OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Toluene
Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Public Review Draft

December 2017

Air, Community, and Environmental Research Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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Appendix D1

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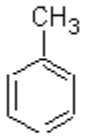
List of Acronyms

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

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.*). These guidelines 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 acutely high 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. Children may be more sensitive to the effects of toluene because of their variability in toluene metabolism compared to adults.

This review includes relevant material published through June 2016 and is a technical review of those studies specifically applicable to developing non-cancer acute, 8-hour, and chronic inhalation RELs for toluene.

1.1 Toluene Acute REL

Reference exposure level	**3,900 µg/m^3 (1,000 ppb)**
Critical effect(s)	Headache, dizziness, slight eye and nose irritation.
Hazard index target(s)	Nervous system; eyes; respiratory system.

1.2 Toluene 8-hour REL

Reference exposure level	**830 µg/m^3 (220 ppb)**
Critical effect(s)	Acquired color vision impairment (dyschromatopsia)
Hazard index target(s)	Eyes

1.3 Toluene Chronic REL

Reference exposure level	**415 µg/m^3 (110 ppb)**
Critical effect(s)	Acquired color vision impairment (dyschromatopsia)
Hazard index target(s)	Eyes

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

Description	Colorless liquid
Molecular formula	C_7H_8
Molecular weight	92.14 g/mol
Density	0.8636 g/cm^3
Boiling point	110.6 °C
Melting point	-94.9 °C
Vapor pressure	28.4 mm Hg at 25°C
Odor threshold	11 mg/m^3 (2.9 ppm) (Amoore and Hautala, 1983) sweet, pungent, benzene-like odor
Solubility	Soluble in most common organic solvents, considered insoluble in water (0.0526 g/100ml at 25 °C).
Conversion factor	3.76 mg/m^3 = 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 an intermediate in benzene production and 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). Toluene is one of the most frequently identified indoor residential chemical risk factors for asthma and allergy in infants and children (Mendell, 2006).

In 2005, the California statewide mean outdoor monitored concentration of toluene was approximately 2.34 µg/m^3 (0.62 ppb) (CARB, 2015). Estimates for toluene emissions from the Statewide 2008 California Toxics Inventory (CTI) were 12,327 tons from stationary sources, 5,063 tons from area-wide sources, 13,647 tons from on-road mobile sources, 7,765 tons from other mobile sources, and 46 tons from natural sources (CARB, 2008). Among the U.S. general public, the mean toluene blood concentration was 1.96 µg/m^3 (0.52 ppb) in adults and 0.53 µg/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 µg/m^3 (0.35, 2.88, and 8.4 ppb) respectively (USEPA, 1988). A geometric mean concentration of 9.8 µg/m^3 (2.6 ppb) (range: 2.6–16.9 µg/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 µg/m^3 (1.19 ppb) (range 0.44–200 µg/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. Walser et al. (2014) showed that the concentration of toluene in the homes released from rotogravure printed matter such as magazines can surpass the US Environmental Protection Agency (US EPA) Reference Dose (RfD) (69 µg/kg/day) in worst-case scenarios. 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 µg/m^3 (1.25 – 5.68 ppb) (Shendell et al., 2004).

