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

Scientific Review Panel Review Draft

May 2019

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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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. The authors noted that a significant toluene-induced hearing loss was provoked in these exposed guinea pigs, but the associated auditory system histopathology has not been demonstrated in studies using other toluene-exposed species, including rats. Histological examination showed that only the stria vascularis and the spiral fibers were disrupted in the apical coil of the cochlea of the guinea pigs. The authors concluded that guinea pigs can metabolize toluene more efficiently than rats, probably because of a higher level of hepatic P-450.

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

Samuel-Herter et al. (2014) studied the possibility of age-dependent neurobehavioral sensitivity to toluene among inhalant abusers using a rodent model to assess the effects of acute binge-like toluene inhalation exposures. Male Long-Evans rats (18/exposure group) were exposed to approximately 5000 ppm (18,800 mg/m^3) for 15 or 30 min in whole-body exposure chambers. The following age-groups of rats were used in the study: adolescent (1 month), young adult (2-3 months), adult (5-6 months), and older adult (10-12 months). Motor

functions evaluated included ambulatory activity, vertical exploration, grooming, balance, gait and neurological functions. An adolescent group of rats were not exposed to 30 min of toluene due to a pilot study result showing that rats in this age group required much longer time to recover any degree of motor function (reference not given). The general results showed that acute toluene exposure impaired both motor and neurological functions in all age groups of rats, with adolescent and young adult rats needing significantly longer recovery times than older rats ($p < 0.05$). The authors claimed that these results suggested an age-dependent vulnerability to the intoxicating effects of toluene. However, the possible cause of the adolescent and young adult age-groups of rats receiving higher doses (per unit body weight) was not discussed.

7. Developmental and Reproductive Toxicity

7.1 Human Studies

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

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

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

An increased incidence of spontaneous abortions was also reported among occupationally exposed women Ng et al. (1992) conducted a study on rates of menstrual disorders among female workers exposed exclusively to toluene at a factory where audio coils and speakers were assembled. The menstrual function questionnaire results of 231 female production workers exposed to high toluene exposure (50-150 ppm (190-570 mg/m^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/m³) and 1,000 ppm (3,800 mg/m³) 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/m³) 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/m³ (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/m^3) 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/m^3) (Hudak and Ungvary, 1978). Fetuses from the 1,500 ppm (5,700 mg/m^3) 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/m^3) 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 the 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.

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

8. Derivation of Reference Exposure Levels

8.1 Toluene Acute Reference Exposure Level

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
Toxicodynamic (UF_{h-d})	10
Cumulative uncertainty factor	30
Reference Exposure Level	5,000 µg/m^3 (1,300 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.

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

Due to the concentration-dependent nature of chemically-related sensory irritation, no time-adjusted exposure was applied for extrapolation to a 1-hour exposure. Supporting evidence for no time-adjusted exposure was observed in the animal study by Oshiro et al. (2011). In this study, some behavioral effects related to neurotoxicity following acute exposure were better predicted by the brain concentration of toluene rather than by cumulative inhaled dose ($C \times t$).

In contrast, our original 1999 acute REL for toluene was based on the same study of Andersen et al. (1983). With a time-adjusted concentration of 98 ppm (370 mg/m³), an interspecies uncertainty factor of 1 and an intraspecies uncertainty factor of 10, the resulting acute REL for toluene was 37,000 µg/m³ (9,800 ppb). In contrast, the new acute, 8-hour and chronic RELs for toluene described in this document were derived using the methodology described in the noncancer TSD adopted in December 2008 (OEHHA, 2008). This methodology explicitly considers possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25). As a result, the intraspecies uncertainty factor changed from 10 to 30 including a toxicodynamic component of 10 to account for the potentially greater susceptibility of children to neurotoxic effects. Meanwhile, the point-of-departure changed from a time-adjusted value of 370 mg/m^3 to the NOAEL value of 150 mg/m^3 since the critical effect of sensory irritation is concentration-dependent and not time-dependent. As described above, the exposure was not time-adjusted for extrapolation to a 1-hour exposure.

