification.

Orbaek et al. (1998) examined subjective reactions to toluene and n-butyl acetate among subjects with chemical sensitivity. Two exposure groups were used. The first group employed subjects with symptoms and neuropsychological test results compatible with toxic encephalopathy (TE) type 2A (TE-2A) and 2B (TE-2B) following long-term occupational exposure to solvents. The second group (controls) consisted of previously unexposed referents. All subjects were men between the ages of 28 and 66, and each group consisted of 12 subjects. Both groups were exposed over a period of 70 minutes in exposure chambers to 11 to 180 mg/m<sup>3</sup> (3 to 48 ppm) toluene, which is below previously reported thresholds for acute neurotoxic effects and trigeminal irritation (750-1125 mg/m<sup>3</sup>, 200-300 ppm).

The test results showed that the TE groups were more sensitive to toluene exposure than the referents. Ratings of smell intensity, which was measured on a 7-step category scale from none to extremely strong and represented by regression coefficients (mean  $\pm$  SD), were higher for the TE-2B group ( $1.05 \pm 0.12$ ) than for the TE-2A ( $0.73 \pm 0.32$ ) and referent ( $0.79 \pm 0.15$ ) groups during toluene exposure ( $p < 0.001$ ). Mean regression coefficients for mucous membrane irritation from toluene exposure, which was rated on a visual analogue scale from 0 to 100, were higher for the TE-2A ( $7.8 \pm 5.0$ ,  $p = 0.009$ ) and TE-2B ( $7.8 \pm 5.5$ ,  $p = 0.008$ ) groups than for the referent group ( $2.1 \pm 2.4$ ). Both TE groups had higher ratings for fatigue from toluene exposure than the referent group: mean regression coefficients were  $1.0 \pm 1.5$  for the referent group and  $7.8 \pm 6.1$  and  $6.8 \pm 5.7$  for the TE-2A and TE-2B groups, respectively ( $p < 0.001$ ).

Little et al. (1999) studied the effects of toluene on human subjects clinically sensitive to toluene. Twenty toluene-sensitive patients ( $n = 11$  females, 9 males; average age = 39.5 years) were exposed in a specially designed testing booth to 15 ppm (56 mg/m<sup>3</sup>) toluene for 20 minutes along with 16 reportedly healthy controls who had no history of adverse chemical exposure ( $n = 8$ /sex; average age = 32 years). The subjects were assessed through neurobehavioral and/or blood chemistry tests before and after the toluene exposure. The battery of neurobehavioral tests evaluated visual acuity (Focal Length), immediate and delayed verbal memory recall (Babcock stories; prose memory), information processing time (Simple Reaction Time; SRT), concentration and attention (Letter Cancellation and STROOP Color Word), and psychomotor coordination (Symbol Digit). Blood serum was used to determine whether toluene exposures resulted in B- or T-cell-mediated immune responses. To this end, levels of B-cell-derived immunoglobulins (IgGs) and T-cell-derived antigen-specific binding molecules (TABMs) were measured via an enzyme-linked immunosorbent assay (ELISA).

Results indicated that the toluene-sensitive patients had moderate to severe clinical reactions with typical symptoms including fatigue, poor concentration, headache, and muscle pains upon exposure to toluene. Neuropsychological testing revealed worsening performance on post-versus pre-exposure Babcock ( $p \leq 0.009$ ), Symbol Digit ( $p = 0.0099$ ), and Letter Cancellation ( $p = 0.0380$ ) tests. Patients exhibited significantly ( $p = 0.002$ ) greater numbers of TABMs to the BA HSA antigen compared to controls. The study results suggested that acute, low-concentration (15 ppm; 56 mg/m<sup>3</sup>) toluene exposure produced deficits in memory, coordination, and mental focus with primarily T- versus B-cell mediators in sensitive individuals. In this study, 15 ppm is a LOAEL. The occurrence of any potential gender-specific differences in neurobehavioral test results was not addressed by the authors.

Osterberg et al. (2000) evaluated psychological test performance of chemically sensitive human subjects exposed to toluene or n-butyl acetate. This publication supplements the previous one

of Orbaek et al. (1998) described above, providing additional data from neuropsychological test results collected during the exposure chamber sessions described by Orbaek et al. (1998) for the same study population. Overall, results from the three psychological performance tests administered (the digit symbol test, the Automated Psychological Test System [APT] two-way reaction-time test, and the APT inhibition test) showed that the TE subjects were not more affected than healthy reference subjects by toluene exposures at the test concentrations of 11 to 180 mg/m<sup>3</sup> (3 to 48 ppm).

Osterberg et al. (2003) studied subjective responses and psychological test performance of chemical-sensitive human subjects upon exposure to toluene or n-butyl acetate. Ten women with symptoms compatible with multiple chemical sensitivity (the test group) and 20 healthy women (the control group) were exposed to five concentrations of toluene, from 11 to 180 mg/m<sup>3</sup> (3 to 48 ppm), over a period of 70 minutes in exposure chambers. The results showed that there were steeper increases of ratings for mucous membrane irritation and fatigue in the test group than the control group, while the ratings of smell intensity and smell annoyance were similar in the two groups. For the three psychological performance tests (the digit symbol test, the Automated Psychological Test System [APT] two-way reaction-time test, and the APT inhibition test), while reductions in test performance were observed in both groups, the decline observed in the test group was more prominent ( $p < 0.05$ ).

To study the influence of the inhalation exposure pattern on the toxic effect of toluene, Lammers et al. (2005) designed a human volunteer study to compare the neurobehavioral effects of exposure to regularly occurring peak concentrations and constant exposure at the same average level. Eleven healthy men (age 20-49 yrs) were exposed for 4 hr on two different days separated by a seven-day wash-out period. One exposure was to a constant concentration of 40 ppm (150 mg/m<sup>3</sup>) toluene; the other exposure was to a time-weighted average dose close to 40 ppm, but included three 30-min exposures to a peak concentration of 110 ppm (410 mg/m<sup>3</sup>). The results showed no clear changes in neurobehavioral function, including tests of motor performance, attention, perceptual coding, and memory. No clear changes in mood and effect were observed. The authors concluded that these conditions did not induce significant acute changes in central nervous system function as shown at much higher concentrations in animals.

### Visual effects

The Baelum et al. (1985) study described above, where middle-aged workers (43 per group) were recruited for an acute toluene exposure study (Group 1 had previous occupational exposure to solvents and Group 2 did not), also included an evaluation of color vision effects resulting from toluene exposure. The study results showed statistically significant decrements in both groups for color discrimination and accuracy in visual perception with the Landolt's ring test. A statistically significant decrease in visuomotor function was observed with the peg board test, but the effect was restricted to toluene-exposed workers. In addition, a trend ( $0.05 < p < 0.10$ ) toward decreased vigilance with the lamp test was observed in both exposed groups. The authors concluded that acute exposure to 100 ppm (380 mg/m<sup>3</sup>) toluene adversely affects persons irrespective of former occupational solvent exposure.

The effects of acute toluene exposure on color vision were studied in a group of eight rotogravure printing workers that had been employed and occupationally exposed to toluene for an average of 9.8 years (Muttray et al., 1999). In rotogravure printing, toluene is generally the

only solvent used. The color vision acuity of the workers before and after an acute toluene exposure (28 – 41 minutes in duration, 300-400 ppm, 1100 – 1500 mg/m<sup>3</sup> of toluene) was evaluated using the Farnsworth panel D-15 test, the Lanthony desaturated panel D-15 test, and the Standard Pseudoisochromatic Plates test part 2. A control group of 8 unexposed workers (from a metal-working factory) was also tested. One exposed worker left the room due to headache. Acute exposure to toluene did not impair color vision or cause other narcotic symptoms. Print worker performance prior to acute toluene exposure was similar to controls on the Farnsworth panel D-15 and Standard Pseudoisochromatic Plates part 2 tests. However, print worker performance on the Lanthony desaturated panel D-15 test prior to exposure was slightly worse (suggesting chronic effects) than that of controls, with median scores of 1.18 and 1.05 for exposed and controls (higher number indicates degraded performance), respectively. The difference was of borderline statistical significance ( $p = 0.06$ ). The authors noted that the small number of subjects ( $n = 8$ ) limited the statistical power of the study.

The Baelum et al. (1990) study described above also included a color test to evaluate the effect of 7-hour, TWA 100 ppm (380 mg/m<sup>3</sup>) toluene exposure on visual performance for 32 males and 39 females from a random sample of the general population. In the color test the subjects compared a list of 20 color samples with a reference table (20 color specimens test). The number of correct answers together with the cumulated deviation according to color and intensity were used as score. The result showed no effect of toluene exposure in color test results, indicating only minimal if any effect on visual performance. However, as noted above, females performed better overall on the color test compared to males.

Muttray et al. (1995) examined the subacute effect of toluene on color vision in 59 rotogravure workers (mean age 36 yrs, mean rotogravure working duration = 10 yrs) without a control group. The work-shift exposure was analyzed by taking venous blood samples immediately before color vision testing and measuring both toluene and ethanol (a potential confounder) concentrations. However, the authors did not measure workplace air toluene concentrations. Color vision tests were performed on Monday before shift following 2 to 3 days of exposure-free time, and on Friday after shift. Color vision tests used included the Ishihara plates, the Velhagen plates, the standard Pseudoisochromatic Plates part 2, the Farnsworth panel D-15 test and the Lanthony desaturated panel D-15 test. The average toluene concentration in blood ranged from < 0.22 to 7.37 mg/L, and the color vision tests did not show any influence of toluene concentration on the color confusion indices. The authors concluded that there was no increase in acquired color vision impairment over the work week with toluene exposure partially exceeding 1.7 mg/L (threshold for biomonitoring in Germany).

Both human and animal studies revealed that acute toluene exposure can disturb different neurotransmitter systems. To investigate whether the visual attention processes is a target of toluene in humans, Kobald et al. (2015) applied a visual change detection task to 17 young healthy human volunteers (mean age of 24.12 yr), using electroencephalography (EEG) to measure neurobehavioral and neurophysiological effects of a single peak exposure of 200 ppm (750 mg/m<sup>3</sup>) toluene for 40 min at light physical activity to mimic a real-life working situation, with 16 volunteers (mean age of 25.25 yr) as control group. The behavioral results showed that toluene impairs the rate of correct responses especially in task conditions in which an irrelevant distractor is given, while the response times did not differ between the experiment and control

groups. The neurophysiological results implied a less efficient visual processing of relevant stimuli and an increased distractibility by irrelevant stimuli.

### Pulmonary effects

Stewart et al. (1975) exposed groups of male subjects in a chamber to a different concentration of toluene (0, 20, 50 or 100 ppm, 0, 80, 190 or 380 mg/m<sup>3</sup>) each week over four-week period. Three different groups of 2-4 subjects each, consisting of 1-, 3-, and 7.5-hour exposure groups, were exposed each day for up to 5 days per week. During the fifth week, three groups of male subjects (2-4 subjects per group), divided into 1-, 3-, and 7.5-hour exposure groups, were exposed to a fluctuating concentration of toluene between 50 and 150 ppm (190 and 570 mg/m<sup>3</sup>) each day for 5 days. In addition, three groups of female subjects (2 or 4 subjects per group) were divided into 1-, 3- and 7.5-hour exposure groups and exposed to 100 ppm (380 mg/m<sup>3</sup>) toluene each day for 5 days. Pulmonary function tests (forced expiratory vital capacity [FVC], forced expiratory volume in one second after maximal expiration [FEV<sub>1</sub>], peak expiratory flow rate [PEFR] and flow rate at 50% of FVC [MMEF]) on two subjects per group in the Stewart et al. (1975) study did not reveal any changes due to the toluene exposures. Likewise, regulation of ventilation, heart rate and alveolar gas exchange in the subjects examined (n = 4) were unaffected by toluene exposure.

### Renal effects

Nielson et al. (1985) investigated the renal effects from acute exposure to toluene in the same groups of workers exposed to toluene by Baelum et al. (1985). The exposure protocol of 0 and 100 ppm (0 and 380 mg/m<sup>3</sup>) toluene for 6.5 hours is described in detail above in Baelum et al. (1985). Changes in the excretion rate of albumin and  $\beta_2$ -microglobulin were used as indicators of glomerular and renal tubular damage, respectively. No significant changes in renal excretion rates of either protein were found following acute exposure to 100 ppm (380 mg/m<sup>3</sup>) toluene suggesting that acute exposure to toluene has no nephrotoxic effect by these measures.

### Other Studies

There are a number of older human inhalation studies of varying quality investigating the acute toxicity of toluene. The following human studies support the association between toluene exposure and the known acute effects. However, the value of these studies is limited by issues such as poorly described or unconventional health endpoints, inadequate descriptions of the methodology, and questionable toluene exposure concentrations used.

Male volunteers were exposed to toluene via mouthpiece in which the concentration increased in a stepwise fashion from zero to 900 mg/m<sup>3</sup> (240 ppm) over 40 minutes (Horvath et al., 1981). The concentration was then held at 240 ppm for 30 min for a total exposure time of 70 min. The air was perfumed presumably to hide the odor of toluene. A total of 23 subjects were exposed, approximately half of which received a capsule containing diazepam, and the other half a placebo. These groups were compared to groups given only the placebo or diazepam. Testing for alertness consisted of spatial discrimination of acoustical clicks and a continuous visual feedback task. No decrement in vigilance performance was observed during exposure in the toluene + placebo group, although the toluene + diazepam group showed a worsening of



performance ( $p < 0.05$ ). However, both toluene exposure groups showed a significant decrease in performance when measured 70-140 min following cessation of toluene exposure.

In order to elucidate the mechanism of toluene reproductive toxicity, Luderer et al. (1999) studied the reproductive endocrine effects of toluene acute exposures in healthy human subjects 10 males and 20 females aged 19-45 yrs. Women were divided into two groups those in follicular phase and those in the luteal phase of the menstrual cycle. A 3-hour exposure to 50 ppm ( $190 \text{ mg/m}^3$ ) toluene through a mouthpiece did not result in alterations of their plasma luteinizing hormone (LH) or follicle stimulating hormone (FSH) secretion profile. However, subtle effects on LH secretion were identified, a greater decline in LH pulse frequency for women in the luteal phase ( $p = 0.06$ ) and a greater LH decline in men ( $p < 0.05$ ) than their respective control groups. There was no effect on blood testosterone levels in men. The authors concluded that the clinical relevance of the subtle effects on LH secretion was unclear.

The controlled human acute exposure studies with known exposure concentrations are summarized in Table 1.

**Table 1. Summary of the principal studies for the acute toxicity of toluene in human adults.**

Study	Exposure duration and Concentration (ppm)	Subjects / Effects (endpoints)	NOAEL ppm (mg/m <sup>3</sup> )	LOAEL ppm (mg/m <sup>3</sup> )
Von Oettingen et al. (1942)	8 hr at 50, 100, 200, 300, 400, 600, and 800 ppm	3 healthy human beings Minor symptoms of fatigue, headache and drowsiness at 50 and 100 ppm. Impairment of coordination and reaction time beginning at 200 ppm.	100 (380)	200 (750)
Longley (1967)	~ 30 min at estimated 10,000-30,000 ppm	29 (accident 1), 7 (accident 2) Euphoria, drunkenness, dizziness, nausea, loss of consciousness, confusion, incoordination, drowsiness	*	10,000 (38,000)
Gamberal & Hulttengren (1972)	20 min at 100, 300, 500, and 700 ppm with 5 min break between 300 and 500 ppm	12 healthy males. Impaired simple reaction time beginning at 300 ppm. Impaired perceptual speed at 700 ppm.	100 (380)	300 (1,100)
Stewart et al. (1975)	1-hr, 3-hr, and 7.5-hr per day exposures, up to 5 d/wk. Males: 0, 20, 50, 100 ppm, and variable 50-150 ppm groups Females: 0 and 100 ppm groups.	2-4 males, and 2-4 females per group At 100 ppm: Eye, nose and throat irritation in 3-hr group only. Inflamed nostrils in 7.5-hr group on day 5 of exposure. Change in visual evoked response in one subject. Increased errors in alertness in 7.5-hr females	50 (190)	100 (380)
Horvath et al. (1981)	3 30-min sessions at 240 ppm	11 males age 20-21. Impaired vigilance during third session	*	240 (900)
Andersen et al. (1983)	6 hr at 0, 10, 40, and 100 ppm	16 young healthy males At 100 ppm: increased eye and/or nose irritation (10 of 16); headache, dizziness, and feeling of intoxication. Significant correlation for increased odor and bad air with increasing toluene concentration. Borderline correlation ( $0.05 < p < 0.1$ ) for decrement in 3 of 8 psychometric performance tests. No effect on nasal flow resistance, nasal mucus flow and lung function.	40 (150)	100 (380)

Abbreviation: \* not observed.

**Table 1. Summary of the principal studies for the acute toxicity of toluene in human adults (continued).**

Study	Exposure duration and Concentration (ppm)	Subjects / Effects (endpoints)	NOAEL ppm (mg/m <sup>3</sup> )	LOAEL ppm (mg/m <sup>3</sup> )
Dick et al. (1984)	2-min (placebo) exposure at 25 ppm or 100 ppm for 4 hours	12 (placebo) or 30-32 males and females age 18-38 yrs	*	2-min (placebo) exposure at 25 ppm or 100 ppm for 4 hours
Olson et al. (1985)	4 hr at 80 ppm	16 males age 23-38yr No effect on subjective symptoms; no impairment in 3 psychomotor tests	80 (300)	*
Baelum et al. (1985)	6.5 hr at 0 ppm (n = 23 G1 workers and 22 G2 workers) or 100 ppm (n = 20 G1 workers and 21 G2 workers).	G1 = 43 workers with previous solvent exposure. G2 = 43 workers with no previous solvent exposure. At 100 ppm: Subjective results: Increased eye (G2 only), nose, and throat (G2 only) irritation, increased strong odor, and low air quality, increased feeling of fatigue and intoxication. Objective findings: decreased color discrimination, visual perception (Landolt's ring test), and visuomotor function (peg board test, G1 only).	*	100 (380)
Nielsen et al. (1985)	Same as Baelum et al. (1985)	Same as above. At 100 ppm, no significant changes in excretion rate of albumin and $\beta$ 2-microglobulin, no nephrotoxic effect.	100 (380)	*
Echeverria et al. (1989, 1991)	7 hr at 0, 75, and 150 ppm	42 young men & women Neurobehavioral results: At 75 ppm – decreased visual perception. At 150 ppm – Decreased verbal short term memory, visual pattern memory, visual perception, and manual dexterity - all showing a linear trend. Objective findings: Dose-response increase in headache, eye irritation and sleeping episodes with increasing concentration.	75 (280)	150 (570)

Abbreviation: \* not observed.

**Table 1. Summary of the principal studies for the acute toxicity of toluene in human adults (continued).**

Study	Exposure duration and Concentration (ppm)	Subjects / Effects (endpoints)	NOAEL ppm (mg/m <sup>3</sup> )	LOAEL ppm (mg/m <sup>3</sup> )
Baelum et al. (1990)	7 hr at 0, 93, and TWA 103 ppm (50 ppm with 300 ppm peaks)	32 males 39 females age 31-50.  For both toluene exposure groups: Increased subjective response to odor, bad air, irritation of nose and lower airway, feeling of intoxication and dizziness. Decreased performance in one (vigilance test) of four psychomotor tests ( $0.05 < p < 0.10$ ).	*	100 (380)
Little et al. (1999)	20 min at 15 ppm	20 toluene-sensitive patients (9 male, 11 female).  Moderate to severe clinical reactions with symptoms including fatigue, poor concentration, headache, and muscle pains; worsened neuropsychological test performances after 15 ppm toluene exposure.	*	15 (56)
Luderer et al. (1999)	3 hr at 50 ppm	10 male 20 female age 19-45.  No change in plasma LH or FSH secretion profile; greater decline in LH pulse frequency for women in the luteal phase, and a greater LH decline in men.	50 (190)	*
Muttray (1999)	28-41 min at 200-400 ppm	8 toluene-exposed workers, 8 controls.  No effect on color vision at test exposure in either group.  Borderline impairment ( $p = 0.06$ ) of color vision in toluene-exposed workers prior to test exposure.	400 (1500)	*
Lammers et al. (2005)	4 hr at 40 ppm.  (TWA ~40ppm) plus 3 30-min peaks of 110 ppm.	11 healthy adult males age 20-50.  No changes in neurobehavioral tests of motor performance, attention, perceptual coding, and memory, and no changes in mood.	40 (150)	*

Abbreviation: \* not observed.

## Acute Toxicity to Infants and Children

Embryopathy due to toluene exposure was first suggested in 1979 in an infant with phenotypic features similar to the fetal alcohol syndrome (FAS), born to a woman who abused toluene-based solvents during pregnancy (Toutant and Lippmann, 1979). Since that time, similar cases resulting from toluene-based solvent abuse have been reported in the literature.

