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# Reproductive and Developmental Toxicity of Toluene: A Review

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**Toluene is a widely used industrial solvent, and humans may also have high exposures to toluene from the deliberate inhalation ("sniffing") of paint reducer, paint thinner, or paint for their narcotic effects. A number of case reports describe neonatal effects that have been attributed to toluene abuse during pregnancy. These effects may include intrauterine growth retardation, premature delivery, congenital malformations, and postnatal developmental retardation. The possibility of exposures to other fetotoxic agents, either as impurities or admixtures in toluene-containing products, or by deliberate or accidental exposures to other chemicals or drugs, cannot be excluded in these cases. The fetotoxic effects of toluene have been demonstrated in controlled studies in animals and are comparable to those observed in 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 some evidence for effects on postnatal physical and possibly neurobehavioral development. Estimated daily exposures from experimental studies in animals are compared to estimated human dailly intakes at the occupational permissible exposure level and at the level reported to produce euphoria in humans. Acceptable human intakes under California's Proposition 65 and under U.S. Environmental Protection Agency procedures are discussed.**

## Introduction

A subject of current concern to many people is environmental and occupational exposures to agents that may cause reproductive and developmental toxicity. This is evidenced in California by the passage of Proposition 65 (the Safe Drinking Water and Toxics Enforcement Act of 1986), which requires regulation of agents known to the State to be reproductive/developmental toxicants (*1*). The evidence for reproductive/developmental toxicity of toluene in animals and humans is reviewed here. Exposures to high levels of toluene occur through the practice of deliberate inhalation ("sniffing") for the narcotic effects of the chemical (*2,3*). Sniffing of toluene by pregnant women has also resulted in prenatal toluene exposure in human infants, and there are a number of case reports of effects such as intrauterine growth retardation among infants of mothers with such exposures (Table 1). Therefore, reason for concern exists about the potential reproductive/developmental toxicity of toluene.

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## Production, Use, and Exposure to Toluene

Toluene (toluol; methylbenzene; phenylmethane), an aromatic hydrocarbon similar to benzene, is used mainly (92%) as a component of gasoline, which contains 5 to 7% toluene by weight (*4,5*). Production in the U.S. was $6.9 \times 10^9$ lb in 1988 (*6*), and 0.1% of this amount (approximately 7 million lb) was released into the environment in California during the same year (*7*). Toluene is used in the production of a number of industrial chemicals (benzene, toluene diisocyanate, phenol, benzyl and benzoyl derivatives, benzoic acid, toluene sulfonates, nitrotoluenes, vinyl toluene, and saccharin) and is a byproduct of styrene production and coke-oven operations (*4,8*). Toluene is also used as a solvent for paints, lacquers, and adhesives.

Inhalation of airborne toluene is the main source of human exposures, which may occur during the production, transport, and use of gasoline or toluene or by deliberate inhalation. The greatest risk of accidental exposures to toluene are likely to occur among paint workers, dye makers, and workers in the chemical and petrochemical industry. Consumer exposure may occur through the use of toluene-containing products such as gasoline, cosmetics, rubber cement, nail polish, stain removers, paint brush cleaners, fabric dyes, inks, adhesives, and cigarette smoke (*4*). In addition, the practice of intentional inhalation exposure for narcotic effects may produce prolonged exposures to greater than

**Table 1. Studies or reports of reproductive and/or developmental toxicity of toluene in humans.**

| Authors | Type of study | Evaluated population | Route of exposure | Adverse effects |
|---|---|---|---|---|
| Epidemiological study | | | | |
| McDonald et al., 1987 (*14*) | Case-referent study of occupationally exposed women | 301 infants with a congenital deformity paired with 301 normal infants | Occupational exposures of the mothers to chemicals | Kidney/urinary, gastrointestinal and cardiac anomalies |
| Case reports | | | | |
| Hersh, 1989 (*15*) | Case reports: developmental | Offspring (3) of three women exposed during pregnancy | Sniffing of paint reducer (all cases) and paint (2 cases) | Premature delivery, malformations, retarded physical and cognitive development |
| Goodwin, 1988 (*16*) | Case reports: developmental | Offspring (5) of five women exposed during pregnancy | Sniffing of paint | IUGR,[a] premature delivery, malformations, retarded cognitive and motor development |
| Hersh et al., 1985 (*17*) | Case reports: developmental | Offspring (2) of two women exposed during pregnancy | Sniffing of toluene paint reducer | IUGR, malfomations, retarded physical and cognitive development |
| Suzuki et al., 1983 (*3*) | Case report: adult male | Single adult male | Sniffing of thinner (toluene) | Testicular atrophy, suppressed spermatogenesis |

[a]IUGR, intrauterine growth retardation.

