2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO TOLUENE IN THE UNITED STATES

Toluene is a clear colorless liquid possessing high vapor pressure and low to moderate water solubility. It is used as a solvent and as an additive in gasolines to improve octane ratings. It is also frequently used to produce other chemicals such as benzene and toluene diisocyanate.

Given its vapor pressure, toluene tends to partition to the atmosphere. Automobile emissions are the principal source of toluene in ambient air, with levels fluctuating in proportion to automobile traffic. Toluene can also be a common indoor contaminant, and indoor air concentrations are often several times higher than outside air. This is likely due to release of toluene from common household products (paints, paint thinners, adhesives, and nail polish in which it is used as a solvent) and from cigarette smoke.

In the atmosphere, toluene is principally degraded by reaction with photochemically generated hydroxyl radicals, but may also degrade through reaction with nitrate radicals and ozone. When released to water surfaces, toluene is expected to volatilize quickly. It may also be biodegraded under aerobic and anaerobic conditions, but hydrolysis is not an important environmental fate process. Toluene does not bioconcentrate or bioaccumulate significantly in aquatic organisms. If released to soil, toluene is expected to volatilize quickly. In the case of a large spill, some toluene may leach into groundwater because it possesses high mobility in soils. Biodegradation in soils may also occur with half-lives ranging from a few hours to several days depending upon the environmental conditions.

The general population is primarily exposed to toluene through the inhalation of ambient air. Ingestion of toluene from contaminated water and food is possible; however, this is a less likely exposure route since toluene is not frequently detected in drinking water and food. Dermal exposure from gasoline or solvents that contain toluene is also possible. Occupational exposure to toluene is expected to be greater than general population exposure for persons employed in heavy traffic occupations (e.g., toll attendants, automobile workers etc.) and persons who frequently use solvents or other products that contain toluene.

2.2 SUMMARY OF HEALTH EFFECTS

Death. Case studies that reported on deaths in humans due to exposure to toluene have generally not provided information on dose. In one instance, ingestion of approximately 625 mg/kg resulted in death

within 30 minutes. The cause of death appeared to be profound disruption of central nervous system function.

An acute 7-hour inhalation LC_{50} value of 5,320 ppm has been reported for mice and acute oral LD_{50} values in adult rats ranged from 5,500 to 7,400 mg/kg. In 13-week gavage studies, all rats and mice that received 5,000 mg/kg died within the first week. Mortality was also high for groups receiving 2,500 mg/kg, with 8/10 male rats, 1/10 female rats, and 4/10 male and female mice dying before the end of the study. A dose of 1,250 mg/kg/day was lethal in 1/10 female mice, but no deaths occurred in male mice or in rats of either sex.

Systemic Effects.

Respiratory Effects. The primary effect of toluene on the respiratory tract following inhalation is irritation. Studies with volunteers and exposed workers have demonstrated that toluene is a mild-to-moderate respiratory irritant. Early animal studies reported respiratory irritation and pulmonary lesions in rats exposed to high concentrations of toluene. These findings are supported by more recent observations of nasal lesions (including metaplasia of olfactory epithelium and degeneration of respiratory epithelium) in rats exposed to concentrations ranging from 600 to 1,200 ppm, 6.5 hours/day, 5 days/week for 2 years. Mice exposed by the same exposure protocol to a similar range of concentrations, however, did not display upper or lower respiratory tract lesions. In shorter duration studies, reversible nasal olfactory degeneration was observed in mice exposed to 1,000 ppm, 5 hours/day, 5 days/week for 4 weeks and inflammatory cell infiltration in peribronchial and alveolar regions, alveolar edema, and interstitial fibrosis and necrosis was observed in rats exposed to 3,000 ppm, 8 hours/day, 6 days/week for 12 weeks.

Only limited data regarding respiratory effects following oral exposure to toluene were located. Following lethal ingestion of approximately 625 mg/kg toluene, lung congestion and hemorrhage were reported in an adult male. Mucosal lesions and pronounced edema were observed during a bronchoscopy following a nonlethal ingestion of paint thinner by a 15-month-old girl. No respiratory effects were reported in mice or rats after oral exposure to toluene at dosage levels up to 2,500 mg/kg/day for 13 weeks or 590 mg/kg/day for 6 months. No changes in lung weight or histology were reported in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls.

Cardiovascular Effects. Inhalation exposure to toluene at concentrations >1,000 ppm has been associated with alterations of the heart rhythm in both humans and animals, but exposure of rats or mice

to concentrations as high as 12,000 ppm (3 hours/day) for intermediate durations and up to 1,200 ppm (6–6.5 hours/day) for chronic durations produced no histological changes in heart tissue. Additionally, no histological changes in the heart were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (6 hours/day; pre-mating and mating, gestation, and lactation) in a multigenerational study. There may be intraspecies differences in the cardiac response to toluene that make some individuals more susceptible than others to potentially fatal arrhythmias; the degree of hypoxia may also be important.

Cardiac effects have been noted following oral exposure to doses >1,200 mg/kg. Cardiac edema and congestion were observed in rats given single gavage doses of 5,200 mg/kg, compared with controls. Increased relative heart weights were noted in rats exposed to toluene at 1,250 mg/kg/day for 13 weeks and myocardial degeneration was present in mice exposed to 5,000 mg/kg/day. All of the mice receiving 5,000 mg/kg/day died during the first weeks of exposure. No effects on the weight or gross morphology of the heart were noted in rats receiving 590 mg/kg/day for 6 months, and no significant treatment-related findings were observed in electrocardiograms or cardiac histology in rats given single oral doses of up to 1,000–1,200 mg/kg.

Gastrointestinal Effects. Gastric pain was reported by a man who accidently ingested 30 mL of an organic solvent containing toluene and other chemicals. Gastrointestinal effects were not reported in other case studies of oral exposure.

The only gastrointestinal effect reported after exposure to toluene was ulceration of the forestomach of rats exposed to 600 and 1,200 ppm by inhalation for 2 years. Similar effects were not seen in mice exposed under the same conditions or in rats or mice orally exposed to 2,500 mg/kg/day for 13 weeks. Additionally, no gastrointestinal effects were observed in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (6 hours/day; pre-mating and mating; gestation, and lactation) in a multigenerational reproductive toxicity study.