Hoyme et al. (1993) reported 12 children whose mothers abused toluene-based spray paint by inhalation during pregnancy. Fifty-eight percent had intrauterine growth retardation, and 3 of 4 followed beyond the neonatal period showed postnatal growth deficiency. Seventy-five percent had craniofacial features consistent with FAS. Three of the 12 had hydronephrosis. Analysis of the pattern and nature of associated malformations suggests a common mechanism of teratogenesis for toluene and alcohol, namely, a deficiency in craniofacial neuroepithelium and mesodermal components due to increased embryonic cell death.

The brain continues to develop in children through adolescence. As a result, children are likely to be more susceptible to the neurotoxic effects of toluene due to less cell differentiation and increased cell division compared to adults (OEHHA, 2008). Undifferentiated cells tend to be more prone to injury than differentiated cells. As will be summarized below, animal studies have shown that the young are more susceptible to the neurotoxic effects of toluene compared to adults.

As indicated in Section 4, infants under one year of age may not metabolize toluene to benzyl alcohol as quickly as adults. The parent compound, toluene, is thought to be the main form that results in toxic effects. Therefore, slower metabolism of toluene may result in longer, more potent deleterious effects in exposed infants.

## Acute Toxicity to Experimental Animals

### *Neurobehavioral Effects*

Dose- and age-dependent decreases in behavioral performance and depression of the central nervous system were observed in mice and rats exposed by inhalation to toluene at concentrations ranging from 2600 to 12,000 ppm (9800 to 45,000 mg/m<sup>3</sup>) for up to 3 hours (Bruckner and Peterson, 1981). Younger animals were more susceptible to toluene toxicity and mice were more sensitive than rats of the same age (statistical analysis not performed by authors). Four-week-old mice were depressed more rapidly than were 8- and 12-week-old mice exposed to toluene concentrations of 2600, 5200, and 12,000 ppm (9800, 19,600 and 45,000 mg/m<sup>3</sup>). Four-week-old rats were also slightly more sensitive than were older animals. Although mice and rats were narcotized similarly after 2 to 3 hr of toluene inhalation, mice appeared to succumb more rapidly than did rats of the same age.

To assess how behavioral effects differ in adolescents compared to adults, Batis et al. (2010) exposed 72 adolescent (postnatal day [PN] 28) and 72 adult (PN 90) male rats to toluene for two 15-min durations separated by a 120-min interval (30 min/day) over 12 days at concentrations of 0, 8000 or 16,000 ppm (0, 30,000 or 60,000 mg/m<sup>3</sup>). Locomotor activity was measured during toluene exposures and 30 min after the final daily exposures. Compared to adults, adolescents displayed greater locomotor activity on the first day and generally greater

increases in activity over subsequent days during toluene exposure. Adults showed greater locomotor activity than adolescents in the “recovery” period following exposure on the first and subsequent days. The results are consistent with dose-dependent shifts in sensitivity and sensitization or tolerance to repeated toluene in the adolescent animals compared to the adult animals.

Batis et al. (2010) noted that toluene and many of the other commonly abused volatile organic solvents resulted in concentration-dependent effects on activity in adult rodents characterized as progressing from initial and low-dose motor excitation, then to sedation, motor impairment and anesthesia as concentration and duration of exposure increase. In rodents, the authors pointed out that these results are in general agreement with previous studies using adult rats (Hinman, 1987; Yavich et al., 1994) and adult mice (Bowen and Balster, 1998; Wood and Colotla, 1990) in which the acute locomotor effects of inhaled toluene were shown to be biphasic, with excitation at concentrations below 4000 ppm and sedation with motor impairment at concentrations above 6000 ppm. Similar biphasic action on locomotor activity was observed with gaseous anaesthetics and barbiturates (Evans and Balster, 1991). Many animal studies have described critical periods for cognitive development in the young that would increase their sensitivity to toluene exposure relative to adults (Kalsbeek et al., 1989; Frohna et al., 1995; Joyce, 1996; Lipska and Weinberger, 2002; Schwabe et al., 2004). These studies identified the anatomical areas that send axonal projections and neurotransmitters to connect with those in other areas of the brain to perform specific cognitive functions. It is reasonable to extrapolate these results to human children because rats are generally considered good models of both human brain dysfunction and normal learning processes (Loupe et al., 2002; Smidt et al., 2003; Vitalis et al., 2005), and rat brain development parallels human brain development in all but timeline and complexity of the human cortex (Nieoullon and Coquerel, 2003; Juraska and Markham, 2004; Vidair, 2004). Investigations of the developmental effects of toluene in animal models are summarized in Section 7, Developmental and Reproductive Toxicity, below.

### *Other Neurological Effects*

The 1-hour LC<sub>50</sub> for toluene in the rat was estimated at 26,700 ppm (100,000 mg/m<sup>3</sup>) (Pryor et al., 1984). The 6-hour LC<sub>50</sub>s in rats and mice were 4618 ppm (17,320 mg/m<sup>3</sup>) and 6949 ppm (26,060 mg/m<sup>3</sup>), respectively (Bonnet et al., 1982). An 8-hour LC<sub>50</sub> was estimated to be 5300 ppm (19,900 mg/m<sup>3</sup>) in the mouse. By inhalation, toluene has been reported to be more acutely toxic in animals than the similar compound benzene (Svirbely et al., 1943).

As evaluated using multisensory Conditioned Avoidance Response (CAR) Task training that involves the use of behavioral audiometry and electrophysiologic audiometry, hearing loss was observed in groups of rats after exposure to various exposure scenarios: 1000 ppm (3760 mg/m<sup>3</sup>) toluene, 14 hours per day for 2 weeks; 1500 ppm (5700 mg/m<sup>3</sup>) for 14 hours per day for three days; 2000 ppm (7500 mg/m<sup>3</sup>) for 8 hours per day for three days; and intermittent exposure to 3000 ppm (11,000 mg/m<sup>3</sup>) for 30 minutes every hour, 8 hours per day for 2 weeks (Pryor et al., 1984). However, groups of rats exposed to single exposures of 4000 ppm (15,100 mg/m<sup>3</sup>) for 4 hours or 2000 ppm (7500 mg/m<sup>3</sup>) for 8 hours did not develop ototoxicity.

Kishi et al. (1988) used the shock avoidance response test to study behavioral effects in rats. Inhalation exposure to 125, 250, or 500 ppm (470, 940 or 1900 mg/m<sup>3</sup>) toluene for 20 minutes resulted in decreased conditioned avoidance response that was reversible. However, exposure to 1000 ppm (3800 mg/m<sup>3</sup>) toluene for about four hours and 2000 ppm (7500 mg/m<sup>3</sup>) for two hours produced a concentration-related increase in incorrect responses and a considerable decrease in the effective avoidance response rate.

Rogers et al. (1999) examined the effect of neurobehavioral sensitization to toluene in two groups of 8 rats each, with two other groups of 8 rats each as controls. The first group was exposed to one acute exposure of 1600 ppm (6000 mg/m<sup>3</sup>) toluene for 6 hrs on one day (acute group); the second group was chronically exposed to 80 ppm (300 mg/m<sup>3</sup>) for 6 hr/day for 4 weeks (repeat group). After 17 days of no exposure, a subsequent very low exposure (10 ppm, 38 mg/m<sup>3</sup>; termed a triggering dose) was given to see if there were differences in operant performance between the acute and chronic exposure groups. One of the two control groups was exposed to 10 ppm (38 mg/m<sup>3</sup>) toluene as well (trigger group), while the other group was exposed to clean air. Trigger and sham exposures and operant testing were continued 5 days/week for 17 sessions. The operant response was to press a lever for food the correct number of times, with the number of lever presses for food changing at specific intervals. The investigation was duplicated with one replicate of 32 female rats and then another of 32 male rats. An increased number of incorrect responses, were seen in the acute, repeat, and trigger groups compared to the control. Both the acute and repeat males and females were adversely affected by the initial toluene exposure. The females treated with a trigger dose demonstrated no deficits, but their male counterparts were adversely affected by the trigger exposures.

To evaluate the rewarding effects of toluene inhalation, Funada et al. (2002) put 5 groups of male ICR mice into an airtight inhalation shuttlebox and tested their toluene inhalation-related place preference. Conditioning (training) sessions of 20 min each were given twice daily (one for toluene and one for air) for 5 days, with a minimum of 7 hr between sessions. The 5 groups of mice were exposed to 0 (control), 350, 700, 2500, or 3200 ppm (1320, 2600, 9400, or 12,100 mg/m<sup>3</sup>) toluene (measured by GC) in one of the two compartments of the same shuttleboxes, respectively. Test sessions were one day after the final conditioning session with no toluene

exposure. The time each mouse spent in each compartment during a 20-min session was measured using a digital video camera. The results showed the exposure to toluene  $\geq 700$  ppm (2600 mg/m<sup>3</sup>) produced a significant place preference (to avoid toluene exposure) in mice. In this study, 700 ppm (2600 mg/m<sup>3</sup>) is a LOAEL.

Using well-established pattern-elicited visual evoked potentials (VEPs) and a physiologically based pharmacokinetic (PBPK) model to estimate the toluene brain concentration during and after inhalational exposure to toluene, Boyes et al. (2007) demonstrated that toluene impaired visual function in rats. Adult male Long-Evans rats were exposed by inhalation to 1000 ppm (3800 mg/m<sup>3</sup>) toluene for 4 hours, 2000 ppm (7500 mg/m<sup>3</sup>) for 2 hours, 3000 ppm (11,300 mg/m<sup>3</sup>) for 1.3 hours, or 4000 ppm (15,100 mg/m<sup>3</sup>) for 1 hour. Brain neurophysiological function was measured using VEP recorded from electrodes located over visual cortex of the rats. The VEP amplitude of the major spectral component was reduced by toluene exposure. The experiment data showed a logistic fit with a significant correlation between VEP amplitude reduction and brain toluene concentration. The authors also concluded that the acute neurotoxic effects of toluene are caused by perturbations of various neurotransmitter systems and ion channels involved in neurotransmission.

Acute exposure to toluene results in neurotoxicity including alterations in visual function. N-methyl-D-aspartate (NMDA)-glutamate receptors are widely present in the visual system and contribute to pattern-elicited VEPs in rodents. To elucidate the mechanisms underlying the visual neurotoxicity of toluene, Bale et al. (2007) studied whether acute toluene effects on NMDA-glutamate receptors contribute to toluene-induced alterations in VEPs of rats. Long-Evans rats were exposed to 2000 ppm (7500 mg/m<sup>3</sup>) toluene by inhalation, and VEPs were measured during toluene exposure in the presence or absence of NMDA (agonist) or MK801 (antagonist). The results showed that the amplitude of VEPs, which strongly rely on glutamatergic neurotransmission, decreased after the exposure to toluene and that this effect could be reduced by pre-administration of MK801. The authors claimed that the data support the hypothesis that especially early visual processing is partly inhibited by toluene.

Bowen et al. (2010) compared four mouse strains (three inbred strains, Balb/cByj, C57BL/6J and DBA/2J, and one outbred strain, Swiss Webster) of five groups in their sensitivity to changes in locomotor activity following acute exposure [30 min, 0, 100, 2000, 8000, and 10,000 ppm (0, 380, 7500, 30,000 and 38,000 mg/m<sup>3</sup>)], and then each group with repeated [8000 ppm (30,000 mg/m<sup>3</sup>), 30 min/day for 14 consecutive days] toluene exposure. With acute exposure, qualitatively similar acute behavioral effects were observed in all four strains of mice, in that a biphasic effect on spontaneous locomotor activity was produced in the mice. At 2000 ppm (7500 mg/m<sup>3</sup>) toluene increased ambulatory distance while the concentrations  $\geq 8000$  ppm (30,000 mg/m<sup>3</sup>) induced temporally biphasic effects of initial increases in activity followed by hypoactivity. There were evident differences between groups in absolute locomotor activity levels. The repeated exposure revealed sensitization developed in locomotor activities that was significantly higher in each group, and that there were time course changes. These differences in acute sensitivity and the differential shifts in sensitivity after repeated exposures among the mouse strains suggest a genetic basis for the behavioral effects to toluene.

Demir et al. (2017) studied the effects of acute toluene toxicity on different regions of the rabbit brain. Ten male New Zealand rabbits (healthy, average age 24 months) were given a single dose of 876 mg/kg toluene by intraperitoneal (IP) injection, with another 10 rabbits serving as



the control group. Five hours after the injection, the blood samples were collected and the animals were euthanized. The brain tissues were removed and processed for biochemical, histopathological and immunohistochemical examinations. In the toluene-administered rabbits, areas of focal vacuolar degeneration, gliosis, perivascular demyelination, and many pyknotic cells and necrosis were detected in the brain cortex. Compared to the control group, a remarkable excessive expansion of the blood vessels, severe degeneration of the compensation in the cells, and almost dispersed cell borders were observed. Abnormal malformations of the nuclei structure of the oligodendrocyte cells and structural damage of the bodies of the sequential neurons of the hippocampus were seen as well. The authors concluded that biochemical and histopathological methods facilitated the observation of the main toluene exposure brain damage effects of astrocyte activation and gliosis. High levels of proapoptotic proteins in the different regions of rabbit brain showed that toluene may trigger apoptosis in about 3 hours.

Oshiro et al. (2011) studied the comparative abilities of the  $C \times t$  and brain toluene concentration ( $Br[ Tol ]$ ) dose metrics to accurately predict behavioral effects of the solvent in rats upon exposures to toluene lasting up to 24 hours. This study was performed in order to determine if Haber's rule was applicable to toluene neurobehavioral effects. Four key experiments were conducted involving: 1) the evaluation of the ability of a previously published physiologically-based toxicokinetic (PBTK) model to predict  $Br[ Tol ]$  in rats performing a lever-pressing task (LPT) during toluene exposure; 2) use of rat telemetry data to refine the PBTK model; 3) comparison of the  $C \times t$  and  $Br[ Tol ]$  dose metrics as they related to rat performance in visual signal detection tasks (SDTs) during and shortly after toluene exposure for  $\geq 21$  hours; and 4) examination of the ability to quantitatively extrapolate 1-hour exposure patterns to longer ( $\geq 21$ -hour) exposures. Details of these four experiments are listed in Table 2 below. Male Long-Evans rats (age  $\sim 12$  weeks;  $350 \pm 10$  g bodyweight) were used. The results suggested that  $Br[ Tol ]$  appeared to be a more accurate dose metric than  $C \times t$  in predicting neurological effects of exposures  $\leq 24$  hours. However, the authors also noted that behavioral tolerance occurred after 24-hours of toluene exposure, suggesting a potential confounder in extrapolating these effects from a 24-hour exposure to a one-hour exposure.

**Table 2. Summary of acute animal studies that have a NOAEL and/or LOAEL.**

Study	Exposure duration and Concentration (ppm)	Experimental animal / Effects (endpoints)	NOAEL (ppm)	LOAEL (ppm)
Pryor et al. (1984)	0 or 1000 ppm for 4 hrs; 0 or 2000 ppm for 2 hrs; 2000 ppm for 8 hrs/day for 3 days	Rats / Hearing loss as measured by the Conditioned Avoidance Response Task	1000 and 2000 ppm single exposure	2000 ppm, 8 hrs/day for 3 days
Kishi et al. (1988)	125, 250, or 500 ppm for up to 3 hrs by inhalation	Rats / decreased conditioned avoidance response	NA	125

Study	Exposure duration and Concentration (ppm)	Experimental animal / Effects (endpoints)	NOAEL (ppm)	LOAEL (ppm)
Rogers et al. (1999)	1600 ppm for 6 hrs, then 17 days of clean air, followed by 10 ppm “trigger” exposures	Rats / neurobehavioral sensitization as measured by lever press operant testing	NA	1600 ppm with 10 ppm trigger dose
Funada et al. (2002)	0 (control), 350, 700, 2500, or 3200 ppm for 20 min	5 groups of ICR mice / toluene inhalation-related place preference	350	700
Bowen et al. (2010)	0, 100, 2000, 8000, and 10,000 ppm for 30 min	5 groups of mice / increased ambulatory distance	100	2000

Abbreviations: NA – not applicable

**Table 2. Summary of acute animal studies that have a NOAEL and/or LOAEL (continued).**

Study	Exposure duration and Concentration (ppm)	Experimental animal / Effects (endpoints)	NOAEL (ppm)	LOAEL (ppm)
Oshiro et al. (2011) Experiment 1	775 or 1225 ppm ± 10 ppm for 1 hr (Groups 1 and 2), 6 hrs (Groups 3 and 4) or 24 hrs (Groups 5 and 6) with necropsy immediately post exposure or toluene exposure as above for 24 hrs (Groups 7 and 8) + fresh air exposure for 6 hours prior to necropsy	40 LPT-trained rats, age = 7.5 - 8.5 months, divided into eight separate groups (n = 5/group)  PBPK model over-estimated Br[tol] values in groups (5-8) exposed to toluene for longer periods.	NA	NA
Experiment 2	0 (fresh air only) or 1125 ± 10 ppm for exposures lasting 6 or 24 hours, with necropsies immediately following, or after a 6-hour post-exposure period in a fresh-air environment	14 of 32 LPT-trained rats (age = 4-5 months) implanted with radio telemeters and exposed and tested similar to Experiment 1  Consistently elevated (~10% higher) heart rates in toluene-exposed rat groups versus controls. >4-fold increases in the PROD activity of the two groups exposed to toluene for 24 hours versus the control.	NA	NA

Abbreviations: Br[tol] – brain toluene concentration; C – concentration; LPT - lever-pressing task; NA – not applicable; PROD - pentoxyresorufin-O-dealkylase enzyme; t – time.

**Table 2. Summary of acute animal studies that have a NOAEL and/or LOAEL (continued).**

Study	Exposure duration and Concentration (ppm)	Experimental animal / Effects (endpoints)	NOAEL (ppm)	LOAEL (ppm)
Oshiro et al. (2011) Experiment 3	0, 1125, or 1450 ppm for exposures lasting 24 hours and 1660 ppm for those lasting 21 hours	16 SDT-trained rats (age = 11 – 13 months)  Overall response accuracy decreased and latency increased as functions of the $C \times t$ and Br[ <i>tol</i> ] dose metrics; however, Br[ <i>tol</i> ] was a better predictor of behavior. Data used to estimate a “mild effect level” for a 1 hour exposure (i.e., a doubling of the control response latency)	NA	1450  (mild effect level for a 1 hour exposure)
Experiment 4	Same as Experiment 3	Animal data from Experiment 3 compared to that from the 1-hr exposure study by Busnell <i>et al.</i> (2007)  1-hr data overestimated accuracy and latency effects of the 24-hour exposures regardless of the dose metric used	NA	NA

Abbreviations: Br[*tol*] – brain toluene concentration; C – concentration; NA – not applicable; SDT – signal detection task; t – time.

## 6. Chronic Toxicity of Toluene

### Chronic Toxicity to Adult Humans

The substantial body of studies examining the subchronic and chronic effects of toluene in occupationally-exposed humans indicate a relationship between neurological effects and long-term occupational exposures to toluene (e.g.  $\geq 20$  ppm (75 mg/m<sup>3</sup>)). The weight of evidence from these studies indicates that various neurological effects (i.e., impaired color vision, impaired hearing, decreased performance in neurobehavioral analysis, changes in motor and sensory nerve conduction velocity, headache, and dizziness) are the most sensitive endpoints. Chronic inhalation exposure of humans to toluene has also resulted in irritation of the upper respiratory tract and eyes, sore throat, and difficulty with sleep (USEPA, 2005).

## *Abuse Toxicity*

CNS depression and other severe neurological symptoms have been reported in chronic abusers exposed to high levels of toluene, resulting in progressive and irreversible changes in brain structure and function (Spencer and Schaumburg, 1985). However, exposure to other solvents in these types of studies cannot be discounted. Early neurobehavioral changes include anxiety, irritability, mood swings, and forgetfulness. Further exposure causes nystagmus, slurring of speech, bilateral hearing impairment, titubation (head tremor and disequilibrium upon standing), and a wide-based ataxic gait. A number of studies found permanent changes in brain structure (loss of grey and white matter differentiation; cerebral, cerebellar and brainstem atrophy) which correlated with brain dysfunction as measured by magnetic resonance imaging (MRI), and brainstem auditory evoked response (BAER) evaluations have also been observed in chronic toluene abusers (Rosenberg et al., 1988a; Rosenberg et al., 1988b; Filley et al., 1990; Ikeda and Tsukagoshi, 1990; Yamanouchi et al., 1995; Caldemeyer et al., 1996).