500 ppm, the level at which narcotic effects have been reported (*9*). The current permissible exposure limit (PEL) for toluene in air established by the U.S. Occupational Safety and Health Administration (OSHA) and by the California Division of Occupational Safety and Health is 100 ppm (375 mg/m$^3$). This value refers to a time-weighted average exposure over an 8-hr work day.

Environmental release of toluene may result from automobile exhaust, the production and use of toluene-containing products, or contamination from disposal of toluene or toluene-containing products at hazardous waste sites. During a 1988 EPA survey of hazardous waste sites, toluene was detected at levels of 7.5 ppb in surface waters, 21 ppb in groundwaters, and 77 ppb in soil (*10*). Toluene has not been observed to be a major contaminant in food or drinking water but is known to be an indoor air pollutant. Atmospheric contamination of toluene has usually been found to be at levels lower than 1 ppm (*4*). Toluene in the environment is rapidly biodegraded and does not tend to bioaccumulate.

# Absorption, Metabolism, and Toxicity of Toluene

During prolonged inhalation exposures to toluene, 75 to 80% of the inhaled dose is initially absorbed, dropping to approximately 50% absorption 2 to 3 hr later (*9*). Between 4 and 18% of toluene is expired unchanged through the lungs, and < 1% is excreted unchanged in the urine (*9,11*). Absorption of ingested toluene is reported to be up to 100% (*11*). Toluene is metabolized primarily in the liver by pathways that are similar in humans and in other mammalian species. The half-life of elimination of toluene from blood in humans is approximately 3.4 hr, compared to 1 hr in rats and mice (*9*).

The most commonly recognized toxic effect of toluene is neurotoxicity, with effects on liver, heart, and kidney also having been reported (*11–13*). The toxic effects of toluene do not appear to be mediated through a biologically active metabolite.

# Review of Reproductive/Developmental Toxicity in Humans

Reviews of studies of reproductive and developmental toxicity in both humans and animals are based on literature identified by means of computerized searches of relevant data bases. The designs and end points of human epidemiological studies and case reports identified in this way are summarized in Table 1.

## Epidemiological Studies

Increased incidences of urogenital, gastrointestinal, and cardiac anomalies among offspring of toluene-exposed mothers were indicated by a case-referant study of women with occupational exposures to organic solvents (toluene and other aromatic hydrocarbons) (*14*). Although the authors considered toluene the most likely causative agent, concurrent exposures to other solvents limit the confidence that can be placed in this finding.

## Case Reports

Deliberate inhalation by pregnant women of products such as paint reducer, paint thinner, and paint, which are reported by the manufacturers (personal communication) to contain as much as 99% toluene, is associated with a number of case reports of adverse reproductive outcome (*15–17*). A frequently reported effect is intrauterine growth retardation, which has been recorded in seven offspring of women who abused toluene-containing products during pregnancy (*16,17*). Cases of premature delivery (*15,16*), congenital cranio-facial, limb, cardiac, renal, and central nervous system (CNS) malformations (*15–17*), and a syndrome considered by the authors to be similar to fetal alcohol syndrome (*16*) have also been reported, as were adverse effects on postnatal neurobehavioral development (*15–17*). The durations and levels of exposure required to produce these effects are not known, although available information on toluene abuse in adults suggests that exposures typically last for 6 to 7 hr/session (*2*). Supporting the conclusion that major exposures to toluene were occurring, a number of women had renal tubular acidosis, a rare condition associated specifically with toluene (*16*).

These cases provide evidence of an association between prenatal exposures to toluene and developmental toxicity in humans. This evidence is limited, however, by the potentially confounding effects of other fetotoxic agents (e.g., tobacco smoke, alcohol) to which the subjects may have been exposed.

Also of concern are exposures to other potentially fetotoxic substances that occur as deliberate or accidental admixtures in the toluene-containing products.

There are no reports of reproductive toxicity in males, with the exception of a single case report suggesting that prolonged abuse of toluene may produce testicular atrophy and reduced spermatogenesis (*3*). A 28-year-old male who had been chronically abusing toluene for 10 years, and who apparently died as a result of excessive sniffing, was found to have degeneration of the spermatogonia and Sertoli cells and showed evidence of faulty or suppressed spermatogenesis. Evidence of cerebral and cerebellar degeneration and mild liver disease was also reported.