Hematological Effects. Before the mid-1950s, chronic occupational exposure to toluene was associated with hematological effects. However, these effects are now attributed to benzene, a common contaminant of toluene at that time. More recent studies of workers exposed to toluene or to mixed solvents containing toluene have not found consistent evidence for abnormal hematological parameters. Following acute exposures, no effects on leukocyte counts were observed in volunteers exposed to 800 ppm toluene for 3 hours, and two workers accidentally exposed to about 1,862 ppm for 2–3 hours had

normal values for hematological variables. Decreased leukocyte and white blood cell counts were observed in dogs and rats repeatedly exposed to airborne toluene, but have not been observed consistently in other studies of rats and mice repeatedly exposed by inhalation or by oral administration. In one study, rats exposed to high concentrations (2,500 or 5,000 ppm) of toluene for 7 hours each day had decreased leukocyte counts following exposure; however, the leukocyte numbers generally returned to normal by the next day. The toxicological significance of a transitory decrease in numbers of leukocytes is not apparent. In chronic-duration studies, rats exposed to 100 or 300 ppm toluene had significantly reduced hematocrit levels, but no consistent effects on hematological variables were reported for mice or rats exposed to toluene at levels up to 1,200 ppm for 15 months or 2 years.

Musculoskeletal Effects. Rhabdomyolysis (an acute disease of the skeletal muscles leading to breakdown of muscle tissue, leading to release of myoglobin into the blood and urine) was reported in two case studies of chronic toluene abuse: a man who had been sniffing glue containing toluene for 18 years, and a 48-year-old man who was a chronic toluene abuser who had been inhaling one tube of toluene-containing glue per day in the month preceding admission.

No musculoskeletal effects were reported in mice or rats after inhalation exposure up to 1,200 ppm for 15 months or 2 years or oral exposure to toluene at dosage levels up to 2,500 mg/kg/day for 13 weeks. Additionally, no musculoskeletal effects were reported in F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation in a multigenerational study. However, bone mineral density and bone mineral content were significantly ($p<0.05$) decreased in the right femoral neck of mice exposed to 300 ppm toluene 6 hours/day for 8 weeks.

Hepatic Effects. Studies of chronic toluene abusers, occupationally exposed workers, and laboratory animals have provided little support for irreversible liver damage due to inhaled toluene. Some studies of workers who were occupationally exposed to average concentrations between about 30 and 350 ppm toluene reported liver effects such as increased serum levels of alkaline phosphatase (AP), but others recorded no adverse effects on serum liver enzyme levels. Results from studies of animals exposed by inhalation for acute, intermediate, or chronic durations indicate that daily 6–8-hour exposures to concentrations above 300 ppm, but not below, can lead to increased liver weights and induction of hepatic cytochrome P450 levels. There are a few reports of toluene-induced effects that may be associated with liver damage (e.g., increased serum levels of liver enzymes in rats exposed to 2,000 ppm for 48 hours, rats exposed to 3,000 ppm, 1 hour/day for 30 days, and rats exposed to 300 ppm, 6 hours/day for 4 weeks;

increased hepatic fibrosis and apoptosis in rats exposed to 3,000 ppm, 1 hour/day for 30 days or 8 hours/day, 6 hours/week for 12 weeks; and increased endoplasmic reticulum in hepatocytes after exposure of rats, mice, and rabbits to 795 ppm 8 hours/day for 7 days), but no significant histopathological liver changes or liver weight changes were observed in well-conducted chronic-duration studies in which rats and mice were exposed to concentrations as high as 1,200 ppm, 6.5 hours/day, 5 days/week for 2 years. Results from intermediate-duration oral studies in rats and mice support the idea that toluene does not cause degenerative liver effects, but, at sufficiently high doses, produces liver weight increases that are likely associated with enzyme induction.

Studies of liver effects following oral exposure in humans are limited to two case studies. The liver of an adult male who died from toluene ingestion (625 mg/kg) was found to be enlarged on autopsy; however, clinical chemistry did not reveal abnormal liver function in a 15-month-old girl following accidental ingestion of paint thinner. Evidence from intermediate-duration animal studies indicates that exposure to toluene results in increased liver weights; however, reported effective dose levels vary widely between studies, species, and sex. Increased liver weight has been reported in male mice exposed to 105 mg/kg/day in drinking water for 28 days, but not at 5, 22, or 84 mg/kg/day. Following exposure to 0, 312, 625, 1,250, 2,500, or 5,000 mg/kg/day via gavage for 12 weeks, significant increases in liver weight were observed in male and female rats exposed to ≥625 and ≥1,250 mg/kg/day, respectively, and male and female mice exposed to ≥1,250 and ≥312 mg/kg/day, respectively. No treatment-related changes in liver weight were observed in female rats exposed to 590 mg/kg/day via gavage for 6 months. No treatment-related gross or histopathological lesions of the liver were reported in any of these intermediate-duration oral studies in laboratory animals.

An acute oral study in rats reports that single gavage doses of 5,200 mg/kg produced slight degeneration of hepatocytes, mononuclear cell infiltration, increased apoptosis, and increased serum levels of AST and ALT; however, no alterations in liver weight or histology were reported in pregnant rats exposed to 1,250 mg/kg/day toluene via gavage from gestation day (GD) 16 to 19 or female mice exposed to 600 mg/kg/day toluene via gavage for 14 days. Increased hepatic cell apoptosis was also reported in rats exposed to 650 mg/kg/day via gavage for 45 days.

Renal Effects. Studies of chronic toluene abusers, occupationally exposed workers, and laboratory animals have provided little support for irreversible kidney damage due to inhaled toluene. Chronic abuse of toluene can produce acidosis, but in most cases, renal dysfunction is transient, and normal function returns when exposure ceases. Studies of workers occupationally exposed to 100–200 ppm toluene,

which assessed changes in tests of kidney function, have not shown consistent effects across studies. Animal studies indicate that inhalation of toluene causes concentration-dependent kidney damage in rats, but only after repeated exposure to concentrations ≥600 ppm for at least 6 hours/day.

The majority of oral exposure studies in animals do not report renal effects. No treatment-related changes in kidney weight were reported in female rats exposed to 590 mg/kg/day via gavage for 6 months, male and female mice exposed up to 2,500 mg/kg/day via gavage for 13 weeks, male mice exposed to 5–105 mg/kg/day in drinking water for 28 days, or female mice exposed to 600 mg/kg/day via gavage for 14 days. However, following exposure to 0, 312, 625, 1,250, 2,500, or 5,000 mg/kg/day via gavage for 12 weeks, significant increases in kidney weight were observed in male and female rats exposed to ≥625 and ≥1,250 mg/kg/day, respectively. No changes in histopathology or renal function were reported in any intermediate-duration study. However, evidence for renal pathology was reported in dams exposed to 1,250 mg/kg/day toluene via gavage from GD 16 to 19. Kidneys from toluene-exposed dams demonstrated swollen tubules, tissue adhesion to Bowman's capsule, and areas of solidification within glomeruli that were not observed in control dams. No exposure-related changes were observed in kidney weight.