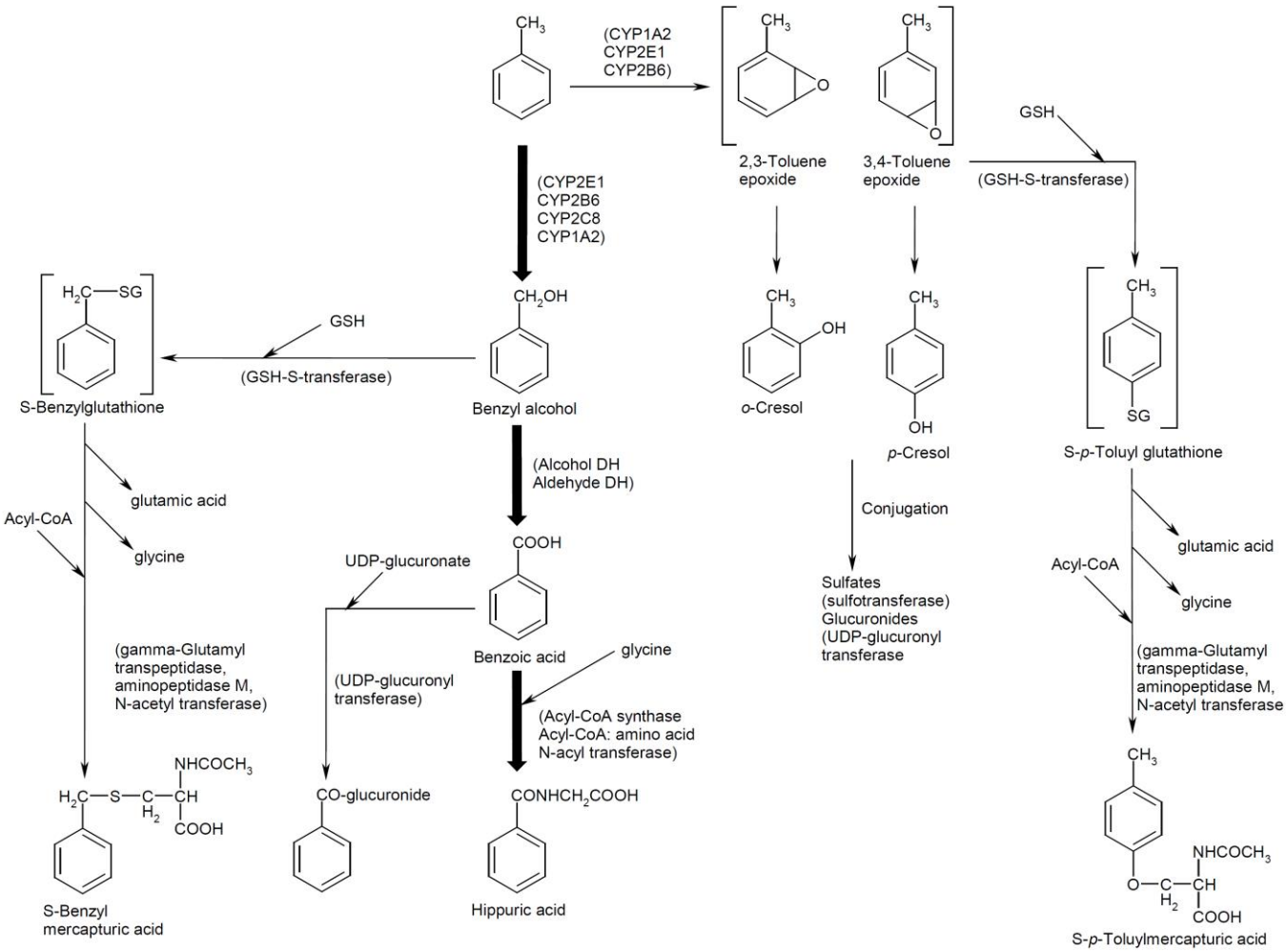
4. Metabolism

Studies in human subjects and laboratory animals indicate that toluene is readily absorbed from the respiratory and gastrointestinal tracts and, to a lesser extent, through the skin. 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 4.1. 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-toluylmercaptic 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).

Tardif et al. (1995) used a 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 threshold limit values of toluene (50 ppm or 190 mg/m^3) and xylene (100 ppm) would not result in significant pharmacokinetic interferences, implying 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

Figure 4.1. 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

Source: USEPA, 2005; Angerer et al. 1998; IARC 1999; Nakajima and Wang 1994; Nakajima et al. 1997; Tassaneeyakul et al. 1996.

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 95th percentile value over the 50th percentile value for the adult and children 1-11 years of age).

Nong et al. (2006) used a previously validated physiologically-based pharmacokinetic (PBPK) model to evaluate the magnitude of interindividual variability in the internal dose of toluene in children of various age groups, on the basis of subject-specific hepatic CYP2E1 (Cytochrome P-450 type 2E1) content and physiology. This study represents the first use of child-specific physiology and data on hepatic CYP2E1 content within a

PBPK modeling framework to simulate the pharmacokinetic profiles and quantify inter-child variability in internal dose of an environmental contaminant (toluene). CYP2E1 is the primary and most active isozyme in metabolizing toluene to benzyl alcohol, particularly at low concentrations. The intrinsic clearance for hepatic metabolism (CL_{int}) was expressed in terms of the CYP2E1 content. The adult toluene PBPK model, with enzyme content-normalized CL_{int}, facilitated the calculation of child-specific CL_{int} based on knowledge of hepatic CYP2E1 protein levels. The resulting model was used to simulate the blood concentration profiles in children exposed by inhalation to 1 ppm toluene for 24 hr. For this exposure scenario, the area under the venous blood concentration vs. time curve (AUC) ranged from 0.30 to 1.01 µg/ml × hr in neonates (<1 month old) with low CYP2E1 concentration (<3.69 pmol/mg protein).

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^3) toluene, a scenario previously evaluated in human volunteers. The adult-child metabolism variability factor, calculated as the ratio of the 95th percentile value for the following age groups over the 50th 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^3) toluene in the air through inhalation over 24 hours. The AF_{HK} values were calculated as the ratio between 95th percentile of the surrogate dose in each respective age group and the median (50th) 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.

5. Acute Toxicity of Toluene

5.1 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. 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^3) 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. (2013) 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 acute 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. His 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 ED for deteriorating mental status and unsteady gait for two months, due to painting work for long hours of 5 days in a poorly air conditioned room. His electroencephalography disclosed diffuse cortical dysfunction, and cerebral MRI showed bilateral periventricular white matter change with corpus callosum involvement. His urine sample was confirmed with toluene intoxication. The authors discussed the extra involvement over the corpus calluosum on brain MRI in both cases which was uncommon to toluene-intoxicated patients, as well as hypothesized possible neuropathological mechanism in these two cases.

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 µg/g.

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 – 3,000 mg/m^3) 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^3) 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^3), 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 (2,300 and 3,000 mg/m^3) 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^3) toluene produces definite impairment of coordination and reaction time.

A number of acute exposure studies in human subjects have been conducted to investigate the nervous system, sensory and other effects of toluene. 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, 1,100, 1,900 and 2,600 mg/m^3) in a successively increasing manner, each for a 20-minute interval with a pause of 5 min between 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 toluene. A statistically significant impairment in perceptual speed was observed at 700 ppm (2,600 mg/m^3) toluene. No effects were observed at 100 ppm (380 mg/m^3).

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^3) 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^3) 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^3) 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) 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 subjects exposed to 100 ppm (380 mg/m^3) 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^3) 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^3) 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^3) 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^3) exposure level. Exposures to 10 and 40 ppm (38 and 150 mg/m^3) toluene were without subjective irritation effects. During the 100 ppm (380 mg/m^3) 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^3) 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 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^3). 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_1) and in forced expiratory flow during exhalation of the middle part of FVC (FEF_{25-75}). 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^3) is only slightly irritating to the mucous membranes, reduces perceived air quality, is moderately odorous, and causes some minor, nonsignificant changes in performance.

Two groups of 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 (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^3) 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. Ten different performance tests were used to measure psychomotor skills, perceptual skills and vigilance. Six tests were on different aspects of visuomotor coordination, the other four 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.