## Review of Reproductive/Developmental Toxicity in Animals

A number of experimental studies investigating the reproductive and developmental toxicity of toluene have been conducted using rats, mice, and rabbits. These studies provide convincing evidence that exposures of dams to toluene during gestation cause fetotoxicity. The evidence for female reproductive toxicity (i.e., adverse effects of toluene on female reproductive function) is limited, and there is no evidence in animals that toluene exposures are associated with adverse reproductive effects in males. Designs and end points of the animal studies are summarized in Table 2.

### Transplacental Toxicokinetics

While there are no quantitative data for the transplacental transfer of toluene in humans, there is one case report of toluene being detected in the serum of a newborn infant (*16*). In mice, one study demonstrated that approximately 10% of an inhaled dose of toluene was rapidly distributed to the fetuses. Tissue distribution within the fetus appeared to vary with time of administration during the course of gestation. Administration at day 11 of

**Table 2. Studies of reproductive and/or developmental toxicity of toluene in animal models.**

| Authors | Type of study | Species (strain) | Exposure parameters |
|---|---|---|---|
| Toxicokinetic studies | | | |
| Ghantous and Danielsson, 1986 (*18*) | Placental transfer and distribution | Mouse (C57BL) | 2000 ppm by inhalation day 11, 14, or 17 gestation (10 min) (dams) or 115 μg by subcutaneous injection (4-day-old pups) |
| Teratology/fetotoxicity studies (inhalation) | | | |
| International Research and Development Corporation (for the American Petroleum Institute), 1985 (*19*) | Reproductive and developmental toxicity | Rat (CR.CD) | 100, 500, or 2000 ppm by inhalation daily from 80 days before mating through lactation (6 hr/day) (dams all concentrations, sires at highest concentration only) |
| Ungvary and Tatrai, 1985 (*20*) | Teratology and fetotoxicity | Mouse (CFLP) | 133, 266, or 399 ppm by inhalation on days 6–15 gestation (3 × 4 hr/day) (dams) |
| | | Rabbit (NZ) | 133 or 266 ppm by inhalation days 7–20 gestation (24 hr/day) (dams) |
| Tatrai et al., 1980 (*21*) | Teratology and fetotoxicity | Rat (CFY) | 266 ppm by inhalation, days 7–14 gestation (24 hr/day) (dams) |
| Hudak and Ungvary, 1978 (*22*) | Teratology and fetotoxicity | Mouse (CFLP) | 133 or 399 ppm by inhalation, days 6–13 gestation (24 hr/day) (dams) |
| | | Rat (CFY) | 399 ppm by inhalation, days 9–14 gestation (24 hr/day) or 399 ppm by inhalation, days 1–8 gestation (24 hr/day) (dams) or 266 ppm by inhalation, days 1–21 gestation (8 hr/day) (dams) |
| Courtney et al., 1986 (*24*) | Teratology and postnatal development (lactic dehydrogenase activity) | Mouse (CD-1) | 200 or 400 ppm by inhalation, days 7–16 gestation (7 hr/day) (dams) |
| Shigeta et al., 1981 (*25*) | Teratology and fetal development | Mouse (ICR) | 100 or 1000 ppm by inhalation, days 1–17 gestation (6 hr/day) (dams) |
| Litton Bionetics, 1978 (*26*) | Teratology | Rat (CRL:COBS CD (SD) BR) | 100 or 400 ppm by inhalation, days 6–15 gestation (6 hr/day) (dams) |
| Teratology/fetotoxicity studies (gavage) | | | |
| Nawrot and Staples, 1979 (*23*) | Teratology and fetotoxicity | Mouse (CD-1) | 0.9, 1.5, or 3.0 mg/kg/day, days 6–15 gestation or 3.0 mg/kg/day, days 12–15 gestation |
| Postnatal neurobehavioral studies | | | |
| Shigeta et al., 1986 (*27*) | Postnatal cognitive function | Rat (high avoidance or Wistar) | 100 ppm by inhalation, day 13 gestation until day 48 postnatal |
| Kostas and Hotchin, 1981 (*28*) | Postnatal neurobehavioral development | Mouse (Nya: NYLAR) | 16, 80, or 400 ppm in drinking water throughout gestation, lactation, and postweaning |

gestation resulted in even distribution among tissues, while later administrations (days 14 or 17 of gestation) produced accumulation in fetal liver (*18*).