Endocrine Effects. Current data do not provide consistent evidence of endocrine disruption in toluene-exposed humans. Elevated plasma levels of triiodothyronine (T3), but not free thyroxine (T4) or thyroid stimulating hormone (TSH), were observed in male printers exposed to 36 ppm toluene compared to an unexposed referent group. No change was observed in serum prolactin levels. Studies of blood levels of reproductive hormones in repeatedly exposed workers or acutely exposed human subjects have not provided strong and consistent evidence of exposure-related endocrine effects.

Similarly, evidence for endocrine effects in animals following acute- or intermediate-duration inhalation exposure to toluene is not consistent across studies and does not clearly identify toluene as an endocrine disrupting chemical. Elevated prolactin levels were reported in rats after exposure to 80 ppm toluene 6 hours/day, 5 days/week for 4 weeks or 80–1,000 ppm 6 hours/day for 3 days. However, no changes in prolactin levels were found in rats after exposure to 40–320 ppm 6 hours/day, 5 days/week for 4 weeks, 500 ppm toluene 6 hours/day for 3 days, or 1,000 ppm 6 hours/day for 5 days. No statistically significant, dose-related changes in serum levels of, luteinizing hormone (LH), follicle stimulating hormone (FSH), corticosterone levels, growth hormone, or TSH were reported in male Sprague-Dawley rats exposed up to 3,000 ppm toluene 6 hours/day for 3 days or 1,000 ppm 6 hours/day for 5 days. Increased serum adrenocorticotropic hormone (ACTH) and corticosterone levels, along with increased adrenal weight and

adrenocortical cell size, were observed in male rats exposed to 1,500 ppm 4 hours/day for 7 days. This exposure scenario was shown to cause, in companion studies, neuronal damage and an increase in glucocorticoid receptor in the hippocampus, suggesting a possible disruption in the neuroendocrine axis. However, no effects on endocrine glands (pancreas, adrenal, or thyroid) were reported in other studies of rats exposed to 200–5,000 ppm toluene for 7 hours/day for 5 weeks, F0 and F1 parental rats or F1 and F2 weanlings exposed to 100–2,000 ppm toluene for 95 days (pre-mating and mating), gestation, and lactation, mice exposed to up to 2,500 ppm for 14 weeks, rats exposed to up to 3,000 ppm for 15 weeks, or mice and rats exposed to up to 1,200 ppm for 2 years.

Limited data are available regarding endocrine effects following oral exposure. Serum corticosterone and ACTH levels were significantly elevated in male mice exposed to 105 mg/kg/day toluene in drinking water for 28 days, compared with controls. Levels were not significantly elevated following exposure to 5 or 22 mg/kg/day. Microscopic examination revealed no effects on the adrenal or thyroid glands in rats and mice administered 312–2,500 mg/kg/day toluene by gavage for 13 weeks.

Dermal Effects. Skin irritation can occur in humans and animals dermally exposed to toluene. In humans, this may be due to the degreasing action of toluene and its removal of protective skin oils. However, exposure to toluene vapors of 100–2,000 ppm for 95 days (pre-mating, mating, gestation, and lactation) in a multigenerational study had no effects on the skin in F0 and F1 parental rats or F1 and F2 weanlings. Repeated or continuous contact with undiluted toluene in guinea pigs and mice leads to swelling, inflammatory cell infiltration, and increased epidermal thickness.

Ocular Effects. Humans have reported eye irritation following exposure to toluene vapors at concentrations ≥100 ppm. This is probably the result of direct contact of toluene vapor with the outer surface of the eye and thus, is not a true systemic effect. Slight to moderately severe irritation of rabbit eyes has been reported following direct application of toluene to the conjunctiva. Reports of color vision deficits in occupationally exposed workers have been postulated to involve toluene interference with dopaminergic mechanisms of retinal cells or toxic demyelination of optic nerve fibers.

Body Weight Effects. Findings regarding body weight effects in animals following inhalation exposure are inconsistent across studies. Weight loss has been reported to occur in rats following acute-duration exposure of 1,500–2,000 ppm; and intermediate-duration exposure (3–23 weeks) to toluene concentrations ranging from 200 to 12,000 ppm. In mice, weight loss has been reported following intermediate-duration exposure (8–14 weeks) to toluene concentrations ranging from 100 to 12,000 ppm.

In contrast, no exposure-related effects on body weights were observed in rats or mice following intermediate-duration exposure (20–95 days) at concentrations ranging from 1,000 to 6,000 ppm or chronic-duration exposure (2 years) up to 1,200 ppm.

The majority of oral exposure animal studies do not report body weight effects. No dose-related changes in body weight were reported in male rats following single gavage administration of up to 1,000 mg/kg, female mice exposed to 600 mg/kg/day via gavage for 14 days, male mice administered 5–105 mg/kg/day toluene in their drinking water for 28 days, or female rats and female mice given gavage doses of up to 2,500 mg/kg/day for 13 weeks. However, body weights were 16% lower in male mice given 1,250 mg/kg/day and 19% lower in male rats given 2,500 mg/kg/day by gavage for 13 weeks. Maternal weight gain was 24% lower in rats given 520 mg/kg/day toluene by gavage from GD 6 to 19, compared with control rats, but there was no change in maternal body weight gain in rat dams exposed to 1,250 mg/kg/day toluene via gavage from GD 16 to 19.

Immunological and Lymphoreticular Effects. Only limited data are available on the immunological effects of toluene in humans. These studies do not identify consistent or strong evidence for toluene effects on immune system end points such as counts of blood lymphocytes or levels of blood immunoglobulins or development of autoimmune disorders.