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 was 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^3 (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. Isoamylacetate was

administered in the chamber during the control exposures to disguise the absence of solvent. However, 12 of 16 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 exposed to 0, 75, and 150 ppm (0, 280, and 560 mg/m^3) 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 each day over 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 was 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 exposures. A significant difference was also noted between the 0 and 75 ppm exposure groups for the pattern recognition latency test.

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^3).

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^3).

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^3)

toluene, or a fluctuating concentration of toluene between 300 and 50 ppm (TWA of 102 ppm, 380 mg/m^3) for 7 hours. Specifically, fluctuating exposures consisted of 14 episodes of 30 min with an increasing concentration reaching a peak of 300 ppm (1,100 mg/m^3) after 5 min, then decreasing to 50 ppm (190 mg/m^3) 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 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.

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^3) 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^3). 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.

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 5 concentrations of toluene, from 11 to 180 mg/m^3 (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 ($p < 0.05$).

Visual effects

The Baelum et al. (1985) study described above also included an evaluation of color vision effects resulting from toluene exposure, which 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^3) 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, 1,100 – 1,500 mg/m^3 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 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.

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^3) 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

Pulmonary function tests (forced expiratory vital capacity [FVC], forced expiratory volume in one second after maximal expiration [FEV_1], 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^3) toluene for 6.5 hours is described in detail above in Baelum et al. (1985). Changes in the excretion rate of albumin and $ß_2$-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^3) 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^3 (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 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 Concentration (ppm)	Subjects / Effects (endpoints)	NOAEL ppm (mg/m^3)	LOAEL ppm (mg/m^3)
Longley 1967	~ 30 min 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)**
Von Oettingen et al., 1942	8 hr 50, 100, 200, 300, 400, 600, 800 ppm	3 healthy human beings 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)**
Luderer et al., 1999	3 hr 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)**	
Gamberal & Hulttengren 1972	20 min at each dose with 5 min break between 300 and 500 ppm 100,300,500,700 ppm	12 healthy males Impaired simple reaction time beginning at 300 ppm Impaired perceptual speed at 700 ppm	**100 (380)**	**300 (1,100)**
Lammers et al., 2005	4 hr 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)**	
Baelum et al., 1990	7 hr 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)**
Andersen et al., 1983	6 hr 0, 10, 40, 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)**
Anshelm Olson et al., 1985	4 hr 80 ppm	16 males age 23-38yr No effect on subjective symptoms; no impairment in 3 psychomotor tests	**80 (300)**	

Study	Exposure duration Concentration (ppm)	Subjects / Effects (endpoints)	NOAEL ppm (mg/m^3)	LOAEL ppm (mg/m^3)
Stewart et al., 1975	1 hr, 3-hr and 7.5-hr per day exposure groups, up to 5 d/wk 0, 20, 50, 100 ppm and variable 50-150 ppm male groups 0 and 100 ppm female 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 240ppm	11 males age 20-21 Impaired vigilance during third session		**240 (900)**
Baelum et al 1985	6.5 hr G1: 43 workers with previous solvent exposure G2: 43 workers with no solvent exposure 0 ppm (23 G1 workers and 22 G2 workers) 100 ppm (20 G1 workers and 21 G2 workers)	At 100 ppm: Subjective results: Increased eye (G2 only), nose, and throat (G2 only) irritation, increased strong odor and low air quality, increase 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 above	Same as above At 100 ppm: No significant changes in excretion rate of albumin and ß2-microglobulin, no nephrotoxic effect	**100 (380)**	
Muttray 1999	28-41 min 300-400 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 (1,500)**	*
Echeverria et al., 1989; 1991	7 hr 0, 75, 150 ppm	42 young men & women Neurobehavioral results: At 150 ppm – Decreased verbal short term memory, visual pattern memory, visual perception, and manual dexterity - all showing a linear trend. At 75 ppm – decreased visual perception. Objective findings: Dose-response increase in headache, eye irritation and sleeping episodes with increasing concentration.	**75 (280)**	**150 (570)**

* 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.

5.2 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 2,600 to 12,000 ppm (9,800 to 45,000 mg/m^3) for up to 3hours (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 2,600, 5,200, and 12,000 ppm (9,800, 19,600 and 45,000 mg/m^3). 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 for two 15-min durations separated by a 120-min interval (30 min/day) over 12 days to toluene concentrations of 0, 8,000 or 16,000 ppm (0, 30,000 or 60,000 mg/m^3). 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.

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 LC50 for toluene in the rat was estimated at 26,700 ppm (100,000 mg/m³) (Pryor et al, 1978). The 6-hour LC50s in rats and mice were 4,618 ppm (17,320 mg/m³) and 6,949 ppm (26,060 mg/m³), respectively (Bonnet et al., 1982). An 8-hour LC50 was estimated to be 5,300 ppm (19,900 mg/m³) 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). Macaque monkeys were exposed by inhalation for 50 minutes to one of six concentrations of toluene (0, 100, 200, 500, 1,000, 2,000 or 4,500 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/m³) using a repeated measures analysis of variance test with 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/m³)) with increasing toluene concentration. The authors noted this type of curve suggests behavioral stimulation at lower concentrations and behavioral sedation at higher concentrations.