For example, Filley et al. (1990) studied 14 chronic toluene abusers using MRI and neuropsychological evaluations. Duration of abuse was from 24 to 252 months, with a mean of 105 months. The clinical assessment of overall neuropsychological functioning, was composed of 12 neuropsychological tests: expanded Halstead-Reitan Battery (HRB), Wechsler Adult Intelligence Scale (WAIS), modified Reitan's Story Memory Test, visual reproduction component of the Wechsler Memory Scale, the Boston Naming Test, word discrimination and complex material tests from the Boston Diagnostic Aphasia Examination, the Thurstone Word Fluency Test, the Peabody Individual Achievement Test, the Digit Vigilance Test, the Wisconsin Card Sorting Test, the Tonal Memory Test, and the Grooved Pegboard and Steadiness Tests. The assessment results indicated that three patients functioned normally, three were in a borderline range, and eight were impaired. Independent analyses of white matter changes on MRI demonstrated that the degree of white matter abnormality was strongly correlated ( $p < 0.01$ ) with neuropsychological impairment. The authors concluded that dementia in toluene abuse appears to be related to the severity of cerebral white matter involvement.

Mild effects on the kidneys and liver have also been reported in solvent abusers chronically exposed to toluene vapor, but are confounded by probable exposure to multiple solvents (NTP, 1990).

## *Community Epidemiological Studies*

Several studies examined the health impact of ambient exposures to toluene below the U.S. EPA RfC of 5.0 mg/m<sup>3</sup> (1.3 ppm), particularly among vulnerable subpopulations such as children and the elderly. However, these studies are examinations on ambient mixtures of air toxics and therefore are less likely to provide data for developing RELs.

Toluene is one of the most frequently identified indoor residential chemical risk factors for asthma and allergy in infants and children (Mendell, 2006). However, there is a lack of evidence that toluene on its own acts as a sensitizing agent that leads to asthma in children. More likely, co-exposures to other antigenic materials (e.g. dust mite antigen) can lead to asthma, and toluene is simply acting as a marker for another correlated compound or substance.

Rumchev et al. (2004) surveyed 88 children aged 6 months to 3 years in Australia who were diagnosed with asthma, together with 104 children of the same age group without an asthma diagnosis (controls). Questionnaire information collected included the health status of the children, exposure to VOCs including toluene, average temperature and relative humidity in the living room of each participating family. The median concentration of indoor toluene was 17.1 (range 0.01 – 153.9)  $\mu\text{g}/\text{m}^3$  (4.54 ppb, 0.0027 – 40.84 ppb). The result showed that the children with asthma were exposed to significantly higher VOCs than controls ( $p < 0.01$ ), and toluene provided the third highest odds ratio for asthma. The risk of asthma increased two-fold for every 10  $\mu\text{g}/\text{m}^3$  (2.7 ppb) increase in the indoor concentration of toluene. The authors concluded that indoor exposure to VOCs, including toluene, at levels lower than current recommended value may still increase the risk of childhood asthma.

Delfino et al. (2003a and 2003b) investigated the correlation between asthma symptoms in children and ambient air VOCs including toluene. Twenty-one Hispanic children with mild asthma from a Los Angeles community with high VOC levels provided symptom diaries and peak expiratory flow (PEF) data daily for three months, and their exhaled VOC samples were analyzed by GC-MS. Toluene was shown to have a positive association with asthma symptoms.

Hulin et al. (2010) compared the asthmatic effects of indoor air pollutants in urban homes with those in rural houses, involving two populations of children living in a city (32 asthmatics and 31 controls) and in the countryside (24 asthmatics and 27 controls). The pollutants, including toluene, were assessed at the homes for 1 week. The urban homes were shown to have up to 2 times higher pollutant levels than rural homes. In both populations, toluene was significantly related to a higher risk of asthma.

In the study of Bentayeb et al. (2013), 567 buildings in Metropolitan areas of France were randomly selected and 1,012 inhabitants over 15 years of age, including 144 individuals over age 65, were surveyed for breathlessness and chronic bronchitis, together with the indoor concentration of aldehydes and 20 VOCs in their dwelling. While similar levels of indoor air pollutants were found for the elderly and other inhabitants, increased toluene concentrations were significantly associated with breathlessness and chronic bronchitis in the elderly but not in the rest of the population. Adjusted odds ratios (95% confidence interval) of 3.36 (1.13, 9.98) were observed in the elderly, in comparison with 0.91 (0.59, 1.39) in the other inhabitants.

Xu et al. (2009) found that blood toluene levels were correlated with increased odds of cardiovascular disease (CVD). The authors used the 1999–2004 National Health and Nutrition Examination Survey (NHANES) data to examine the relationship between alkylbenzene levels (toluene, styrene, ethylbenzene, and the xylenes) and CVD prevalence. Levels of all five alkylbenzenes demonstrated linear dose-response trends. For toluene, 389 subjects had an average blood concentration of 0.751 ng/mL, the odds ratio was 2.30 (50<sup>th</sup>–85<sup>th</sup> percentiles) and 3.49 ( $\geq 85^{\text{th}}$  percentiles). Further studies are needed to explore associations between these highly prevalent pollutants and CVD.

## *Occupational Studies*

### *Neurological effects (CNS, sensory irritations, neurobehavioral and psychometric tests)*

Wilson (1943) reported that occupational exposure of workers to concentrations of commercial toluene ranging from 50 to 200 ppm (200 to 750 mg/m<sup>3</sup>) for periods of 1 to 3 weeks resulted in headaches, lassitude, and loss of appetite. At 200 to 500 ppm (750 to 2000 mg/m<sup>3</sup>), symptoms of nausea, bad taste in the mouth, slightly impaired coordination and reaction time, and temporary memory loss were also observed. Exposure to 500 to 1500 ppm (2000 to 5600 mg/m<sup>3</sup>) resulted in palpitations, extreme weakness, pronounced loss of coordination, and impaired reaction time. Red blood cell counts were decreased and there were 2 cases of aplastic anemia. Commercial toluene likely contained significant levels of benzene, which may have caused the red blood cell effects.

Matsushita et al. (1975) evaluated the clinical and subjective responses of industrial workers exposed to toluene vapor. Subjects included 38 female shoemakers occupationally exposed to toluene (and slight amounts of gasoline) and a control group of 16 female workers from the same manufacturing plant who had never been exposed to any solvent. The mean duration of solvent exposure was 3 years and 4 months, and the mean age of the exposed subjects was 20.7 ± 5.2 years, which was similar for the control group. Toluene concentrations in air in the workroom of the exposed group ranged between 56 to 380 mg/m<sup>3</sup> (15 and 100 ppm) in February and September, with an average of 226 to 263 mg/m<sup>3</sup> (60 to 70 ppm), and between 38 and 750 mg/m<sup>3</sup> (10 and 200 ppm) with an average of 100 ppm in August of the study period. A health questionnaire was administered to collect subjective symptoms, and clinical findings were drawn from urinalysis and tests for liver and neuromuscular function.

Subjective symptoms of the exposed subjects were significantly different from the controls with respect to dysmenorrhea ( $p < 0.05$ ) and feeling uneasy about solvent vapor ( $p < 0.01$ ); exposed subjects also reported a sensation of general weakness and itching and dermatitis of hands, but these did not reach statistical significance by  $\chi^2$ -test ( $p < 0.1$ ). The only significant hematological difference between groups was a marked increase in the appearance rate of Mommson's toxic granules in peripheral neutrophils in the exposed group (7.6 ± 5.6%, 13/38 subjects;  $p < 0.05$ ) relative to controls (3.8 ± 3.4%, 1/16 subjects). Excretion of hippuric acid in urine was significantly elevated in the exposed group (3.26 ± 0.82 mg/ml) compared to controls (0.36 ± 0.24 mg/ml). Neuromuscular function tests revealed a significant difference between the exposed and control groups with abnormal tendon reflex of the patella ( $p < 0.05$ ); other differences reported (abnormal tendon reflex of the ankle) were not statistically significant by application of t- and  $\chi^2$ -tests ( $p < 0.1$ ). No significant correlation was found between the duration of toluene vapor exposure and the appearance rate of abnormal findings.

Up to 101 solvent workers exposed to toluene in shoe-making factories were examined for subjective symptoms, and changes in hematology and serum and urine biochemistry (Yin et al., 1987). The mean TWA toluene exposure of the subjects was 42.8 ppm (161 mg/m<sup>3</sup>) and the average exposure duration was 6.8 years. Concurrent exposure to benzene (TWA 1.3 ppm) also occurred in these workers. Compared to a group of 127 control workers, the prevalence of subjective symptoms was greater ( $p < 0.01$ ) in the toluene-exposed workers during work and in the past 6 months. The most common symptoms were sore throat, dizziness, and headache. Nose and/or eye irritation during work and difficulty in sleeping were also reported. When the toluene-exposed workers were separated into a low exposure group (< 40 ppm, 151 mg/m<sup>3</sup>) and a high exposure group (≥ 40 ppm, 151 mg/m<sup>3</sup>), the prevalence of the three most common

symptoms appeared to be dose-related. Their hematology was essentially normal and serum and urine biochemistry was unremarkable.

Orbaek and Nise (1989) examined the neurological effects of toluene on 30 rotogravure printers, 33-61 years of age (mean 50), employed at two Swedish printing shops for 4-43 years (median 29) in 1985. Mean toluene exposure levels at the two printing shops were 43 mg/m<sup>3</sup> (11.4 ppm) and 157 mg/m<sup>3</sup> (41.8 ppm); however, before 1980 the mean exposure levels had exceeded 300 mg/m<sup>3</sup> (79.8 ppm) in both shops. The authors noted that rotogravure printing provides an occupational setting with toluene exposure not confounded by exposures to other solvents. Comparisons were made to a reference group of 72 men aged 27-69 yrs (mean 47 yrs). The alcohol consumption of both the workers and referents was also determined (< 200 g/week or > 200 g/week). Neurological function in the workers and referents was evaluated using interviews and psychometric testing; the results from each of the two printing shops were pooled. The printers reported statistically significantly ( $p < 0.05$ ) higher occurrences of fatigue (60%), recent short-term memory problems (60%), concentration difficulties (40%), mood lability (27%), and other neurasthenic symptoms. The printers also scored significantly worse than referents in a number of psychometric tests, including synonym, Benton revised visual retention, and digit symbol tests, even after adjustment for age. For all comparisons, tests for interaction between the effects of toluene exposure and alcohol consumption were not statistically significant.

A battery of neurobehavioral tests was performed in 30 female workers exposed to toluene vapors in an electronic assembly plant (Foo et al., 1990). The average number of years worked was  $5.7 \pm 3.2$  (mean  $\pm$  standard deviation (SD)) for the exposed group and  $2.5 \pm 2.7$  years for a control group. Study subjects did not smoke tobacco or drink alcohol, were not taking any medications, and had no prior history of central or peripheral nervous system illness or psychiatric disorders. The exposed group of workers inhaled a time-weighted average (TWA) of 88 ppm (330 mg/m<sup>3</sup>) toluene while the control workers inhaled 13 ppm (49 mg/m<sup>3</sup>). The control group, matched for age, sex and ethnicity, was selected from another section of the facility where toluene was not used, but had low levels of toluene due to cross contamination of work areas. Post-shift blood toluene concentrations were also determined in the workers. Mean blood toluene levels were 1.25 mg/l in the exposed group and 0.16 mg/l in the control group. In a previous study of factory workers at the same plant, Foo et al. (1988) observed a linear correlation between blood toluene concentration and TWA exposure.

Foo et al. (1990) conducted the neurobehavioral tests during the work week on Wednesdays and Thursdays prior to start of the work shift. A statistically significant decrease in neurobehavioral performance was observed in the exposed workers for 6 out of 8 tests ( $p < 0.05$ , Student's t test). Analysis of covariance showed that the performance in each of these six positive tests was related to the TWA concentration of toluene. However, no statistically significant relationship was found between duration of exposure and performance in the neurobehavioral tests. Decreased performance of exposed workers were observed for the grooved peg board test that measures manual dexterity, the trail making, visual reproduction, Benton visual retention, and digit symbol tests that measure visual scanning ability, and the digit span test that measures verbal memory. Simple reaction time and finger tapping tests, which measure motor speed, were not affected by toluene exposure. The authors noted that the lack of effects on motor speed, while other neurobehavioral tests of toluene exposure exhibited



decreased performance, matches the results of the acute toluene exposure study by Echeverria et al. (1989).

Irritant effects of toluene in the workers were not discussed by Foo et al. (1990), although the study states that no clinical symptoms or signs were observed. This finding suggests that the neurobehavioral effects observed were subclinical. Potential concurrent exposures to other chemicals were not specifically addressed. In this study, 88 ppm was considered a LOAEL for central nervous system effects. However, the workers designated by the authors to be controls did not comprise a true control group, since they were exposed to an average of 13 ppm (49 mg/m<sup>3</sup>) toluene. This may have resulted in an underestimation of the effects of exposure to 88 ppm (330 mg/m<sup>3</sup>) toluene, in particular because the authors noted a dose-response relationship between TWA toluene air concentration and neurobehavioral performance in most of the tests.

Murata et al. (1993) investigated peripheral neurotoxicity in toluene-exposed workers. Subjects included 10 male workers who were between the ages of 20 and 77 years (mean age 39 years) and exposed to toluene at a rotogravure printing company in Saitama, Japan for 1-36 years (mean 11 years) at an estimated exposure level of 41-224 ppm (mean 83 ppm) toluene. These men ingested alcohol (0-480 ml/week, mean 88), smoked tobacco (0-30 cigarettes/day, mean 7), and had no history of cardiovascular or neurologic disease or alcohol dependency. Control subjects were 10 healthy, unexposed men of similar economic status who were not significantly different in age, skin temperature, or alcohol and tobacco consumption from the exposed workers. Blood and urine samples were collected for analyses of toluene and hippuric acid levels, respectively. Electrophysiological tests were conducted to measure cardiac autonomic function as follows: coefficient of variation in electrocardiographic R-R intervals (CV<sub>RR</sub>), distribution of nerve conduction velocities (DCV), maximal motor and sensory nerve conduction velocities (MCV and SCV) in the median nerve, and the high-frequency (HF) and low-frequency (LF) components of the CV<sub>RR</sub> corresponding with parasympathetic (C-CV<sub>HF</sub>) and sympathetic (C-CV<sub>LF</sub>) activities, respectively.

Blood toluene concentrations in the exposed workers averaged 526 µg/L (range, 147 to 1,119) and urinary hippuric acid levels averaged 1.61 g/L (range, 0.94 to 3.9). Blood toluene and urinary hippuric acid concentrations were not provided for the control group. Relative to unexposed controls, the CV<sub>RR</sub> and C-CV<sub>HF</sub> were significantly lower in the exposed workers ( $p < 0.05$ ), suggesting reduced nerve conduction velocities. The other significant finding was a reduction of the MCV in the forearm and the SCV in the palm in the exposed workers compared with controls, with observed matched differences of  $-3.7 \pm 1.9$  m/sec ( $p < 0.01$ ) and  $-4.6 \pm 4.4$  m/sec ( $p < 0.05$ ), respectively. Heart rate did not differ between exposed subjects and controls, and no significant association was found for electrophysiological data and blood toluene or urinary hippuric acid levels, or duration of toluene exposure among exposed subjects.

Vrca et al. (1995) studied the effects of toluene on visual evoked potentials (VEPs), which are measurements of electrical signals produced in the brain in response to light stimuli. The exposed group included 46 male and 3 female employees (total  $n = 49$ ) of a printing-press factory in which toluene was used exclusively as an organic solvent for 30 years prior to the study. Average airborne toluene concentrations ranged from 226 to 301 mg/m<sup>3</sup> (60 to 80 ppm) in the printing-press factory; average employee age was  $42.3 \pm 6.8$  years (range = 24 - 55 years); and average employment length was  $21.4 \pm 7.4$  years (range = 4 - 30). The control group ( $n = 59$ ; 54 males; 5 females) included factory workers producing rotating coils for electric

motors who were not occupationally exposed to any neurotoxic substances known to the study authors. Average age was  $43 \pm 7.2$  years (range = 23 - 55 years); and average employment length was  $20.6 \pm 7.7$  years (range = 4 - 32 years).

Subject VEPs were measured with a brain imager (electroencephalograph; EEG) with electrodes placed at the occiput (the location of the visual cortex) for recording VEPs. According to the authors, N75, P100, and N145 waves of VEPs were analyzed by summing up the same values for the right and left eye in each subject and dividing by two. Measured brain wave parameters included the time of onset, attainment of maximal amplitude (wave latency), offset, and total duration (offset minus onset latency), as increases in latency and decreases in amplitude could indicate pathology/dysfunction.

Toluene exposure was determined using the peripheral blood concentration of toluene, and the urinary creatinine-adjusted concentrations of hippuric acid and ortho-cresol in 36 exposed and 27 unexposed workers (sex ratios not stated). The results suggested that pre-work-shift concentrations of toluene in blood were significantly greater ( $p < 0.00096$ ) in the exposed workers relative to their unexposed counterparts, as were the pre ( $p < 0.05$ ) and post ( $p < 0.001$ ) work-shift hippuric acid levels in urine. Toluene-exposed workers exhibited significantly ( $p \leq 0.01$ ) higher wave (P100, N45, and N175) amplitudes and significantly longer P100 latency ( $p < 0.05$ ) than unexposed workers. Though the authors stated the former may be due to more excitable wave generators in the visual pathway, they also recognized that P100 latency was a more sensitive toluene-induced VEP parameter, and suggested the increased latency may be due to reduced wave conductivity in certain segments of the visual pathway.

Using the same subjects from their 1995 study, Vrca et al. (1996) evaluated the effects of occupational toluene exposure (60-80 ppm) on brainstem auditory evoked potentials (BAEPs). As above, workers were asked to refrain from taking medicine for 5 days prior to testing. BAEPs were measured on a Monday, prior to the start of the work-shift, by subjecting each ear to testing. Subjects were tested in a dark, temperature-controlled (range = 19 - 21°C) room, and asked to keep their eyes closed. While one (test) ear was stimulated with a click stimulus [2048 times, 15 stimuli/second at 70 decibel sound pressure level (dB SPL)], the other (unstimulated ear) was simultaneously subjected to masked noise (50 dB SPL). Brain responses occurring within the first millisecond after commencement of stimulation were recorded by EEG. Waves P1 - P5 were analyzed by amplitude, latency, and interpeak latency. The wave forms had abscissae (horizontal, x-axis measures) and ordinates (vertical, y-axis measures). The values for the left and right ear in each subject were summed and divided by 2, and data were statistically analyzed as above.

Results indicated that all wave latencies were significantly longer ( $p \leq 0.01$ ) and amplitudes were significantly smaller ( $p \leq 0.05$ ) in exposed versus unexposed workers. According to the authors, smaller wave amplitudes suggested changes to the whole length of the auditory pathway, through the brainstem. Significantly ( $p \leq 0.01$ ) longer inter-peak latencies were also observed for exposed workers when compared to their unexposed counterparts. These latencies included P3 - P4, P1 - P5, P3 - P5, and P1 - P4. Given the interpeak latencies and that of the P1 wave, specifically, the authors suggested that the sites most affected by toluene may be the extramedullary part of the auditory pathway (the Corti organ and auditory nerve) and the high medullary part up to the level of the auditory colliculus, with the intervening section of the pathway more resistant to the effects of toluene.

A group of 49 printing-press workers occupationally exposed to toluene for approximately 21.6 years was studied by Vrca et al. (1997a). Toluene exposure levels were determined from blood toluene and urinary hippuric acid levels, and were estimated to range from 40-60 ppm (151-226 mg/m<sup>3</sup>). No control group was used. Brain-evoked auditory potential (BEAP; similar to BAER) and visual evoked potential (VEP) measurements were performed on a Monday morning after a nonworking weekend. There was a significant increase in the latencies of all the BEAP waves examined (except for P2 waves), as well as in the interpeak latency (IPL) P3-P4, while IPL P4-P5 decreased significantly with the length of exposure. No correlation was noted between the amplitude of BEAP waves and the length of exposure. The amplitude but not the latency of all the VEPs examined decreased significantly with the length of exposure.

The Vrca et al. (1997b) report expanded upon the Vrca et al. 1995 publication by reporting the effects of toluene on the P300 wave. This wave is not like the P100 wave, which is generated by an external stimulus, but rather is generated by a person's reaction to a stimulus as part of a decision-making process. For example, to measure the P100 waves, VEPs were provoked using alternating checkerboard patterns on a computer screen. To measure P300 waves, subjects are presented with a sequence of events such that each event can be categorized as "frequently" or "infrequently" occurring. In the 1997 study by Vrca et al., the alternating checkerboard pattern fell into the former category. A separate "rare" stimulus generated the P300 wave about 300 ms after stimulus onset. As P300 is involved with the process of memory modification or learning, and stimuli appear to be learned if, and only if, they are novel. The P300 wave is smaller in subjects with decreased cognitive ability relative to age-matched normal subjects. Results from this study suggested that toluene caused delayed VEPs (increased latency) during mental engagement (i.e. presentation of the "rare" stimulus, which may reflect adverse changes to intracranial structures like the brainstem and hypothalamus.