## Fetal Development

Reduction of late fetal body weight and retardation of skeletal development were the most consistent fetotoxic effects demonstrated in animals. Convincing evidence for retardation of somatic development comes from a study in which rat dams of strain CR.CD were exposed in inhalation chambers to 0, 100, 500, or 2000 ppm toluene (> 99.99% pure) for 6 hr/day (begining 80 days prior to mating, excluding day 20 of gestation) and sacrificed on day 21 of gestation (*19*). The mean body weights of fetuses from dams exposed to 2000 ppm toluene were significantly lower than controls (Table 3). There was no evidence of maternal toxicity in these dams (Table 4). This study used appropriate controls and adequate numbers of animals and was conducted according to Good Laboratory Practice protocols. The no-observed-adverse-effect level (NOAEL) in this study was 500 ppm; however, it is possible that the true threshold for adverse effects detectable in a study such as this may lie between 500 ppm and the high dose (2000 ppm) that was associated with fetotoxicity.

The association between toluene exposure and fetotoxicity is also supported by effects on fetal somatic development in several other studies (*20–24*), which are summarized in Table 3. Effects of toluene exposure on late fetal weights and skeletal maturation were reported in rats and mice; data in rabbits are weak. Potential methodological issues limit the value of these studies These issues include questionable exposure parameters (e.g., exposure in inhalation chambers for 24 hr/day for several consecutive days), incomplete reporting of parameters of maternal toxicity, use of individual pups rather than litters as the basis for statistical analyses, and purity of the test material.

In one of these studies, fetuses of rat dams exposed to 399 ppm toluene for 24 hr/day on days 1 to 8 of gestation had lower body weights than any other group including two control groups (*22*). The percentage of weight-retarded (i.e., < 3.3 g body weight) fetuses among these dams was also increased. An appropriate control group for this experimental group was not included in this study, however, and it is not clear which control group was used for statistical analyses. Additionally, 5 of the original 14 dams in this treatment group died prior to delivery (Table 4), although the remaining dams were reported to show no signs of toxicity. The fetotoxic effect observed may be time dependent: fetuses of dams exposed to the same concentration of toluene for 24 hr/day on days 9 to 14 instead of days 1 to 8 of gestation were not reported to show any indications of developmental retardation. The mechanism by which such a time-dependent effect might operate is unclear, since early embryonic exposures might be expected to produce malformations rather than developmental retardation. Further studies are required to resolve this issue.

Reductions in fetal weights at term were also demonstrated in two studies in which pregnant mice were exposed by inhalation to toluene at 133 ppm for 24 hr/day on days 6 to 13 gestation (*22*) or by exposure to 1.5 or 3.0 mL/kg via gavage on days 6 to 15 gestation (*23*). Data from these studies are presented in Table 3. [The study by Nawrot and Staples (*23*) has been reported in the literature only in abstract form, but additional data in Table 3 were supplied by one of the authors (R. E. Staples, personal communication)]. A further study by Ungvary and Tatrai demonstrated an increase in the incidence of weight-retarded fetuses (i.e., < 0.9 g body weight) among dams exposed to 266 ppm toluene via inhalation chambers 3 periods of 4 hr on each day between days 6 to 15 of gestation (*20*). This index of developmental retardation was also increased in the fetuses of dams exposed to 133 ppm toluene in the other study where mean fetal weight was reduced (*22*).

The study by Nawrot and Staples demonstrated decreased fetal weights among dams exposed to 1.5 or 3.0 mL toluene/kg body weight on days 6 to 15 of gestation, while no effect on fetal weights was observed in dams exposed to 3.0 mL/kg on days 12 to 15 of gestation (*23*). Maternal toxicity was reported to be absent in these dams, although exposure to 3.0 mL toluene/day on days 12 to 15 of gestation in another group of pregnant animals did result in decreased weight gain among those dams. One of the authors has questioned the reported absence of maternal toxicity in this study (R. E. Staples, personal communication).

With the possible exceptions of some groups in the Nawrot and Staples study (*23*) and the group of rats exposed to 399 ppm toluene on days 1 to 8 of gestation in the study by Hudak and Ungvary (*22*), there was no evidence for a significant degree of maternal toxicity in rats or mice in any of these studies. Maternal toxicity in rabbits did result from exposure to 266 ppm toluene for 24 hr/day on days 7 to 20 of gestation (*20*). Indices of possible maternal toxicity were generally poorly reported in these studies, however, and the available data are summarized in Table 4.