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: 1,000 ppm (3760 mg/m³) toluene, 14 hours per day for 2 weeks; 1,500 ppm (5,700 mg/m^3) for 14 hours per day for three days; 2,000 ppm (7,500 mg/m^3) for 8 hours per day for three days; and intermittent exposure to 3,000 ppm (11,000 mg/m^3) 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 4,000 ppm (15,100 mg/m^3) for 4 hours or 2,000 ppm (7,500 mg/m^3) 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 1,900 mg/m^3) toluene for 20 minutes resulted in decreased conditioned avoidance response that was reversible. However, exposure to 1,000 ppm (3,800 mg/m^3) toluene for about four hours and 2,000 ppm (7,500 mg/m^3) 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 1,600 ppm (6,000 mg/m^3) toluene for 6 hrs on one day (acute group); the second group was chronically exposed to 80 ppm (300 mg/m^3) 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^3) (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^3) 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, 5 sessions for toluene and 5 sessions for air counter-balanced, were given twice daily for 5 days, with a minimum of 7 hr between sessions. The 5 groups of mice were exposed to 0 (control), 350, 700, 2,500, or 3,200 ppm (1,320, 2,600, 9,400, or 12,100 mg/m^3) 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 ≥ 700 ppm (2,600 mg/m^3) produced a significant place preference (to avoid toluene exposure) in mice. In this study, 700 ppm (2,600 mg/m^3) 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 (3,800 mg/m^3) toluene for 4 hours, 2000 ppm (7,500 mg/m^3) for 2 hours, 3000 ppm (11,300 mg/m^3) for 1.3 hours, or 4000 ppm (15,100 mg/m^3) 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 (7,500 mg/m^3) 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 [30 min, 0, 100, 2,000, 8,000 and 10,000 ppm (0, 380, 7,500, 30,000 and 38,000 mg/m^3)] and then each group with repeated [8,000 ppm (30,000 mg/m^3), 30 min/day for 14 consecutive days] toluene exposure. With acute exposure, concentrations of toluene of 2,000 ppm (7,500 mg/m^3) increased ambulatory distance while the concentrations of ≥ 8,000 ppm (30,000 mg/m^3) 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 that sensitization developed in locomotor activities was significantly higher in each group and 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.

6. Chronic Toxicity of Toluene

6.1 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. ≥ 20 ppm (75 mg/m^3)). 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^3 (1.3 ppm), particularly among vulnerable subpopulation 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.

Rumchev et al. (2004) surveyed 88 children aged 6 months to 3 years from a hospital in Australia who were diagnosed with asthma (subjects), 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) µg/m^3 (4.54 ppb, 0.0027 – 40.84 ppb) The result showed that the subjects children were exposed to significantly higher VOCs than controls ($p < 0.01$), and toluene was of the third highest odds ratio for asthma, the risk of asthma increased two times for every 10 µg/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 associations 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 homes for 1 week and urban homes were shown to have higher pollutant levels than rural homes, up to 2 times. 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 others, increased toluene concentrations were significantly associated with breathlessness and chronic bronchitis in the elderly but not in the rest of the population, with adjusted odds ratios (95% confidence interval) of 3.36 (1.13, 9.98) in elderly, in comparison with 0.91 (0.59, 1.39) in the others.

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 exposure concentration of 0.751 ng/mL, the odds ratio was 2.30 (50^{th}-85^{th} percentiles) and 3.49 (>=85^{th} percentiles), respectively. 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/m³) for periods of 1 to 3 weeks resulted in headaches, lassitude, and loss of appetite. At 200 to 500 ppm (750 to 2,000 mg/m³), 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 1,500 ppm (2,000 to 5,600 mg/m³) 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.

Up to 101 solvent workers exposed to toluene in shoe-making factories were examined for subjective symptoms, 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^3) 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^3) and a high exposure group (≥ 40 ppm, 151 mg/m^3), 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 exposure levels at the two printing shops were 43 mg/m^3 (11.4 ppm) and 157 mg/m^3 (41.8 ppm) of toluene, respectively; however, before 1980 the mean exposure levels had exceeded 300 mg/m^3 (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 ± 3.2(mean ± standard deviation (SD)) for the exposed group and 2.5 ± 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^3) toluene while the control workers inhaled 13 ppm (49 mg/m^3). A significant decrease in

neurobehavioral performance was observed in the exposed workers for 6 out of 8 tests. Irritant effects were not examined, and concurrent exposures to other chemicals were not 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^3) toluene. This may have resulted in an underestimation of the effects of exposure to 88 ppm (330 mg/m^3) toluene.

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^3) 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^3). 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.

A group of 49 printing-press workers occupationally exposed to toluene for approximately 21.6 years was studied by Vrca et al. (1997). 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^3). 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.

Eller et al. (1999) evaluated the chronic effects on the central nervous system of exposure to toluene on 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^3) 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^3) 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^3) for at least 4 years (range: 4-27 years) was associated with impaired neuropsychological function.

Chouaniere et al. (2002) tested 128 toluene-exposed printing workers (14 women and 114 men) from two plants 48 hours after their shift ended for psychometric testing 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^3), although past exposures were estimated to range from 0 to 179 ppm (675 mg/m^3). 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^3) and a mean current exposure of 24.7 ppm (93.1 mg/m^3), and a “low dose” group of 124 workers (end-processing area) had a mean LWAE of 9.3 ppm (35 mg/m^3) and a mean current exposure of 3.3 ppm (12 mg/m^3). 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 exposure at a current exposure level of 1-88 ppm with performance variables.

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^3). Current exposure levels were grouped as "high" (printing area, average concentration 26 ppm (98 mg/m^3)) or "low" (end-processing, average concentration 3 ppm (11 mg/m^3)). Past exposure levels (LWAEs) were grouped as "high" (45 ppm (170 mg/m^3)) or "low" (9 ppm (34mg/m^3)). 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^3) 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^3) and LWAE of 45 ppm (170 mg/m^3), while the "low" group had 86 workers, current exposure of 3 ppm (11 mg/m^3) and LWAE of 9 ppm (34 mg/m^3). 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.

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. This kind of color vision deficit is often used as a sign of the toxicity of industrial chemicals (Iregren et al., 2002).

