

CHRONIC TOXICITY SUMMARY

NAPHTHALENE

(*naphthene, NCI-C5290, albocarbon, dezodorator, moth balls, moth flakes, tar camphor, white tar, naphthalin, naphthaline*)

CAS Registry Number: 91-20-3

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	9 µg/m³
<i>Critical effect(s)</i>	Respiratory effects (nasal inflammation, olfactory epithelial metaplasia, respiratory epithelial hyperplasia) in mice
<i>Hazard index target(s)</i>	Respiratory system, blood systems

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	White crystalline powder
<i>Molecular formula</i>	C ₁₀ H ₈
<i>Molecular weight</i>	128.6 g/mol
<i>Density</i>	4.42 g/cm ³ @ 20°C
<i>Boiling point</i>	218°C
<i>Melting point</i>	80.5 °C
<i>Vapor pressure</i>	0.087 mm Hg
<i>Conversion factor</i>	5.26 µg/m ³ per ppb at 25°C

III. Major Uses or Sources

Naphthalene is a natural constituent of coal tar (approximately 11%) (HSDB, 1995). Naphthalene is used as a moth repellent, though this use is decreasing in favor of p-dichlorobenzene (HSDB, 1995). It has also been used in the manufacture of phthalic anhydride, phthalic and anthranilic acids, naphthols, naphthylamines, 1-naphthyl-n-methylcarbamate insecticide, beta-naphthol, naphthalene sulfonates, synthetic resins, celluloid, lampblack, smokeless powder, anthraquinone, indigo, perylene, and hydronaphthalenes (NTP, 1992; HSDB, 1995).

IV. Effects of Human Exposure

Nine persons (eight adults and one child) were exposed to naphthalene vapors from several hundred mothballs in their homes. Nausea, vomiting, abdominal pain, and anemia were reported (Linick, 1983). Testing at one home following the incidence indicated an airborne naphthalene concentration of 20 ppb (105 $\mu\text{g}/\text{m}^3$). Symptoms abated after removal of the mothballs.

Workers occupationally exposed to naphthalene fumes or dust for up to five years were studied for adverse ocular effects (Ghetti and Mariani, 1956). Multiple pin-point opacities developed in 8 of 21 workers. Vision did not appear to be impaired.

Cataracts and retinal hemorrhage were observed in a 44 year old man occupationally exposed to powdered naphthalene, and a coworker developed chorioretinitis (van der Hoeve, 1906).

Wolf (1978) reported that a majority of 15 persons involved in naphthalene manufacture developed either rhinopharyngolaryngitis and/or laryngeal carcinoma.

Ingestion of naphthalene or p-dichlorobenzene mothballs is a frequent cause of accidental poisoning of children (Siegel and Wason, 1986). Infants exposed to naphthalene vapors from clothes or blankets have become ill or have died (U.S. EPA, 1990). The effects in infants have been associated with maternal naphthalene exposure during gestation (U.S. EPA, 1990).

Deaths have been reported following ingestion of naphthalene mothballs. A 17-year old male ingested mothballs, developed gastrointestinal bleeding, hematuria, and coma, and died after five days (Gupta *et al.*, 1979). A 30-year old female ingested 30 mothballs and died after five days (Kurz, 1987).

Acute hemolytic anemia was reported among 21 infants exposed to naphthalene vapors from nearby mothball-treated materials (Valaes *et al.*, 1963). Increased serum bilirubin, methemoglobin, Heinz bodies, and fragmented red blood cells were observed. Kernicterus was noted in eight of the children, and two of the children died. Ten of these children had a genetic deficiency in glucose-6-phosphate dehydrogenase.

A 12-year old male ingested 4 g of naphthalene and 20 hours later developed hematuria, anemia, restlessness, and liver enlargement (Manchanda and Sood, 1960). The patient recovered after 8 days.

A 69-year old female developed aplastic anemia two months after several weeks exposure to naphthalene and p-dichlorobenzene (Harden and Baetjer, 1978).

V. Effects of Animal Exposure

Male and female B6C3F1 mice were exposed to naphthalene (>99% pure) vapor for 6 hours per day, 5 days per week over 104 weeks (NTP, 1992). Concentrations used were 0 (150 mice), 10 (150 mice), or 30 ppm (300 mice) naphthalene. (Table 1). Lesions were observed in the nose and lungs of exposed mice, including increased incidences of chronic nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia.

Table 1. Incidence of respiratory tract lesions in mice (male and female combined) chronically exposed to naphthalene vapors (NTP, 1992).

	<i>0 ppm</i>	<i>10 ppm</i>	<i>30 ppm</i>
<i>Nasal inflammation</i>	3/139	34/134	108/270
<i>Olfactory epithelial metaplasia</i>	0/139	131/134	269/270
<i>Respiratory epithelial hyperplasia</i>	0/139	131/134	269/270

CD-1 mice were administered 5.3, 53, or 133 mg/kg/day naphthalene by gavage over 90 days (Shopp *et al.*, 1984). The only effect noted was inhibition of aryl hydrocarbon hydroxylase activity. No increase in mortality or changes in body weight were noted. Reduced spleen weights were noted in females exposed to the highest dose. No changes were noted in serum enzyme levels or electrolytes. The researchers did not conduct a histopathological examination.

B6C3F1 mice were administered 200 mg naphthalene/kg/day by gavage for 5 days per week over 13 weeks. No adverse effects were observed (U.S. EPA, 1990).

Developmental effects of naphthalene ingestion in Sprague-Dawley CD rats was studied by Navarro and associates (1991). The lowest dose tested (50 mg/kg/day by gavage) was associated with signs of CNS depression for the first 3 days. Fetal growth, survival, and morphological development were not significantly affected at 450 mg/kg/day compared with control animals, although a trend toward decreased fetal weight and increased malformations was observed.

Harris and associates (1979) intraperitoneally administered 395 mg/kg/day naphthalene to Sprague-Dawley rats over days 1 through 15 of gestation. Fetuses had a 50% increase in incidence in delayed cranial ossification and heart development.

New Zealand white rabbits were given 0, 40, 200, or 400 mg/kg/day by gavage over days 6 through 18 of gestation (U.S. EPA, 1986a). A dose-dependent increase in grooming, vocalization, aggression, diarrhea, dyspnea, and ocular and nasal discharge were noted at all doses. No statistically significant increase in malformations or developmental abnormalities was observed.

Sprague-Dawley rats were administered 0, 100, 300, or 1000 mg/kg/day of naphthalene via dermal application (U.S. EPA, 1986b). No effects were reported at 100 or 300 mg/kg/day. At the high dose a slight decrease in testes weight was noted.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	NTP (1992)
<i>Study population</i>	B6C3F1 mice (75 or 150/group/sex)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposures to 0, 10, or 30 ppm naphthalene vapor
<i>Critical effects</i>	Nasal inflammation, olfactory epithelial metaplasia, and respiratory epithelial hyperplasia
<i>LOAEL</i>	10 ppm (96% incidence for males and 100% incidence for females)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hours/day for 5 days/week
<i>Average experimental exposure</i>	1.8 ppm (10 ppm x 6/24 x 5/7) for LOAEL group
<i>Exposure duration</i>	104 weeks
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	10
<i>Interspecies uncertainty factor (UF)</i>	10
<i>Intraspecies uncertainty factor (UF)</i>	10
<i>Cumulative uncertainty factor</i>	1000
<i>Inhalation reference exposure level</i>	0.002 ppm (2 ppb, 0.009 mg/m ³ , 9 µg/m ³)

The NTP study was chosen for the REL derivation since it is the only available lifetime animal inhalation bioassay and because no adequate epidemiological studies of long-term human exposure are available. The study was judged to be of adequate study design. The complete lack of nasal effects among control animals and the nearly total effect among animals exposed at 2 different concentrations strongly indicates a causal relationship between naphthalene exposure and nasal effects. The effects seen are consistent with those reported among exposed workers, who developed rhinopharyngolaryngitis or laryngeal carcinoma (Wolf, 1978). However, the hematological effects observed in humans have not been reported in laboratory animals, which raises the possibility that humans may be significantly more sensitive to naphthalene.

The most important limitation of the study is that the lowest concentration tested caused adverse effects in most (≥96%) of the animals tested. Thus the study amply demonstrates the risk of lifetime exposures to 10 ppm, but is uninformative regarding the concentration-response relationship at lower concentrations. Only a general assumption can be drawn on the magnitude of uncertainty factor needed to predict a concentration at which adverse effects would most likely not be observed. Lacking specific guidance or relevant research for this situation, the default 10-fold factor was applied.