A supporting human exposure study by Echeverria et al. (1989) with a similar study design provided a LOAEL of 150 ppm (570 mg/m^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) (ANOVA p values = 0.045 and 0.021 for effect and trend, respectively) was the only one among the battery of 27 tests within seven psychometric performance measures. Subjective symptoms of eye irritation and headache increased with dose but statistical analysis was not provided. A dose-dependent increase ($p < 0.001$) in the number of subjects observed sleeping was reported and noted by the authors as the best evidence for neurological effects from toluene exposure. The evidence by Echeverria et al. suggests that 75 ppm (280 mg/m^3) exposure for 7 hours is near the threshold for the NOAEL.

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

Here in this derivation, an intraspecies uncertainty factor with a toxicodynamic component (UF_{H-d}) of 10 is applied for the use of human studies with normal adult subjects and to address the human variation in response to substances with nervous system effects, including sensitive subpopulations such as children (OEHHA 2008), resulting in an overall UF of 30.

8.2 Toluene 8-hour Reference Exposure Level

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 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 ($BMCL_{05}$)	45.1 mg/m^3 (12 ppm)
Time-adjusted exposure	32.3 mg/m^3 (8.6 ppm) (12 ppm × 8/8hr × 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).

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

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

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

The study by Zavalic et al. (1998c) was selected as the best available study because it employed human subjects, used a sensitive endpoint (acquired color vision impairment (dyschromatopsia)), and two toluene exposure concentrations of 35 and 156 ppm (132 and 587 mg/m^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 2).

Use of a $BMCL_{05}$ in a REL derivation takes into account some of the inter-individual variability within a population, generally resulting in a reduction of the standard intraspecies uncertainty factor. However, a worker population such as that used in the Zavalic study is considered healthier than the human population as a whole (i.e., healthy worker effect). Thus, to be adequately protective of vulnerable subpopulations, an intraspecies toxicodynamic ($UF_{h\text{-}d}$) 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 ($UF_{H\text{-}k}$) for which a PBPK model including measured inter-individual variability is applied.

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

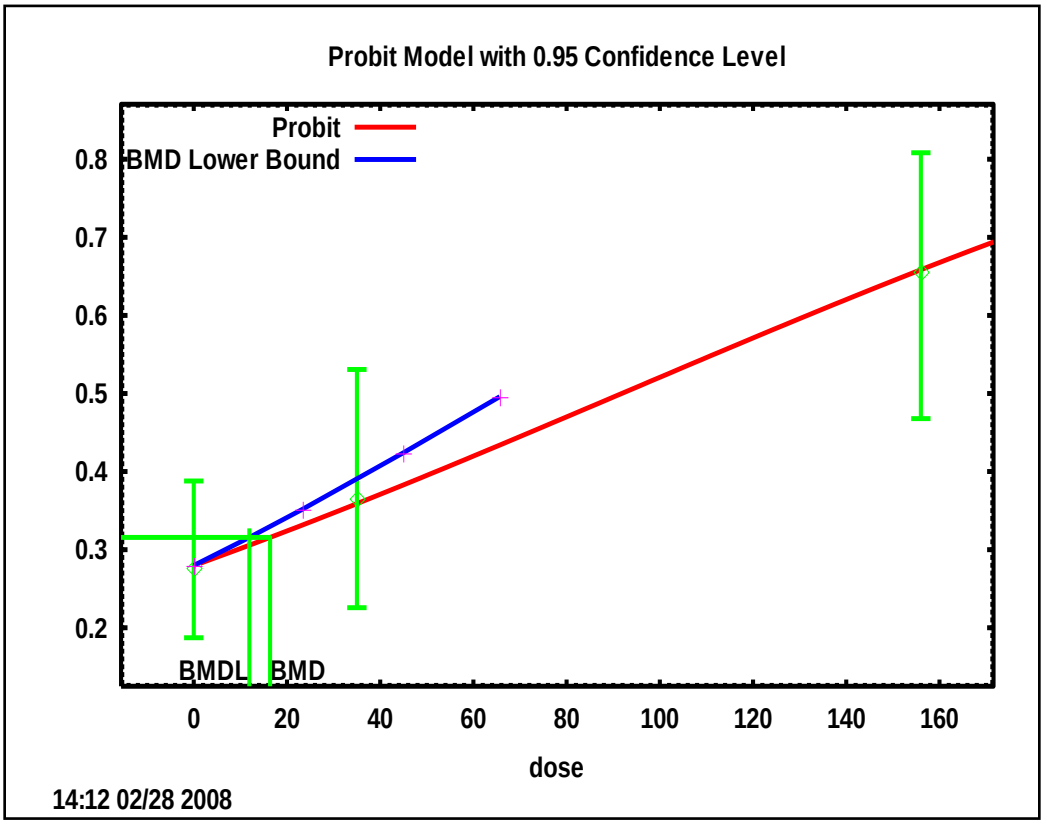
2429 Table 4. Benchmark dose analysis (USEPA BMDS 1.3.2) of data from Zavalic et
2430 al., 1998c