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 other than damage to ocular structures (Zavalic et al. 1998c). However, it has also been proposed 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). Studies have showed that color vision impairment progressed with increasing cumulative exposure to neurotoxic chemicals including toluene. However, whether the effect was reversible or long-lasting was not clear (Gobba and Cavalleri, 2003).

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 was exposed to a geometric mean toluene air concentration of 35 ppm (132 mg/m^3) (range 11.3–49.3 ppm (42.6–186 mg/m^3)) for an average of 16.21 $\pm$ 6.10 yr and the second exposed group (E2) of 32 subjects was exposed to a geometric mean toluene air concentration of 156 ppm (590 mg/m^3) (range 66.0–250.0 ppm (250–940 mg/m^3)) for an average of 18.34 $\pm$ 6.03 yr. The nonexposed group (NE) comprised 83 subjects. 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^3) and a LOAEL of 156 ppm (590 mg/m^3).

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^3), 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 possibly longer than 64 hours.

Cavalleri et al. (2000) evaluated color vision impairment in 33 toluene-exposed workers. Toluene exposure was estimated by measuring urinary excretion of the unmetabolized toluene (i.e. TolU). 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^3) 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.

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^3) as the geometric mean concentration); the rest were exposed to either tetrachloroethylene alone (13 ppm), or a mixture of tetrachloroethylene (12 ppm) and trichloroethylene (7 ppm). The exposure duration history of the workers was not provided. Color vision loss was first screened using Lanthony's new color test and finally 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.

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 ± 21 ppm (98 ± 79 mg/m^3)) or “low-level” (end-processing, mean current exposures of 3 ± 4 ppm (11 ± 15 mg/m^3)), with mean exposure durations of 23 ± 6 years (long-term) or 7 ± 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^3) 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 $\pm$ 8.5 years, working tenure of 7.7 $\pm$ 6.1 years, noise exposure of 7.7 $\pm$ 6.0 years, and solvent exposure of 6.5 $\pm$ 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^3 (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^3) 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^3) and 82 ± 7 dB(A) for high-toluene-exposed printers, and 10 ± 7 ppm (38 ± 26 mg/m^3) 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^3) and 81 ± 4 dB(A) for printers, and 3 ± 3 ppm (11 ± 11 mg/m^3) 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, 50 ppm (188 mg/m^3) 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^3) (1–108 ppm (4–410 mg/m^3)) and 47 ppm (180 mg/m^3) (6–142 ppm (23–550 mg/m^3)) at the time of study sampling, respectively. The time weighted average air toluene concentration was below 80 ppm (300 mg/m^3) (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 20 rotogravure male printing workers (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^3) (range 8–111 ppm (30–420 mg/m^3)), 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. There were no significant correlations between any hormone level and 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 from exposure to low concentration 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^3) 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 (γ-GTP) activities in the male test group, but significantly higher serum GPT and γ-GTP activities in the female test group than those in respective control groups, which suggested that toluene exposure below 100 ppm (380 mg/m^3) was possibly causing the change in liver functions.

The studies investigating long-term exposure of toluene in humans are summarized below in Table 2.

Table 2. Summary of toluene occupational exposure studies in adult workers with identified NOAEL or LOAEL.

Studies	Number of Subjects	Exposure Duration (average years ± SD)	Concentration (TWA in ppm)	Critical Effects (endpoints)	NOAEL ppm (mg/m^3)	LOAEL ppm (mg/m^3)
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 and B 19 exposed (A) 11 exposed (B) 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±3.2 2.5±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 1997	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±3.5	90.9 0 ppm	Significant decrease in neurobehavioral performance (9 of 14 tests)	*	90.9 (340)
Eller 1999	19 controls 30 with low exposure 49 with high exposure	7.7±3.5 (range:1-12) 25.5±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)
Zavalic 1998a,c	83 controls 41 low exp 32 high exp	20.91(work) 15.60±4.61 19.86±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±5.94	0 ppm 120	Color vision impairment in exposed group	*	120 (450)

* not observed

** indirect exposure estimate: based on urinary levels of metabolites and toluene blood levels for the Vrca study, and based on urinary excretion of toluene plus data from previous air toluene measurements for the Cavalleri study.

nd No data

6.2 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^3 (1.3 ppm)). Delfino et al. (2003a and 2003b), Rumchev et al. (2004), and Hulin et al. (2010) showed that toluene was associated with increased odds of asthma or asthma symptoms in children. 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.

6.3 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 by actual differences in biochemical assays in neonatal animals and in adult animals (von Euler et al., 1989; Riegel et al., 2004) or by toluene's effects on dopamine-dominated functions, such as hyper-locomotion (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 respiratory 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 1,100 mg/m^3) 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_2 agonist binding in the rat caudate-putamen was observed with exposure to 80 ppm (300 mg/m^3) 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^3) 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^3) 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

serotoninergic neurotransmission activity was significantly altered in various brain regions of rats exposed to 40 ppm (150 mg/m^3) toluene for 16 weeks.

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 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 1,200 ppm (4,500 mg/m^3) 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 1,200 ppm (4,500 mg/m^3) 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 (6,800 mg/m^3) 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^3) 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, toluene exposure affected the rats' spatial memory 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_2 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 1,000, 3,600 or 6,000 ppm (3,800, 13,600, or 22,600 mg/m^3) 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 (6,000 ppm (22,600 mg/m^3)) showed a dramatic decrease in the number of rewards received, while those exposed to 3,600 ppm (13,600 mg/m^3) of toluene had significantly more responses. Mice exposed to the lowest level (1,000 ppm (3,800 mg/m^3)) 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 1,000 ppm (0, 38, 380, or 3,800 mg/m^3)) 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 1,000 ppm (3,800 mg/m^3) 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$_1$ 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 1,200 ppm (2,300 and 4,500 mg/m^3) for rats, and 120, 600 and 1,200 ppm (450, 2,300 and 4,500 mg/m^3) 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 1,200 ppm (4,500 mg/m^3). 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^3) 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.