VII. References

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Determination of Noncancer Chronic Reference Exposure Levels
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CHRONIC TOXICITY SUMMARY

NICKEL AND NICKEL COMPOUNDS
NICKEL OXIDE

<i>Molecular Formula</i>	<i>Molecular Weight</i>	<i>Synonyms</i>	<i>CAS Registry Number</i>
Ni	59	elemental nickel	7440-02-0
NiO	74.69	nickel oxide	1313-99-1
NiCl ₂	129.6	nickel chloride nickel dichloride	7718-54-9
NiSO ₄	154.75	nickel sulfate nickelous sulfate	7786-81-4
NiCO ₃	118.7	nickel carbonate carbonic acid nickel salt	3333-67-3
Ni ₃ S ₂	240.19	nickel subsulfide trinickel disulfide heazlewoodite	12035-72-2

I. Chronic Toxicity Summary

A. Nickel and Nickel Compounds (except nickel oxide)

<i>Inhalation reference exposure level</i>	0.05 µg Ni/m³
<i>Critical effect(s)</i>	Lung, nasal epithelial and lymphatic pathology in male and female rats
<i>Hazard index target(s)</i>	Respiratory system; immune system

B. Nickel Oxide

<i>Inhalation reference exposure level</i>	0.10 µg Ni/m³
<i>Critical effect(s)</i>	Lung and lymphatic pathology in male and female rats
<i>Hazard index target(s)</i>	Respiratory system; immune system

II. Physical and Chemical Properties (from HSDB, 1995)

<i>Description</i>	Ni metal: Silvery metal; NiCl ₂ : deliquescent crystals (U.S.EPA, 1985)
<i>Molecular formula</i>	See above
<i>Molecular weight</i>	See above
<i>Density</i>	8.9 g/cm ³ @ 20°C (Ni)
<i>Boiling point</i>	2730°C (Ni)
<i>Vapor pressure</i>	Not applicable
<i>Solubility</i>	Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water soluble.
<i>Conversion factor</i>	Not applicable for fumes and dusts

III. Major Uses and Sources

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent (Ni²⁺) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). Nickel is also used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985). Nickel dust or powder is flammable (CDTSC, 1985). Due to its unique toxicological and physico-chemical properties, nickel carbonyl is not included in this summary.

IV. Effects of Human Exposure

Several studies have indicated that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey *et al.* (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell *et al.*, 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by FEV₁ with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal values. Dyspnea, non-productive cough, chest-tightness, and wheezing were reported as symptoms by this worker during the work period.

A group of 7 metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel and other metals (Cirla *et al.*, 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the 7 asthmatics exhibited significantly decreased FEV₁ (> 15%) when exposed to 0.3 mg/m³ nickel sulfate for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV₁.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis (Muir *et al.*, 1993). An occupational epidemiology report by Broder *et al.* (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter, however a healthy worker effect was observed in this study.

V. Effects of Animal Exposure

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

A 2-year inhalation study of nickel oxide in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m³ (0, 0.5, 1.0, or 2.0 mg Ni/m³) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m³ or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m³ for both sexes, and in males exposed to 1.0 mg Ni/m³.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m³. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m³ group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 µg Ni/m³ as nickel oxide for 2 years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were observed in the animals exposed to nickel. Only 1 rat per group survived the nickel exposures to the end of the experiment.

A 2-year study on the effects of nickel subsulfide in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg Ni₃S₂/m³ (0, 0.11, or 0.73 mg Ni/m³) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg Ni/m³ compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy was observed in both sexes exposed to 0.73 mg Ni/m³.

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg Ni₃S₂/m³ (0, 0.44, or 0.88 mg Ni/m³) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg Ni/m³ and the 0.88 mg Ni/m³ concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

An exposure of rats to either 0 or 0.97 mg Ni₃S₂/m³ (0 or 0.71 mg Ni/m³) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs due to Ni (Ottolenghi *et al.*, 1974).

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg NiSO₄/m³ (0, 0.03, 0.06, or 0.11 mg Ni/m³) for 6 hours/day, 5 days/week for 104 weeks. Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg Ni/m³ or greater.

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg Ni/m³ as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg Ni/m³ or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg Ni/m³.

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide 6 hours/day, 5 days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg Ni/m³ or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as low as 0.02 mg Ni/m³. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m³.

A similar study by Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m³ as Ni₃S₂; 0.47, 2.0, or 7.9 mg Ni/m³ as NiO; and 0.027, 0.11, and 0.45 mg Ni/m³ as NiSO₄ for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m³ as nickel subsulfide. In contrast, AFC responses in the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m³ as nickel chloride 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

VI. Derivation of Chronic Reference Exposure Level (REL)

A. Nickel and Nickel Compounds (except nickel oxide)

<i>Study</i>	National Toxicology Program, 1994c
<i>Study population</i>	Male and female F344/N rats (52-53 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy
<i>LOAEL</i>	60 µg Ni/m ³ (as nickel sulfate hexahydrate)
<i>NOAEL</i>	30 µg Ni/m ³
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	104 weeks
<i>Average experimental exposure</i>	5.4 µg Ni/m ³ for NOAEL group
<i>Human equivalent concentration</i>	1.6 µg Ni/m ³ for NOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m ² , DEP(PU) = 0.024)
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.05 µg Ni/m ³

B. Nickel Oxide

<i>Study</i>	National Toxicology Program, 1994c
<i>Study population</i>	Male and female F344/N rats (52-53 per group)
<i>Exposure method</i>	Discontinuous inhalation
<i>Critical effects</i>	Pathological changes in lung and lymph nodes: (1) active pulmonary inflammation, (2) lymph node hyperplasia Adrenal medullary hyperplasia (females)
<i>LOAEL</i>	500 µg Ni/m ³
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hours/day, 5 days/week
<i>Exposure duration</i>	104 weeks
<i>Average experimental exposure</i>	89.5 µg Ni/m ³ for NOAEL group
<i>Human equivalent concentration</i>	30 µg Ni/m ³ for NOAEL group males (particulate with respiratory effects, RDDR = 0.29 based on MMAD = 2.5, sigma g = 1.26, male rat body weight = 380 g, SA(PU) = 0.34 m ² , DEP(PU) = 0.024)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.10 µg Ni/m ³

The studies conducted by NTP (1994 a,b, & c) all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or animals, therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel subsulfide. The higher chronic REL value for nickel oxide of 0.1 µg/m³ reflects these dose response differences. Furthermore, while it is based upon a LOAEL, the lower severity of the

adverse health effects at the LOAEL mitigates some of the uncertainty associated with use of a LOAEL rather than a NOAEL. OEHHA therefore concludes that 0.1 µg/m³ is an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species.

Derivation of U.S. EPA Reference Dose (RfD)

<i>Study</i>	Ambrose et al., 1976
<i>Study population</i>	Rats
<i>Exposure method</i>	Diet
<i>Critical effects</i>	Decreased body and organ weights
<i>LOAEL</i>	1000 ppm (50 mg/kg-day)
<i>NOAEL</i>	100 ppm (5 mg/kg-day)
<i>Exposure continuity</i>	Continuous
<i>Exposure duration</i>	Lifetime
<i>Average exposure</i>	5 mg/kg-day
<i>Human equivalent concentration</i>	5 mg/kg-day
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	10
<i>Intraspecies uncertainty factor</i>	10
<i>Special uncertainty factor</i>	3 (inadequacies of reproductive studies)
<i>Cumulative uncertainty factor</i>	300
<i>Oral reference exposure level</i>	0.02 mg/kg-day

The oral REL for nickel is the U.S. EPA's oral Reference Dose (RfD). U.S.EPA assumed that rat consumption of 1 ppm Ni in the feed resulted in a dose of 0.05 mg/kg/day. An uncertainty factor of 10 was used for interspecies extrapolation and another of 10 to protect sensitive human populations. An additional uncertainty factor of 3 was used to account for inadequacies in reproductive studies of nickel.

VII. References

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Determination of Noncancer Chronic Reference Exposure Levels

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CHRONIC TOXICITY SUMMARY

PHENOL

(Carbolic acid, phenylic acid, phenyl hydroxide)

CAS Registry Number: 108-95-2

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	600 µg/m³
<i>Critical effect(s)</i>	Twitching, muscle tremors, neurological impairment; elevated serum liver enzymes in rats
<i>Hazard index target(s)</i>	Alimentary system; circulatory system; kidney; nervous system

II. Physical and Chemical Properties (From HSDB, 1995; ATSDR, 1989)

<i>Description</i>	Colorless to light pink solid
<i>Molecular formula</i>	C ₆ H ₅ OH
<i>Molecular weight</i>	94.11 g/mol
<i>Density</i>	1.0576 g/cm ³ @ 20° C
<i>Boiling point</i>	181.75° C
<i>Vapor pressure</i>	0.3513 torr @ 25° C
<i>Solubility</i>	86,000 ppm in water, very soluble in alcohol, carbon tetrachloride, acetic acid and liquid sulfur dioxide; soluble in chloroform, ethyl ether, carbon disulfide; slightly soluble in benzene
<i>Henry's Law Constant</i>	3.97 x 10 ⁻⁷ ATM-m ³ /mol (25 °C)
<i>Conversion factor</i>	1 ppm = 3.85 mg/m ³

III. Major Uses or Sources (HSDB, 1995)

Phenol is obtained from coal tar and is widely used as a disinfectant for industrial and medical applications. It also serves as a chemical intermediate for manufacture of nylon 6 and other man-made fibers and for manufacture of epoxy and other phenolic resins and as a solvent for petroleum refining. Approximately half of the U.S. consumption is directly related to the housing and construction industries, in applications such as germicidal paints and slimicides.

IV. Effects of Human Exposures

The information that is available concerning the health effects of phenol exposure to humans is almost exclusively limited to case reports of acute effects of oral exposure (Bruce *et al.*, 1987), dermal exposure (Griffiths, 1973), or occupational exposures, including some exposure by inhalation (Dosemeci *et al.*, 1991; Ohtsuji and Ikeda, 1972; Connecticut Bureau of Industrial Hygiene, undated). Data in animals are consistent with human data and show phenol to be well absorbed by oral, dermal, and inhalation routes of exposure. Severe chronic poisoning manifests in systemic disorders such as digestive disturbances including vomiting, difficulty swallowing, ptyalism (excess secretion of saliva), diarrhea, and anorexia (Bruce *et al.*, 1987; Baker *et al.*, 1978). Phenol poisoning is associated with headache, fainting, vertigo, and mental disturbances (Bruce *et al.*, 1987; Gosselin *et al.* 1984) which are likely symptoms of neurological effects well documented in animal studies. Ochronosis, or discoloration of the skin, and other dermatological disorders may result from dermal phenol exposure (Deichmann and Keplinger, 1962; Bruce *et al.*, 1987). Several investigators (Truppman and Ellenby, 1979; Warner and Harper, 1985) have reported that the use of phenol in the surgical procedure of skin peeling can produce cardiac arrhythmias although specifics of dose received were not determined and would be expected to be high.