Boey et al. (1997) examined 29 electronic assembly plant workers chronically exposed to toluene ( $4.9 \pm 3.5$  years) for neurobehavioral deficits on a midweek morning (i.e., more than 12 hours but less than 24 hours after exposure ceased). The exposed workers had an 8-hour TWA toluene exposure of 90.9 ppm (343 mg/m<sup>3</sup>) and were compared to a matched control group of 29 workers from the same electronics factory that had a low level of toluene exposure (8-hour TWA 12.2 ppm, 46.0 mg/m<sup>3</sup>). Neurobehavioral effects were investigated using the logical memory, digit span, visual reproduction, trail making, symbol digit modality, and grooved pegboard tests. Significant decrements were observed in 9 of 14 examiner-administered tests of exposed workers compared to referents analyzed by ANOVA with years of education as a covariate.

Eller et al. (1999) evaluated the chronic effects of toluene exposure on the central nervous system of workers in a rotogravure plant. Ninety-eight male workers from a selection pool of 107 (92%) underwent neuropsychological examination using a Cognitive Function Scanner, and neurological examination by computerized methods measuring coordination ability, tremor and position stability. In addition, measures of symptoms and former exposure were obtained by questionnaire. The workers were divided into three groups: Group 0 with no exposure to organic solvents (n = 19); Group 1 with exposure to TWA <20 ppm (75 mg/m<sup>3</sup>) of toluene for less than 13 years (n = 30, average exposure time 7.7 yr) and Group 2 with exposure for more than 12 years (n = 49, average exposure time 25.5 yr). Within Group 2, all workers had been exposed to

levels exceeding 100 ppm (380 mg/m<sup>3</sup>) for at least 4 years, with 37 of the workers (75%) exposed for 10 or more years before 1983 at that level.

Among the findings by Eller et al. (1999), no significant differences were found between Group 0 and Group 1 regarding symptoms and the results of neuropsychological and neurological function tests. However, Group 2 differed significantly from the other two groups for increased symptom index score ( $p = 0.04$ ), particularly with answers on the questionnaire regarding the ability to concentrate, and reduced memory and fatigue. Group 2 scored much poorer on neuropsychological tests compared to group 0 for visuospatial function ( $p = 0.06$ ), number learning ( $p = 0.04$ ) and word recognition ( $p = 0.02$ ), while only one marginal deficit ( $p = 0.05$ ) in the neurological function tests (finger tap, left hand) was observed. The authors concluded that the exposure to toluene in Group 2 for over 12 years with an estimated TWA over 100 ppm (380 mg/m<sup>3</sup>) for at least 4 years (range: 4-27 years) was associated with impaired neuropsychological function.

Deschamps et al. (2001) studied the cognitive function impairment from long-term toluene exposure on 72 workers (42 men, 30 women) compared with 61 non-exposed workers. Toluene exposure dosage from personal samplers and atmospheric sampling showed 9 to 467 ppm (34 to 1,756 mg/m<sup>3</sup>), and the mean duration of exposure was  $19.9 \pm 0.9$  years. The results from a subjective questionnaire and psychometric tests showed no significant differences between exposed and non-exposed workers except that the mucosal irritation score was significantly higher in exposed than in non-exposed workers ( $p < 0.005$ ). According to the authors, several workers exposed to high atmospheric concentrations of toluene did not show statistically different results in the subjective questionnaire for cognitive and neurological functions or for the psychometric tests.

Neubert et al. (2001) studied a large cohort of 1,290 rotogravure printers in Germany occupationally exposed to toluene for an undefined number of years, for whom questionnaire on subjective symptoms and psycho-physiological and psycho-motoric tests were assessed, compared with 200 unexposed workers. No neurological symptoms or impairment on overall performance was observed in the tests, except for the flicker fusion frequency test. A significant decrease effect on visual perception was found among a subgroup of 47 workers exposed to an estimated 70–80 ppm (263–301 mg/m<sup>3</sup>) toluene for 6 hours during a daily shift. In this study, a LOAEL for impaired visual perception was estimated as 75 ppm (282 mg/m<sup>3</sup>).

Gericke et al. (2001) studied the effect of long-term (at least 20 years) low-concentration toluene exposure on 1,077 male rotogravure factory workers. Toluene exposure concentrations were 24 ppm (90 mg/m<sup>3</sup>) for printers and 4.5 ppm (17 mg/m<sup>3</sup>) for non-printers (N = 109). Physical exam, neuropsychological tests and a questionnaire of subjective symptoms showed no significant increase in neurological symptoms or overall performance on neuropsychological or psychomotor tests from the exposed workers compared with unexposed workers.

Chouaniere et al. (2002) examined 128 toluene-exposed printing workers (14 women and 114 men) from two plants 48 hours after their shift ended for psychometric changes using the Neurobehavioral Evaluation System (NES) tests. Worker exposure was monitored for 3 or 4 days prior to testing to estimate exposure based on workshop, job type, and shift in the plants. The average exposure duration was  $14 \pm 10$  years with current TWA toluene exposures ranging

from 0 to 27 ppm (102 mg/m<sup>3</sup>), although past exposures were estimated to range from 0 to 179 ppm (675 mg/m<sup>3</sup>).

Multiple regression analysis found a statistically significant dose-effect relationship between toluene exposure and decrements for the Digit Span Forwards ( $p = 0.04$ ) and Digit Span Backwards tests ( $p = 0.01$ ) (both measures of short-term memory performance), after correction for the confounders of sex, age, synonym score (for education), history of CNS diseases, alcohol consumption, psycho-active drugs used in last day, concentration in performing tests and computer experience. Neurotoxic symptoms were obtained through a questionnaire (EUROQUEST), including 83 items within 5 categories: (1) neurological symptoms and psychosomatic symptoms; (2) acute symptoms; (3) mood, memory, concentration, fatigue, sleep disturbances; (4) environmental susceptibility; (5) anxiety, perception of health status and life. The results showed that the neurotoxic symptoms were not significantly correlated with current exposure, and no association was found between estimated cumulative exposure and either psychometric performances or neurotoxic symptoms. Although specific NOAELs and/or LOAELs were not determined in this study, the results indicate low current exposures to toluene were associated with decrements of memory test performance.

Zupanic et al. (2002) investigated the possible effects of long-term occupational exposure to toluene below 100 ppm on psychological performance and subjective symptoms. Male workers (N=278, mean duration 14.9 years) from 14 rotogravure printing plants in Germany were examined. A “high dose” group of 154 workers (printing area) had a mean lifetime weighted average exposure (LWAE) of 45.1 ppm (170 mg/m<sup>3</sup>) and a mean current exposure of 24.7 ppm (93.1 mg/m<sup>3</sup>), and a “low dose” group of 124 workers (end-processing area) had a mean LWAE of 9.3 ppm (35 mg/m<sup>3</sup>) and a mean current exposure of 3.3 ppm (12 mg/m<sup>3</sup>). Examinations were performed on psychomotor performance (steadiness, line tracing, aiming, tapping, and peg board) and subjective symptoms. Analysis of variance (ANOVA) found no significant differences between the two groups. There was no significant correlation between long-term toluene exposures and adverse psychological performance or subjective symptoms of exposure at levels ranging between 1 - 88 ppm.

Seeber et al. (2004) reported a study using the same data set from Zupanic et al. (2002) above, with a subsample of 192 workers that went through all 4 repeated examinations for the cognitive function effects of occupational exposure to toluene lower than 50 ppm (188 mg/m<sup>3</sup>). Current exposure levels were grouped as “high” (printing area, average concentration 26 ppm (98 mg/m<sup>3</sup>)) or “low” (end-processing, average concentration 3 ppm (11 mg/m<sup>3</sup>)). Past exposure levels (LWAEs) were grouped as “high” (45 ppm (170 mg/m<sup>3</sup>)) or “low” (9 ppm (34 mg/m<sup>3</sup>)). Exposure durations were grouped as “long-exposure” (average exposure 21 years) and “short-exposure” (average exposure 6 years). Psychological tests included tests of attention, memory, and psychomotor functions. The results showed neither past exposure nor current exposure resulted in significant impacts on the psychological test performance. The author’s conclusion was that long-term toluene exposure below 50 ppm (188 mg/m<sup>3</sup>) did not show psychological effects on cognitive functions of the above printing workers.

Seeber et al. (2005) further analyzed the above dataset of 192 workers for 4 examinations, with more details on sensory functions. The “high” group had 106 workers, current toluene exposure of 26 ppm (98 mg/m<sup>3</sup>) and LWAE of 45 ppm (170 mg/m<sup>3</sup>), while the “low” group had 86 workers, current exposure of 3 ppm (11 mg/m<sup>3</sup>) and LWAE of 9 ppm (34 mg/m<sup>3</sup>). Measured sensory

functions included vibration thresholds, color discrimination and auditory thresholds. Psychological performance tests included attention, memory and psychomotor functions. An odds ratio statistical analysis revealed no significant relationship between long-term toluene exposure below 50 ppm and impaired psychological functions among the “high” exposure group workers.

Kang et al. (2005) examined 54 workers in Korea (average 35.3 years of age, 11.7 years of education) from three different industries for the effects of chronic exposure to toluene. The workers were divided into three exposure groups, according to the periodic work environment measurement of toluene concentrations: high exposure group, 20 workers, exposed for  $5.4 \pm 5.3$  years to 70-80 ppm (263-301 mg/m<sup>3</sup>) of toluene; moderate exposure group, 13 workers, exposed for  $8.3 \pm 9.5$  years to 20-30 ppm (75-113 mg/m<sup>3</sup>) of toluene; low exposure group, 21 workers, exposed for  $10.9 \pm 6.4$  years to less than 1 ppm (3.8 mg/m<sup>3</sup>) of toluene. The results of a battery of neurobehavioral tests on these workers showed significant impairments on the finger tapping (ANOVA,  $p = 0.002$ ) and selective attention tests for the high exposure group of workers compared with the low exposure group ( $p = 0.000$ ), while the test results for the moderate exposure group of workers did not differ significantly from the low exposure group ( $p > 0.05$ ).

Nordling Nilson et al. (2010) conducted a longitudinal follow-up study to examine effects of long-term toluene exposure on cognitive functions later in life. Study participants, who were initially examined and studied in 1983/1984, were re-examined 20 years later and included 12 male rotogravure printers previously exposed to toluene and 19 male referents. The follow-up study population was much smaller than the initial study (originally comprised of 30 male printers and a reference group of 50 sugar refinery workers and 22 railway carriage repair shop workers), after applying exclusion criteria of present/past solvent exposure, diagnosis of disease affecting central nervous system, and non-age-matched referents among available (living) subjects. The mean ages of the printers and referents were  $70 \pm 6.8$  years (range, 59 to 80) and  $67 \pm 7.3$  years (range, 57 to 84), respectively. Most of the subjects were retired, and the mean number of years of education for printers and referents was 9 and 11, respectively. There was no significant difference in cognitive performance between printers and referents at the time of the initial study in 1983/1984.

Cumulative exposure assessments for solvents and potential neurotoxicants were conducted for each subject based on data collected during the initial and follow-up studies. Among printers, toluene was the primary chemical of exposure, which occurred before 1985 for an average of 35 years. Estimated retrospective toluene exposure levels were 400 ppm (1500 mg/m<sup>3</sup>) during the 1950s–1960s, after which exposures gradually declined due to preventive measures. At the two printing plants where the printers were employed the mean toluene levels were 43 and 157 mg/m<sup>3</sup> by the mid-1980s when their occupation as printers ended. No exposure to toluene or other organic solvents occurred during the follow-up period; however, 4 printers were exposed after 1992. The referent group had no significant exposure to toluene or other solvent at any time, nor did the printers have any significant exposure to solvents during their leisure time relative to the general population. Low-level lead exposure was reported for one printer who sealed packages for two years in the late 1930s, and for 3 referents while plumbing up to 1968 and using paint in the mid-1940s.

Each subject underwent extensive clinical interviews and medical examinations that focused on signs of neurological or psychiatric impairment and alcohol and drug use. Physical examination and standard screening blood tests were included. No disease or medical discrepancy relevant to cognitive function was found. No alcohol overuse was reported, and there were 4 smokers and 6 ex-smokers among the printers and 2 smokers and 10 ex-smokers among the referents. Study participants were administered questionnaires regarding social interactions and subjective cognitive impairments, as well as the same neuropsychological tests used in the initial study and additional tests sensitive to the influence of aging.

There was a decline over time observed on test performance from the initial study to follow-up in all except one test (reasoning) for both printers and referents. However, the decline in reasoning was evident only in the printers while performance of the referents remained stable. A dose-effect relationship was evident in lower performance with increasing cumulative toluene exposure for reasoning [ $F(1, 8) = 6.99, p = 0.03$ ]. Additionally, the decline in associative learning over time was more notable in printers than in referents, and a significant interaction effect of time and exposure group was found ( $p < 0.05$ , analysis of variance (ANOVA)). Performance of printers was significantly worse than referents in sustained attention ( $p < 0.05$ ) and verbal memory related to categories involved in cued recollection of organizable words ( $p = 0.05$ ). Also noteworthy were an observed decline in attention and memory in 75% of printers vs. 25% of referents, and a somewhat higher rating for depression among printers relative to referents ( $p = 0.05$ ). Printers did not indicate more subjective cognitive impairments than referents, nor were there significant differences between the two groups regarding ratings for social interactions.

### Color vision impairment

The physiology of color perception and color vision abnormalities were reviewed by Iregen et al. (2002). Cones, one of the two main types of visual receptor cells in the human eye, are responsible for the perception of color. Among the cone cells, short wavelengths (i.e., the color blue) are perceived by S cones, which represent less than 10% of the total cone population. S cones are believed to be more sensitive to diseases of the eyes and exposure to various drugs and chemicals. Thus, acquired color vision defects regarding blue-yellow dimensions are often reported. Color discrimination abilities seem to be especially sensitive to impairment following exposure to industrial chemicals including toluene (Geller and Hudnell, 1997). Occupation-related color vision impairment usually results in blue-yellow color discrimination loss (Type III dyschromatopsia) or, less frequently, a combination of blue-yellow and red-green loss (Type II dyschromatopsia), while congenital dyschromatopsias more frequently result in red-green deficits (Type I dyschromatopsia) (Gobba and Cavalleri, 2003).

However, the pathogenesis of occupational color vision loss remains unclear. It is probably a result of damage to the optic nerve rather than damage to ocular structures (Zavalic et al. 1998c). It has also been proposed that the color loss is due to a direct action of neurotoxins on receptors, possibly on the cone's membrane metabolism, and/or to an interference with neurotransmitters within the retina (Gobba and Cavalleri, 2003). As a generalized mechanism of action, the liposoluble nature of toluene might play an important role in affecting the visual system, such as visual pathway degeneration, direct impact on photoreceptor function, and cortical or retinal changes in the neurotransmitter system (Costa et al. 2012). Additionally, color vision impairment may involve toluene interference with dopaminergic mechanisms of retinal

cells or demyelination of optic nerve fibers, which have a high lipid content (Zavalic et al., 1998b; Mutti and Franchini, 1987). Finally, lipophilic solvents such as toluene can alter membrane permeability, which involves intercalation of toluene into the lipid bilayer of nerve membranes producing an anesthetic action (Bowen et al., 2006; Evans and Balster, 1991). However, membrane alteration appears to occur at concentrations higher than those that result in neuronal excitability and neurotoxicity.

Studies have found that color vision impairment progressed with increasing cumulative exposure to toluene and other neurotoxic chemicals. However, whether color vision impairment due to toluene exposure is reversible or long-lasting beyond a long weekend of non-exposure is not clear. Studies have been conducted with other solvents that have examined the reversibility of this effect. Styrene is one of the most investigated compounds with regard to color vision loss. In their review, Gobba and Cavalleri (Gobba and Cavalleri, 2003) noted that the cumulative data on the reversibility of color vision loss following chronic styrene exposure is conflicting. For example, a one-month interruption in exposure to styrene did not improve color vision in one study, while another study observed complete recovery. In studies that followed a reduction in styrene exposure of 1 year or more, no improvement was found in one study while another did see an improvement. Gobba and Cavalleri (2003) suggested longer exposure duration and higher exposure concentration may be a factor leading to long-lasting color vision loss following cessation of exposure.

Nakatsuka et al. (1992) examined color vision loss in two groups of workers: one group of 261 workers with previous occupational exposure to solvents and a second group of 120 (48 men and 72 women) non-exposed control workers. Among the solvent workers, 63 men and 111 women were exposed to predominantly toluene (46 ppm (170 mg/m<sup>3</sup>) as the geometric mean concentration); the rest were exposed to either tetrachloroethylene alone (13 ppm, 49 mg/m<sup>3</sup>), or a mixture of tetrachloroethylene (12 ppm, 45 mg/m<sup>3</sup>) and trichloroethylene (7 ppm, 26 mg/m<sup>3</sup>). The exposure duration history of the workers was not provided. Color vision loss was first screened using Lanthony's new color test, and then confirmed by Ishihara's color vision test. The only instances of color vision loss that were detected in either the exposed workers or the controls were six cases of red-green loss (all in men), which was probably congenital in nature rather than acquired through workplace exposure. Further examination for distribution of red-green loss cases among men showed no correlation to toluene exposure. Additionally, no gender-related differences in color vision loss were observed.

Zavalic et al. (1996) examined color vision in 41 female shoe workers exposed to toluene and 29 non-exposed female workers as controls. For the exposed group, average toluene air concentration throughout the working week was 35 ppm (132 mg/m<sup>3</sup>) and average toluene concentration in blood on Wednesday before work was 0.36 ppm (1.33 mg/m<sup>3</sup>), average exposure duration (years of work) was 19.90 ± 7.10 years. Color vision was tested through the Lanthony-D-15 desaturated panel in standard conditions. Since alcohol can be a major confounding factor in acquired color vision impairment, the color confusion index (CCI) was corrected for alcohol intake with a formula residual CCI (RCCI) = CCI – (1.129 + 0.000595 × alcohol intake). The results showed that both CCI and RCCI values were significantly higher in the exposed group than in the non-exposed ( $p < 0.05$ ).

Valic et al. (1997) studied acquired color vision impairment (dyschromatopsia) in 138 persons who were exposed to solvents (toluene, xylene, trichloroethylene and tetrachloroethylene)



combined with alcohol consumption, against 100 nonexposed controls. The gender of the subjects was not provided. Of those subjects, 105 had toluene or xylene exposure. Color vision loss was tested with Lanthony's D-15 method and assessed based on Bowman's color confusion index (CCI). The results showed that more than 250 g per week alcohol consumption combined with solvent exposure significantly elevated CCI ( $p = 0.0044$ ).

Zavalic et al. (1998a, 1998c) investigated color vision impairment in three groups of workers, two groups occupationally exposed to toluene and a control group. The first exposed group (E1) of 41 workers (initially 45 women and 3 men, with 7 subsequently excluded due to medical issues or insufficient exposure duration) was exposed to a geometric mean toluene air concentration of 35 ppm (132 mg/m<sup>3</sup>) (range 11.3–49.3 ppm (42.6–186 mg/m<sup>3</sup>)) for an average of  $16.21 \pm 6.10$  yr and the second exposed group (E2) of 32 subjects (initially 36 men and 3 women, with 7 subsequently excluded) was exposed to a geometric mean toluene air concentration of 156 ppm (590 mg/m<sup>3</sup>) (range 66.0–250.0 ppm (250–940 mg/m<sup>3</sup>)) for an average of  $18.34 \pm 6.03$  yr. Color vision impairment is dependent on age and alcohol consumption, so these factors were controlled for in the color vision impairment study. The nonexposed group (NE) comprised 83 subjects (initially 61 men and 31 women, with 9 subsequently excluded). Color vision was evaluated by the Lanthony D-15 desaturated test according to Verriest's classification: type I, loss in the red-green range; type II, loss in the blue-yellow and red-green ranges, and type III, loss in the blue-yellow range. Subjects were classified as dyschromates if specific acquired loss was determined in at least one eye. In both exposed groups, exposure was evaluated by measurement of the concentration of toluene in the ambient air and in the blood. In group E2, levels of hippuric acid and orthocresol in urine after the work shift were also determined.

The prevalences of the total dyschromatopsia (type III + type II) in the three groups of subjects were analyzed and there was a statistically significant difference between group E2 and group E1 ( $p < 0.05$ ), and between group E2 and group NE ( $p < 0.005$ ), whereas no significant difference was found between groups E1 and NE. In group E2, total dyschromatopsia correlated significantly with toluene in ambient air and in blood (both  $p < 0.05$ ) as well as with hippuric acid in urine after the work shift ( $p < 0.001$ ). This study indicates that toluene can impair color vision in exposed workers, and provides a NOAEL of 35 ppm (132 mg/m<sup>3</sup>) and a LOAEL of 156 ppm (590 mg/m<sup>3</sup>). The authors did not comment on any potential gender-related differences in acquired color vision impairment, even though Group E1 consisted of mostly female workers and Group E2 consisted of mostly male workers.