In animals, there is evidence that toluene may lead to immune depression. A series of studies evaluated immune end points in male CD-1 mice (5/group) administered toluene in their drinking water for 28 days at concentrations of 0, 5, 22, or 105 mg/kg/day or 0, 22, or 84 mg/kg/day. In one study, significantly decreased thymus weight and significantly depressed immune responses were observed in all *in vitro* immune assays (mitogen-stimulated lymphocyte proliferation, mixed lymphocyte reaction, interleukin 2 [IL-2] production assay, and antibody plaque-forming cell [PFC] response) at 105 mg/kg/day, compared with controls. IL-2 production and mitogen-stimulated lymphocyte proliferation were also significantly increased at 22 mg/kg/day, compared with controls. Significantly depressed immune responses were observed in the PFC assay and mixed lymphocyte reaction at 84 mg/kg/day. The mixed lymphocyte reaction was also significantly depressed at 22 mg/kg/day. In another study, the IL-2 production assay was significantly depressed at 105 mg/kg/day. Taken together, these studies consistently reported diminished immune responses in multiple *in vitro* immune assays following *in vivo* exposure to 84–105 mg/kg/day in drinking water for 28 days, compared with controls. A couple of immune assays were altered at 22 mg/kg/day, but findings were not consistent between the Hsieh studies. Additionally, the antibody PFC assay was significantly altered at 84 and 105 mg/kg/day, but not at 22 mg/kg/day. The

PFC *in vitro* assay is considered the most predictive assay of impaired immune function. Collectively, results from these studies support a no-observed-adverse-effect level (NOAEL) of 22 mg/kg/day for immune effects. Decreased resistance to mortality from respiratory infection by *Streptococcus zooepidemicus* was observed in a study of mice exposed for 3 hours to toluene concentrations as low as 2.5 ppm, but not 1 ppm. However, in an acute oral study, exposure to 600 mg/kg/day via gavage for 14 days did not diminish immune response in *in vitro* immune assays or decrease host resistance to *Listeria monocytogenes, Streptococcus pneumoniae, Plasmodium yoelii*, B16F10 melanoma cells, or PYB6 fibrosarcoma in female mice, compared with controls.

No evidence for exposure-related adverse changes in weight or histology of the spleen or thymus has been reported in animals exposed by inhalation for intermediate or chronic durations. Thymus weight was significantly decreased in male mice exposed to 105 mg/kg/day via gavage for 28 days, but not to 5–84 mg/kg/day. No changes in spleen weight were observed at any dose. No changes in thymus or spleen weight were observed in female mice exposed to 600 mg/kg/day via gavage for 14 days or rats or mice exposed up to 2,500 mg/kg/day via gavage for 13 weeks. No gross or histopathological lesions of the spleen or thymus were reported in any oral study.

Neurological Effects. Dysfunction of the central nervous system is a critical human health concern following acute, intermediate, or chronic inhalation exposure to toluene. Chronic toluene abuse in humans has been associated with neurotoxic symptoms, narcosis, permanent damage to the central nervous system, and death. Self-reported neurological symptoms, reduced ability in tests of cognitive and neuromuscular function, and hearing and color vision loss have been observed in humans occupationally exposed to average concentrations ranging from 35 to 200 ppm; several occupational studies identify NOAELs for these effects in the range of 20–187 ppm toluene. Performance deficits in tests of neurobehavior have also been observed in volunteers acutely exposed to controlled concentrations >50 ppm.

Numerous studies in animals have also reported clinical signs of neurotoxicity and neurobehavioral alterations following acute, intermediate, or chronic inhalation exposure to toluene. Consistently reported effects following acute exposure include overt signs of neurotoxicity (ataxia, tremors, inability to walk); increased, followed by decreased, locomotor activity at ≥500 ppm; impaired learning and/or memory at 125–4,000 ppm; and impaired motor coordination and reflexes at ≥100 ppm. However, studies of rodents exposed for intermediate durations to concentrations as high as 1,000 ppm have not found strong and consistent evidence for exposure-related changes for these neurological end points. Following repeated

abuse-like exposures (>1,000 ppm), neurobehavioral alterations have been observed in several animal studies.

Various other neurological effects have also been reported in animal inhalation studies. Hearing loss in animals has been observed following acute- and intermediate-duration exposure to toluene at concentrations of ≥250 ppm in guinea pigs and ≥1,000 ppm in rats. Observed hearing loss may not be solely due to neurological damage, as animal studies indicate that exposure to 500–2,000 ppm damages the cells in the inner ear (cochlea) that are responsible for amplifying incoming sound waves prior to initiation of the nerve signal from the ear to the brain. Other effects that have been reported include alterations in visual-evoked brain potentials (VEPs) or electroretinograms (ERGs), altered pain perception, decreased olfactory sensitivity, altered sleep patterns, altered brain weight and volume in rats, altered levels of glial fibrillary acidic protein (GFAP) and markers of oxidative stress, and altered levels of neurotransmitters, precursors, and receptors.

Limited data are available regarding neurological effects following oral exposure. Neurological effects were reported in three case reports of toluene ingestion: severe depression of central nervous system function was the probable cause of death for a 51-year-old man who ingested approximately 60 mL (625 mg/kg) of toluene; a man who accidently ingested 30 mL of an organic solvent containing toluene and other chemicals was drowsy and complained of dizziness; and depressed consciousness, lethargy, hypotonia, and nystagmus were observed in a 15-month-old girl following accidental ingestion of paint thinner. Effects reported following acute-duration oral exposure in animals include transient increases in motor activity in rats; decreased in the flash-evoked brain potential (FEP) wave pattern amplitudes in male rats; and outer hair cell (OHC) loss in the cochlea of rats. However, no changes in brain weight or histology were reported in female mice exposed to 600 mg/kg/day via gavage for 14 days, compared with controls. Effects reported following intermediate-duration oral exposure in animals include regional specific neurotransmitter alterations; increased relative brain weights in male and female rats and male, but not female, mice; cellular necrosis in the hippocampus and cerebellum of male and female rats; and overt signs of neurotoxicity.

Reproductive Effects. Current data do not provide convincing evidence that acute or repeated inhalation exposure to toluene may cause reproductive effects in humans. Limited evidence in humans indicates that occupational exposure to toluene (and other solvents) may lead to an increased incidence of spontaneous abortion or decreased fecundity in female workers. One study reports increased risk of preterm birth with increasing environmental toluene exposure; however, concurrent exposure to multiple

pollutants limits the conclusions that can be drawn from this study. A few studies in animals exposed to toluene via inhalation at concentrations ≥2,000 ppm reported effects on male and female reproductive tissues, including abundant vacuoles, lytic areas, and mitochondrial degeneration in the antral follicles of the ovaries of female rats and reduced sperm count, motility, and quality and altered reproductive organ weight and histology in male rats. However, changes in sperm count and epididymis weight were not accompanied by any change in indices of reproductive performance (e.g., fertility) in male rats exposed to 2,000 ppm for 60 days before mating. The majority of animal studies provide little evidence for toluene reproductive toxicity. Studies in rats exposed repeatedly by inhalation to toluene, including a 2-generation reproductive toxicity study, have shown no evidence of adverse effects on mating or fertility at tested concentrations as high as 1,200–2,000 ppm. In addition, the majority of numerous gestational exposure studies in rodents reported no exposure-related changes in reproductive indices.