Human exposure studies in which populations were exposed to phenol over longer periods of time (subchronic and chronic) are limited and have serious deficiencies including multiple chemical exposures, in many cases small size of exposed populations, and lack of information on dose received.

Occupational studies make up the majority of subchronic/chronic studies available on human health effects associated with phenol exposure. Merliss (1972) described muscle pain and weakness of unknown etiology, enlarged liver, and elevated serum enzymes (LDH, GOT, and GPT) characteristic of liver damage in an individual with intermittent inhalation and dermal exposures to phenol, cresol and xylenol. Bruze (1986) noted that a number of phenol-formaldehyde based resins are dermal irritants and contact sensitizers. Johnson *et al.* (1985) examined 78 iron and steel foundry workers with multiple chemical and aerosol exposures that included phenol and found more respiratory symptoms in the phenol exposed group. However, multiple exposure to diphenyl methane diisocyanate, formaldehyde, and silica containing aerosols prevented determination of the effects of phenol. Baj *et al.* (1994) examined twenty-two office workers exposed for six months via inhalation to a commercial product containing formaldehyde, phenol and chlorohydrocarbons. At the end of the six month period the indoor air of the workers contained 1,300 $\mu\text{g}/\text{m}^3$ of formaldehyde and 800 $\mu\text{g}/\text{m}^3$ of phenol. The eight workers with the highest concentrations of phenol in their urine had decreased erythrocyte and T-helper lymphocyte numbers and increased numbers of eosinophils and monocytes compared to controls. The multiple chemical exposure of this study prevents concluding that these effects are attributable to phenol exposure. In a study of hospital workers Apol and Cone (1983) documented dermal effects in workers exposed to a number of chemicals including phenols contained in disinfectants. This study however could not document any differences in urinary levels of phenol metabolites between control populations and exposed populations and could not assign any of the dermal effects seen to phenol or other substances in the work environment. Dosemeci *et al.* (1991) conducted a follow-up study to evaluate mortality in 14,861 workers in

five manufacturing facilities producing or using phenol and formaldehyde. Arteriosclerotic heart disease, emphysema, disease of the digestive system, and cirrhosis of the liver were inversely related to the extent of phenol exposure. Due to multiple chemical exposures the effects of phenol alone could not be identified with any certainty.

Baker *et al.* (1978) completed a study of 39 individuals exposed to drinking water contaminated with phenol for a period of 4-8 weeks. Doses of phenol were estimated to range between 10 mg/day and 240 mg/day. Effects seen included increased incidence of diarrhea, mouth sores and irritation of the oral cavity.

Two occupational studies are of note since they reported NOAELs. Workers exposed continuously for an unspecified period of time to an average air concentration of 4 ppm phenol experienced no respiratory irritation (Connecticut Bureau of Industrial Hygiene, undated). No adverse effects were reported among workers in a Bakelite factory who were exposed to 3.3 ppm (Ohtsuji and Ikeda, 1972). In this study urinary phenol levels were measured and were observed to return to pre-exposure levels within 16 hours after exposure indicating a relatively rapid clearance of phenol from the body that was confirmed in a study by Piotrowski (1971).

V. Effects of Animal Exposures

In animal studies a number of subchronic and chronic studies employing oral and inhalation routes of exposure are available as well as shorter term studies using the dermal route of exposure. Responses observed in animal studies include: pulmonary damage (inhalation exposure), myocardial injury (inhalation and dermal exposure), liver damage (inhalation exposure), renal damage (inhalation exposure), neurological effects (inhalation exposure), developmental effects (oral exposure) and dermal effects (dermal exposure). Comparison of the three routes of exposure found that oral exposure was less effective at producing systemic toxic effects possibly due to the rapid metabolism of phenol to sulfate and glucuronide conjugates by the gastrointestinal tract. Comparison of health effects among studies using dermal, oral and inhalation routes of exposure finds that inhalation is a sensitive route of exposure for laboratory animals.

Several subchronic inhalation studies of health effects from phenol exposure are available but no inhalation studies longer than 90 days could be identified. Deichmann *et al.* (1944) exposed guinea pigs, rats, and rabbits to concentrations of phenol between 26 and 52 ppm for 28-88 days depending on species. Guinea pigs exposed for 7 hours per day, five days per week, for four weeks, displayed signs of respiratory difficulty and paralysis primarily of the hind quarters, indicating neurological effects. Five of twelve animals exposed at this concentration died at 28 days. At necropsy, extensive myocardial necrosis, lobular pneumonia, fatty degeneration of the liver, and centrilobular hepatocellular necrosis were observed in all animals exposed at this level. Guinea pigs sacrificed at 41 days also exhibited pulmonary inflammation, pneumonia, bronchitis, endothelial hyperplasia, and capillary thrombosis. Rabbits exposed at these same concentrations did not exhibit any signs of discomfort, but showed similar findings at necropsy at 88 days. Rats were less sensitive in this study with an apparent NOAEL of 26 ppm phenol for these effects. In this study, guinea pigs were the most sensitive species. Limitations of the Deichmann study

include the range of exposure concentrations and the lack of a control group. Sandage (1961) exposed Sprague-Dawley rats, mice and rhesus monkeys for 90 days continuously to 5 ppm phenol. Sandage found no effects on pulmonary, cardiovascular, hematological, hepatic, or renal systems, thus defining free-standing NOAELs for these systemic effects in these species. Limitations of this study include absence of guinea pigs previously identified as the most sensitive species in the Deichmann study and lack of a demonstrated dose response to the effects of phenol. Dalin and Kristofferson (1974) examined the effects of phenol on the nervous system in rats exposed continuously for 15 days to a concentration of 26 ppm phenol and found muscle tremors, twitching and disturbances in walking rhythm and posture after 3-5 days exposure. After 15 days exposure, severe neurological impairment as measured by decreased performance on tilting plane test was found. The Dalin and Kristofferson (1974) study also documented elevated serum concentrations of LDH, GOT, GPT, and GDH indicative of liver damage in animals exposed to 26 ppm phenol continuously for 15 days.

The NCI (1980) study of the carcinogenicity of phenol is the most complete chronic study using the oral route of exposure. Mice and rats were exposed for 103 weeks to concentrations of phenol in their drinking water of 100, 2500, 5000, and 10,000 ppm. NOAELs in the mouse of 523 mg/kg/day (5000 ppm in drinking water) and NOAELs in the rat of 630 mg/kg/day (5000 ppm in drinking water) were observed for effects on the respiratory system, cardiovascular system, gastrointestinal system, hepatic system, renal system, and the brain based on histological examination of tissues. Male rats exposed to the 5000 ppm had a higher incidence of kidney inflammation (94%) than controls (74%). No tests of kidney function were performed in this study.

Boutwell and Bosch (1959) reported on the results of a chronic study in mice involving skin painting of 1.2 mg phenol or 2.5 mg phenol for a 52 week period. A NOAEL of 1.2 mg/animal for a 52 week exposure for dermal effects was found.

No multi-generational studies evaluating reproductive or developmental effects under chronic exposure conditions could be identified. Jones-Price *et al.* (1983a) reported that pregnant rats dosed orally with 0, 30, 60, and 120 mg/kg/day on gestation days 6-15 exhibited reduced fetal weight in a dose-related manner. However, no teratogenic effects or fetal deaths were observed. In a following study Jones-Price *et al.* (1983b) reported that pregnant mice dosed orally with 0, 70, 140, and 280 mg/kg/day on gestation days 6-15 exhibited decreased maternal weight gain, tremors, and increased maternal mortality at the 280 mg/kg/day dose. In the fetus reduced growth, decreased viability, and increased incidence of cleft palate were seen at the 280 mg/kg/day dose.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Sandage, 1961; Dalin and Kristofferson, 1974
<i>Study population</i>	Mice, Sprague Dawley rats and rhesus monkeys
<i>Exposure method</i>	Continuous inhalation
<i>Critical effects</i>	Systemic effects including liver and nervous system effects
<i>LOAEL</i>	26 ppm (Dalin and Kristofferson, 1974)
<i>NOAEL</i>	5 ppm (Sandage, 1961)
<i>Exposure Continuity</i>	Continuous
<i>Average exposure concentration</i>	5 ppm for NOAEL group
<i>Human equivalent concentration</i>	5 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>Exposure duration</i>	90 days
<i>Subchronic uncertainty factor</i>	1
<i>LOAEL uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.2 ppm (200 ppb; 0.6 mg/m ³ (600 µg/m ³))

No suitable human studies were available for use since all exposures were either short term or occupational in nature or did not determine dose. Of the three routes of exposure available, inhalation appears to be the most sensitive based on the number and intensity of systemic effects noted (Deichmann *et al.*, 1944) relative to oral exposure (NCI, 1980). In support of this, ATSDR (1989) notes that the gastrointestinal tract has a large capacity to metabolize phenol to sulfate and glucuronide conjugates which appear likely to be less toxic than the parent compound, thus NOAELs derived from oral studies may not be applicable for other routes of exposure. The Deichmann *et al.* (1944) study identified guinea pigs as being the most sensitive species, however, this study had a number of serious deficiencies including absence of controls, significant variability in the concentrations of phenol used in their exposure, and exposure that was not continuous. Since alternative studies using guinea pigs could not be identified, the rat was chosen as an alternative species since the rat has the most similar metabolic profile for metabolism of phenol to that of humans (ATSDR, 1989; Capel *et al.*, 1972). The Sandage (1961) study was chosen over other available studies since it was the longest in duration (90 days), had a continuous exposure, and evaluated three species (rats, mice, monkey). NOAELs determined in the Sandage study for systemic effects in all three species examined were 5 ppm, consistent with the idea that 5 ppm is a NOAEL for a number of species. Although this is a free-standing NOAEL, a subsequent study in rats indicated that nervous system and hepatic effects occur at a concentration of 26 ppm after several days (Dalin and Kristofferson, 1974).