In addition to decreased body weight, fetal skeletal development was also consistently affected. The International Research and Development Corporation (IRDC) study demonstrated a non-significant increase in incidence of skeletally-retarded fetuses (SRF) from rat dams exposed subchronically to 2000 ppm toluene for 6 hr/day (*19*) (Table 3). Significant increases in incidences of SRF among pregnant rats were reported from a variety of exposure scenarios (266 ppm toluene for 8 hr/day on days 1 to 21 of gestation (*22*) or for 24 hr/day on days 7 to 14 of gestation (*21*) and to 399 ppm toluene for 24 hr/day on days 1 to 8 of gestation (*22*).

In mice, a significant increase was also reported in the incidence of SRF resulting from exposures to 266 ppm toluene for 12 hr/day on days 6 to 15 of gestation (*20*). A significant increase in the incidence of fetuses with a 13/13 rib profile from dams that had been exposed to 400 ppm toluene for 7 hr/day on days 7 to 16 of gestation was also reported (*24*). It is unclear whether this result is adverse, since this profile is considered normal. In conjunction with a significant increase in the incidence of fetuses with enlarged renal pelves in the 200 ppm treatment group, the authors speculated that this finding suggested desynchronization of growth and maturation.

## Teratology

There is little evidence that exposure to toluene produces teratogenic effects. A significant increase in incidence of fetuses with extra 14th ribs in dams exposed to 1000 ppm toluene for 6 hr/day on days 1 to 17 of gestation was reported by Shigetta et al. (*25*). A significant increase in the incidence of cleft palate in mouse fetuses from dams receiving 3 doses/day of 1.0 mL toluene/kg via gavage was also reported by Nawrot and Staples (*23*). A study by Litton Bionetics, Inc. for the American Petroleum Institute (*26*), using GLP procedures and standard

**Table 3. Summary of weight and skeletal retardation in fetuses of dams exposed to toluene.**

| Reference | Species | Strain | Fetal exposure | No. of dams | Weight, g[a] | Weight-retarded fetuses, % | Skeletal-retarded fetuses, % |
|---|---|---|---|---|---|---|---|
| (*19*) | Rats | CR.CD | Control | 18 | 3.6 ± 0.25 | NR[b] | 14.3 |
| | | | 2000 ppm (6 hr/day, 80 days before mating through lactation) | 18 | 3.3 ± 0.22 ($p < 0.01$) | NR | 24.4 |
| (*21*) | Rats | CFY | Control | 21 | 3.94 ± 0.02 | 2.8 | 13 |
| | | | 266 ppm (24 hr/day, 7–14 dg)[c] | 18 | 3.91 ± 0.02 | 3.3 | 31 ($p < 0.05$) |
| (*22*) | Rats | CFY | Control (24 hr/day, 9–14 dg) | 26 | 3.76 ± 0.02 | 6.9 | 6.5 |
| | | | 399 ppm (24 hr/day, 9–14 dg) | 19 | 3.75 ± 0.03 | 17.3 | 6 |
| | | | Control (8 hr/day, 1–21 dg) | 10 | 3.81 ± 0.03 | 7.2 | 0 |
| | | | 266 ppm (8 hr/day, 1–21 dg) | 10 | 3.61 ± 0.03 | 16.0 | 25 ($p < 0.05$) |
| | | | 399 ppm (24 hr/day, 1–8 dg) | 9 | 3.31 ± 0.08 ($p < 0.05$) | 46.0 ($p < 0.05$) | 17 ($p < 0.05$) |
| (*26*) | Rats | Crl:COBS CD (SD) BR | Control | 27 | 3.6 | NR | NR |
| | | | 100 ppm (6 hr/day, 6–15 dg) | 27 | 3.5 | NR | No effect |
| | | | 400 ppm (6 hr/day, 6–15 dg) | 27 | 3.8 | NR | No effect |
| (*20*) | Mice | CFLP | Control | 115 | NR | 7 | 5 |
| | | | 133 ppm (3 × 4 hr/day, 6–15 dg) | 15 | NR | 10 | 8 |
| | | | 266 ppm (3 × 4 hr/day, 6–15 dg) | 15 | NR | 29 ($p < 0.05$) | 12 ($p < 0.05$) |
| | | | 399 ppm (24 hr/day, 6–15 dg) | 15 | — | — | — (All dams died) |
| (*22*) | Mice | CFLP | Control | 14 | 1.07 ± 0.01 | 6.5 | 5.0 |
| | | | 133 ppm (24 hr/day, 6–13 dg) | 11 | 0.96 ± 0.01 ($p < 0.01$) | 27.6 ($p < 0.05$) | 1.9 |
| | | | 399 ppm (24 hr/day, 6–13 dg) | 15 | — | — | — (All dams died) |
| (R. E. Staples, personal communication; *23*) | Mice | CD-1 | Control | 95 | 0.93 | NR | NR |
| | | | 0.9 mL/kg (3 × 0.3 mL, 6–15 dg) | 24 | 0.92 | NR | NR |
| | | | 1.5 mL/kg (3 × 0.5 mL, 6–15 dg) | 26 | 0.87 (Significantly lower) | NR | NR |
| | | | 3.0 mL/kg (3 × 1.0 mL, 6–15 dg) | 25 | 0.79 (Significantly lower) | NR | NR |
| | | | 3.0 mL/kg (3 × 1.0 mL, 12–15 dg) | NR | No effect | NR | NR |
| (*24*) | Mice | CD-1 | Control | 15 | 1.11 | NR | NR |
| | | | 200 ppm (7 hr/day, 7–16 dg) | 16 | 1.11 | NR | NR |
| | | | 400 ppm (7 hr/day, 7–16 dg) | 16 | 1.07 | NR | NR |
| (*20*) | Rabbits | NZ | Control (24 hr/day, 7–20 dg) | 60 | 33.0 ± 1.06 (M)<br>32.7 ± 0.86 (F) | NR<br>NR | 40 |
| | | | 133 ppm (24 hr/day, 7–20 dg) | 10 | 33.2 ± 1.88(M)<br>31.9 ± 1.75 (F) | NR<br>NR | 54 |
| | | | 266 ppm (24 hr/day, 7–20 dg) | 8 | All fetuses died | | |