Available data from oral exposure studies in animals do not provide evidence of reproductive effects following toluene exposure. No significant differences in the mean number of implantations per dam, corpora lutea per dam, live fetuses per litter, total number of resorptions per dam, and/or pre- or post-implantation loss were reported when rats and mice were exposed to toluene during gestation. Increased relative testicular weights were reported in male mice exposed to 1,250 and 2,500 mg/kg/day by gavage for 13 weeks. However, no effects on the weight of the prostate, testes, uterus, or ovaries were observed in rats and female mice exposed to 312–2,500 mg/kg/day. Reproductive performance was not evaluated in these 13-week studies.

Developmental Effects. There are a number of published reports of birth defects, similar to those associated with fetal alcohol syndrome, that have been described in children born to women who intentionally inhaled large quantities of toluene or other organic solvents during pregnancy. Defects described include microcephaly, central nervous system dysfunction, growth deficiency, cranofacial and limb abnormalities, and reversible renal tubular acidosis. Studies of women exposed during pregnancy to much lower concentrations of toluene in the workplace are restricted to a retrospective study of 14 women in Finland occupationally exposed to mixed solvents that suggested that solvent exposure may increase risk for central nervous system anomalies and neural tube closure defects.

The reports of birth defects in solvent abusers suggest that high-level exposure to toluene during pregnancy can be toxic to the developing fetus. The available human data, however, do not establish causality between low-level or occupational exposure to toluene and birth defects, because of the small sample size and the mixed solvent exposure experienced by the subjects, the lack of other studies of

possible birth defects in children of occupationally exposed women, and the likelihood that the high exposure levels experienced by pregnant solvent abusers (4,000–12,000 ppm) overwhelm maternal protection of the developing fetus from absorbed toluene. Experiments with pregnant mice demonstrated that 10-minute exposures to 2,000 ppm resulted in low uptake of toluene into fetal tissue and suggest that, at lower exposure levels, absorbed toluene is preferentially distributed to maternal adipose tissue before distribution to the developing fetus.

A number of developmental toxicity studies with rats, mice, and rabbits involving toluene exposure by inhalation during gestation have been conducted to further describe developmentally toxic effects from toluene and exposure-response relationships. The results indicate that toluene did not cause maternal or developmental toxic effects in animals at exposure levels <1,000 ppm administered for 6–7 hours/day during gestation. Predominant effects reported at concentrations ranging from 1,000 to 3,000 ppm include retarded fetal growth and skeletal development and altered development of behavior in offspring; these effects were almost always accompanied by signs of maternal toxicity. Other animal studies reported that continuous, 24-hour/day exposure during gestation caused maternal body weight depression and effects on fetuses including depressed body weight and delayed skeletal ossification at toluene concentrations as low as 133–399 ppm in rats, mice, and rabbits. Impaired learning and memory, increased malformations, and fetal death have been observed when animals were exposed during gestation to higher concentrations modeling solvent abuse (8,000–16,000 ppm, 15–30 minutes/day).

In animal studies of oral exposure during gestation, toluene was not a developmental toxicant when administered orally at 1,800 or 2,350 mg/kg/day to pregnant mice during the period of organogenesis in two developmental screening studies. In a comprehensive developmental toxicity study in rats, a statistically significant increase in the incidence of a dilated renal pelvis in the left kidney was observed in fetuses from dams exposed to 1,250 mg/kg on GDs 16–19 via gavage, compared with controls. No changes were observed in any other developmental end point. In other studies, exposure of pregnant rats to gavage doses of 650 mg/kg/day toluene in corn oil on GDs 6–19 produced offspring with decreased body weights, delayed ossification, smaller brain volumes, decreased forebrain myelination per cell, and decreased cortical cell proliferation and migration, compared with controls.

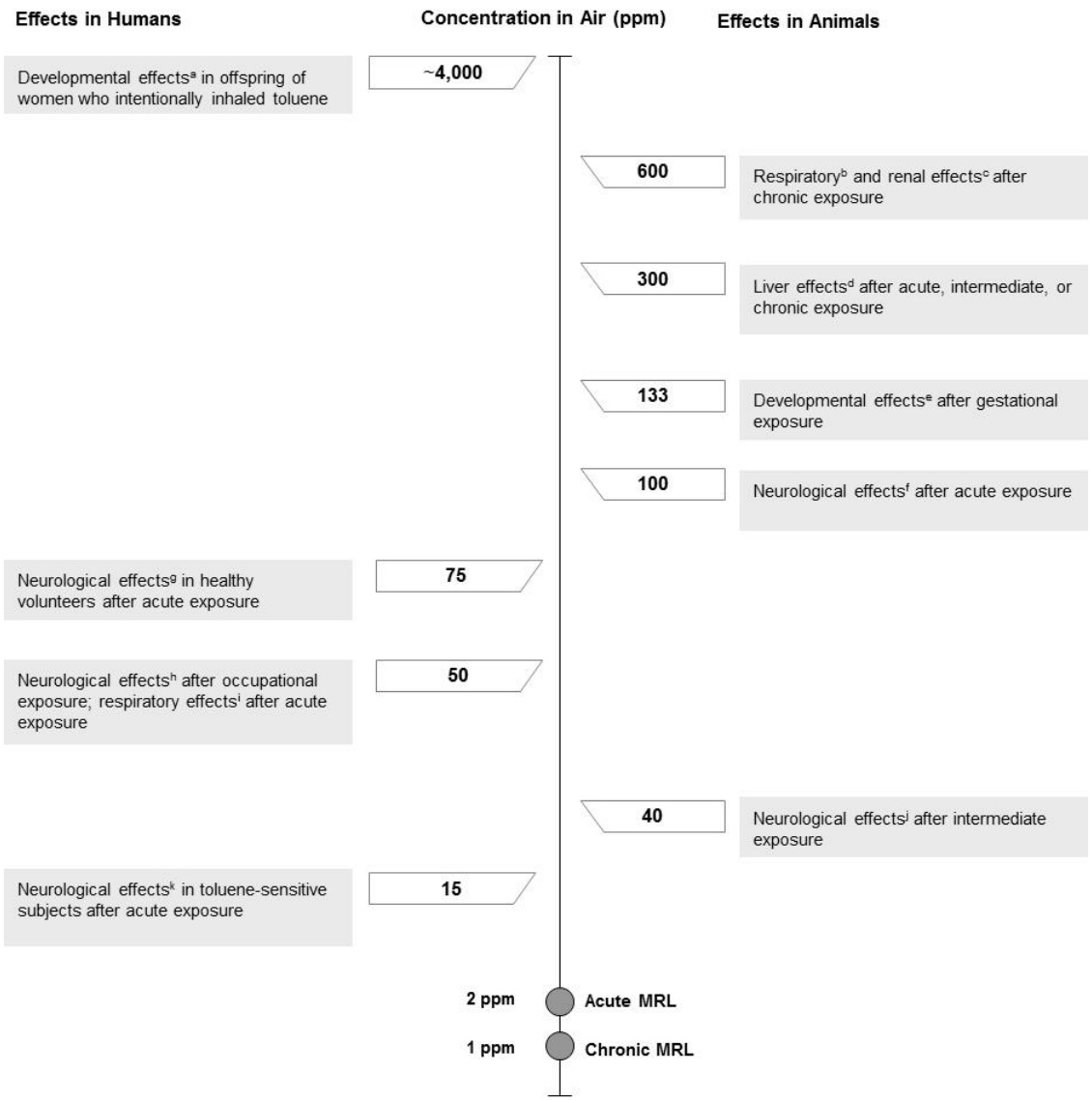
Performance deficits in a few neurobehavioral tests were observed in one study in offspring of pregnant mouse dams exposed by inhalation to 2,000 ppm, but not 200 or 400 ppm, for 60 minutes 3 times/day on GDs 12–17. Performance deficits were not observed in offspring of pregnant rat dams exposed by inhalation to up to 2,000 ppm for 6 hours/day during gestation. Drinking water exposure during gestation