The 5.0 ppm standard for phenol in the workplace (ACGIH, 1988; OSHA, 1985; NIOSH, 1976) is considered protective of the health of workers exposed occupationally but does not consider sensitive populations and is not for continuous exposure conditions.

The major strength of the key study is the observation of a NOAEL from a continuous exposure study involving exposure of several different species. The primary uncertainties are the lack of adequate human health effects data, the lack of multiple concentration inhalation exposure studies demonstrating a dose-response relationship, and the lack of studies with guinea pigs which have previously been identified as a sensitive species.

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Determination of Noncancer Chronic Reference Exposure Levels

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CHRONIC TOXICITY SUMMARY

PHOSPHORIC ACID

(Orthophosphoric acid)

CAS Registry Number: 7664-38-2

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	10 µg/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Bronchiolar fibrosis of the respiratory tract in rats
<i>Hazard index target(s)</i>	Respiratory system

II. Chemical Property Summary (HSDB, 1995)

<i>Description</i>	Clear syrupy liquid or unstable crystals; odorless
<i>Molecular formula</i>	H ₃ PO ₄
<i>Molecular weight</i>	98
<i>Boiling point</i>	213°C
<i>Vapor pressure</i>	0.03 mm Hg @ 20°C
<i>Solubility</i>	Very soluble in hot water; 548 g/100 ml cold water; soluble in alcohol
<i>Conversion factor</i>	4.0 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

Phosphoric acid has varied uses (HSDB, 1995). In manufacturing, it is a chemical intermediate or reagent in the production of numerous phosphate fertilizers, agricultural feeds, waxes, polishes, soaps, and detergents. It is added to foods as a preservative, acidifying agent, flavor enhancer, and clarifying agent. Phosphoric acid is also used in processes such as the coagulation of rubber latex, electropolishing, soil stabilization, and as a catalyst in the production of propylene and butene polymers, ethylbenzene, and cumene. By far, largest use of phosphoric acid comes in the production of fertilizers (>75%).

Airborne phosphoric acid can be produced by the hydrolysis of phosphorus oxides generated from either the spontaneous ignition of white phosphorus in air or the combustion of red phosphorus (Burton *et al.*, 1982; US Department of Defense (US DOD), 1981).

IV. Effects of Human Exposures

The toxic effects to 48 workers exposed (28 unexposed control workers) to oxidation products of phosphorus during the course of phosphorus production were reported (Hughes *et al.*, 1962). Exposure duration ranged from 1 to 17 years. No differences were observed between exposed and control workers with respect to leukocyte count, an effect observed in acute intoxications, or hand bone density, an effect observed in experimentally exposed animals (Inuzuka, 1956).

A prospective study of 131 workers exposed to several compounds including phosphoric acid, phosphorus pentoxide, fluorides and coal tar pitch in the air was conducted at an industrial refinery (Dutton *et al.*, 1993). Mean duration of exposure (employment) was 11.4 years and the maximum exposure level measured was 2.23 mg/m³ (phosphorus pentoxide). Pulmonary function tests were performed annually over a 3 to 7 year period. No significant residual effect was found after adjusting for age and smoking status.

V. Effects of Animal Exposures

Two 13-week inhalation studies of the effects of exposure to the combustion products of 95% red phosphorus and 5% butyl rubber were conducted in male Sprague-Dawley rats, with the first group exposed to 0, 300, 750, or 1200 mg/m³ combustion products, and the second exposed to 0, 50, 180, or 300 mg/m³ combustion products (Aranyi *et al.*, 1988a; Aranyi *et al.*, 1988b). Group numbers in the first study were 176, 84, 176, and 176, respectively. The second study used 40 animals/group. Animals were exposed for 2¼ hours/day on 4 consecutive days/week. Control animals were exposed to filtered air only. Daily particle measurements showed MMADs of 0.49-0.65 µm and σ_gs of 1.56-1.83. Fractional content of phosphoric acid in the aerosol was 71-79%. Nineteen of the 176 animals in the 1200 mg/m³ dose group died of treatment related effects. Post-mortem examination of animals that died during the course of the study showed damage to the laryngeal mucosa, which was probably contributory to mortality. The two highest dose groups in the first study also showed decreased weight gain. Twelve animals from each dose group in the first study were examined histologically and neurobehavioral studies were conducted on other animals. Half the animals in the second study were examined strictly for toxic effects on the respiratory tract, with examination of the trachea, 2 sections of the nasal turbinates, and 5 lobes of the lung. Surviving animals in the high-dose study were observed to have moderate to severe fibrosis of the terminal bronchioles, with minimal severity of this effect in the animals in the low-dose study. The reported incidence of this lesion was 9/20 at 300 mg/m³, 4/20 at 180 mg/m³, and 0/20 at 50 mg/m³. Little to no involvement of pulmonary tissue was observed.

The effects of acid aerosols (particularly sulfuric and phosphoric acid) were studied by U.S. EPA (1989). The respiratory tract was the primary target of toxicity resulting from the irritational effect of the acid on the tissues of the larynx and trachea. The nature of the effect was dependent upon the aerosol particle size, duration of exposure, and the hygroscopic character of the acid.

Sprague-Dawley rats were exposed to the smoke and combustion products of white phosphorus in felt pellets at 192.5 (18 animals/sex), 589 (24 animals/sex), or 1161 mg/m³ (34 males, 43 females) phosphoric acid equivalents for 15 minutes/day, 5 days/week, for 13 weeks (US Department of Defense (US DOD), 1981). Control animals numbering half the size of the treated groups were exposed to air only. Groups of animals were sacrificed at 6 and 13 weeks, and 4 weeks post-exposure. Endpoints examined included: hematology, clinical chemistry, gross- and histo-pathology, ECG, pulmonary function, and behavior. Of the animals in the highest dose group, 56% died as a result of exposure, with the only other death occurring in the control group. Findings were restricted to effects on the respiratory system, with tracheitis and laryngitis incidences of 2/35, 32/47, and 28/31 among surviving animals in the three dose groups. In the post-exposure examination, bronchiolitis occurred with a frequency of 0/12, 5/24, and 6/16 in the three dose groups.

The toxicity of the combustion products of 95% amorphous red phosphorus and 5% polyvinyl butyral BL18 to female Wistar rats, Porton-strain mice, and guinea pigs was reported (Marrs *et al.*, 1989). Rats (50/group), mice (100/group), and guinea pigs (42-48/group) were exposed to concentrations of 0, 16, or 128 mg/m³ for 1 hour/day, 5 days/week for 36 weeks (mice) or 40 weeks (rats and guinea pigs), with an examination conducted at 19 months or when animals appeared unhealthy. All groups, including controls, showed high mortality. Mice showed accumulation of alveolar macrophages with incidences of 2/41, 9/37, and 9/22 in the control, low-, and high-dose groups, respectively. Guinea pigs appeared to be particularly intolerant to the effects of the smoke.

Female rabbits and rats (10/group) were examined for acute toxic effects of smoke generated by the combustion of either 95% red phosphorus / 5% butyl rubber (Smoke I) or 97% red phosphorus / 3% butadiene styrene (Smoke II) (Marrs, 1984). Animals were exposed for 30 minutes and examined one and 14 days later. Smoke I produced inflammation of the larynx and trachea in rats at 1 day with some inflammation still observed at 14 days. Tracheal inflammation was also reported in rabbits exposed to Smoke I. Four of the rats exposed to Smoke II died within the first day, with severe pulmonary congestion observed in the animals.

One hour exposure to the combustion products of 95% red phosphorus / 5% butyl rubber (plus 1% mineral oil) produced epiglottal deformation, laryngeal edema, and laryngeal and tracheal lesions in rats (Burton *et al.*, 1982). A four-hour exposure produced more severe effects of a similar nature plus some hemorrhaging.

Rats (number unspecified) exposed to 150-160 mg/m³ elemental phosphorus for 30 minutes/day for 60 days were examined for toxic effects (Inuzuka, 1956). Limb bone abnormalities were noted and effects included delayed ossification, widening of the epiphysis, and abnormal axial development.

Two studies have addressed the reproductive and developmental toxicity from exposure to the combustion products of white phosphorus and felt for 15 minutes/day during gestational days 6-15 in rats (24/group) (US Department of Defense (US DOD), 1981; US Department of Defense (US DOD), 1982). Fetal effects included increased incidence of some visceral variations and hypoplasia of the xiphoid process although data were incompletely reported. Another study,

which exposed dams 3 weeks prior to mating, throughout gestation, and through lactation and males for 10 weeks prior to and during mating, showed decreased pup body weight, 24-hour and 21-day survival, and lactation. An oral study in which elemental phosphorus was administered to male and female rats by gavage in corn oil showed no statistically significant effects (Condray, 1985).