[a]Means ± SD.
[b]NR, data not reported.
[c]dg, days of gestation.

protocols, found no evidence of teratogenicity following maternal exposures to 100 or 400 ppm toluene by inhalation for 6 hr/day on days 6 to 15 gestation.

## Postnatal Maturation

Adverse effects on postnatal maturation are associated with gestational exposures to toluene at certain levels of exposure. Data on postnatal development from the study by IRDC (*19*) are summarized in Table 5. Prolonged maternal exposure to 2000 ppm toluene for 6 hr/day before and during gestation clearly had a marked effect on postnatal weight gain of rat pups up to weaning. In contrast, maternal exposures to 400 ppm for 6 hr/day on days 7 to 16 of gestation in the study by Courtney et al. (*24*) had no significant effect on pup body weights, although the toluene-treated pups were somewhat heavier than controls (Table 5). There is also limited evidence for retardation of postnatal physical development as assessed by appearance of developmental landmarks such as hair eruption and eye opening (*27*). Postnatal neurobehavioral and cognitive development has also been shown to be affected by prenatal and postnatal exposures to toluene in rats (*27*) and in mice (*28*).

**Table 4. Indices of maternal toxicity in rats, mice, and rabbits.**

| Reference | Species | Exposure group, ppm | Maternal mortality, % | Maternal weight gain through gestation, % | Maternal liver weight, relative % |
|---|---|---|---|---|---|
| (*19*) | Rat | Control | 0 | 45.1 | 4.5 |
| | | 100 | 0 | 44.3 | NR[a] |
| | | 500 | 0 | 42.5 | NR |
| | | 2000 | 0 | 43.9 | 4.9 |
| | | 0 | 0 | 41.2 | NR |
| | | 2000 | 0 | 42.6 | NR |
| (*20*) | Mouse | Control | 0 | NR | NR |
| | | 133 | 0 | NR | NR |
| | | 266 | 0 | NR | NR |
| | | 399 | 100 | NR | NR |
| | Rabbit | Control | 0 | 12.7 | 3.0 |
| | | 133 | 0 | 9.0 | 3.0 |
| | | 266 | 25 | 5.0* | 3.6* |
| (*21*) | Rat | Control | 0 | 68.8 | 4.25 |
| | | 266 | 0 | 65.8 | 4.37* |
| (*22*) | Rat | Control[b] | 0 | 52.4 | NR |
| | | 399[b] | 9.5 | 41.8 | NR |
| | | Control[c] | 0 | 46.6 | NR |
| | | 266[c] | 0 | 44.1 | NR |
| | | 399[d] | 35.7 | 44.0 | NR |
| | Mouse | Control | 0 | NR | NR |
| | | 133 | 0 | NR | NR |
| | | 399 | 100 | — | NR |
| (*23*) | Mouse | Control | NR | NR | NR |
| | | 0.9 mL/kg[e] | NR | NR | NR |
| | | 1.5 mL/kg[e] | NR | NR | NR |
| | | 3.0 mL/kg[e] | NR | NR | NR |
| | | 3.0 mL/kg[f] | NR | NR[g] | NR |
| (*24*) | Mouse | Control | 0 | 12.7[h] | 7.69 |
| | | | 0 | 11.7[h] | 7.23[i] |
| | | | 0 | 10.6[h] | 6.93[i] |