Zavalic et al. (1998b) examined the effects of chronic occupational toluene exposure on color vision using a group of 45 exposed male workers (mean toluene exposure concentration = 119.6 ppm (450 mg/m<sup>3</sup>), duration =  $16.8 \pm 5.94$  yr) and 53 controls. Although not specified in the study, the workers appear to be a sub-group of the same workers investigated in the other Zavalic et al. (1998a, c) studies. Color vision was evaluated using the Lanthony desaturated panel D-15 test on Wednesday morning before work and repeated on Monday morning, at least 64 hours after exposure; test scores were age and alcohol consumption-adjusted. Color vision was significantly impaired in toluene-exposed workers ( $p < 0.0001$ ) compared to controls. There was no significant difference between test scores of the exposed workers on Monday morning (prework) and Wednesday morning. The authors stated that the effect of toluene on color vision can be chronic with a recovery period of possibly longer than 64 hours.

Cavalleri et al. (2000) evaluated color vision impairment in 33 toluene-exposed workers. Toluene exposure was estimated to be 42 ppm by measuring urinary excretion of the unmetabolized toluene (i.e. ToU). Color vision was tested with the Lanthony D-15 desaturated panel, and the outcomes were expressed quantitatively with two indices of color perception, the color confusion index (CCI) and the total confusion index (TOTCI). Toluene-exposed workers had a subclinical reduction in color vision, compared with 16 referents ( $p < 0.01$  and  $p < 0.001$  for CCI and TOTCI, respectively). This effect was found to be related to cumulative solvent exposure - estimated as the product of urinary excretion of unmodified toluene and previous toluene exposure duration (Cavalleri et al., 2000). This analysis supported the hypothesis that color vision impairment progresses as exposure continues. In the examined group of workers, toluene exposure was within the occupational limit (50 ppm, 190 mg/m<sup>3</sup>) proposed by the American Conference of Governmental Industrial Hygienists (ACGIH, 1997). The observed loss in color vision raised doubts on the protection afforded by this limit with respect to the color vision impairment health endpoint.

Campagna et al. (2001) examined 72 printers exposed to an average of 36 ppm (136 mg/m<sup>3</sup>) toluene, 34 workers exposed to an average ambient concentration of 9 ppm (32 mg/m<sup>3</sup>) toluene, and 19 non-exposed workers (controls) for acquired color vision loss analysis. The three groups' average employment durations were 18, 19 and 8 years, respectively. Age and alcohol consumption, both color vision-dependent factors, were controlled for in the study. Color vision was assessed with the Lanthony D-15 desaturated panel method. Workers from the two exposed groups had significantly higher CCI values than from the control group ( $p < 0.01$ , Wilcoxon rank test). The authors suggested that the Lanthony D-15 desaturated panel detects early neurotoxic effects among workers exposed to toluene.

Schaper et al. (2004) conducted a four-year study of three repeated examinations on correlation between human occupational exposures to toluene and color vision impairment. A total of 189 workers were grouped as "high-level" (printing, mean current exposures of  $26 \pm 21$  ppm ( $98 \pm 79$  mg/m<sup>3</sup>)) or "low-level" (end-processing, mean current exposures of  $3 \pm 4$  ppm ( $11 \pm 15$  mg/m<sup>3</sup>)), with mean exposure durations of  $23 \pm 6$  years (long-term) or  $7 \pm 2$  years (short-term). Color vision was tested with Lanthony desaturated color vision test D-15d and a CCI was calculated. Repeated analyses of covariance (complete repeated dataset of 162 subjects) and multiple regressions (highest available subjects of 267) did not demonstrate a significant effect of toluene on color vision function.

Paramei et al. (2004) performed a meta-analysis of chronic toluene exposure on human color vision impairment using effect sizes approach, which requires means and standard deviations from the individual studies. Among the 11 studies from existing peer-reviewed human studies, 4 studies were included in the meta-analysis as fulfilling all three criteria: (1) use of the common color discrimination test Lanthony Panel D-15d; (2) arithmetic means and standard deviations of CCI available for both an exposed and unexposed groups; (3) documentation of exposure level for the exposed group. The meta-analysis results showed generally higher CCI values for the exposed groups and positive effect sizes for 3 of the 4 studies for toluene, indicating that color discrimination was inferior for exposed groups in the majority of the studies. By applying a random effects model, an average effect size of 0.15 was obtained ( $p = 0.44$ ) with a weighted mean exposure of 30 ppm of toluene, indicating an inferior performance of the exposed subjects. None of the values reached significance at the 5%-level.

### Auditory effects

Abbate et al. (1993) evaluated alterations induced in the auditory nervous system by exposure to toluene in a group of rotogravure workers. A sample of 40 workers of normal hearing ability was selected from a group of 300 workers who were apparently in good health but were professionally exposed to toluene (12 – 14 years exposure, 97 ppm (370 mg/m<sup>3</sup>) average exposure). They were subjected to an adaptation test utilizing a BAEP (Brainstem Auditory Evoked Potential) technique with 11 and 90 stimulus repetitions a second. The results were compared with an age and sex-matched control group not professionally exposed to solvents. A statistically significant alteration in the brainstem auditory evoked response (BAER) results was noted in the toluene-exposed workers with both 11 and 90 stimuli repetitions. Since alterations of the BAEPs have been demonstrated to be positively correlated to otoneurotoxicity in animals, the authors suggested that these results can be explained as a toluene-induced effect on the auditory system, even in the absence of any clinical sign of neuropathy. Furthermore, this effect was observed in the responses of the entire auditory system, from peripheral receptors to brainstem nuclei.

Morata et al. (1997) studied the effects of organic solvents (mainly toluene) and noise on the hearing of rotogravure printing workers. Pure-tone audiometry and immittance audiometry testing were conducted with 124 workers who were occupationally exposed to various levels of noise and an organic solvent mixture of toluene, ethyl acetate, and ethanol. The subjects were fairly young employees, with the following average characteristics: age of 33.8 ± 8.5 years, working tenure of 7.7 ± 6.1 years, noise exposure of 7.7 ± 6.0 years, and solvent exposure of 6.5 ± 6.0 years. Using biological monitoring of hippuric acid and creatinine in the urine, 109 solvent-exposed workers had their total toluene exposure assessed for statistical analysis. The levels of toluene in the air ranged from 0.14 to 919 mg/m<sup>3</sup> (0.037 to 244 ppm). The measured toluene air concentrations and levels of toluene urinary metabolites were found to be correlated. The results of this study showed that forty-nine percent of the workers had hearing loss. Among the numerous variables that were analyzed, age, and toluene urinary metabolites were shown to be statistically significant on the development of hearing loss. The findings suggested that exposure to toluene has a toxic effect on the auditory system. However, the authors did not identify the specific concentration of urinary hippuric acid corresponding to a NOAEL or LOAEL for the end point of hearing loss, and no control group was used for comparison.

Schaper et al. (2003, 2008) studied the ototoxicity of occupational exposure to toluene below 50 ppm (188 mg/m<sup>3</sup>) with 333 male workers from rotogravure printing plants. The mean past lifetime weighted average exposures (LWAE) measures to toluene and noise were 45 ± 17 ppm (170 ± 64 mg/m<sup>3</sup>) and 82 ± 7 dB(A) for high-toluene-exposed printers, and 10 ± 7 ppm (38 ± 26 mg/m<sup>3</sup>) and 82 ± 4 dB(A) for low-toluene-exposed end-processors. The mean current exposures to toluene and noise were 26 ± 20 ppm (98 ± 75 mg/m<sup>3</sup>) and 81 ± 4 dB(A) for printers, and 3 ± 3 ppm (11 ± 11 mg/m<sup>3</sup>) and 82 ± 4 dB(A) for end-processors. The auditory thresholds were measured with pure tone audiometry. Statistical analyses did not reveal significant effects of toluene concentration, exposure duration and interactions between toluene intensity and noise intensity. In this study, 45 ppm (169 mg/m<sup>3</sup>) was a NOAEL.

### Neuroendocrine effects

Svensson et al. (1992a) studied the neuroendocrine effect of toluene on the plasma concentration of LH and testosterone in 47 male workers from two rotogravure printing companies, with 46 metal workers as a control group. The average toluene exposure at the two printing companies was 11 ppm (41 mg/m<sup>3</sup>) (1–108 ppm (4–410 mg/m<sup>3</sup>)) and 47 ppm (180 mg/m<sup>3</sup>) (6–142 ppm (23–550 mg/m<sup>3</sup>)) at the time of study sampling, respectively. The time weighted average air toluene concentration was below 80 ppm (300 mg/m<sup>3</sup>) (Swedish threshold limit value) for all 47 subjects, while the median historical cumulative exposure (ppm x years) was 2,896 ppm-yr. Increasing exposures were significantly associated with decreasing plasma concentration of LH ( $p = 0.003$ ) and testosterone ( $p = 0.02$ ). Cumulative exposure had no correlation with plasma hormone concentration. The authors concluded that low toluene exposure had an effect on the hypothalamus-pituitary axis.

Svensson et al. (1992b) also studied the neuroendocrine effects of occupational toluene exposure in a different group of rotogravure male printing workers ( $n = 20$ , exposure group), compared with 44 male industrial workers without toluene exposure (control group). The median individual time-weighted toluene concentration in air was 36 ppm (136 mg/m<sup>3</sup>) (range 8–111 ppm (30–420 mg/m<sup>3</sup>)), while the median historical cumulative exposure was 5,630 ppm-year for the printing workers. The hormone assays showed lower median plasma levels of FSH ( $p = 0.02$ ) and LH ( $p = 0.05$ ), and also lower serum level of free testosterone ( $p = 0.05$ ) for the exposure group, but the decreases of hormone levels were not significantly correlated to airborne toluene concentration. In 8 out of the 20 exposed workers, the levels of FSH and LH increased during a 4 week vacation, which the authors concluded was a slight and reversible effect of toluene on pituitary function in addition to a general depression of brain functions. However, no solvent-induced cases of toxic encephalopathy in the exposed group were verified.

### Other chronic effects

Wang et al. (1996) showed decreased liver and kidney function in workers due to exposure to low concentrations of toluene. The liver function test results for 153 workers (108 males and 45 females) exposed to 1.0–60.4 ppm (4.0–228 mg/m<sup>3</sup>) toluene for at least 2–5 years (male and female test groups) were compared with those for 420 workers (238 males and 182 females) who had never been occupationally exposed to solvents (male and female control groups). The results showed significantly lowered serum glutamic-pyruvic transaminase (GPT) and gamma-glutamyl transpeptidase ( $\gamma$ -GTP) activities in the male test group, but significantly higher serum GPT and  $\gamma$ -GTP activities in the female test group, compared to those in their respective control groups. These effects suggested that toluene exposure below 100 ppm (380 mg/m<sup>3</sup>) was possibly causing the changes in liver function. Body mass index and alcohol consumption were ruled out as possible confounders for the observed difference. However, the authors suggested that other life-style factors and nutritional status may be factors for liver enzyme differences between groups.

The studies investigating long-term exposure of toluene in humans are summarized below in Table 3.

**Table 3. Summary of toluene occupational exposure chronic studies in adult workers with identified NOAEL and/or LOAEL.**

Studies	Number of Subjects	Exposure Duration (average years $\pm$ SD)	Concentration (TWA in ppm)	Critical Effects (endpoints)	NOAEL ppm (mg/m <sup>3</sup> )	LOAEL ppm (mg/m <sup>3</sup> )
Yin (1987)	101 exposed, 127 controls	6.8	42.8	Sore throat, dizziness, headache	*	42.8 (161)
Orbaek & Nise (1989)	30 pooled workers from two shops, A (n = 19) and B (n = 11). 50 controls	29 (median)	11.4 (A) 41.8 (B)	Fatigue, memory problems, concentration difficulties.  Marginal effects on performance in psychometric tests	*	<41.8 (158)
Foo (1990)	30 exposed, 30 controls	5.7 $\pm$ 3.2, 2.5 $\pm$ 2.7	88 (exposed), 13 (controls)	Significant decrease in neurobehavioral performance (6 out of 8 tests)	*	88 (330)
Nakatsuka (1992)	174 exposed, 120 controls	nd	46 (geometric mean), 0 ppm	No measured effect on color vision	46 (170)	*
Abbate (1993)	40 exposed, 40 controls	12-14	97, 0 ppm	Auditory nervous system (BAER) 28% increase of latency shift	*	97 (370)
Vrca et al. (1995)	59 unexposed workers (54 males, 5 females), age 43 $\pm$ 7.2 years or 49 toluene-exposed workers (46 males, 3 females), age 42.3 $\pm$ 6.8 years	0 ppm for 20.6 $\pm$ 7.7 years, or 60-80 ppm for 21.4 $\pm$ 7.4 years	0 ppm, or 80 ppm	In exposed workers, higher VEP wave amplitudes and longer wave latency indicative of more excitable wave generators in the visual pathway and reduced wave conductivity in certain segments of the visual pathway, respectively.	ND	60-80 (230-300)
Vrca et al. (1996)	Same as above	Same as above	Same as above	Longer BAEP wave latencies and smaller wave amplitudes in exposed versus unexposed workers suggesting changes to the auditory pathway.	Same as above	Same as above
Vrca et al. (1997b)	Same as above	Same as above	Same as above	Increased VEP wave latency during mental engagement suggesting adverse changes to intracranial structures like the brainstem and hypothalamus.	Same as above	Same as above

Abbreviations: BAEP – brainstem auditory evoked potential; BAER – brainstem auditory evoked response; ND – not determined; VEP – visual evoked potential.

\* not observed

nd No data

**Table 3. Summary of toluene occupational exposure chronic studies in adult workers with identified NOAEL and/or LOAEL (continued).**

Studies	Number of Subjects	Exposure Duration (average years $\pm$ SD)	Concentration (TWA in ppm)	Critical Effects (endpoints)	NOAEL ppm (mg/m <sup>3</sup> )	LOAEL ppm (mg/m <sup>3</sup> )
Vrca (1997a)	49 exposed, 59 controls	21.6	40-60 (est.), ** 0 ppm	BAEP evoked auditory potential increase in latencies, dose-response	*	40-60 (150-230)
Boey (1997)	29 exposed, 29 controls	4.9 $\pm$ 3.5	90.9, 0 ppm	Significant decrease in neurobehavioral performance (9 of 14 tests)	*	90.9 (340)
Zavalic (1998a,c)	83 controls, 1 low exp, 32 high exp	20.91 (work), 15.60 $\pm$ 4.61, 19.86 $\pm$ 5.61	0 ppm, 35 (11.3-49.3), 156 (66-250)	Color vision impairment in high exposure group	35 (130)	156 (590)
Zavalic (1998b)	53 controls, 45 male workers exposed	22.4 (work), 16.8 $\pm$ 5.94	0 ppm, 120	Color vision impairment in exposed group	*	120 (450)
Eller (1999)	19 controls, 30 with low exposure, 49 with high exposure	7.7 $\pm$ 3.5 (range:1-12), 25.5 $\pm$ 8.9 (range:13-40)	0 ppm, <20, >100	No difference between low exposure group and control group; impaired neuropsychological function in high exposure group	<20 (75)	>100 (380)
Cavalleri (2000)	33 exposed, 16 controls	9.75	42 (est.) **	Increase in color vision impairment test index	*	42 (160)
Chouaniere et al. (2002)	128 workers (114 male, 14 female)	14 $\pm$ 10	0-27	Subjective symptoms and psychometric testing	27 (102)	*
Zupanic et al. (2002)	154 high-dose 124 low-dose	15.3 $\pm$ 9.7 14.5 $\pm$ 8.6	High 45.1 $\pm$ 16.4, Low 9.3 $\pm$ 7.6	psychomotor performance and subjective symptoms	45 (169)	*
Schaper et al. (2003, 2008)	47 short-low, 39 long-low, 59 short-high, 47 long-high	6.3 $\pm$ 2.3, 21.4 $\pm$ 7.4, 5.6 $\pm$ 3.8, 22.0 $\pm$ 7.5	Hi 44.7 $\pm$ 17.1, Low 9.5 $\pm$ 7.3	ototoxicity	45 (169)	*
Schaper et al. (2004)	41 short-low, 28 long-low, 54 short-high, 39 long-high	8.11 $\pm$ 2.23, 21.82 $\pm$ 5.31, 6.82 $\pm$ 2.42, 22.36 $\pm$ 7.44	Hi 42.66 $\pm$ 15.71, Low 9.16 $\pm$ 7.90	Color vision impairment	43 (162)	*
Seeber et al. (2004, 2005)	47 short-low, 39 long-low, 59 short-high, 47 long-high	6.6 $\pm$ 2.1, 21.3 $\pm$ 6.0, 5.5 $\pm$ 3.6, 21.2 $\pm$ 7.0	Hi 45 $\pm$ 17, Low 9 $\pm$ 7	Psychological tests and psychomotor functions	45 (169)	*
Kang et al. (2005)	20 high, 13 medium, 21 low	5.4 $\pm$ 5.3, 8.3 $\pm$ 9.5, 10.9 $\pm$ 6.4	High 70-80, Medium 20-30, Low < 1	Neurobehavioral tests	~20 (75)	~75 (282)

Abbreviations: BAEP - brainstem auditory evoked potential; ND – not determined; VEP – visual evoked potential.

\* not observed; \*\* indirect exposure estimate: based on urinary levels of metabolites and toluene blood levels for the Vrca 1997a study, and based on urinary excretion of toluene plus data from previous air toluene measurements for the Cavalleri study.

## Chronic Toxicity to Infants and Children

Several studies examined the health impact of ambient exposures of children to mixtures of air toxics including toluene (below the U.S. EPA RfC of 5.0 mg/m<sup>3</sup> (1.3 ppm)). Individual study summaries can be found under “Community Epidemiological Studies” in section 6.1 above. Since these studies are examinations on ambient mixtures of air toxics, they are less likely to provide data for developing RELs.

Toluene abuse by pregnant women has resulted in infants born with phenotypic features similar to fetal alcohol syndrome, such as postnatal growth deficiency, craniofacial malformations and hydronephrosis (Hoyme et al., 1993) and is discussed in Section 7.1 below.

## Chronic Toxicity to Experimental Animals

### Neurobehavioral effects

Toluene affects dopamine levels in the brain, the key neurotransmitter of working memory in the prefrontal cortex. Toluene has been implicated for many years in the changes of dopamine levels in the brain, as measured in exposed neonatal and adult animals (von Euler et al., 1989; Riegel et al., 2004) or by toluene’s effects on dopamine-dominated functions, such as hyperlocomotion (von Euler et al., 1993; Riegel and French, 1999; Riegel et al., 2003) and activation of mesolimbic reward pathways (Riegel and French, 2002).

Effects on the CNS have been observed in studies of animals chronically exposed to toluene by inhalation. Inflammation and degeneration of the nasal and airway epithelium and pulmonary lesions have also been observed in rats and mice chronically exposed to high levels of toluene by inhalation. Adverse effects on the liver, kidneys, lungs and auditory system (i.e., high-frequency hearing loss) have been reported in some chronic inhalation studies of rodents.

In a comprehensive chronic exposure study, male and female Fischer-344 rats (120/group/sex) were exposed to 30, 100, or 300 ppm (110, 380, or 1100 mg/m<sup>3</sup>) toluene for 6 hrs/day, 5 days/week for two years (Gibson and Hardisty, 1983). Pathologic, hematologic, clinical blood chemistry, urinalysis, and ophthalmologic examination did not reveal any injury considered to be evidence of chemical toxicity. Body weights were elevated in exposed male and female rats, but no clear-cut dose response relationship was apparent.

A persistent increase in the affinity of dopamine D<sub>2</sub> agonist binding in the rat caudate-putamen was observed with exposure to 80 ppm (300 mg/m<sup>3</sup>) 6 hr/day, 5 days a week for 4 weeks (TWA 14 ppm/week), followed by a post-exposure period of 29–40 days. However, similar exposure of adult rats to 40 ppm (150 mg/m<sup>3</sup>) toluene for 4 weeks did not result in these specific effects (Hillefors-Berglund et al., 1995). In contrast, longer duration (16-week, 104 hr/week) inhalation exposure of adult rats to 40 ppm (150 mg/m<sup>3</sup>) toluene produced behavioral neurotoxicity and alterations in neurotransmitters (Berenguer et al., 2003). Neurobehavioral alterations were determined by assaying locomotor activity and rearing activity. Both exposed males and females had significant differences in rearing activity compared to control animals.

Dopaminergic and serotonergic neurotransmission activity was significantly altered in various brain regions of rats exposed to 40 ppm (150 mg/m<sup>3</sup>) toluene for 16 weeks.

Macaque monkeys were exposed by inhalation for 50 minutes to one of six concentrations of toluene (0, 100, 200, 500, 1000, 2000 or 4500 ppm; 6 animals/treatment group) twice/week for 6 weeks (Taylor and Evans, 1985). Attention deficits and impairment of cognitive and motor abilities were observed beginning at 2,000 ppm (7,500 mg/m<sup>3</sup>) using a repeated measures analysis of variance test with a statistical significance of  $p < 0.05$ . Expired carbon dioxide showed an inverted-U shaped response initially increasing above control levels, and then decreasing below control levels (primarily at 4,500 ppm (17,000 mg/m<sup>3</sup>)) with increasing toluene concentration. The authors noted this type of curve suggests behavioral stimulation at lower concentrations and behavioral sedation at higher concentrations.