VI. Derivation of the U.S. EPA RfC

<i>Study</i>	Aranyi <i>et al.</i> , 1988a
<i>Study population</i>	Male Sprague-Dawley rats
<i>Exposure method</i>	Discontinuous whole body inhalation
<i>Critical effects</i>	Bronchiolar fibrosis of the respiratory tract
<i>LOAEL</i>	180 mg/m ³
<i>NOAEL</i>	50 mg/m ³
<i>BMC₁₀</i>	100 mg/m ³
<i>Exposure continuity</i>	2¼ hours/day, 4 days/week
<i>Exposure duration</i>	13 weeks
<i>Average experimental exposure</i>	2.7 mg/m ³ for NOAEL group (estimated as 5.4 mg/m ³ at BMC ₁₀)
<i>Human equivalent concentration</i>	3.3 mg/m ³ at BMC ₁₀ (particle with respiratory effects, RDDR = 0.63)
<i>LOAEL uncertainty factor</i>	1 (BMC ₁₀ assumed to be similar to NOAEL)
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Reference exposure level</i>	0.01 mg/m ³ (10 µg/m ³)

The U.S. EPA has used a benchmark dose methodology for the derivation of the reference concentration (RfC) for phosphoric acid from the toxicity data in the Aranyi *et al.* (1988) study (U.S. EPA, 1995). The RfC is restricted to “aerosols of phosphoric acid and phosphorus oxidation products and does not apply to elemental phosphorus or other forms of phosphorus, such as phosphorus salts”. The Aranyi *et al.* (1988a) study represents the most adequate study for the quantitative evaluation of the toxicity of phosphoric acid. It was conducted with a large number of animals with multiple doses, produced good dose-response data, and examined likely targets of toxicity (respiratory system) of smoke generated from the combustion of phosphorus and butyl rubber. Uncertainties associated with these data, however, include that (1) the study used combustion products of phosphorus rather than phosphoric acid itself, (2) the total exposure time was relatively short and discontinuous over the duration of the experiment, and (3) only one species/strain/sex was studied.

The U.S. EPA, using the Weibull model, estimated the lower 95% confidence level bound on the maximum likelihood estimate (MLE = 150 mg/m³) resulting in 10% incidence of lesions in the tracheobronchiolar region to be 100 mg/m³ (the BMC₁₀). The U.S. EPA considered 10% incidence level to be a correlate to the NOAEL, based on a precedent in the analysis of data with

developmental toxicity endpoints (Allen *et al.*, 1994; Faustman *et al.*, 1994). After correction for exposure continuity, a regional deposited dose ratio (RDDR) for the tracheobronchial region of 0.64 was applied due to the availability of data concerning the growth and deposition of phosphoric acid aerosol particles in humans and the similarities in the effects of phosphoric and better-characterized sulfuric acid aerosols. Key assumptions in the generation of this factor include: (1) the lowest σ_g of 1.56 μm cited in the study was used in the calculation; (2) geometric rather than aerodynamic diameter approximations were used; (3) particles of this size reach the deposition / lesion site (bronchioles); 4) these hygroscopic particles become more uniform with growth; and (5) particle growth is similar in humans and rodents. An uncertainty factor of 10 was applied because of the subchronic duration of the study. A factor of 3 was applied for interspecies extrapolation in light of the fact that some correction for human equivalency was made with the RDDR. Finally, a factor of 10 was applied for protection of potentially sensitive human subpopulations. The resulting chronic REL for phosphoric acid is 0.01 mg/m^3 .

The strengths of the inhalation REL include the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis and the observation of a NOAEL. Major areas of uncertainty are the lack of adequate human exposure data, the lack of chronic inhalation exposure studies and the discontinuous nature of exposures (only 2 1/4 hours per day).

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CHRONIC TOXICITY SUMMARY

PROPYLENE

(1-propene; 1-propylene; propene; methylethene; methylethylene)

CAS Registry Number: 115-07-1

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	3,000 µg/m³
<i>Critical effect(s)</i>	Squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity in Fischer 344/N rats
<i>Hazard index target(s)</i>	Respiratory system

II. Chemical and Physical Properties (HSDB, 1995)

<i>Description</i>	Colorless gas; practically odorless.
<i>Molecular formula</i>	C ₃ H ₆
<i>Molecular weight</i>	42.08
<i>Boiling point</i>	-48°C
<i>Vapor pressure</i>	8690 mm Hg at 25°C
<i>Solubility</i>	Soluble in alcohol and ether.
<i>Conversion factor</i>	1.72 µg/m ³ per ppb at 25°C

III. Major Uses and Sources (HSDB 1995)

Propylene is produced primarily as a by-product of petroleum refining and of ethylene production by steam cracking of hydrocarbon feedstocks. Propylene is a major chemical intermediate. The most important derivatives of chemical and polymer grade propylene are polypropylene, acrylonitrile, propylene oxide, isopropanol and cumene. Use of polypropylene in plastics (injection moulding) and fibers (carpets) accounts for over one-third of U.S. consumption. It is also used in the production of synthetic rubber and as a propellant or component in aerosols. In 1994, propylene was ranked seventh among the top 50 chemicals produced domestically (C&EN, 1995). In the environment, propylene occurs as a natural product from vegetation. It is also a product of combustion of organic matter (biomass burning, motor vehicle exhausts and tobacco smoke) and is released during production and use. The most probable route of exposure to humans is by inhalation. Propylene has been detected in the atmosphere over both metropolitan (2.6 to 23.3 ppb) and rural (0.007 to 4.8 ppb) areas (Cox *et al.*, 1976; Leonard *et al.*, 1976).

IV. Effects of Human Exposures

No data were available on the absorption, distribution or excretion of propylene in humans. However, hemoglobin (Hb) adducts of the metabolite intermediate propylene oxide have been used to monitor the internal dose of propylene (Tornqvist and Ehrenberg, 1990). The background level of the 2-hydroxypropyl adduct to the N-terminal valine of hemoglobin was found to be about 2 pmol/g Hb. This was estimated to be equivalent to smoking 10 cigarettes per day; cigarette smoking is a source of propylene. Occupational exposure to propylene at 1 ppm (1.72 mg/m³) was assumed to be associated with an increment of 5 pmol/g Hb (Kautiainen and Tornqvist, 1991).

No data were available on the chronic effects of propylene in humans.

V. Effects of Animal Exposures

In rats and mice, most propylene inhaled into the lungs is exhaled again and does not reach the blood to become systemically available (Golka *et al.*, 1989; Svensson and Osterman-Golkar, 1984). Once absorbed, a major route of metabolism for propylene is through the cytochrome P-450 system to propylene oxide, a known carcinogen in experimental animals. Cytochrome P-450 enzymes in both the liver and nasal epithelium (Maples and Dahl, 1991) can convert propylene to its toxic metabolite. However, in rats, propylene metabolism becomes increasingly saturated at concentrations above 50 ppm (86 mg/m³) in the atmosphere (Golka *et al.*, 1989), which limits the amount of propylene oxide produced. Therefore, the amount of absorbed propylene may not reach high enough levels in classical long-term inhalation studies (Quest *et al.*, 1984) to show positive carcinogenic or serious chronic effects.

The only chronic toxicity investigation found for propylene was a comprehensive 2-year study in F344/N rats and B6C3F₁ mice (Quest *et al.*, 1984; NTP, 1985). Groups of 50 rats and 50 mice of each sex were exposed to concentrations of 0, 5000, and 10,000 ppm for 6 hr/day, 5 days/week, for 103 weeks. (Mean daily concentrations were 0, 4985, and 9891 ppm, respectively, for the rat study; and 0, 4999, and 9957 ppm, respectively, for the mouse study.) In exposed rats, treatment-related chronic effects were observed in the nasal cavity. In female rats, epithelial hyperplasia occurred in the high dose group and squamous metaplasia occurred in both dosage groups. In male rats, squamous metaplasia was seen only in the low dose group, but both dosage groups had inflammatory changes characterized by an influx of lymphocytes, macrophages and granulocytes into the submucosa and granulocytes into the lumen. Nasal lesions were not observed in mice. The inflammatory lesions were more severe in the high dose group. Very mild focal inflammation was observed in the kidneys of treated mice but the relationship to propylene exposure was unclear. No other treatment-related effects, including clinical signs, mortality, mean organ and body weights and histopathology, were observed.

In a long-term carcinogenicity study, Sprague-Dawley rats and Swiss mice (100-120 animals/group/sex) were exposed by inhalation to 0, 200, 1000 and 5000 ppm propylene 7 hr/day, 5 days/week, for 104 weeks (rats) or 78 weeks (mice) (Ciliberti *et al.*, 1988). No body weight differences were observed between treated and control animals of either species.

Mortality was reported to be slightly increased in male rats in the 1000 and 5000 ppm groups and in male mice in the 5000 ppm group, but numerical values of mortality were not presented in the report. Therefore, it is assumed that mortality differences were insignificant. Other possible general body system or nonneoplastic effects were not reported and assumed to have not been investigated.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Quest <i>et al.</i> , 1984
<i>Study population</i>	50 rats/group/sex, 300 total.
<i>Exposure method</i>	Discontinuous whole body inhalation exposure (0 or 4,985 or 9,891 ppm).
<i>Critical effects</i>	Respiratory system; squamous metaplasia (males and females), epithelial hyperplasia (females only), and inflammation (males only) of the nasal cavity
<i>LOAEL</i>	4,985 ppm (8,570 mg/m ³)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	2 years
<i>Average experimental exposure</i>	890 ppm for LOAEL group
<i>Human equivalent concentration</i>	190 ppm (gas with extrathoracic respiratory effects, RGDR = 0.21, based on BW = 305 g, MV = 0.21 L/min, SA(ET) = 15 cm ²)
<i>LOAEL uncertainty factor</i>	3 (low severity)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	2 ppm (2,000 ppb, 3 mg/m ³ , 3,000 µg/m ³)

Strengths of the propylene REL include the availability of a long-term, controlled exposure study in large groups of experimental animals that included extensive histopathological analyses.