and lactation at doses of 106 mg/kg/day resulted in changes in postweaning open-field locomotor activity in rat offspring.

Cancer. Human and animal studies generally do not support a concern for the carcinogenicity of toluene. Numerous human epidemiology studies were located that assessed toluene exposure as a possible risk factor for cancer. Five of the studies examined workers exposed predominantly to toluene, whereas the remainder of the human studies primarily involved subjects exposed to mixtures of solvents including toluene. Cancers of most sites were not significantly associated with toluene exposure, and there was weak consistency in the findings of those studies that did find association of a particular cancer type with toluene exposure. The information from these studies is inadequate to assess the carcinogenic potential of toluene, predominantly because of the lack of consistent findings across the studies and the likelihood that many of the studied groups were exposed to multiple chemicals. The validated animal inhalation bioassays were negative; however, one available oral study showed a nondose-related increase in a variety of tumors. Dermally administered toluene markedly inhibits skin tumorigenesis in the two-stage mouse model utilizing phorbol-12-myristate-13-acetate (PMA) as a promoter. The reduction in tumorigenesis was observed in mice initiated with dermal applications of benzo(a)pyrene or 7,12-dimethylbenz(a)anthracene. Thus, the data do not support a firm conclusion regarding the carcinogenicity of toluene. As such, the EPA determined that there is inadequate information to assess the carcinogenic potential of toluene, IARC determined that toluene is not classifiable as to its carcinogenicity in humans (Group 3), and ACGIH determined that toluene is not classifiable as a human carcinogen (A4). The NTP has not considered the carcinogenic potential of toluene.

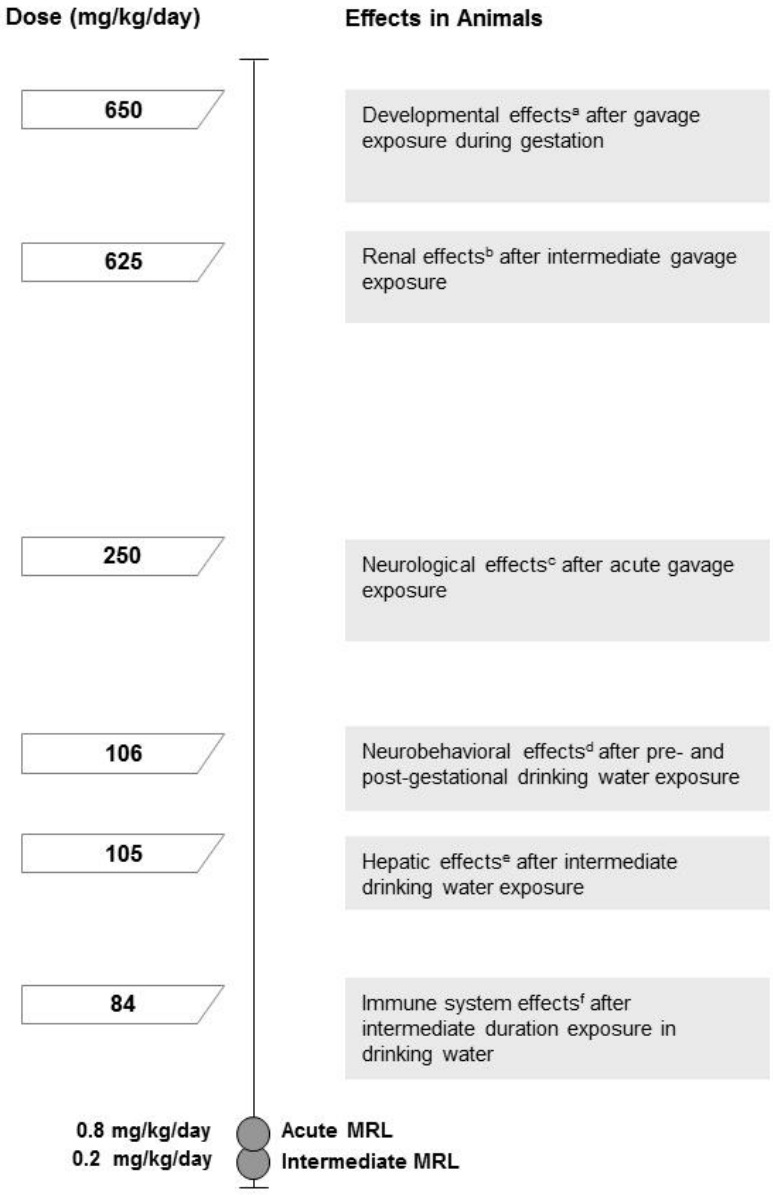
Major health effects of toluene inhalation in humans and animals and ingestion in animals and the lowest concentrations at which these effects have been observed are shown in Figures 2-1 and 2-2. An estimate of exposure levels posing minimal risk to humans (MRL) are also presented in these figures. An MRL is an estimate of the daily human exposure that is likely to be safe over a certain period of exposure. MRLs are not intended to define clean-up or action levels, but are intended only to serve as a screening tool to help public health professionals decide where to look more closely. Therefore, MRLs are set at levels well below those where effects have been observed.