[a]NR, data not reported.
[b]Exposed 24 hr/day on days 9–14 of gestation.
[c]Eposed 8 hr/day on days 1–21 of gestation.
[d]Exposed 24 hr/day on days 1–8 of gestation.
[e]Dose administered by gavage on days 6–15 of gestation.
[f]Dose administered by gavage on days 12–15 of gestation.
[g]Reported to be significantly lower than control value, but magnitude of effect or probability value not reported and no parallel control group included in study.
[h]Calculated on the basis of the reported increase in body weight (grams) and the mean initial body weight of the dams.
[i]Reported to be significantly lower than control value.
*$p < 0.05$.

## Fetal and Pup Viability

Exposures to 266 ppm toluene for 24 hr/day on days 7 to 20 of gestation was associated with complete reproductive failure in rabbits (*20*). Of eight pregnant does, two completely resorbed their litters, four aborted their litters, and two died. Only one doe aborted in a group of ten animals exposed to 133 ppm toluene for the same period, and one of the concurrent control group of sixty pregnant does resorbed her entire litter.

Significant increases in embryonic lethality were reported to have occurred at all dose levels (0.9, 1.5, or 3.0 mg/kg/day) in the study reported in abstract by Nawrot and Staples (*23*). No data were presented in support of this conclusion.

These results could be considered either as developmental effects or as female reproductive effects. No other effects on female reproduction were reported in these studies.

# Discussion

It is clear from the results of studies in animals that toluene is associated with developmental toxicity. Subchronic exposure to toluene via inhalation for 6 hr/day at dose levels producing no significant maternal toxicity in a well-controlled and well-conducted study caused retardation of both fetal and postnatal development (*19*). Results from this study are supported by consistent effects from other, less robust studies (*20–23*). Associations between toluene exposures and adverse effects on postnatal development have also been suggested by a number of studies, although the evidence is limited at present (*19,27,28*). Some of the adverse outcomes in offspring ascribed to toluene sniffing by pregnant women, such as intrauterine growth retardation and delayed postnatal development, are similar to the prenatal and postnatal developmental retardation associated with prenatal toluene exposures in experimental animal studies. Other effects that occur in humans, such as facial and visceral malformations, have not been reported in nonhuman species. It is clear, however, that exposure to toluene may present a hazard to the developing organism.

The NOAEL from the IRDC study in rats is 500 ppm (*19*). Exposure of rats to 500 ppm of toluene in air for 6 hr/day is estimated to result in a total daily intake of 112.5 mg/kg/day (Table 6). Adverse effects (low fetal weights at term) were reported to

**Table 5. Postnatal pup mean body weights (grams) for rats (*19*) and mice (*24*).**

| Postnatal day (sex) | | Toluene concentration, ppm | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 (M+F)[a] | 100 (M+F) | 500 (M+F) | 2000 (M+F) | 2000 (M) | 2000 (F) |
| Rats | | | | | | | |
| 1 | (M) | 6.5 | 6.4 | 6.4 | 5.7† | 6.4 | 5.9* |
| | (F) | 6.0 | 5.9 | 6.2 | 5.3† | 6.1 | 5.5* |
| 4 BR[b] | (M) | 9.0 | 8.9 | 8.9 | 7.8* | 9.3 | 8.1 |
| | (F) | 8.7 | 8.4 | 8.7 | 7.2† | 8.9 | 7.6† |
| 4 AR | (M) | 9.0 | 8.8 | 8.8 | 7.8† | 9.3 | 8.1* |
| | (F) | 8.6 | 8.4 | 8.7 | 7.2† | 8.9 | 7.6* |
| 14 | (M) | 22.8 | 23.9 | 22.0 | 19.6* | 23.9 | 19.7† |
| | (F) | 22.0 | 22.8 | 21.3 | 18.3† | 22.9 | 18.2† |
| 21 | (M) | 36.1 | 38.9 | 35.8 | 31.1† | 38.9 | 30.8† |
| | (F) | 34.8 | 37.4 | 35.0 | 29.5* | 37.3 | 28.8† |

| Mice | Toluene concentration, ppm | |
|---|---|---|
| | 0 | 400 |
| 1 | 1.36 | 1.52 |
| 21 | 6.53 | 7.15 |

[a]Parental exposure, male (M) or female (F), parental groups that are not indicated were not exposed to toluene.
[b]BR, before reduction in litter size to eight pups/litter; AR, after reduction in litter size.
*$p < 0.05$.
†$p < 0.01$.