Toluene exposure produced neurological impairments in the ability to create new strategies after investigators made changes in the presence or placement of the hidden platform in the Morris water maze (Hass et al., 1999; Hougaard et al., 1999; von Euler et al., 2000). These studies were performed on young rats with prenatal, postnatal, and adolescent exposure, and at widely varying doses. The lowest dose tested in the Morris water maze assay was 80 ppm (300 mg/m<sup>3</sup>) for 4 weeks in adolescent rats in the study by von Euler et al. (2000), which also caused significantly reduced performance in beam-walk test, used to detect neurological deficits in sensory, balance, or motor performance (detailed below).

Hass et al. (1999) exposed female rats to 0 or 1200 ppm (4500 mg/m<sup>3</sup>) toluene for 6 hours per day from day 7 of pregnancy until day 18 postnatal. Developmental and neurobehavioral effects in the offspring were investigated using a test battery including assessment of functions similar to those in the proposed Organization for Economic Cooperation and Development (OECD) Testing Guidelines for Developmental Neurotoxicity Study (OECD, 2006) (physical development, reflex development, motor function, motor activity, sensory function, and learning and memory). The exposure did not cause maternal toxicity or decreased offspring viability. However, lower birth weight, delayed development of reflexes, and increased motor activity in the open field were noted in the exposed offspring. The exposed female offspring had poorer scores on a Morris water maze test (they took longer to locate a hidden platform after platform relocation) at the age of 3.5 months indicating impaired cognitive function. The difference was not related to impaired swimming capabilities since swim speeds were similar to control values. The authors stated that exposure to 1200 ppm (4500 mg/m<sup>3</sup>) toluene during brain development caused long-lasting developmental neurotoxicity in rats.

Hougaard et al. (1999) studied the development and neurobehavioral effects of prenatal exposure to toluene by exposing 16 pregnant rats (Mol:WIST) to 1800 ppm (6800 mg/m<sup>3</sup>) of toluene in whole body inhalation chambers for 6 h per day, 2 weeks on days 7–20 of gestation. Body weights of exposed pups were lower until day 10 after parturition. Neurobehavioral tests included neuromotor abilities (rotarod), activity level (open field), reactivity, habituation and prepulse inhibition (acoustic startle), sensory function (auditory brain stem response), and learning and memory ability (Morris water maze). Evaluation of the pups revealed no effects on motor function, activity level, acoustic startle, and prepulse inhibition. Auditory brain stem response measurements of hearing function revealed small effects in male exposed offspring. Morris water maze performance during initial learning indicated some impaired cognitive



functions and was confirmed during further testing, especially in reversal and new learning. Effects on cognitive functions seemed most marked in female offspring.

Von Euler et al. (2000) investigated the effect of inhalation exposure to 0 ppm and 80 ppm (0 and 300 mg/m<sup>3</sup>) toluene for 4 weeks (6 hr/day, 5 days/week) on the behavior and brain features in 60 male Sprague-Dawley rats (about 50 days of age). At least 4 weeks after the final exposure, the rats' spatial memory was altered by toluene, in that they spent a longer time in the initial quadrant of a Morris swim maze. Toluene-exposed rats also showed trends for increases in both locomotion and rearing behaviors and a significantly reduced beam-walk performance. Magnetic resonance imaging of living rats and autoradiograms of frozen brain sections showed a decreased area of the cerebral cortex, especially the parietal cortex, by 6–10%. The biochemical receptor binding assays indicated a persistent effect of toluene selectively binding to dopamine D<sub>2</sub> receptors. They concluded that low concentrations of toluene exposure led to persistent effects on cognitive, neurological, and brain-structural properties in the rat.

Bowen and McDonald (2009) studied the behavioral effects of repeated toluene binge exposure (high dose of toluene as to mimic toluene abuse) on cognitive function (behavioral impulse control) of Swiss Webster mice using a “wait-for-reward” operant task. After being trained on fixed-ratio schedule wait task, groups of 40 mice were exposed to 1000, 3600 or 6000 ppm (3800, 13,600, or 22,600 mg/m<sup>3</sup>) toluene for 30 min per day for 40 days. Repeated toluene exposure decreased response rates and resulted in a higher response-to-reinforcer ratio than the control group exposed to air for the same duration. Mice receiving the highest exposure level (6000 ppm (22,600 mg/m<sup>3</sup>)) showed a dramatic decrease in the number of rewards received, while those exposed to 3600 ppm (13,600 mg/m<sup>3</sup>) of toluene had significantly more responses. Mice exposed to the lowest level (1000 ppm (3800 mg/m<sup>3</sup>)) showed little change in the number of rewards. The authors concluded that repeated binge exposures to high concentrations of toluene can significantly interfere with behavioral performance, suggesting a significant impact on cognitive and/or psychomotor function.

To determine the neurobehavioral effects of subchronic exposure to toluene, groups of 160 adult male Long-Evans rats inhaled toluene vapor (0, 10, 100, or 1000 ppm (0, 38, 380, or 3800 mg/m<sup>3</sup>)) for 6 hr/day, 5 days/week for 13 weeks and were evaluated on a series of behavioral tests beginning 3 days after the end of exposure (Beasley et al. 2010). Toluene delayed appetitively-motivated acquisition of a lever-press response in all treatment groups ( $p < 0.05$ ), but did not affect the responses on motor activity, anxiety-related behavior in the elevated plus maze, trace fear conditioning, acquisition of an appetitively-motivated visual discrimination, or performance of a visual signal detection task. They concluded that these results were consistent with a pattern of subtle and inconsistent long-term effects of daily exposure to toluene vapor, in contrast to robust and reliable effects of acute inhalation of toluene.

### Other CNS effects

Airborne pollutants and toxics such as toluene interact with chemoreceptors in the nasal cavity, especially trigeminal and olfactory receptors. To elucidate the influence of toluene inhalation on the mitral and granular neurons in olfactory bulbs and the pyramidal cells in hippocampus of rats, Gelazonia et al. (2006a, 2006b) exposed two age groups of rats (one and two months old), 5 per group, to air saturated with toluene vapor (concentration unknown) in closed glass chambers for 40 days (6 days/week, 3-4 minutes per day until the rats attained a sidewise

laying position). Another two groups of rats, 5 each, of either one or two months of age, were exposed to air only, as control groups. The results showed that, compared with the respective control groups, the exposed rats had a significantly decreased number of mitral neuron cells (43% reduction for one-month rats and 28% reduction for two-month ones), while the granular cells remained unaltered in both age groups of exposed rats. The number of pyramidal neurons in the hippocampus decreased by 26% in one-month exposed rats only, which induced deterioration of the hippocampal neural circuits and destruction of memory and learning processes.

To evaluate the potential modifications of subchronic exposure to inhaled toluene on behavior and olfactory functioning, Jacquot et al. (2006) exposed mice to 1000 ppm (3800 mg/m<sup>3</sup>) of toluene for 5 hr/day, 5 days/week for 4 weeks, and assessed their behavioral (sensitive and perceptive) and histological (cellular level) changes. Tests were administered during the 4-week exposure (W1-W4) and up to 4 weeks following exposure (W5-W8). Behavioral evaluation (T-maze test) of mice sensitivity toward toluene (as a repulsive odor) showed a constant decrease (less sensitive) during the 4 weeks of exposure, which continued for 2 weeks after the exposure (W5, W6). During the last two weeks of the study (W7, W8), the sensitivity of mice to toluene returned to normal. On the cellular level, the density of olfactory epithelium cells decreased markedly during W3 and W4 and increased significantly in the first week of the recovery period (W5). The thickness of olfactory neuroepithelium decreased at W1, followed by an increase at W2 and W3 (suggesting an inflammatory process), but decreased abruptly at W4, followed by a gradual return to normal at W5 through W8.

### Respiratory effects

The National Toxicology Program exposed F344/N rats and B6C3F<sub>1</sub> mice (60 males and 60 females of each species) to toluene 6.5 hrs/day, 5 days/week for up to two years (NTP, 1990). Toluene levels were 600 and 1200 ppm (2300 and 4500 mg/m<sup>3</sup>) for rats, and 120, 600 and 1200 ppm (450, 2300 and 4500 mg/m<sup>3</sup>) for mice. At the 15-month interim sacrifice, incidences and severity of nasal cavity lesions, including degeneration of olfactory and respiratory epithelium and goblet cell hyperplasia, were increased in exposed rats at both dose levels. Minimal hyperplasia of the bronchial epithelium was seen in female mice at 1200 ppm (4500 mg/m<sup>3</sup>). Severity of nephropathy, but not incidence, was slightly increased in exposed female rats at both dose levels. Following two years of exposure, erosion of olfactory epithelium and degeneration of respiratory epithelium were increased in exposed rats. Inflammation of nasal mucosa and metaplasia of olfactory epithelium were increased in exposed female rats. These lesions were not seen in mice. No biologically important non-neoplastic lesions were observed in mice. Nephropathy was seen in almost all rats, and severity was somewhat increased in exposed rats.

To investigate the effect of long-term, low level toluene exposure on airway inflammatory responses in mouse lung, Fujimaki et al. (2007) exposed female C3H mice to 0 or 50 ppm (0 or 190 mg/m<sup>3</sup>) of toluene or air for 6 hr/day on 5 days/week for 6 or 12 weeks in whole-body exposure chambers. One day after the last toluene exposure, they collected bronchoalveolar lavage (BAL) fluid from each mouse and examined cellular infiltration and production of cytokines, chemokines, neurotrophins and substance P with the ELISA method. They found that the number of total cells and macrophages increased significantly ( $p < 0.05$ ) in mice of both 6- and 12-week-exposure. The production of interferon-gamma and substance P were decreased

significantly. In addition, neurotrophin-3 production in BAL fluid was significantly increased only in 12-week-exposed mice. This study suggested long-term, low-level toluene exposure modulates airway inflammatory response in mice through neurological signaling.

Shwe et al. (2007) exposed male C3H mice to 0, 9 and 90 ppm (0, 34 and 340 mg/m<sup>3</sup>) toluene for 30 min by nose-only inhalation on days 0, 1, 2, 7, 14, 21, and 28 to study the effects of volatile organic compounds in the indoor air on the induction or augmentation of airway inflammatory responses (general neuroimmune interaction). One day after the 28-day exposure period, bronchoalveolar lavage (BAL) fluid was collected for analysis of inflammatory cell influx, while lung tissue and blood samples were collected to determine cytokine, neurotrophin mRNA, protein expressions, and plasma antibody titers. Exposure of mice to 9 ppm (34 mg/m<sup>3</sup>) or 90 ppm (340 mg/m<sup>3</sup>) toluene both resulted in increased inflammatory cell infiltration in BAL fluid, increased IL-5 mRNA, decreased nerve growth factor receptor tropomyosin-related kinase A and brain-derived neurotrophic factor mRNAs in lung, and increased IgE and IgG1 antibodies and nerve growth factor content in the plasma. Even though there was no pathological endpoint in this study, these findings suggested that low-level toluene exposure aggravates the airway inflammatory responses.

### Auditory Effects

Hearing loss was observed in groups of rats after exposure to various toluene exposure scenarios: 1000 ppm (3800 mg/m<sup>3</sup>), 14 hours per day for 2 weeks; 1500 ppm (5700 mg/m<sup>3</sup>) for 14 hours per day for three days; 2000 ppm (7500 mg/m<sup>3</sup>) for 8 hours per day for three days; and intermittent exposure to 3000 ppm (11,300 mg/m<sup>3</sup>) for 30 minutes every hour, 8 hours per day for 2 weeks (Pryor et al., 1984). However, groups of rats exposed to lower concentrations (400 and 700 ppm (1500 and 2600 mg/m<sup>3</sup>)) for 14 hours per day did not have hearing loss even after 16 weeks of exposure, and single exposures to 4000 ppm (15,100 mg/m<sup>3</sup>) for 4 hours or 2000 ppm (7500 mg/m<sup>3</sup>) for 8 hours were also not ototoxic.

Toluene has been shown to disrupt the auditory system in rats but not in guinea pigs, whose high amount of hepatic cytochrome P-450s and high concentration of glutathione in the cochlea likely play a key role in its auditory resistance to long-term, high-level toluene exposure. Waniusiow et al. (2009) tested toluene-induced hearing loss in glutathione-depleted guinea pigs whose P-450 activity was partly inhibited. The animals were exposed to 1750 ppm (6600 mg/m<sup>3</sup>) toluene 6 hr/day, 5 days/week for 4 weeks. Auditory function was tested by electrocochleography and supported by subsequent histological examination. The authors noted that a significant toluene-induced hearing loss was provoked in these exposed guinea pigs, but the associated auditory system histopathology has not been demonstrated in studies using other toluene-exposed species, including rats. Histological examination showed that only the stria vascularis and the spiral fibers were disrupted in the apical coil of the cochlea of the guinea pigs. The authors concluded that guinea pigs can metabolize toluene more efficiently than rats, probably because of a higher level of hepatic P-450.

**Table 4. Summary of chronic animal studies with identified NOAEL and/or LOAEL.**

Study	Exposure duration and Concentration (ppm)	Subjects / Effects (endpoints)	NOAEL ppm (mg/m <sup>3</sup> )	LOAEL ppm (mg/m <sup>3</sup> )
Gibson and Hardisty (1983)	30, 100, or 300 ppm 6 hrs/day, 5 days/week for two years	male and female Fischer-344 rats (120/group/sex) / no effect on pathology, blood chemistry, urinalysis, and ophthalmologic examination	300 (1130)	NA
Taylor and Evans (1985)	0, 100, 200, 500, 1000, 2000 or 4500 ppm for 50 min/day, 2x/week for 6 weeks	Macaque monkeys / Attention deficits and impairment of cognitive and motor abilities	1000 (3800)	2000 (7600)
Von Euler et al. (2000)	0 ppm and 80 ppm for 4 weeks (6 hr/day, 5 days/week)	60 male Sprague-Dawley rats / Altered spatial memory and changes in brain structural features	NA	80 (300)
Beasley et al. (2010)	0, 10, 100, or 1000 ppm for 6 hr/day, 5 days/week for 13 weeks	160 adult male Long-Evans rats / battery of neurobehavioral tests – only finding was delayed appetitively-motivated acquisition of a lever-press response	NA	10 (38)
Jacquot et al. (2006)	1000 ppm for 5 hr/day, 5 days/week for 4 weeks	Mice / behavioral (sensitive and perceptive) and histological (cellular level) changes	NA	1000 (3800)
NTP (1990)	600 and 1200 ppm for rats, and 120, 600 and 1200 ppm for mice, 6.5 hrs/day, 5 days/week for up to two years	F344/N rats and B6C3F <sub>1</sub> mice (60 males and 60 females of each species) / nasal cavity lesions, erosion of olfactory epithelium and degeneration of respiratory epithelium	NA for rats, 600 for mice (2300)	600 for rats (2300), 1200 for female mice (4500)
Fujimaki et al. (2007)	0 or 50 ppm for 6 hr/day on 5 days/week for 6 or 12 weeks	C3H mice / cellular infiltration in bronchoalveolar lavage (BAL) fluid	NA	50 (190)
Shwe et al. (2007)	0, 9 and 90 ppm for 30 min by nose-only inhalation on days 0, 1, 2, 7, 14, 21, and 28	C3H mice / induction or augmentation of airway inflammatory responses in BAL	NA	9 (34)
Waniusiow et al. (2009)	1750 ppm for 6 hr/day, 5 days/week for 4 weeks	glutathione-depleted guinea pigs / Auditory function loss	NA	1750 (6580)

### Other chronic effects

To investigate the adverse effects of toluene inhalation on bone morbidity and bone mineralization, Atay et al. (2005) exposed 10 4-wk-old male Swiss albino BALB/c mice to 300 ppm (1100 mg/m<sup>3</sup>) (static) toluene 6 hr per day for 8 weeks and measured the bone mineral density and bone mineral content in the femoral neck by dual X-ray absorptiometry bone densitometer. They found that the bone mineral density was significantly reduced compared to a control group of another 10 mice of the same type. They concluded that chronic exposure to toluene affected bone metabolism and could contribute to bone resorption and inhibition of bone formation.

Toluene has also been shown to impair visual functions in animal studies. Boyes *et al.* (2016) examined visual function of male Long-Evans rats by recording visual evoked potentials (VEP) and / or electroretinograms (ERG) in four sets of experiments. First set exposed 40 rats per group to 0, 10, 100, 1000 ppm (0, 38, 380, 3,800 mg/m<sup>3</sup>) toluene in controlled inhalation chambers, 6h/d 5d/wk for 13 weeks, and one week after the exposure completion their VEPs were recorded, which were not significantly changed by toluene exposure. Four to five weeks after exposure ended, their ERGs were recorded and showed that only the visual function of rats exposed to 1000 ppm (3800 mg/m<sup>3</sup>) toluene were reduced.

A second set of approximately 40 rats per group were exposed concurrently with the first set for 13 weeks. One year after the exposure ended, their ERGs were recorded and again only rats exposed to 1000 ppm (3800 mg/m<sup>3</sup>) toluene were shown to have visual function negatively affected. A third set of approximately 40 rats per group were exposed to the same concentrations of toluene for 4 weeks.

A fourth set of approximately 20 rats per group exposed to 0 or 1000 ppm (0 or 3800 mg/m<sup>3</sup>) toluene for 4 weeks were tested 1 year after the exposure termination. ERGs of rats exposed for 4 weeks were not significantly reduced. The reductions of ERGs after 13 weeks of exposure and persisting for 1 year suggest alterations in rat retina. The authors concluded that repeated toluene exposure may lead to subtle persistent changes in rat visual function, and particularly the rat retina may be more sensitive to toluene exposure than visual cortex.

### Young / Adult Animal Comparisons

Samuel-Herter et al. (2014) studied the possibility of age-dependent neurobehavioral sensitivity to toluene among inhalant abusers using a rodent model to assess the effects of acute binge-like toluene inhalation exposures. Male Long-Evans rats (18/exposure group) were exposed to approximately 5000 ppm (18,800 mg/m<sup>3</sup>) for 15 or 30 min in whole-body exposure chambers. The following age-groups of rats were used in the study: adolescent (1 month), young adult (2-3 months), adult (5-6 months), and older adult (10-12 months). Motor functions evaluated included ambulatory activity, vertical exploration, grooming, balance, gait and neurological functions. An adolescent group of rats were not exposed to 30 min of toluene due to a pilot study result showing that rats in this age group required much longer time to recover any degree of motor function (reference not given). The general results showed that acute toluene exposure impaired both motor and neurological functions in all age groups of rats, with adolescent and young adult rats needing significantly longer recovery times than older rats ( $p < 0.05$ ). The authors claimed that these results suggested an age-dependent vulnerability to the intoxicating

effects of toluene. However, the possible cause of the adolescent and young adult age-groups of rats receiving higher doses (per unit body weight) was not discussed.

## 7. Developmental and Reproductive Toxicity

### Human Studies

Toluene has been listed under Proposition 65 as known to the State of California to cause developmental toxicity (OEHHA, 2015). Most of the information concerning the adverse developmental effects of toluene in humans comes from case reports of children born to organic solvent “sniffers”, in which toluene was often the primary solvent inhaled. Children whose mothers had inhaled large quantities of toluene during pregnancy were found to have microencephaly, facial and limb abnormalities, attention deficits, hyperactivity, developmental delay with greater language impairment, and growth retardation similar to effects of alcohol abuse (Hersh et al., 1985; Hersh, 1989).

In other studies, hyperchloremic acidosis along with growth retardation and craniofacial abnormalities were observed in the children of women with severe renal tubular acidosis induced by chronic paint sniffing (Goodwin, 1988). Preterm delivery, perinatal death, and growth retardation were significantly increased in a study of 21 newborns exposed to toluene as a result of maternal inhalation abuse (Wilkins-Haug and Gabow, 1991).

In a case referent study of women occupationally exposed to organic solvents including toluene, increased incidences of urogenital, gastrointestinal, and cardiac anomalies were reported in their children (McDonald et al., 1987). Paternal occupational toluene exposure (in which the mothers had no exposure) increased the odds ratio for spontaneous abortions; however, these observations cannot be clearly ascribed to toluene because of the small number of cases evaluated and the large number of confounding variables (Lindbohm et al., 1992).