To investigate the effects of volatile organic compounds in the indoor air on the induction or augmentation of airway inflammatory responses (neuroimmune interaction in general), Shwe et al. (2007) exposed male C3H mice to 0, 9 and 90 ppm (0, 34 and 340 mg/m^3) toluene for 30 min by nose-only inhalation on days 0, 1, 2, 7, 14, 21, and 28. 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^3) or 90 ppm (340 mg/m^3) 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: 1,000 ppm (38,00 mg/m^3), 14 hours per day for 2 weeks; 1,500 ppm (5,700 mg/m^3) for 14 hours per day for three days; 2,000 ppm (7,500 mg/m^3) for 8 hours per day for three days; and intermittent exposure to 3,000 ppm (11,300 mg/m^3) 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 (1,500 and 2,600 mg/m^3)) for 14 hours per day did not have hearing loss even after 16 weeks of exposure, and single exposures to 4,000 ppm (15,100 mg/m^3) for 4 hours or 2,000 ppm (7,500 mg/m^3) 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 1,750 ppm (6,600 mg/m^3) toluene 6 hr/day, 5 days/week for 4 weeks. Auditory function was tested by electrocochleography and supported by subsequent histological examination. A significant toluene-induced hearing loss was provoked in these exposed guinea pigs, but was different from that observed in 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.

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 (1,100 mg/m^3) (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^3) 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 (3,800 mg/m^3) 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 (3,800 mg/m^3) 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 3,800 mg/m^3) 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

To investigate the possibility of age-dependent neurobehavioral sensitivity to toluene among inhalant abusers, Samuel-Herter et al. (2014) used a rodent model to assess the effects of acute binge-like toluene inhalation exposures (~5000 ppm (18,800 mg/m^3), 15 or 30 min in the chambers with the total amount of liquid toluene for evaporization. It is assumed, but not stated the chambers were the same size.) on motor functions including ambulatory activity, vertical exploration, grooming, balance, gait and neurological functions for the following age-groups of rats: adolescent (1 month), young adult (2-3

months), adult (5-6 months), and older adult (10-12 months). 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

7.1 Human Studies

Toluene has been listed under Proposition 65 as known to the State of California to cause developmental toxicity (OEHHA, 2015). Toluene is also considered most likely to be a teratogenic agent, primarily as a result of inhalant abuse in humans, although concurrent exposures to other developmental toxicants make this conclusion difficult to confirm (OEHHA, 2008). 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^3), mean 88 ppm (330 mg/m^3), 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^3), 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.

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 8,181 children with term LBW (>=37 weeks' completed gestation and birth weight <2,500 g) and 370,922 term normal-weight children in Los Angeles (LA) Country 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 <= 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.

7.2 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^3) and 1,000 ppm (3,800 mg/m^3) 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 1,000 ppm (3,800 mg/m^3) 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 1,000 mg/m^3 (265 ppm) toluene for 24 hours per day on days 7–14 of gestation (Tatrai et al., 1980).

Fused sternebrae and extra ribs were observed in rats exposed to 400 ppm (1,500 mg/m³) 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 (1,000 mg/m³) toluene for 8 hours per day on days 1–21 of gestation and to 400 ppm (1,500 mg/m³) 24 hours per day on days 1–8. This same group exposed mice to 400 ppm (1,500 mg/m³) or to 133 ppm (500 mg/m³) toluene for 24 hours per day on days 6–13 of gestation. All dams died at 400 ppm (1,500 mg/m³) and a statistically significant decrease in fetal weight was observed at 266 ppm (1,000 mg/m³). In another set of experiments, continuous exposure of pregnant rats to higher concentrations of 1,000 and 1,500 ppm (3,800 and 5,700 mg/m³) toluene on days 9 to 14 of gestation resulted in the death of two dams out of 19 during the exposure to 1,500 ppm (5,700 mg/m³) (Hudak and Ungvary, 1978). Fetuses from the 1,500 ppm (5,700 mg/m³) 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 1,000 ppm (3,800 mg/m³) 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 1,100 mg/m³), 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 1,900 mg/m³) 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 (1,500 mg/m³) 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 (1,500 mg/m³) 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 2,000 ppm (0, 380, 1,900, or 7,500 mg/m³) toluene during an 80-day premating 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 2,000 ppm (7,500 mg/m³) were significantly decreased compared to controls. No maternal toxicity was reported. Exposure at 2000 ppm (7,500 mg/m³) 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 (7,500 mg/m^3) 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 (1,900 mg/m^3).

In a more recent teratogenicity study, Ono et al. (1995) exposed pregnant Sprague-Dawley rats to 600 or 2,000 ppm (2,300 or 7,500 mg/m^3) toluene for 6 h/day from day 7 to day 17 of pregnancy. The control group inhaled clean air. Maternal exposure to 2,000 ppm (7,500 mg/m^3) 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 (2,300 mg/m^3) is a NOAEL in this study.

Da Silva et al. (1990) exposed pregnant rats and hamsters to 0 or 800 mg/m^3 (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^3 (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 1,200 ppm (0 or 4,500 mg/m^3) 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 1,200 ppm (4,500 mg/m^3) 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 inhalation route, and were categorized into the following groups: 0–500 ppm (0–1,884 mg/m^3), 501–2000 ppm (1,888–7,537 mg/m^3), 2001–5000 ppm (7,541–18,842 mg/m^3), 5001–7500 ppm (18,846–28,263 mg/m^3), and 7500 ppm (28,263 mg/m^3) 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.