**Table 6. Estimated total daily intakes for humans, mice, and rats exposed to toluene.**

| Species | Exposure | Duration per day, hr | Estimated absorption, % | Body weight, kg | Total daily intake, mg/kg/day |
|---|---|---|---|---|---|
| Human[a] | 100 ppm (inhalation) (375 mg/m³) | 8 | 50 | 55 | 34 |
| | 500 ppm (inhalation)[b] (1875 mg/m³) | 6 | 50 | 55 | 127.5 |
| Mouse[c] | 200 ppm (inhalation) (750 mg/m³) | 7 | 50 | 0.03 | 157.5[d] |
| | 133 ppm (inhalation) (500 mg/m³) | 12 | 50 | 0.03 | 180 |
| | 400 ppm (inhalation) (1500 mg/m³) | 6 | 50 | 0.03 | 270 |
| | 400 ppm (inhalation) (1500 mg/m³) | 7 | 50 | 0.03 | 315[d] |
| | 133 ppm (inhalation) (500 mg/m³) | 24 | 50 | 0.03 | 360[d] |
| | 266 ppm (inhalation) (1000 mg/m³) | 12 | 50 | 0.03 | 360[d] |
| | 399 ppm (inhalation) (1500 mg/m³) | 24 | 50 | 0.03 | 1080[d] |
| | 0.9 mL/kg (gavage)[e] | | 100 | 0.03 | 780[d] |
| | 1.5 mL/kg (gavage) | | 100 | 0.03 | 1300[d] |
| | 3.0 mL/kg (gavage) | | 100 | 0.03 | 2600[d] |
| Rat[f] | 100 ppm (inhalation) (375 mg/m³) | 6 | 50 | 0.3 | 22.5 |
| | 266 ppm (inhalation) (1000 mg/m³) | 8 | 50 | 0.3 | 80[d] |
| | 500 ppm (inhalation) (1875 mg/m³) | 6 | 50 | 0.3 | 112.5 |
| | 266 ppm (inhalation) (1000 mg/m³) | 24 | 50 | 0.3 | 240[d] |
| | 399 ppm (inhalation) (1500 mg/m³) | 24 | 50 | 0.3 | 360[d] |
| | 2000 ppm (inhalation) (7500 mg/m³) | 6 | 50 | 0.3 | 450[d] |

[a]Human respiratory volume estimated to be 1250 L/hr.
[b]Exposure level reported to cause euphoria in humans.
[c]Mouse respiratory volume estimated to be 1.8 L/hr.
[d]Total daily intakes reported to cause toxicity in the fetuses, dams, or both.
[e]One milliliter of toluene = 866.9 mg.
[f]Rat respiratory volume estimated to be 6 L/hr.

have occurred in rats of a different strain at an total daily intake of 80 mg/kg/day (*22*). The questionable quality of this and other studies that provide apparent NOAELs that are lower than 112.5 mg/kg/day make it inappropriate to use such data in calculating an exposure level that would be considered acceptable for humans.

If 112.5 mg/kg/day was treated as a no-observed-effect level (NOEL) for the purposes of California's Proposition 65, a mandatory safety factor of 1000 would be applied, resulting in an acceptable daily intake level for humans of 0.1125 mg/kg/day. Using the procedures developed by the U.S. Environmental Protection Agency (EPA), an uncertainty factor of 100 (a 10-fold factor for intraspecies variability × a 10-fold factor for interspecies variability) could be applied to this NOAEL, resulting in a reference dose (RfD) of 1.125 mg/kg/day in humans (*29*).

Table 6 compares the estimated daily absorbed doses for a 55-kg human female at the permissible exposure level of 100 ppm and from the minimum exposure expected to occur during inhalation abuse of toluene, with estimates of absorbed doses for mice and rats at the exposure regimens employed in the studies reviewed here. The human exposure level of 34 mg/kg/day estimated from the U.S. OSHA and the California OSHA per-

missible exposure levels of 100 ppm is within an order of magnitude of the exposure levels that cause adverse effects in rats and mice. It is also apparent that abuse of toluene for its euphoric effects will result in exposure levels that match or exceed those producing adverse effects in these animal models.

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