An increased incidence of spontaneous abortions was also reported among occupationally exposed women. Ng et al. (1992) conducted a study on rates of menstrual disorders among female workers exposed exclusively to toluene at a factory where audio coils and speakers were assembled. The menstrual function questionnaire results of 231 female production workers exposed to high toluene exposure (50-150 ppm (190-570 mg/m<sup>3</sup>), mean 88 ppm (330 mg/m<sup>3</sup>), average employment duration 6.0 years) were compared with those for 58 female workers in the same factory with low toluene exposure (0-25 ppm (0-94 mg/m<sup>3</sup>), average employment duration 6.7 years) (factory controls) and 187 working class women receiving routine care at maternal and child health centers (external community controls). Dysmenorrhea occurred more frequently in women exposed to high concentration of toluene compared with external community controls ( $p < 0.001$ ), but not compared to factory controls (not significant at  $p < 0.05$ ). The rates for dysfunctional uterine bleeding were similar in all groups, and there was no evidence that dysfunctional uterine bleeding resulted from exposure to toluene.

An epidemiology study by Plenge-Bonig and Karmaus (1999) involved 150 male and 90 female printers in Germany exposed to toluene, which were compared with non-exposed workers in other industries using an infertility and subfecundity questionnaire. Historical exposure levels

varied from 10 to 200 ppm. The fecundability ratio (FR) was estimated on the basis of time to pregnancy (TTP) or periods of unprotected intercourse not leading to pregnancy (PUNP). After adjustment for confounders such as age, ethnicity, smoking, parity and pelvic inflammatory diseases, 169 periods of TTP or PUNP in men and 100 periods in women were analyzed. The results showed no increased risk to males at any exposure level (FR 1.05, 95% confidence interval 0.93 to 1.19,  $p = 0.43$ ), but fecundity was reduced in females by about half (FR 0.47, 95% confidence interval 0.29 to 0.77). The authors concluded that even though there were possible biases (e.g., participant selection bias, recall bias), low daily exposure to toluene in women seems to be associated with reduced fecundity, likely due to hormonal regulation changes or early fetal loss.

The epidemiology study by Ghosh et al. (2012) examined the associations of low birth weight (LBW) with toxic air pollutants in traffic exhaust including toluene. The data for 8181 children with term LBW ( $\geq 37$  weeks' completed gestation and birth weight  $< 2500$  g) and 370,922 term normal-weight children in Los Angeles (LA) County were compared against land-use-based regression (LUR)-modeled estimates and air toxics exposure, covering 1995 through 2006. Measurements of air toxics including toluene were available for every 12 days from four California Air Resources Board (CARB) air toxics monitoring stations in LA County and their averages were calculated. The geocoded residential addresses of the mothers from the birth certificates who resided  $\leq 5$  miles (8 km) from a CARB air toxics station were overlaid with the LUR model to assign estimated exposures. The results showed BTEX exposures in the third trimester and the last month of pregnancy were particularly associated with odds of term LBW, while no association for first and second trimester and entire pregnancy exposure averages. This study provided evidence for traffic exhaust including toluene's contribution to term LBW. Mothers who deliver at term have greater odds of delivering a low-weight baby when exposed to higher levels of traffic exhaust pollutants including toluene in the third trimester.

## Animal Studies

There are a number of older animal inhalation studies of varying quality investigating the reproductive and developmental toxicity of toluene. The following animal studies support the association between toluene exposure and effects on somatic development of the fetus. However, the value of these studies is limited by issues such as unknown or unconventional exposure durations, inadequate descriptions of maternal toxicity, use of individual offspring instead of litters for statistical analyses, as well as questions about the presence of contaminants in the toluene used (Donald et al., 1991).

Shigeta et al. (1982) reported that in the offspring of mice exposed by inhalation to 100 ppm (380 mg/m<sup>3</sup>) and 1000 ppm (3800 mg/m<sup>3</sup>) toluene for 6 hours per day on days 1–17 of gestation, the number of fetal resorptions increased. However, the increases showed neither a dose-response nor were they statistically significant (no  $p$ -value given). Exposure at 1000 ppm (3800 mg/m<sup>3</sup>) resulted in a statistically significant increase in the incidence of extra ribs. A statistically insignificant increased incidence of extra ribs ( $p < 0.1$ ) was observed in newborn rats exposed by inhalation to 1000 mg/m<sup>3</sup> (265 ppm) toluene for 24 hours per day on days 7–14 of gestation (Tatrai et al., 1980).

Fused sternbrae and extra ribs were observed in rats exposed to 400 ppm (1500 mg/m<sup>3</sup>) toluene for 24 hours per day on days 9–14 of gestation (Hudak and Ungvary, 1978). Skeletal

retardation was observed in rats exposed to 266 ppm (1000 mg/m<sup>3</sup>) toluene for 8 hours per day on days 1–21 of gestation and to 400 ppm (1500 mg/m<sup>3</sup>) 24 hours per day on days 1–8. This same group exposed mice to 400 ppm (1500 mg/m<sup>3</sup>) or to 133 ppm (500 mg/m<sup>3</sup>) toluene for 24 hours per day on days 6–13 of gestation. All dams died at 400 ppm (1500 mg/m<sup>3</sup>) and a statistically significant decrease in fetal weight was observed at 266 ppm (1000 mg/m<sup>3</sup>).

In another set of experiments, continuous exposure of pregnant rats to higher concentrations of 1000 and 1500 ppm (3800 and 5700 mg/m<sup>3</sup>) toluene on days 9 to 14 of gestation resulted in the death of two dams out of 19 during the exposure to 1500 ppm (5700 mg/m<sup>3</sup>) (Hudak and Ungvary, 1978). Fetuses from the 1500 ppm (5700 mg/m<sup>3</sup>) group showed increased incidence of sternebral alterations, extra ribs and missing tails. The same exposure on days 1 through 8 of gestation resulted in 5 deaths out of 14 dams. Fetuses exposed to this treatment showed increased incidence of hydrocephaly and growth retardation compared to controls. A third treatment that exposed pregnant rats to 1000 ppm (3800 mg/m<sup>3</sup>) on days 1 through 21 of gestation resulted in no maternal death, decreased maternal weight gain or fetal loss, but resulted in an increase in the incidence of skeletal variations in the fetuses.

In Klimisch et al. (1992), skeletal retardations were observed in the offspring of 15 pregnant rabbits per group exposed by inhalation to concentrations of toluene ranging from 30 to 300 ppm (110 to 1100 mg/m<sup>3</sup>), 6 hours per day on days 6–18 of gestation, however the frequency of skeletal retardations was not significant compared with corresponding controls. These results were not dose-dependent and no effects were seen in the two additional groups of 20 rabbits, each group exposed to 100 or 500 ppm (380 and 1900 mg/m<sup>3</sup>) toluene.

A statistically significant increase in the number of animals showing a 13/13 rib profile (which is considered within the range of normal development) was observed in offspring of female mice exposed to 400 ppm (1500 mg/m<sup>3</sup>) toluene, 7 hours per day on days 7–16 of gestation (Courtney et al., 1986).

Gleich and Hofman (1983) observed an increased number of resorptions in female mice exposed to 400 ppm (1500 mg/m<sup>3</sup>) toluene on days 6–15 of gestation; the daily exposure duration was not specified.

The best available study relating toluene exposure and retardation of somatic development is one in which adult rats of 2 generations were exposed for 6 hours per day to 0, 100, 500 or 2000 ppm (0, 380, 1900, or 7500 mg/m<sup>3</sup>) toluene during an 80-day pre-mating period and a 15 day mating period (API, 1985). Adult females of both generations were also exposed on days 1–20 of gestation and on days 5–21 of lactation. The mean body weights of fetuses of both generations of dams exposed to 2000 ppm (7500 mg/m<sup>3</sup>) were significantly decreased compared to controls. No maternal toxicity was reported. Exposure at 2000 ppm (7500 mg/m<sup>3</sup>) to the male parent did not result in any adverse effects.

After weaning, the F1 pups were exposed 80 times (6 hrs per day, 5 days per week) to the appropriate exposure level and then randomly mated to members of the same exposure group. The F1 generation exposed to 2000 ppm (7500 mg/m<sup>3</sup>) toluene showed significantly decreased body weight which persisted throughout lactation. No effects were observed on histopathology. No data were presented for the F2 generation. The NOAEL for fetotoxic effects in this study was 500 ppm (1900 mg/m<sup>3</sup>).



In a more recent teratogenicity study, Ono et al. (1995) exposed pregnant Sprague-Dawley rats to 600 or 2000 ppm (2300 or 7500 mg/m<sup>3</sup>) toluene for 6 h/day from day 7 to day 17 of pregnancy. The control group inhaled clean air. Maternal exposure to 2000 ppm (7500 mg/m<sup>3</sup>) caused significant toxic effects such as body weight suppression in dams and offspring, high fetal mortality, and embryonic growth retardation. However, no external, internal, or skeletal anomalies were observed in the fetuses of either treated group. In addition, there were no differences in the results of pre- and post-weaning behavioral tests of the offspring, including surfacing righting, position adjusting, space exploration and spatial learning. No changes which could be related to toluene were apparent in the 600 ppm group. Thus, 600 ppm (2300 mg/m<sup>3</sup>) is a NOAEL in this study.

Da Silva et al. (1990) exposed pregnant rats and hamsters to 0 or 800 mg/m<sup>3</sup> (210 ppm) toluene for 6 hours/day on gestation days 14–20 (rats), or days 6–11 (hamsters). Fetuses of exposed rats demonstrated a significant exposure-related decrease in birth weight compared with controls. In addition to low birth weight, the number of live pups was significantly lower in the 800 mg/m<sup>3</sup> (210 ppm) group. No deficits in any parameter were noted in the hamsters. In this study, offspring of rats and hamsters exposed to toluene performed worse than controls on two neurobehavioral tests – spontaneous alternation test for rats and rotating rod test for hamsters; however, the differences are not statistically significant (i.e.,  $p > 0.05$ ).

Hass et al. (1999) exposed female rats to 0 or 1200 ppm (0 or 4500 mg/m<sup>3</sup>) toluene for 6 hours per day from day 7 of pregnancy until day 18 postnatal. Developmental and neurobehavioral effects in the offspring were investigated using a test battery including assessment of functions similar to those in the proposed Organization for Economic Cooperation and Development (OECD) Testing Guidelines for Developmental Neurotoxicity Study (OECD, 2006) (physical development, reflex development, motor function, motor activity, sensory function, and learning and memory). The exposure did not cause maternal toxicity or decreased offspring viability. However, lower birth weight, delayed development of reflexes, and increased motor activity in the open field were noted in the exposed offspring. The exposed female offspring had poorer scores on a Morris water maze test (they took longer to locate a hidden platform after platform relocation) at the age of 3.5 months indicating impaired cognitive function. The difference was not related to impaired swimming capabilities since swim speeds were similar to control values. The authors stated that exposure to 1200 ppm (4500 mg/m<sup>3</sup>) toluene during brain development caused long-lasting developmental neurotoxicity in rats.

Toluene-based solvents are among the most frequently misused psychoactive substances during pregnancy, and in both animal models and clinical case reports of toluene exposure, the primary physiological outcome measure of prenatal inhalant exposure is low birth weight (BW). To clarify the effect of low BW with prenatal and postnatal toluene exposure, the meta-analysis by Callan *et al.* (2016) investigated toluene exposure-induced BW differences in non-primate mammals by applying a systematic review and meta-analytic techniques to the existing peer-reviewed animal studies modeling prenatal and postnatal exposure to the inhaled solvent toluene. Among the 288 studies from literature screen, 24 studies were included in the meta-analysis with a total of 46 control-to-toluene comparisons differing only in the inhaled concentration of toluene. The software program DSTAT 1.11 was used for analyzing the data and conducting the meta-analysis. DSTAT quantification of the data showed a total of 26 different concentrations of toluene were administered through the inhalation route, and were

categorized into the following groups: 0–500 ppm (0–1884 mg/m<sup>3</sup>), 501–2000 ppm (1888–7537 mg/m<sup>3</sup>), 2001–5000 ppm (7541–18,842 mg/m<sup>3</sup>), 5001–7500 ppm (18,846–28,263 mg/m<sup>3</sup>), and 7500 ppm (28,263 mg/m<sup>3</sup>) and above. The analysis results indicated that the overall weighted effect size (a measure of deviance from the null hypothesis)  $d = -0.39$ , which means that prenatal toluene exposure resulted in decreased BW. The 95% confidence interval (- 0.42 to - 0.35) does not include 0, indicating that the effect was significant. External inhaled concentration, route of administration, day of weighing, and toluene exposure magnitude were identified as modifiers of this correlation.

Callan et al. (2017) studied the effects of prenatal exposure to relatively high concentrations of toluene (~ abuse levels) on spatial memory performance in adolescent rats using a spatial learning and memory task, the Morris Water Maze (MWM). Pregnant Sprague-Dawley rats (N = 56) were exposed to 0, 8000, or 12,000 ppm (0, 30,000 or 45,000 mg/m<sup>3</sup>) of toluene for 15 min twice daily from gestation day 8 (GD8) through GD20. Male and female offspring (N= 104) were observed in the MWM for 5 days beginning on postnatal day (PN) 28 and again on PN44. While prenatal toluene exposed animals did not differ in initial acquisition in the MWM, rats prenatally exposed to 12,000 ppm toluene displayed performance deficits (significantly greater distances from the platform) during a probe trial and in reversal learning on PN44 ( $p < 0.05$ ). The authors concluded that prenatal exposure to repeated inhaled high concentrations of toluene can impair spatial memory function that persists into adolescence.

## 8. Derivation of Reference Exposure Levels

### 8.1 Toluene Acute Reference Exposure Level

<i>Study</i>	Andersen <i>et al.</i> , 1983
<i>Study population</i>	16 male humans, mean age = 24 years
<i>Exposure method</i>	Inhalation chamber, 0, 10, 40 and 100 ppm (0, 38, 150, 380 mg/m <sup>3</sup> respectively)
<i>Duration</i>	6 hours
<i>Critical effects</i>	Impaired reaction time and symptoms of headache, dizziness, feeling of intoxication, sensory irritation (eye and nose irritation)
<i>LOAEL</i>	380 mg/m <sup>3</sup> (100 ppm)
<i>NOAEL</i>	150 mg/m <sup>3</sup> (40 ppm)
<i>Time-adjusted exposure</i>	150 mg/m <sup>3</sup> (40 ppm) (no time adjustment for sensory irritation)
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	3
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Reference Exposure Level</i>	5000 µg/m <sup>3</sup> (5 mg/m <sup>3</sup> ; 1300 ppb; 1.3 ppm)

Reference Exposure Levels are based on the most sensitive, relevant health effect reported in the medical and toxicological literature. Acute Reference Exposure Levels are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (OEHHA, 2008).

The controlled human exposure study by Andersen *et al.* (1983) is the key study used for acute REL derivation. Andersen *et al.* observed nasal mucus flow, lung function, psychometric performance, and subjective responses in 16 male humans (mean age = 24 years, age range 21 – 32 years) exposed to toluene concentrations of 10, 40 and 100 ppm (38, 150 and 380 mg/m<sup>3</sup>) for 6 hours. Exposures to 10 and 40 ppm (38 and 150 mg/m<sup>3</sup>) toluene were without subjective irritation effects of strong odor sensation. Statistically significant ( $p < 0.05$ ) subjective symptomatology included eye and/or nose irritation, headache, and feeling of intoxication among subjects of 100 ppm (380 mg/m<sup>3</sup>) toluene exposure. In the psychometric performance tests, there was a borderline significant correlation ( $0.05 < p < 0.10$ ) for the results on three of the eight

tests for the subjects exposed to 100 ppm (380 mg/m<sup>3</sup>) toluene. In this study, 40 ppm (150 mg/m<sup>3</sup>) was recognized as a NOAEL and 100 ppm (380 mg/m<sup>3</sup>) as a LOAEL to derive an acute REL for toluene.

In the key study by Andersen et al. (1983), the CNS effects (impaired reaction time and symptoms of headache, dizziness, feeling of intoxication) and sensory irritation to eyes and nose were the critical effects for the acute toluene inhalation REL derivation, not odor detection. Odor detection is mediated through the olfactory nerve, having an accommodation process which is the gradual decrease in odor perception with continued exposure (Cometto-Muniz and Abraham, 2016), whereas irritation to eyes and nose is mediated primarily through the trigeminal nerve (Nielsen and Wolkoff, 2017).

Due to the concentration-dependent nature of chemically-related sensory irritation, no time-adjusted exposure was applied for extrapolation to a 1-hour exposure. Supporting evidence for no time-adjusted exposure was observed in the animal study by Oshiro et al. (2011). In this study, some behavioral effects related to neurotoxicity following acute exposure were better predicted by the brain concentration of toluene rather than by cumulative inhaled dose ( $C \times t$ ). In addition, the duration- and concentration-dependent biphasic action of toluene on locomotor activity could confound the derivation in high acute rodent-exposure situations. Our original 1999 acute REL for toluene was based on the same study by Andersen et al. (1983). With a time-adjusted concentration of 98 ppm (370 mg/m<sup>3</sup>), an interspecies uncertainty factor of 1 and an intraspecies uncertainty factor of 10, the resulting acute REL for toluene was 37,000 µg/m<sup>3</sup> (37 mg/m<sup>3</sup>; 9,800 ppb; 9.8 ppm). In contrast, the new acute, 8-hour and chronic RELs for toluene described in this document were derived using the methodology described in the noncancer TSD adopted in December 2008 (OEHHA, 2008). This methodology explicitly considers possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25). As a result, the previous single intraspecies uncertainty factor was divided into separate toxicokinetic and toxicodynamic factors, and the combined value changed from 10 to 30 including a toxicodynamic component of 10 to account for the potentially greater susceptibility of children to neurotoxic effects. A toxicokinetic component of 3 ( $\sqrt{10}$ ) was used in this case to account for the sensory irritation effect which would happen before metabolism. The point-of-departure was changed from a time-adjusted value of 370 mg/m<sup>3</sup> to the NOAEL value of 150 mg/m<sup>3</sup> since the critical effect of sensory irritation is concentration-dependent and not time-dependent. As described above, the exposure was not time-adjusted for extrapolation to a 1-hour exposure.

A supporting human exposure study by Echeverria et al. (1989) with a similar study design provided a LOAEL of 150 ppm (570 mg/m<sup>3</sup>) and a NOAEL of 75 ppm (280 mg/m<sup>3</sup>). Echeverria et al. observed statistically significant decrements in several neurobehavioral tests among a battery of tests conducted at 150 ppm (570 mg/m<sup>3</sup>). The results from one test, pattern recognition latency, were statistically significant at 75 ppm (280 mg/m<sup>3</sup>). The statistically significant finding at 75 ppm (280 mg/m<sup>3</sup>) (ANOVA *p* values = 0.045 and 0.021 for effect and trend, respectively) was the only one among the battery of 27 tests within seven psychometric performance measures. Subjective symptoms of eye irritation and headache increased with dose but statistical analysis was not provided. A dose-dependent increase ( $p < 0.001$ ) in the number of subjects observed sleeping was reported and noted by the authors as the best

evidence for neurological effects from toluene exposure. The evidence by Echeverria et al. suggests that 75 ppm (280 mg/m<sup>3</sup>) exposure for 7 hours is near the threshold for the NOAEL.

In the current Hot Spots noncancer REL methodology, when data for compound-specific models of toxicokinetics are unavailable, an overall intraspecies uncertainty factor ( $UF_H$ ) of 30 rather than 10 (toxicokinetic component,  $UF_{H-k}=10$ ; toxicodynamic component,  $UF_{H-d}=\sqrt{10}$ ) can be used as a default procedure to protect infants' and children's health. An example of this is cases where differences in metabolism and excretion are key to the toxicological activity. In contrast, for direct-acting chemicals whose site of action is the point of first contact, a  $UF_{H-k}$  of  $\sqrt{10}$  may be sufficient. Where significant concern for toxicodynamic differences larger than three-fold is present, a larger UF may be applied, such that the total  $UF_H$  could be larger than 30 (OEHHA, 2008).

Here in this derivation, where the key effect (sensory irritation) is elicited at the point of first contact, a  $UF_{H-k}$  of  $\sqrt{10}$  is used. An intraspecies uncertainty factor with a toxicodynamic component ( $UF_{H-d}$ ) of 10 is applied for the use of human studies with normal adult subjects and to address the human variation in response to substances with nervous system effects, including sensitive subpopulations such as children (OEHHA 2008), resulting in an overall UF of 30.

## 8.2 Toluene 8-hour Reference Exposure Level

<i>Study</i>	Zavalic et al. 1998c
<i>Study population</i>	41 adult workers for NOAEL, 32 adult workers for LOAEL, 83 adult workers for control
<i>Exposure method</i>	Occupational inhalation
<i>Continuity</i>	10 m <sup>3</sup> /day occupational inhalation rate, 8 hours/day, 5 days/week
<i>Duration</i>	15.60 ± 4.61 years (NOAEL); 19.86 ± 5.61 years (LOAEL)
<i>Critical effects</i>	Acquired color vision impairment (
<i>LOAEL</i>	587 mg/m <sup>3</sup> (156 ppm)
<i>NOAEL</i>	132 mg/m <sup>3</sup> (35 ppm)
<i>Benchmark concentration (BMCL<sub>05</sub>)</i>	45.1 mg/m <sup>3</sup> (12 ppm)
<i>Time-adjusted exposure</i>	32.3 mg/m <sup>3</sup> (8.6 ppm) (12 ppm × 8/8hr × 5/7 days/week)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1 (exposure > 12% of a 70-year lifetime )
<i>Interspecies uncertainty factor</i>	1
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	3.9 (Nong et al. 2006)
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	10
<i>Cumulative uncertainty factor</i>	39
<i>Reference Exposure Level</i>	830 µg/m <sup>3</sup> (0.83 mg/m <sup>3</sup> ; 220 ppb; 0.22 ppm)

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (OEHHA, 2008).