Figure 2-1. Health Effects of Breathing Toluene



[a]Birth defects similar to fetal alcohol syndrome
[b]Nasal lesions
[c]Nephropathy
[d]Increased liver weight, induction of hepatic P450 levels, increased serum levels of liver enzymes
[e]Depressed fetal body weight and delayed skeletal ossification
[f] Increased locomotor activity
[g] Impairments in psychomotor testing
[h] Altered measures of visual and auditory electrophysiology
[i] Irritation of nose and throat
[j] Altered open-field behavior
[k] Impairments in neuropsychological testing

Figure 2-2. Health Effects of Ingesting Toluene



[a]Decreased body weights, delayed ossification, smaller brain volumes, decreased forebrain myelination per cell, and decreased cortical cell proliferation and migration

[b]Increased kidney weight

[c]Altered measures of visual electrophysiology

[d]Increased open-field activity (lack of habituation)

[e]Increased liver weight

[f]Diminished immune responses in multiple *in vitro* immune assays following *in vivo* exposure

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been established for toluene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Adverse effects on the nervous system are critical effects of concern from acute, intermediate, or chronic exposure to toluene. Acute exposure is associated with reversible neurological symptoms progressing from fatigue, headaches, and decreased manual dexterity to narcosis with increasing exposure levels. Reversible neurological impairment from acute exposure likely involves the direct interaction of toluene with nervous system membranes. Degenerative changes in white matter regions of the brain have been correlated with the severity of persistent neurological impairment in individuals who abused solvents and have repeatedly inhaled toluene at high exposure levels (4,000–12,000 ppm). Results from studies of groups of occupationally exposed workers suggest that chronic exposure to toluene at lower exposure levels (from about 50 to 200 ppm) can produce subtle changes in neurological functions including cognitive and neuromuscular performance, hearing, and color discrimination. Supporting data come from studies of toluene-exposed animals showing changes in behavior, hearing loss, and subtle changes in brain structure, electrophysiology, and levels of neurotransmitters.

Other effects of concern include immune system effects, liver effects, kidney effects, and developmental effects. Evidence from a few animal studies suggests that repeated exposure to toluene can suppress the

immune system, although current data from human studies are limited and inconclusive. Various reports indicate hepatic and renal effects following inhalation and oral exposure to toluene; however, there is little support for irreversible damage to the liver or kidney. Case reports of birth defects in children of mothers who abused toluene during pregnancy suggest that exposure to high levels of toluene may be toxic to the developing fetus. Results from animal studies indicate that toluene is not a teratogenic agent, but can retard fetal growth and skeletal development, and adversely influence behavior of offspring at exposure levels that produce maternal toxicity.

Available evidence does not support adverse effects on reproductive performance as a noncancer health effect of concern from toluene exposure.

Issues relevant to children are explicitly discussed in Section 3.7, Children's Susceptibility and Section 6.6, Exposures of Children.

Inhalation MRLs

- An MRL of 2 ppm (7.6 mg/m^3) has been derived for acute-duration (14 days or less) inhalation exposure to toluene.

This MRL is based on a study by Little et al. (1999) in which the effects of toluene on human subjects with a history of solvent exposure and adverse reactions to toluene (i.e., clinically sensitive to toluene) were assessed in a battery of neuropsychological tests prior to and after a 20-minute exposure to 15 ppm toluene (see Appendix A). The battery of tests included immediate and delayed prose memory, reaction time, letter cancellations, digit symbol, focal length, and STROOP color and color-word tasks. Statistically significant ($p<0.05$) impairments were measured in immediate and delayed prose memory (number of items recalled decreased 31%), the digit symbol test (number of correct items decreased 11%), and the letter cancellation test (percent correct decreased 5%) following a 15-minute exposure to 15 ppm toluene, compared with pre-exposure scores. A near-significant 15% increase in reaction time was also observed ($p=0.06$). No significant difference between pre- and post-exposure values was found for focal length or the STROOP tests. The minimally adverse lowest-observed-adverse-effect level (LOAEL) of 15 ppm was divided by an uncertainty factor of 9 (3 for use of a minimally adverse LOAEL and 3 to account for human variability [a full uncertainty factor of 10 is not necessary as the observed effects were noted in a susceptible/sensitive group of individuals]) to derive the MRL of 2 ppm. More details of the development of this MRL can be found in Appendix A.

- No MRL has been derived for intermediate-duration (15–364 days) inhalation exposure to toluene.

No data were considered suitable for use in deriving an intermediate-duration MRL for inhalation exposures. ATSDR believes that the chronic inhalation MRL would also be protective for intermediate-duration exposures.

- An MRL of 1 ppm (3.8 mg/m^3) was derived for chronic-duration (365 days or more) inhalation exposure to toluene.

The chronic inhalation MRL is based on a NOAEL of 45 ppm toluene for neurological effects based on a series of studies by the same group of investigators assessing subjective neurological symptoms, performance on psychomotor tasks, color vision, and hearing in groups of German photogravure printers employed for an average duration of 13.5 years (Schäper et al. 2003, 2004, 2008; Seeber et al. 2004, 2005; Zupanic et al. 2002). These studies compared neurological end points in high-exposure printers (n=106–181) and low-exposure tend-processors (n=86–152). Current toluene air exposure levels for printers and end-processors were 24.6–26 and 3–3.5 ppm, respectively (measured twice yearly from 1996 to 2001). Historical exposure levels for printers prior to 1995 and prior to 1975 were 40 and 140 ppm, respectively. Historical exposure levels for end-processors prior to 1995 and prior to 1975 were 5 and 40 ppm, respectively. Using job history and current exposure and historical exposure levels, individual time-weighted average (TWA) exposure levels were calculated. The average TWA levels for printers and end-processors were calculated to be 45 and 10 ppm for subjects included in analyses by Schäper et al. (2003, 2008), 45 and 9 ppm for subjects included in analyses by Seeber et al. (2004, 2005) and Zupanic et al. (2002), and 43 and 9 ppm for subjects included in analyses by Schäper et al. (2004). Schäper et al. (2003, 2008) did not find any statistically significant differences in audiometric readings from four readings over 5 years in 181 printers, compared with 152 end-processors; Schäper et al. (2004) did not find any differences in color vision assessed 4 times over 5 years in 154 printers, compared with 124 end-processors; and Seeber et al. (2004, 2005) and Zupanic et al. (2002) did not find any increase in subjective neurological complaints or decreased performance in psychomotor tasks in 106–154 printers, compared with 86–124 end-processors. The NOAEL of 45 ppm was adjusted for continuous exposure (45 ppm x 5 days/7 days x 8 hours/24 hours = 10.7 ppm) and was divided by an uncertainty factor of 10 to account for human variability to derive the MRL of 1 ppm.