8. Derivation of Reference Exposure Levels

8.1 Toluene Acute Reference Exposure Level

Study	Andersen *et al.*, 1983
Study population	16 male humans, mean age = 24 years
Exposure method	Inhalation chamber, 0, 10, 40 and 100 ppm (0, 38, 150, 380 mg/m^3 respectively)
Duration	6 hours
Critical effects	Impaired reaction time and symptoms of headache, dizziness, feeling of intoxication, sensory irritation (eye and nose irritation)
LOAEL	380 mg/m^3 (100 ppm)
NOAEL	150 mg/m^3 (40 ppm)
Time-adjusted exposure	150 mg/m^3 (40 ppm) (no time adjustment for sensory irritation)
LOAEL uncertainty factor	1
Interspecies uncertainty factor	1
Toxicokinetic (UF_{a-k})	
Toxicodynamic (UF_{a-d})	
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	3.9 (Nong *et al.* 2006)
Toxicodynamic (UF_{h-d})	10
Cumulative uncertainty factor	39
Reference Exposure Level	3,900 µg/m^3 (1,000 ppb)

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^3) for 6 hours. Exposures to 10 and 40 ppm (38 and 150 mg/m^3) toluene were without subjective irritation effects of strong odor sensation. Statistically significant ($p < 0.05$) subjective symptomology included eye and/or nose irritation, headache, and feeling of intoxication among subjects of 100 ppm (380 mg/m^3) 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 of 100 ppm (380 mg/m^3) toluene exposure. In this study, 40 ppm (150 mg/m^3) was recognized as a NOAEL and 100 ppm (380 mg/m^3) as a LOAEL to derive an acute REL for toluene.

This study was also used by the US Agency for Toxic Substances and Disease Registry (ATSDR) to develop a Minimal Risk Level (MRL) of 1 ppm (4 mg/m$^(\)$) for acute-duration (14 days or less) inhalation exposure to toluene (ATSDR 2000). The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over acute-duration of exposure, which is comparable to OEHHA's acute REL.

A supporting human exposure study by Echeverria et al. (1989) with a similar study design provided a LOAEL of 150 ppm (570 mg/m^3) and a NOAEL of 75 ppm (280 mg/m^3). Echeverria et al. observed statistically significant decrements in several neurobehavioral tests among a battery of tests conducted at 150 ppm (570 mg/m^3). The results from one test, pattern recognition latency, were statistically significant at 75 ppm (280 mg/m^3). The statistically significant finding at 75 ppm (280 mg/m^3) 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^3) exposure for 7 hours is near the threshold for the NOAEL/LOAEL.

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 ($C \times t$).

Several studies (Pelekis *et al.* 2001; Price *et al.* 2003; Nong *et al.* 2006) developed physiologically based pharmacokinetic (PBPK) models of inhalation exposure to volatile organic compounds including toluene for children. These models accounted for human inter-individual variability by age. An adult-to-child pharmacokinetic adjustment for neonates about 1 month of age was calculated to have the largest inter-individual variability at 3.9 (Nong et al 2006). Thus, a toxicokinetic $UF_{h\text{-}k}$ of 3.9 is used in the derivation. An intraspecies uncertainty factor – toxicodynamic component ($UF_{H\text{-}d}$) - of 10 is applied for 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).

8.2 Toluene 8-hour Reference Exposure Level

Study	Zavalic et al. 1998c
Study population	41 adult workers for NOAEL, 32 adult workers for LOAEL, 83 adult workers for control
Exposure method	Inhalation
Continuity	10 m^3/day occupational inhalation rate, 8 hours/day, 5 days/week
Duration	15.60 $\pm$ 4.61 years (NOAEL); 19.86 $\pm$ 5.61 ye+ars (LOAEL)
Critical effects	Acquired color vision impairment (dyschromatopsia) (Table 2)
LOAEL	587 mg/m^3 (156 ppm)
NOAEL	132 mg/m^3 (35 ppm)
Benchmark concentration ($BMCL_{05}$)	45.1 mg/m^3 (12 ppm)
Time-adjusted exposure	32.3 mg/m^3 (8.6 ppm) (12 ppm x 8/8hr x 5/7 days/week)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Toxicokinetic (UF_{a-k})	
Toxicodynamic (UF_{a-d})	
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	3.9 (Nong et al. 2006)
Toxicodynamic (UF_{h-d})	10
Cumulative uncertainty factor	39
Reference Exposure Level	830 µg/m^3 (220 ppb)

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).

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^3). The use of two exposure levels made it possible to perform a benchmark dose analysis. A LOAEL of 156 ppm (587 mg/m^3) and a NOAEL of 35 ppm (132 mg/m^3) 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.

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 chronic effects of toluene on CNS. However, in the Eller et al. (1999) study, the time-weighted average level of toluene for the low-exposure group could only be obtained as below 20 ppm (75 mg/m^3), while that for the high-exposure group as exceeding 100 ppm (380 mg/m^3), neither of which is definite. Thus, Zavalic et al (1998c) was chosen over Eller et al. (1999) for OEHHA's 8-hour and chronic RELs derivation for toluene.