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

2431 *Akaike Information Criterion

2432

2433 Figure 2. Probit model fit to Zavalic et al. (1998c) human dyschromatopsia data



2434

2435 From the PBPK modeling study of Nong et al. (2006), the inter-individual
2436 variability factors for child age groupings indicate that the area under the venous
2437 blood concentration vs. time curve (AUC) of toluene varied only by a factor of up
2438 to 3.9 (for neonate group) even though liver CYP2E1 content can vary by a factor
2439 of 20. Due to the age-related changes in other physiological parameters, the PK

variability is less than expected on the basis of age-related change in the levels of hepatic CYP2E1. The magnitude of the inter-individual variability factor, in part, can be explained on the basis of CYP2E1 levels in neonates, children, and adults. The synthesis pathway of the enzyme CYP2E1 is immature at birth followed by rapid onset and eventual maturation by 6 months to 1 year (Vieira et al., 1996; Cresteil, 1998; Nakamura et al., 1998; Tanaka, 1998). Using a more extensive analysis, Johnsrud et al. (2003) observed that maturation of hepatic CYP2E1 content occurred after 3 months, and expression comparable to adult levels after 1 year. A sensitivity analysis by Nong et al. (2006) showed that hepatic metabolism of toluene appears to be limited by enzyme content at birth and its pharmacokinetics evolve gradually to a hepatic blood flow-limited condition with increasing age.

The most recent PBPK models for toluene developed by Mörk et al. (2014) only recognized a slight difference between adults and infants in terms of toluene metabolism, i.e., the adult-to-child pharmacokinetic adjustment factor they developed was close to 1. To be adequately protective for the infants and children, we applied the value of 3.9 derived by Nong et al. (2006) as the intraspecies uncertainty factor – toxicokinetic component ($UF_{H\text{-}k}$). 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 ($BMCL_{05}$)	45.1 mg/m^3 (12 ppm)
Time-adjusted exposure	16.2 mg/m^3 (4.3 ppm) (12 ppm × 10 m^3/20 m^3 × 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	420 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.

US EPA (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.

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

The study LOAEL and NOAEL were 300 mg/m^3 (80 ppm) and 150 mg/m3 (40 ppm), respectively, and the time-adjusted NOAEL was 26 mg/m^3 (7 ppm). A cumulative UF = 100 was used to derive the chronic REL. This cumulative UF consisted of a subchronic UF = 10, interspecies UF = 1 and an intraspecies UF = 10 with both human toxicokinetic ($UF_{h\text{-}k}$) and toxicodynamic ($UF_{h\text{-}d}$) factors = 3. An interspecies UF of 1 (instead of the default of 3 generally used in 2000) was used because a human occupational study (Foo et al., 1990) had a similar LOAEL (243 mg/m^3; 88 ppm).

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

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

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

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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