Lifetime exposure of rats and mice to propylene resulted in adverse effects in the nasal cavity of rats at both exposure levels. Therefore, a NOAEL was not observed. However, the effects observed were mild.

Other weaknesses of the database for propylene include the lack of lifetime toxicity studies in any non-rodent species. Also, no long-term human toxicity or epidemiology studies were located in the literature. Human pharmacokinetic studies to compare with experimental animal pharmacokinetic studies were absent. Another uncertainty is the lack of reproductive and developmental toxicity studies. A comprehensive multi-generation study in an experimental animal species would enhance the development of a propylene REL.

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CHRONIC TOXICITY SUMMARY

PROPYLENE GLYCOL MONOMETHYL ETHER

(1-Methoxy-2-propanol; 1-methoxypropanol; Propapsol solvent M)

CAS Registry Number: 107-98-2

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	2,000 µg/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	CNS effects (mild, reversible sedation) in rabbits
<i>Hazard index target(s)</i>	Nervous system

II. Physical and Chemical Properties (HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₄ H ₁₀ O ₂
<i>Molecular weight</i>	90.14
<i>Density</i>	0.962 g/cm ³ @ 20° c
<i>Boiling point</i>	118-118.5°c
<i>Melting point</i>	-96.7°c
<i>Vapor pressure</i>	11.8 mm Hg @ 25°C
<i>Solubility</i>	Soluble in water, methanol, ether, and other organic solvents
<i>Conversion factor</i>	1 ppm = 3.69 mg/m ³ at 25° C

III. Major Uses or Sources

Propylene glycol monomethyl ether (PGME) is used as a solvent for cellulose, acrylics, dyes, inks and stains (HSDB, 1995). Thus, the primary use of PGME is in lacquers and paints. Use of PGME is anticipated to increase due to its low systemic toxicity.

IV. Effects of Exposures on Humans

No reports or studies of human toxicity following chronic exposure to PGME were located in the literature. Slight eye irritation was reported by two of six human volunteers exposed to 100 ppm PGME for 2 hours (Stewart *et al.*, 1970). These subjects were exposed for a total of 3 1/2 hours during which no decrement in visual acuity, coordination, neurological responses or reaction time was measured. The same experiment exposed 23 subjects to 250 ppm PGME. After 15 to 30 minutes of exposure, 8/23 reported eye irritation and 3/23 reported throat irritation; lacrimation was observed in 3/23 subjects. Three subjects each reported one of the following symptoms: irritation, headache, and nausea. While the subjects frequently reported the odor to be objectionable upon first entering the chamber, the odor was usually undetectable by the end of the exposure. Clinical chemistry and urinalysis completed following exposure was not altered as compared to pre-exposure measurements.

V. Effects of Exposures on Animals

Male and female rats (10 per sex per concentration) and rabbits (7 per sex per concentration) were exposed by inhalation to 300, 1000, or 3000 ppm PGME 5 hours per day, 5 days per week for 13 weeks (Landry *et al.*, 1983). Relative liver weights were statistically significantly higher than controls in both male and female rats exposed to 3000 ppm PGME. Hepatocellular hypertrophy was observed upon histopathologic examination of high dose females. The authors conclude that these effects are the result of physiologic adaptation rather than a manifestation of toxicity. The key observation in this study was sedation of rats and rabbits exposed to 3000 ppm PGME. The sedative effects were no longer apparent after 1-2 weeks of exposure.

Similar findings of mild CNS depression were observed by Hanley *et al.* (1984). Pregnant rats and rabbits were exposed to 500, 1500, or 3000 ppm PGME 6 hours per day either days 6-15 or days 6-18 of gestation, respectively. During the first 4-5 days of exposure, rats in the 3000 ppm PGME exposure group were lethargic and moderately ataxic. Statistically significant decreases in food consumption and maternal body weight gain were also observed during this period. A statistically significant increase in the incidence of delayed sternebral ossification was observed in the 3000 ppm exposure group. Rabbits exposed to 3000 ppm exhibited mild lethargy during the first 1-2 days of exposure with rapid post-exposure recovery. Overall maternal weight gain during the exposure (days 6-18 of gestation) was statistically significantly lower than controls.

No significant effect on fetal birth weight or on pup survival indices (e.g., proportion of pups surviving to day 3 post-delivery) was noted following exposure of pregnant rats to 200 or 600 ppm PGME 6 hours per day on days 6-17 of gestation (Doe *et al.*, 1983). Male rats were exposed to 200 or 600 ppm PGME 6 hours per day for 10 consecutive days. No significant effects on testicular weight or pathology were observed.

Increased liver and kidney weights were observed in male and female rats (10 per sex per concentration) following exposure to 6000 ppm for 7 hours per day, for 81 exposures over a 114-day period (Rowe *et al.*, 1954). No histopathological abnormalities were observed at necropsy.

Ethylene glycol methyl ether (EGME), a structurally-related compound, exerts considerable toxicity on the blood, thymus, testes, and developing fetus. The toxicity of EGME has been linked to its primary metabolite, methoxyacetic acid. Recent comparative toxicity and metabolism studies (Miller *et al.*, 1983, Miller *et al.*, 1984) indicate that the relatively low systemic toxicity exerted by PGME is due to its different metabolites. Following a single oral dose of PGME, the key urinary metabolites identified in rats were propylene glycol and the sulfate and glucuronide conjugate of PGME (Miller *et al.*, 1983).

VI. Derivation of U.S. EPA Reference Concentration (U.S. EPA, 1995)

<i>Study</i>	Landry <i>et al.</i> , 1983
<i>Study population</i>	Fischer 344 rats (10/sex/concentration); New Zealand white rabbits (7/sex/concentration)
<i>Exposure method</i>	Discontinuous whole-body inhalation (0, 300, 1000, or 3000 ppm)
<i>Critical effects</i>	Mild reversible sedation observed in animals exposed to 3000 ppm PGME (This effect was observed for the first 1-2 weeks of exposure only.)
<i>LOAEL</i>	3000 ppm
<i>NOAEL</i>	1000 ppm
<i>Exposure continuity</i>	6 hours per day, 5 days per week
<i>Average experimental exposure</i>	179 ppm for NOAEL group
<i>Human equivalent concentration</i>	179 ppm for NOAEL group (gas with systemic effects, based on RGDR = 1.0 using default assumption that $\lambda(a) = \lambda(h)$)
<i>Exposure duration</i>	13 weeks
<i>LOAEL factor</i>	1
<i>Subchronic uncertainty factor</i>	10
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.6 ppm (600 ppb, 2 mg/m ³ , 2000 µg/m ³)

Strengths of the PGME RfC include the observation of a NOAEL, and the availability of subchronic exposure studies involving multiple concentrations and species. Major areas of uncertainty are the lack of human data, the small groups tested in the study, and the difficulty in interpreting the significance of apparent acutely toxic effects in deriving a value protective for long-term exposures.

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Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and
Emergency Response, Washington, DC. EP/540/1-86/052. [cited in U.S. EPA, 1995].

CHRONIC TOXICITY SUMMARY

PROPYLENE OXIDE

(1-,2-propylene oxide; methyl ethylene oxide; propene oxide)

CAS Registry Number: 75-56-9

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	30 $\mu\text{g}/\text{m}^3$ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Degenerative and hyperplastic changes in the respiratory epithelium of rats
<i>Hazard index target(s)</i>	Respiratory system

II. Physical and Chemical Properties (HSDB, 1994)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	$\text{C}_3\text{H}_6\text{O}$
<i>Molecular weight</i>	58.08
<i>Density</i>	0.83 g/cm^3 @ 20° C
<i>Boiling point</i>	34.23° C
<i>Melting point</i>	-112.13° C
<i>Vapor pressure</i>	445 mm Hg @ 20° C
<i>Solubility</i>	Soluble in water, miscible in acetone, benzene, carbon tetrachloride, methanol, ether
<i>Conversion factor</i>	2.38 mg/m^3 per ppm at 25° C

III. Major Uses or Sources

Propylene oxide is used as a fumigant such as in the sterilization of packaged foods. It is also used as a chemical intermediate in the production of propylene glycol and glycol ethers and as a solvent. Propylene oxide is used in the preparation of surfactants and oil demulsifiers (HSDB, 1994).

IV. Effects of Human Exposures

Conclusive data regarding the effects of occupational exposure to propylene oxide were not located.

An epidemiological study examining mortality among workers with exposure to asbestos and several chemicals, including propylene oxide, identified three deaths due to mesothelioma, a rare cancer associated with asbestos exposure and a statistically significant increase in the number of deaths attributed to forms of heart disease other than ischemia and hypertension (Egedahl *et al.*, 1989). The latter finding was explained by the authors to be the result of differences in diagnostic accuracy between rural and urban, and primary and tertiary medical care settings. A statistically significant decrease in observed deaths was found for all respiratory cancers, cancer of the bronchus and lung, circulatory disease, digestive diseases, cirrhosis and other liver disease, and death due to accidents, poisonings, and violence. These observations may be partially attributed to a “healthy worker effect”.