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, 2007). The BMC models for dichotomous data gave acceptable line fits to the data with BMD_{05} values over a range of 5 to 32 ppm (19 to 121 mg/m^3) (Table 3). 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 $BMCL_{05}$ (12 ppm (45 mg/m^3)) at the lower end of the range (Figure 1). 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 ($UF_{h\text{-}d}$) 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

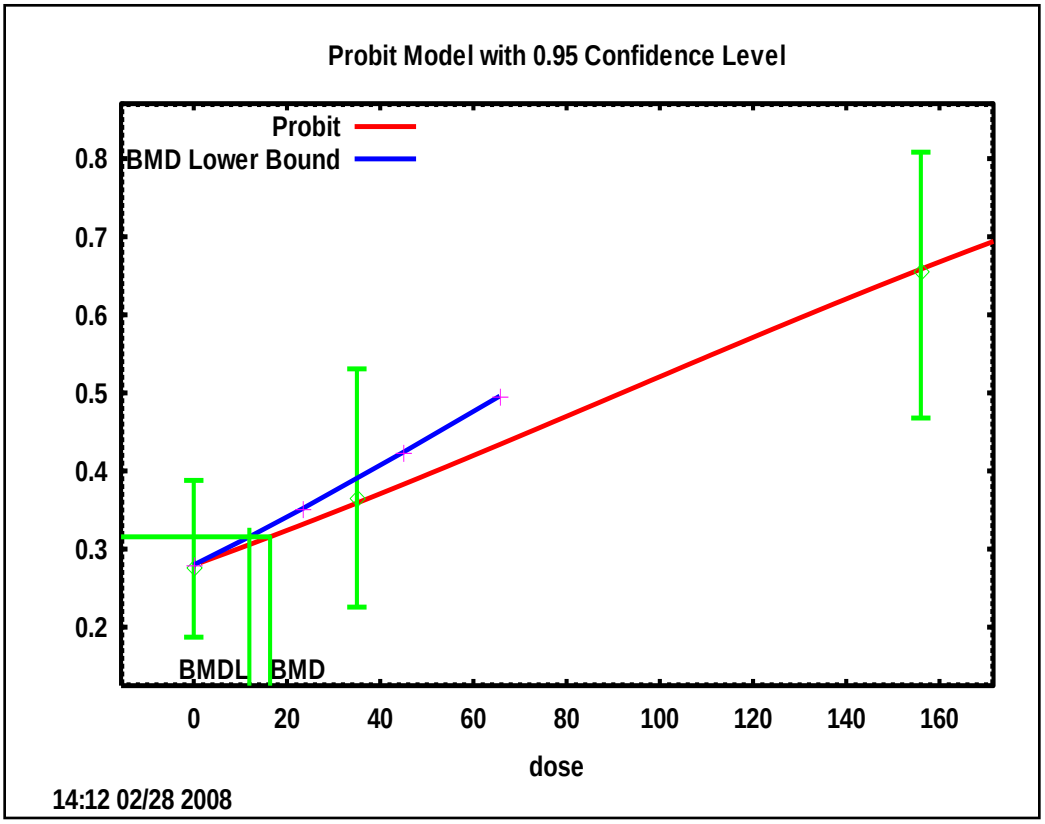
children/infants (Nong et al., 2006). This was used in place of the default intraspecies uncertainty factor – toxicokinetic component (UF_{H-k}) for which a PBPK model including measured inter-individual variability is applied. An intraspecies uncertainty factor – toxicodynamic component (UF_{H-d}) – of 10 is applied to account for the greater susceptibility of children to neurotoxic effects.

Table 3. Benchmark dose analysis (USEPA BMDS 1.3.2) 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
Quantal Quadratic	41.24 (155)	32.05 (121)	0.4726	197.52
Multistage (β=2)	11.01 (41)	6.90 (26)	0.8021	197.07

*Akaike Information Criterion

Figure 1. 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 on 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 3.9 derived by Nong et al. (2006) as the intraspecies uncertainty factor – toxicokinetic component ($UF_{H\text{-}k}$). 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.

8.3 Toluene Chronic Reference Exposure Level

Study	Zavalic *et al.* 1998c
Study population	41 adult workers for NOAEL, 32 adult workers for LOAEL, 83 adult workers for control
Exposure method	Inhalation
Continuity	10 m^3/day occupational inhalation rate, 5 days/week
Duration	15.60 $\pm$ 4.61 years (NOAEL); 19.86 $\pm$ 5.61 years (LOAEL)
Critical effects	Acquired color vision impairment (dyschromatopsia) (Table 2)
LOAEL	587 mg/m^3 (156 ppm)
NOAEL	132 mg/m^3 (35 ppm)
Benchmark concentration (BMC_{05})	45.1 mg/m^3 (12 ppm)
Time-adjusted exposure	16.2 mg/m^3 (4.3 ppm) (12 ppm * 10 / 20 * 5 days/7 days)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	1
Interspecies uncertainty factor	1
Toxicokinetic (UF_{a-k})	
Toxicodynamic (UF_{a-d})	
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	3.9 (Nong et al., 2006)
Toxicodynamic (UF_{h-d})	10
Cumulative uncertainty factor	39
Reference Exposure Level	415 µg/m^3 (110 ppb)

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). 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 (Gobba and Cavalleri, 2003). The resulting time-adjusted exposure is 4.3 ppm (16.2 mg/m^3). The uncertainty factor application is the same for both 8-hr and chronic RELs.

USEPA (2005) derived a chronic inhalation Reference Concentration (RfC) of 5 mg/m^3 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.

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

Proposition 65 provides mechanisms for listing chemicals that are known to the State to cause cancer or reproductive toxicity (Health and Safety Code section 25249.8(b)). Toluene was listed as a developmental toxicant on January 1, 1991 under Proposition 65 based on neonatal effects from maternal toluene abuse during pregnancy.

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). 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). Other neonatal effects from maternal toluene abuse during pregnancy include intrauterine growth retardation, premature delivery, congenital malformations, and postnatal developmental retardation, as well as fetotoxic effects of toluene demonstrated in controlled animal studies 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 wide-spread exposure to toluene as an industrial solvent, and the documented toxicokinetic variability in toluene metabolism by age, 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).

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