Most of the data on health effects in humans chronically exposed to toluene come from occupational studies or medical reports of solvent abusers. In both situations, concurrent exposure to other chemicals

can limit the usefulness of the data for development of guidelines or standards. In addition, there are other confounding variables, especially in the occupational setting, such as alcohol consumption patterns, employment history, diet, use of medications, noise, and fluctuations in atmospheric toluene levels during different portions of the day, all of which complicate evaluation of dose-response patterns. These complexities were considered in selecting the studies for derivation of the MRL (see Appendix A for more details).

EPA (2005a) has recommended a similar chronic RfC of 5 mg/m^3 (1.33 ppm) based on the arithmetic mean (34 ppm) of the NOAELs from a subset of the highest quality studies investigating neurological effects in workers occupationally exposed predominantly to toluene (Abbate et al. 1993; Boey et al. 1997; Cavalleri et al. 2000; Eller et al. 1999; Foo et al. 1990; Murata et al. 1993; Nakatsuka et al. 1992; Neubert et al. 2001; Vrca et al. 1995; Zavalic et al. 1998a). ACGIH (2007) has recommended a Threshold Limit Value (TLV) of 20 ppm toluene based on subclinical changes in blue-yellow color vision and the potential for spontaneous abortion in female workers (Campagna et al. 2001; Cavalleri et al. 2000; Ng et al. 1992b). This value is designed to be protective for healthy adult workers exposed 8 hours/day, 5 days/week for up to 45 years. Adjusting the value for a continuous exposure lasting up to 70 years yields a value of 4 ppm (25 ppm x 5 days/7 days x 8 hours/24 hours x 45 years/70 years = 4 ppm). This figure is slightly higher than the current chronic-duration MRL, but does not include an uncertainty factor to protect susceptible populations. Use of an uncertainty factor of 10 (10 for human variability) would arrive at a value to 0.4 ppm, which is slightly lower than the current MRL.

Oral MRLs

- An MRL of 0.8 mg/kg has been derived for acute (14 days or less) oral exposure to toluene.

This MRL was based on a LOAEL of 250 mg/kg from a study of FEP wave forms in male Long-Evans rats administered doses of 0, 250, 500, or 1,000 mg/kg toluene by gavage (Dyer et al. 1988). FEP tests were administered 45 minutes later as a test of the ability of the nervous system to process visual information. The amplitude of the N3 peak of the FEP was decreased by toluene exposure at all doses ($p<0.0001$). This decrease in peak amplitude was not dose-related. Dyer et al. (1988) also carried out a time-course study in which toluene was administered to male Long-Evans rats (16 per group) at doses of 0 and 500 mg/kg by gavage, and FEP tests were performed 4, 8, 16, and 30 hours later. In the time course study, 500 mg/kg also decreased the amplitude of the FEP; at this dose, little change in magnitude of peak N3 depression had occurred 8 hours posttreatment; by 16 hours, recovery was complete. The LOAEL of

250 mg/kg was divided by an uncertainty factor of 300 (3 for use of a minimally adverse LOAEL, 10 for interspecies extrapolation, and 10 for intraspecies variability) to derive the MRL of 0.8 mg/kg. More details of the development of this MRL can be found in Appendix A.

- An MRL of 0.2 mg/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to toluene.

This MRL was based on a NOAEL of 22 mg/kg/day from a series of studies evaluating immune end points in male CD-1 mice administered toluene in their drinking water for 28 days at concentrations of 0, 5, 22, or 105 mg/kg/day (Hsieh et al. 1989, 1991) or 0, 22, or 84 mg/kg/day (Hsieh et al. 1990a). In Hsieh et al. (1989, 1990a), rats were weighed, sacrificed, and examined for gross pathological lesions at 28 days. Spleen and thymus were weighed and hematology was performed. Spleens were assessed for cellularity, and splenocytes were used in *in vitro* immune assays (mitogen-stimulated lymphocyte proliferation, mixed lymphocyte reaction, IL-2 production assay, and antibody PFC response). Hsieh et al. (1990a) also measured the *in vitro* cell-mediated cytolysis response. In Hsieh et al. (1991), immune function was only assessed using the IL-2 assay in cultured splenocytes. In Hsieh et al. (1989), significantly decreased thymus weight and significantly depressed immune responses were observed in all *in vitro* immune assays at 105 mg/kg/day, compared with controls. IL-2 production and mitogen-stimulated lymphocyte proliferation were also significantly increased at 22 mg/kg/day compared with controls. In Hsieh et al. (1990a), significantly depressed immune responses were observed in the PFC assay and mixed lymphocyte reaction at 84 mg/kg/day. The mixed lymphocyte reaction was also significantly depressed at 22 mg/kg/day. In Hsieh et al. (1991), the IL-2 production assay was significantly depressed at 105 mg/kg/day. Taken together, these studies consistently reported diminished immune responses in multiple *in vitro* immune assays following *in vivo* exposure to 84–105 mg/kg/day in drinking water for 28 days, compared with controls. A couple of immune assays were altered at 22 mg/kg/day, but findings were not consistent between the three Hsieh studies. Additionally, the antibody PFC assay was significantly altered at 84 and 105 mg/kg/day, but not at 22 mg/kg/day (Hsieh et al. 1989, 1990a). The PFC *in vitro* assay is considered the most predictive assay of impaired immune function (Luster et al. 1992). Collectively, results from these studies support a NOAEL of 22 mg/kg/day for immune depression. The NOAEL of 22 mg/kg/day was divided by an uncertainty factor of 100 (10 for interspecies extrapolation and 10 for intraspecies variability) to derive the MRL of 0.2 mg/kg/day. More details of the development of this MRL (including consideration of other effects as bases of the intermediate-duration oral MRL) can be found in Appendix A.

- No MRL was derived for chronic-duration (365 days or more) oral exposures because there were no suitable data for toluene.

EPA (2005a) has recommended a chronic oral reference dose (RfD) of 0.08 mg/kg/day based on a benchmark dose limit (BMDL) of 238 mg/kg/day for increased kidney weight in male rats from the 13-week NTP (1990) study. The derivation included an uncertainty factor of 3,000 (10 for interspecies extrapolation, 10 for intraspecies variability, 10 for use of a subchronic study, and 3 for database uncertainties). If the UF for subchronic to chronic is removed, the value is 0.8 mg/kg/day, which is well within an order of magnitude of the derived intermediate MRL of 0.2 mg/kg/day.