V. Effects of Animal Exposures

Male and female rats were exposed for 124 or 123 weeks (respectively) to 30, 100 or 300 ppm propylene oxide 6 hours per day, 5 days per week (Kuper *et al.*, 1988). Interim sacrifices were performed at 12, 18, and 24 months. Cumulative mortality was statistically significantly different from controls at 115 weeks in rats of both sexes exposed to 300 ppm propylene oxide. Cumulative mortality was also significantly different from controls at 119 weeks in female rats exposed to 100 ppm. However, a contributing factor to the increased mortality in female rats was the presence of mammary tumors. Atrophy of the olfactory epithelium and degenerative changes in the respiratory epithelium were observed in both male and female rats following 28 months of exposure to 30, 100, or 300 ppm propylene oxide. Severe hyperplastic changes in the olfactory epithelium were observed in male and female rats following 28 months exposure to 300 ppm propylene oxide. Mild hyperplastic changes were observed in the olfactory epithelium of female rats exposed to 100 ppm propylene oxide.

Rats and mice were exposed to 200 and 400 ppm propylene oxide 6 hours per day, 5 days per week for 103 weeks (NTP, 1985). Survival in mice was adversely affected in all groups exposed to propylene oxide; a statistically significant decrease in survival was observed in male and female mice exposed to 400 ppm propylene oxide. Survival in rats was not adversely affected by propylene oxide exposure. Rats exhibited exposure-related increases in suppurative inflammation of the nasal cavity, epithelial hyperplasia and squamous metaplasia.

Rats were exposed to 1500 ppm propylene oxide 6 hours per day, 5 days per week for 7 weeks (Ohnishi *et al.*, 1988). After 3-4 weeks of exposure the rats exhibited an awkward gait; the rats were ataxic by the seventh week. Histopathological examination revealed axonal degeneration of myelinated fibers of the hindleg nerve and fasciculus gracilis indicating central-peripheral distal axonopathy.

Artificially inseminated rabbits were exposed to 500 ppm propylene oxide on days 1-19 or 7-19 of gestation (Hardin *et al.*, 1983). Maternal toxicity as indicated by a significant reduction in food intake and a significant decrease in maternal body weight gain was observed in both exposed groups. An increased number of resorptions per litter, with no change in total resorptions, was observed in rabbits exposed on days 1-19 of gestation. Sternebral and limb anomalies (considered minor by U.S. EPA and the authors) were significantly increased in the offspring of rabbits exposed on days 1-19 of gestation.

The same study also reported similar findings in sperm-positive rats exposed to 500 ppm propylene oxide on either days 1-16 or 7-16 of gestation or daily for 3 weeks prior to mating and then daily on days 1-16 of gestation. Reproductive capacity was impaired in rats exposed prior to breeding; the number of corpora lutea, implantation sites, and live fetuses were reduced. Those dams exposed pregestationally to propylene oxide also exhibited more resorptions. Maternal toxicity as indicated by decreased food intake and decreased body weight gain was observed in all exposed rats. Significant reductions in fetal body weight and fetal crown-rump length were observed in all exposed groups. An increased incidence of wavy ribs and reduced ossification were observed in the offspring of rats exposed from days 1-16 of gestation.

VI. Derivation of U.S. EPA Reference Concentration (IRIS, 1995)

<i>Study</i>	Kuper <i>et al.</i> , 1988
<i>Study population</i>	Rats (male and female)
<i>Exposure method</i>	Inhalation (0, 30, 100 or 300 ppm)
<i>LOAEL</i>	30 ppm
<i>Critical effects</i>	Degenerative and hyperplastic changes in the respiratory epithelium
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	6 hr/day for 5 days/week
<i>Exposure duration</i>	124 weeks
<i>Average experimental exposure</i>	5.4 ppm for LOAEL group
<i>Human equivalent concentration</i>	1.2 ppm for LOAEL group (gas with extrathoracic respiratory effects, RGDR = 0.23, based on MV = 0.3 m ³ /day, SA(ET) = 11.6 cm ²)
<i>LOAEL uncertainty factor</i>	3 (mild effects only observed during last 4 months of exposure)
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	3
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.009 ppm (9 ppb, 0.03 mg/m ³ , 30 µg/m ³)

The major strength of the RfC is the use of a well-conducted long-term multi-concentration study with adequate histopathological analyses. Weaknesses include the lack of adequate human data and the lack of a NOAEL observation.

VII. References

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CHRONIC TOXICITY SUMMARY

STYRENE

(ethenylbenzene, phenylethylene, vinylbenzene)

CAS Registry Number: 100-42-5

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	1,000 µg/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effects(s)</i>	Neurotoxicity in humans
<i>Hazard index target(s)</i>	Nervous system

II. Chemical Property Summary

<i>Description</i>	Colorless to slightly yellow liquid
<i>Molecular formula</i>	C ₈ H ₈
<i>Molecular weight</i>	104.16
<i>Boiling point</i>	145.2 °C
<i>Vapor pressure</i>	10 mm at 31°C, polymerizes at 82°C and above (Weast, 1979)
<i>Solubility</i>	310 µg/ml (Dean, 1985)
<i>Conversion factor</i>	4.26 µg/m ³ per ppb at 25°C

III. Major Uses and Sources

The major source of styrene is industrial synthesis in which ethylbenzene is the starting material (ATSDR, 1992). The major uses of styrene are in polystyrene manufacturing, the butadiene-styrene rubber industry, and in the reinforced plastics industry (RPI) (WHO, 1983). Major non-styrene contaminants in the butadiene-styrene rubber industry are butadiene, benzene, carbon disulfide, and trichloroethylene, whereas the main co-contaminants associated with the RPI are glass fibers and acetone (WHO, 1983). Environmental exposures to styrene may result from mainstream cigarette smoke (Newhook and Caldwell, 1993) and newly installed carpets containing a styrene-butadiene rubber latex adhesive (Hodgson *et al.*, 1993). The Third National Health and Nutrition Examination Survey (NHANES) (Ashley *et al.*, 1994) reported a mean blood styrene level among ≥ 600 individuals as 0.074 ppb.

IV. Effects of Human Exposure

Chronic exposures to styrene (to be discussed below) result in central nervous system (CNS) and peripheral nervous system effects, although the latter are not as pronounced (ATSDR, 1992; Rebert and Hall, 1994; Murata *et al.*, 1991). The evidence for styrene induced hepatic changes is either negative or equivocal (ATSDR, 1992). Although increased urinary enzymes suggest renal changes in workers exposed to 212 ppm (8-hour workday), exposure of another workforce to 53 ppm did not result in the urinary excretion of other proteins. Some human studies suggest that chronic exposure to styrene results in reproductive effects, but the limited data are difficult to interpret because of the small sample numbers (Brown, 1991; Lindbohm, 1993). Immunologic alterations (e.g., altered phenotypic profiles among lymphocyte subsets, decreased natural killer cell activity, and decreased chemotaxis) have also been observed, but the limited data prevent quantitative interpretation (Bergamaschi *et al.*, 1995; Governa *et al.*, 1994).

The CNS depressant effects of acute exposures to high styrene levels are probably mediated by the direct effect of the lipophilic, unmetabolized styrene on nerve cell membranes, but long-term effects of styrene exposure may result from the action of one or more metabolites of styrene (Savolainen, 1977; Mutti *et al.*, 1988). In humans, styrene metabolism is initiated by cytochrome P450 (P450)-mediated oxidation of styrene to a reactive metabolite, styrene oxide (SO). The reaction takes place in human liver and, to a minor extent, in lung (Nakajima *et al.*, 1994). The P450 enzymes responsible for the epoxidation of styrene to styrene oxide are also found in human brain, but the brain isozymes have not been tested specifically with styrene as substrate (Bhamre *et al.*, 1993). Styrene may also be oxidized to styrene oxide by enzymes which share specific iron and porphyrin components with P450 and those that utilize active oxygen species (Belvedere *et al.*, 1983; Tursi *et al.*, 1983; Miller *et al.*, 1992).

The major end product of styrene metabolism in humans is urinary mandelic acid (MA) and phenylglyoxylic acid (PGA) (Bardodej and Bardodejova, 1970; Leibman, 1975; Guillemin and Bauer, 1979). Other pathways that may be present in other animals are either absent or are quantitatively negligible in humans, except when high styrene levels are encountered (Guillemin and Berode, 1979; Chakrabarti *et al.*, 1993; Hallier *et al.*, 1995). Confounders of the quantitative relationship between styrene exposure and urinary MA+PGA are the consumption of ethanol (Berode *et al.*, 1986) and exposure to ethylbenzene (Bardodej and Bardodejova, 1970). An important consequence of ethanol related decreased levels of urinary mandelic acid is the potential underestimation of exposure to styrene (Guillemin and Bauer, 1979; Berode *et al.*, 1986). However, the urinary metabolite levels return to control values 4-5 hours after the ethanol consumption (Berode *et al.*, 1986).

Indicators of human styrene exposure include exhaled styrene, blood styrene, urinary MA, and urinary MA+PGA (Guillemin and Berode, 1988). Exposure to styrene by inhalation results in 89 percent absorption (Guillemin and Berode, 1988). In the occupational studies that are the basis for quantifying the relationship between chronic styrene exposure and health effects, end-of-shift or next-morning MA+PGA have been used. The next-morning measurements are more reflective of past exposures due to the high fat solubility of styrene (fat:blood partition coefficient = 94 (Csanady *et al.*, 1994)), the presence of a second, long biological half-life for MA = 25 hours., and a long biological half-life for PGA = 11 hours (Guillemin and Bauer, 1979). Following

inhalation, the half-life for styrene is 41 minutes in blood (Wigaeus *et al.* 1983) and 32-46 hours in fat tissue (Perbellini *et al.*, 1988).