In the Air Toxics Hot Spots Program Technical Support Document (TSD) for the Derivation of Noncancer RELs adopted in 2008, the use of an 8-hour REL was introduced in order to refine the risk assessment approach for the large number of industrial facilities that operate and emit chemicals for 8 hours per day, 5 to 7 days per week and to utilize the advanced features in air-dispersion modeling. The air dispersion modeling in the Hot Spots Program has traditionally

modeled such emissions as if they were uniformly emitted over 24 hours a day, continuously. Advances in computer capabilities have made it feasible to model more accurately the ground level concentrations of these emission scenarios by using meteorology obtained during the time when the facilities are actually operating (generally daytime). The majority of the highly populated areas in California have significant diurnal-nocturnal meteorological differences that can affect the magnitude of the modeled risk and location of receptors (OEHHA, 2008).

The 8-hour REL also is protective of offsite workers and children in schools. The chronic noncancer health impacts on offsite workers (individuals working at other worksites in areas impacted by the facility emissions) have been traditionally assessed with the chronic RELs. Because offsite workers generally work 8 hours and not 24, the eight-hour RELs will ensure a more accurate assessment of the health impacts of their exposures. The eight-hour RELs will also be useful for assessing the health impacts of exposure of children in schools, who usually would not spend more than 8 hours a day in schools. Exposure duration for children and offsite workers will vary, but an eight-hour exposure duration assumption would be reasonable, particularly if children and offsite workers are exposed to facility emissions at their school or place of work and not at their residential locations (OEHHA, 2008).

The study by Zavalic et al. (1998c) was selected as the best available study because it employed human subjects, used a sensitive endpoint (acquired color vision impairment (dyschromatopsia)), and two toluene exposure concentrations of 35 and 156 ppm (132 and 587 mg/m<sup>3</sup>). The use of two exposure levels made it possible to perform a benchmark dose analysis. A LOAEL of 156 ppm (587 mg/m<sup>3</sup>) and a NOAEL of 35 ppm (132 mg/m<sup>3</sup>) were estimated for acquired color vision impairment (dyschromatopsia) using a sensitive color vision testing method (i.e., Lanthony D-15 desaturated test).

Acquired color vision impairment (dyschromatopsia), reflects neural alterations in the peripheral system and can be detected before subjects are aware of functional disability (Grant, 1980). As indicated by Braun et al. (1989), acquired color vision impairment effects can be observed earlier than putative neuropsychotoxic effects in workers exposed to organic solvents including toluene. This conclusion is supported by Gobba et al. (2000), who reviewed more than 50 studies published on color perception in workers exposed to neurotoxic chemicals, and concluded that color vision impairment from chemical exposure is an early effect that can generally be detected at low exposure levels if the method adopted for color vision testing is sensitive enough, such as the Lanthony D-15 desaturated test. With the sensitive and early detectable effects of acquired color vision impairment, the dataset from Zavalic et al. (1998c) provided the possibility of a lower and more protective REL value than studies on other neurological effects.

The primary study by Zavalic et al. (1998c) provided the minimal dichotomous data (two exposure concentrations and a control group) necessary to run a benchmark concentration analysis using U.S. EPA BMDS software (USEPA, 2020). The BMC models for dichotomous data gave acceptable line fits to the data with BMD<sub>05</sub> values over a range of 5 to 32 ppm (19 to 121 mg/m<sup>3</sup>) (Table 4). The probit model was chosen to provide the point of departure for the REL derivation because it had the lowest Akaike Information Criterion (AIC) value, and the highest p-value for goodness-of-fit, and generated a BMCL<sub>05</sub> (12 ppm (45 mg/m<sup>3</sup>)) at the lower end of the range (Figure 2).

Use of a  $BMCL_{05}$  in a REL derivation takes into account some of the inter-individual variability within a population, generally resulting in a reduction of the standard intraspecies uncertainty factor. However, a worker population such as that used in the Zavalic study is considered healthier than the human population as a whole (i.e., healthy worker effect). Thus, to be adequately protective of vulnerable subpopulations, an intraspecies toxicodynamic ( $UF_{h-d}$ ) factor of 10 is used to represent differences within the human population. A factor of 1 was used for the subchronic uncertainty factor because all the worker subjects have been exposed for more than 8.4 years (i.e., >12% of estimated 70 year lifetime, OEHHA 2006), which is considered a chronic human exposure. No adjustment for average experimental exposure duration was applied for occupational exposures of 8 hr/day since the REL is for an 8-hour daily exposure. An adult-to-child pharmacokinetic adjustment factor of 3.9 for neonates about 1 month of age was calculated and represented the largest inter-individual variability between adults and children/infants (Nong et al., 2006). This was used in place of the default intraspecies uncertainty factor – toxicokinetic component ( $UF_{H-k}$ ) for which a PBPK model including measured inter-individual variability is applied.

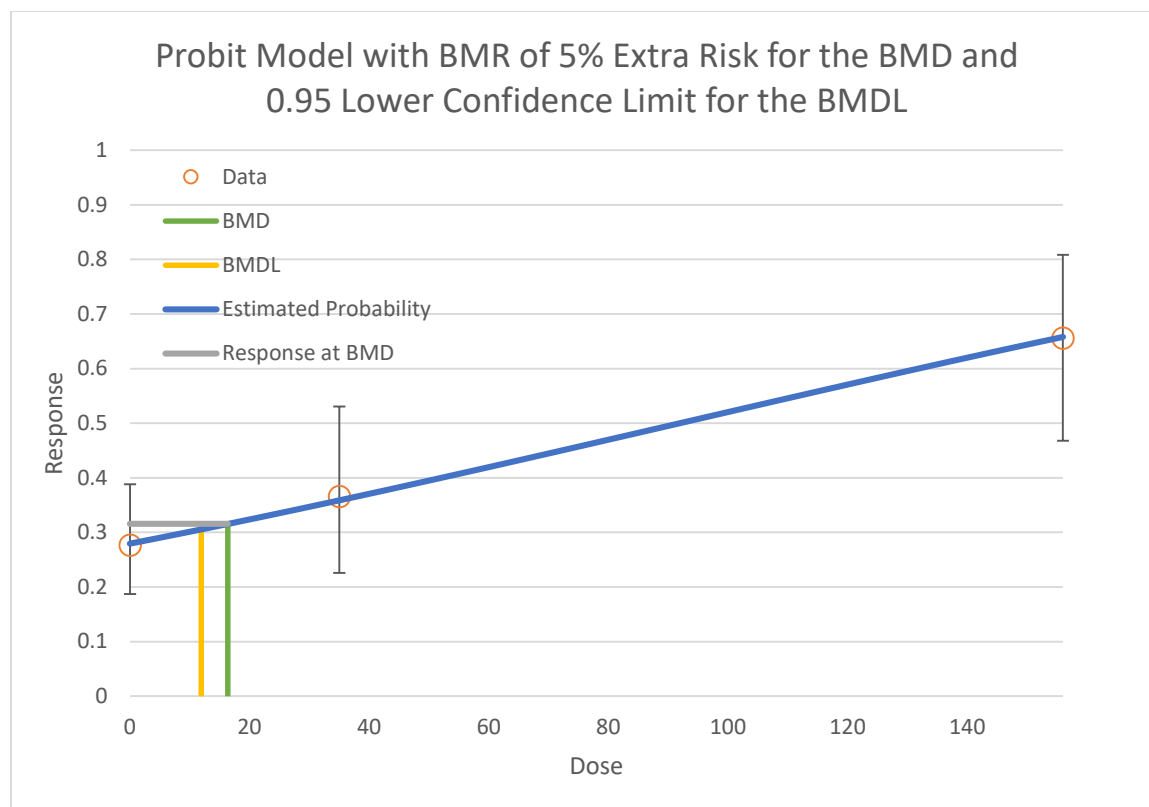
An intraspecies uncertainty factor – toxicodynamic component ( $UF_{H-d}$ ) – of 10 is applied to account for the greater susceptibility of children to neurotoxic effects.

**Table 5. Benchmark dose analysis (USEPA BMDS 3.1.2) of data from Zavalic et al., 1998c.**

Model	$BMC_{05}$ ppm ( $mg/m^3$ )	$BMCL_{05}$ ppm ( $mg/m^3$ )	p-value for fit	AIC*
Probit	16.37 (62)	11.93 (45)	0.9133	197.02
Logistic	16.78 (63)	12.10 (46)	0.8996	197.02
Quantal Linear	11.01 (41)	6.90 (26)	0.8021	197.07
Multistage ( $\beta=2$ )	11.01 (41)	6.90 (26)	0.8021	197.07

\*Akaike Information Criterion





**Figure 2. Probit model fit to Zavalic et al. (1998c) human dyschromatopsia data.**

From the PBPK modeling study of Nong et al. (2006), the inter-individual variability factors for child age groupings indicate that the area under the venous blood concentration vs. time curve (AUC) of toluene varied only by a factor of up to 3.9 (for neonate group) even though liver CYP2E1 content can vary by a factor of 20. Due to the age-related changes in other physiological parameters, the PK variability is less than expected on the basis of age-related change in the levels of hepatic CYP2E1. The magnitude of the inter-individual variability factor, in part, can be explained on the basis of CYP2E1 levels in neonates, children, and adults. The synthesis pathway of the enzyme CYP2E1 is immature at birth followed by rapid onset and eventual maturation by 6 months to 1 year (Vieira et al., 1996; Cresteil, 1998; Nakamura et al., 1998; Tanaka, 1998). Using a more extensive analysis, Johnsrud et al. (2003) observed that maturation of hepatic CYP2E1 content occurred after 3 months, and expression comparable to adult levels after 1 year. A sensitivity analysis by Nong et al. (2006) showed that hepatic metabolism of toluene appears to be limited by enzyme content at birth and its pharmacokinetics evolve gradually to a hepatic blood flow-limited condition with increasing age.

The most recent PBPK models for toluene developed by Mörk et al. (2014) only recognized a slight difference between adults and infants in terms of toluene metabolism, i.e., the adult-to-child pharmacokinetic adjustment factor they developed was close to 1. To be adequately protective for the infants and children, we applied the value of 3.9 derived by Nong et al. (2006) as the intraspecies uncertainty factor – toxicokinetic component ( $UF_{H-k}$ ). Although a toxicokinetic component of 3.9 represents only the first month after birth, we concluded that the most

sensitive members of the population should still be protected from potential adverse effects in the development of 8-hour and chronic RELs.

Among the available human studies on long-term neurological effects of toluene, Zavalic et al. (1998c) is the only study that provided clear data supporting both a NOAEL and LOAEL. Another study that provided both a NOAEL and a LOAEL is Eller et al. (1999), where a control group, a low-exposure group and a high-exposure group of workers were examined for the chronic CNS effects of toluene. However, the time-weighted average level of toluene for the low-exposure group could only be described as below 20 ppm (75 mg/m<sup>3</sup>), while that for the high-exposure group could only be described as exceeding 100 ppm (380 mg/m<sup>3</sup>). Neither exposure level was clearly defined. Foo et al. (1990) provided a LOAEL of 88 ppm (331 mg/m<sup>3</sup>), but its “control group” was actually exposed to a low concentration of toluene, and therefore cannot be regarded as a true control group providing a NOAEL. Thus, Zavalic et al. (1998c) was chosen over Eller et al. (1999) and Foo et al. (1990) for OEHHA’s 8-hour and chronic RELs derivation for toluene. In support of the 8-hour REL, an alternative REL (Table 5) was derived based on the findings of Foo et al. (1990).

**Table 5. Supporting 8-hour REL derivation based on study by Foo et al. (1990)**

<i>Supportive Study</i>	Foo et al. 1990
<i>Study population</i>	30 female electronic assembly workers for LOAEL, 30 female workers for LOAEL
<i>Exposure method</i>	Occupational Inhalation
<i>Continuity</i>	10 m <sup>3</sup> /day occupational inhalation rate, 8 hours/day, 5 days/week
<i>Duration</i>	5.7 ± 3.2 years (NOAEL); 2.5 ± 2.7 years (LOAEL)
<i>Critical effects</i>	Neurobehavioral deficits in 6 out of 8 tests
<i>LOAEL</i>	331 mg/m <sup>3</sup> (88 ppm)
<i>NOAEL</i>	49 mg/m <sup>3</sup> (13 ppm)
<i>Time-adjusted exposure</i>	35.0 mg/m <sup>3</sup> (9.3 ppm) (13 ppm × 8/8hr × 5/7 days/week)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	√10 (8-12% of life time 70 years)
<i>Interspecies uncertainty factor</i>	1
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	3.9 (Nong et al. 2006)
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	10
<i>Cumulative uncertainty factor</i>	123
<i>Reference Exposure Level</i>	285 µg/m <sup>3</sup> (0.285 mg/m <sup>3</sup> ; 76 ppb; 0.076 ppm)

The exposure duration was relatively short in the Foo et al. study, necessitating the use of an uncertainty factor (√10) for lack of chronic exposure. Without this added uncertainty, the supporting 8-hour REL would have been essentially identical to the 8-hour REL derived in the key study by Zavalic et al. (1998c).

### 8.3 Toluene Chronic Reference Exposure Level

<i>Study</i>	Zavalic <i>et al.</i> 1998c
<i>Study population</i>	41 adult workers for NOAEL, 32 adult workers for LOAEL, 83 adult workers for control
<i>Exposure method</i>	Inhalation
<i>Continuity</i>	10 m <sup>3</sup> /day occupational inhalation rate, 5 days/week
<i>Duration</i>	15.60 ± 4.61 years (NOAEL); 19.86 ± 5.61 years (LOAEL)
<i>Critical effects</i>	Acquired color vision impairment (dyschromatopsia) (Table 2)
<i>LOAEL</i>	587 mg/m <sup>3</sup> (156 ppm)
<i>NOAEL</i>	132 mg/m <sup>3</sup> (35 ppm)
<i>Benchmark concentration (BMCL<sub>05</sub>)</i>	45.1 mg/m <sup>3</sup> (12 ppm)
<i>Time-adjusted exposure</i>	16.2 mg/m <sup>3</sup> (4.3 ppm) (12 ppm × 10 m <sup>3</sup> /20 m <sup>3</sup> × 5 days/7 days)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1 (>12% of a 70-year life time exposure)
<i>Interspecies uncertainty factor</i>	1
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	3.9 (Nong <i>et al.</i> , 2006)
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	10
<i>Cumulative uncertainty factor</i>	39
<i>Reference Exposure Level</i>	420 µg/m <sup>3</sup> (0.42 mg/m <sup>3</sup> ; 110 ppb; 0.11 ppm)

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 7 in the Technical Support Document (OEHHA, 2008)).

Both the 8-hr and chronic RELs are based on the study by Zavalic *et al.* (1998c), with Foo *et al.* (1990) as a supporting study. The chronic REL derivation is the same, with the exception that the time-adjusted exposure is based on a 24 hr/day exposure. Studies have shown that color

vision impairment progresses with increasing cumulative exposure to neurotoxic chemicals including toluene. However, it is unclear whether the effect is reversible or long-lasting if exposure is discontinued (Gobba and Cavalleri, 2003). Studies with other solvents indicate that color vision impairment can be a long-term injury (Gobba and Cavalleri, 2003). The resulting time-adjusted exposure is 4.3 ppm (16.2 mg/m<sup>3</sup>). The uncertainty factor application is the same for both 8-hr and chronic RELs.

US EPA (2005) derived a chronic inhalation Reference Concentration (RfC) of 5 mg/m<sup>3</sup> for toluene based on the arithmetic mean of NOAELs (34 ppm) from four studies that measured either neuropsychological tests results or color vision loss. This introduced uncertainty in deriving the point of departure from multiple studies with varied endpoints and varied levels of response. The same time-adjusted exposure was used by both USEPA and OEHHA. However, USEPA applied an intraspecies UF = 3 for adult-to-child variability based on the pharmacokinetic information presented in Pelekis et al. (2001). Another 3-fold UF was applied to account for additional pharmacodynamic and pharmacokinetic factors not accounted for, resulting in a total UF = 10.

In contrast, the prior chronic REL for toluene was 300 µg/m<sup>3</sup> (70 ppb) (OEHHA, 2000). This chronic REL was derived from a rat inhalation study by Hillefors-Berglund et al. (1995). Male Sprague-Dawley rats were exposed to 0, 150, 300, 600 or 1200 mg/m<sup>3</sup> (0, 40, 80, 160 or 320 ppm) for 4 weeks, 6 hours/day, 5 days/week, followed by a postexposure period of 29-40 days. The key effects were decreased brain (subcortical limbic area) weight and altered dopamine receptor (caudate-putamen) binding.

The study LOAEL and NOAEL were 300 mg/m<sup>3</sup> (80 ppm) and 150 mg/m<sup>3</sup> (40 ppm), respectively, and the time-adjusted NOAEL was 26 mg/m<sup>3</sup> (7 ppm). A cumulative UF = 100 was used to derive the chronic REL. This cumulative UF consisted of a subchronic UF = 10, interspecies UF = 1 and an intraspecies UF = 10 with both human toxicokinetic (UF<sub>h-k</sub>) and toxicodynamic (UF<sub>h-d</sub>) factors = 3. An interspecies UF of 1 (instead of the default of 3 generally used in 2000) was used because a human occupational study (Foo et al., 1990) had a similar LOAEL (331 mg/m<sup>3</sup>; 88 ppm).

The Foo et al., 1990 study was not used to develop a chronic REL for toluene because: 1) there was no true NOAEL group in the human study; 2) the neurotoxicity endpoints observed were felt to be more sensitive in the animal study than those observable in human studies; and 3) the Hillefors-Berglund et al. (1995) study was believed to have better exposure characterization than the Foo et al., 1990 study.

However, the Zavalic et al. (1998) occupational exposure study reported both LOAEL and NOAEL concentrations (587 mg/m<sup>3</sup> (156 ppm) and 132 mg/m<sup>3</sup> (35 ppm), respectively). This study also had acceptable exposure characterization and sensitive neurotoxicity endpoints. Since OEHHA prefers to use human data to develop RELs where possible, the Zavalic et al. (1998) occupational exposure study was chosen to develop the current toluene chronic REL.

## 8.4 Toluene as a Toxic Air Contaminant Especially Affecting Infants and Children

Under Health and Safety Code Section 39669.5, OEHHA establishes and maintains a list of Toxic Air Contaminants (TACs) that may disproportionately impact infants and children. OEHHA evaluates TACs for addition to this list as Reference Exposure Levels for TACs are developed. Toluene was identified by CARB as a TAC in accordance with section 39657(b) of the California Health and Safety Code (Title 17, California Code of Regulations, section 93001) (CCR, 2007).

OEHHA considers substances that cause neurotoxicity to disproportionately impact children (OEHHA, 2001). It has been demonstrated in this report that toluene is neurotoxic in human and animal models. Animal neurobehavioral studies have also shown that toluene disproportionately impacts the young, when exposed either prenatally or postnatally, compared to adults (Bruckner and Peterson, 1981; Batis et al., 2010; Hass et al., 1999; Hougaard et al., 1999). In addition, the ability of infants to metabolize and detoxify toluene may not reach adult levels until about one year of age (Cresteil, 1998; Fanucchi, 2006). Thus, infants may be more susceptible to the toxic effects of toluene due to slower toluene metabolism during the early years of life.

Finally, neonatal effects from human maternal toluene abuse during pregnancy include intrauterine growth retardation, premature delivery, congenital malformations, and postnatal developmental retardation. The fetotoxic effects of toluene demonstrated in controlled animal studies are comparable to humans who have abused toluene-containing products before or during pregnancy. Intrauterine developmental retardation is the most clearly established effect in animals, as evidenced by decreased late fetal weight and retarded skeletal development. There is also limited evidence in rodents for skeletal and kidney abnormalities, as well as evidence for effects on postnatal physical and neurobehavioral development (Donald et al., 1991; Grandjean and Landrigan, 2006).

In view of the widespread exposure to toluene as an industrial solvent, increased susceptibility to the neurotoxic effects on the developing brain in children, the possible reduction in the metabolism of toluene in the first years of life, and fetotoxicity in newborns resulting from toluene abuse by pregnant mothers, there is valid concern that toluene exposure may disproportionately impact infants and children. OEHHA recommends that toluene be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

## 9. References

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