One postulated mechanism for the chronic non-cancer toxicity of styrene is the binding of the highly reactive styrene oxide to components of nervous tissue. Another postulated mechanism is an alteration in the levels of circulating catecholamines (e.g., dopamine) due to the binding of PGA to these biogenic amines (Mutti, 1993; Mutti *et al.*, 1984a; Checkoway, 1994) and the subsequent changes in physiological functions that are under biogenic amine control. Although long-term exposures to styrene are associated with decrements in physiological functions, the exact mechanism(s) for these effects have not been clearly established (see reviews by ATSDR, 1992; Mutti, 1993; Rebert and Hall, 1994).

Kolstad *et al.* (1995) estimated excess deaths due to four major non-malignant disease groups for 53,847 male workers in the Danish reinforced plastics industry. Low and high styrene exposures were based on companies with less than 50% (low) and those with 50% or more (high) employees involved with reinforced plastics. An internal comparison was made with workers unexposed to styrene to account for more similar activities and lifestyles. Statistically significant ($p < 0.05$) excess deaths due to pancreatitis and degenerative disorders of the myocardium and non-significant excess deaths due to degenerative diseases of the nervous system were observed. Non-significant excess deaths due to glomerulonephritis were also observed.

Checkoway *et al.* (1994) described a cross-sectional study of 59 male boat plant workers exposed to <1 to 144 (mean = 37.2) ppm styrene. Monoamine oxidase B (MAO-B) activity in platelets was measured as an indicator of catecholamine metabolism. When the styrene exposed workers were divided into quartile exposures, a dose dependent decrease in MAO-B activity was observed after adjustments were made for age, smoking, alcohol and medication use.

Female workers employed in the RPI were studied for levels of substances associated with neuroendocrine function (Mutti *et al.*, 1984a). Serum prolactin, thyroid stimulating hormone, human growth hormone, follicle stimulating hormone, and luteinizing hormone were measured in 30 women who were between the 5th and 15th day of the menstrual cycle. Exposure was based on the next-morning MA+PGA and levels of the neuroendocrine substances were measured in venous blood samples taken the next-morning before the start of work. On the basis of a relationship (not detailed in the report) between urinary metabolites and styrene air concentration, the authors estimated the average styrene TWA/8 hr was about 130 ppm. Controls consisted of women who were factory workers living in the same area as the styrene-exposed women, but who were not knowingly exposed to styrene. After controlling for age and exposure time, the increased prolactin and thyroid stimulating hormone levels were correlated with the concentration of next-morning urinary MA+PGA, although only the increased prolactin levels were statistically significant.

Disturbances to the CNS have been observed in occupational settings. Decreased manual dexterity and increased reaction times were observed by Mackay and Kelman (1986), Flodin *et al.* (1989), and Cherry and Gautrin (1990) for air styrene levels of 25 ppm to more than 100 ppm. However, in each of these studies, there were difficulties in quantifying the effect. The difficulties included small sample size, unknown exposure duration, and either unknown ethanol consumption or lack of adjustment for ethanol consumption. In the Cherry and Gautrin (1990)

investigation, however, the authors determined that ethanol consumption did not reduce the correlation between increased reaction time and exposure.

Decrements in other CNS functions were observed among workers in the well controlled studies of Fallas *et al.* (1992), Chia *et al.* (1994), and Mutti *et al.* (1984b). Fallas *et al.* (1992) studied 60 male workers (average age = 29.5 years, average air styrene = 24.3 ppm). The styrene-exposed population was compared to non-exposed worker controls and matched for age, intellectual level, and ethnic origin. The results from a standardized test battery showed decrements in the aiming response and 22/60 styrene exposed workers exhibited increased reaction times compared to 7/60 controls. Acquired color vision loss (dyschromatopsia) was also observed in the styrene-exposed workers compared to controls. Chia *et al.* (1994) also observed decrements in CNS function as defined by altered visual retention, audio-digit recognition, and digit recognition. However, a dose-response relationship did not exist. These workers also exhibited a statistically nonsignificant dose-dependent dyschromatopsia.

Dyschromatopsia among styrene workers in the reinforced plastics industry was also reported by Gobba and Cavalleri (1993) and Campagna *et al.* (1995). Workers (n=36) exposed to an average of 16 ppm styrene exhibited significantly greater dyschromatopsia than controls, matched for age, ethanol consumption and tobacco smoking (Gobba and Cavalleri, 1993). Among the study population, only 1/36 styrene-exposed workers (compared to 16/36 controls) performed the test with 100 percent accuracy. When a different group of styrene-exposed workers was tested, those exposed to > 50 ppm air styrene exhibited greater dyschromatopsia than those exposed to ≤ 50 ppm, and within this group, a subset exhibited a similar decrement after returning from a one month vacation. In the Campagna *et al.* (1995) study, the test for dyschromatopsia was given to 81 reinforced plastics industry workers (79 male and 2 female) exposed to 4.6, 10.1, and 88.8 ppm styrene (first quartile, median, and third quartile, respectively). No control group was used in this study. Statistical analysis revealed a correlation of color vision loss with exposure to styrene (defined as next-morning urinary mandelic acid), age, and ethanol consumption.

Mutti *et al.* (1984b) studied a group of male styrene workers and a control group of manual workers exposed to styrene for an average of 8.6 years. Eligibility criteria included absence of metabolic, neurologic, or psychiatric disorders, limited ethanol intake, and limited cigarette usage. Styrene exposure was assessed by the next-morning urinary MA+PGA. Workers with metabolite concentrations of up to 150 mmoles/mole appeared to have no significant effects, and this level is therefore designated as the NOAEL in this study. The authors state that this level of urinary metabolites corresponds to a mean daily 8-hour exposure to air styrene of 25 ppm (106 mg/m³). The 95% confidence interval was also calculated for an 8-hour exposure to 100 ppm; the lower limit of the confidence calculation was 88% of the mean styrene exposure. This factor was applied directly to the NOAEL of 25 ppm [25 ppm x 0.88 = 22 ppm (94 mg/m³)]. A battery of neuropsychological tests designed to measure CNS function was given on the morning after the last workday in the week. In addition to matching for age, sex, and educational level, a vocabulary test was included to match for general intelligence. The tests assessed memory and sensory/motor function. The results were expressed as continuous and quantal data. For the continuous data, the degree of abnormal response to each of the eight tests was recorded.

Mutti *et al.* (1984b) expressed the quantal data as the fraction of tested subjects who responded abnormally to ≥ 1 , ≥ 2 , and ≥ 3 tests (see Table 1). When the quantal data were assessed by an analysis of probabilities of proportions (Kirby, 1993), the low dose group was significantly higher than controls ($p=0.001$). In addition, a three-way representation of the data (prevalence (number of respondents for at least one, two or three abnormal tests), duration (years at work), and intensity (metabolite level)) as presented in Mutti *et al.* (1984b), revealed an effect of duration as well as intensity. This study was well designed and executed in terms of experimental protocol and statistical evaluation, which included tests for false positive and false negative responses. While not all confounders (e.g., compensatory mechanisms, biorhythms, workers who leave because of styrene related illness) could be ruled out, this study does show that workers exposed to styrene over a long period of time show evidence of CNS dysfunction.

Table 1. Subjects Classified Positive on Neuropsychological Tests as a Function of Styrene Exposure ^a.

MA+PGA (mmoles per mole creatinine) ^b	Total Subjects	Number of Abnormal Tests		
		≥ 1	≥ 2	≥ 3
Controls	50	4/50	2/50	0/50
< 150 (mean = 75 ± 33)	14	6/14	3/14	2/14
150-299 (mean=216 ± 45)	9	6/9	5/9	3/9
300 - 450 (mean=367 ± 49)	14	10/14	7/14	5/14
> 450 (mean=571 ± 108)	13	11/13	8/13	6/13

^a Data from Table IV in Mutti *et al.* (1984b).

^b “Next-morning” urinary metabolites.

Exposure to styrene may affect the peripheral nervous system (PNS). In a case report (Behari *et al.*, 1986), a man working for 5 years with a photostat process that used styrene was diagnosed with peripheral neuropathy. However, in occupational studies, the relationship between exposure to styrene and PNS effects has been inconsistent (Triebig *et al.*, 1985; Cherry and Gautrin, 1990). A major difficulty in understanding the potential for this relationship is the lack of knowledge about the appropriate surrogate for dose that leads to PNS disturbance (Murata *et al.*, 1991). In one study, however, chronic exposure indices were developed which included work method, years at work, time spent laminating (source of high exposure), styrene air concentration, and end-of-shift urinary mandelic acid (Matikainen *et al.* (1993). Numbness in the extremities increased with the exposure index, although the effect was statistically marginally insignificant ($p < 0.1$). The styrene TWA/8 hr was 32 ppm for the 100 study subjects.

Female reproductive toxicity has been inconsistently reported among humans (Brown, 1991; Lindbohm, 1993). These studies are difficult to interpret because of the high background rates of endpoints such as spontaneous abortion and menstrual disorders in combination with confounding exposures. In those studies that showed no reproductive effects due to styrene exposure, the power of the studies was low due to the small numbers of women. Hence the evidence for any adverse effects of exposure to styrene on female reproductive function is inconclusive.

Immune system alterations were reported in a study conducted by Bergamaschi *et al.* (1995). Reinforced plastics industry workers (n=32 female/39 male, average age = 32 years, average exposure duration = 7 years, tobacco consumption = 7 cigarettes/day, ethanol consumption = 16 glasses/week) were compared with non-styrene exposed factory workers and matched for age, sex, tobacco use and ethanol consumption. Air styrene levels, among the different factories, varied between 10 - 50 ppm, and individual worker exposure was measured by urinary metabolites the morning after the last shift (15 hours post-exposure). Among all workers in the study (median exposure = 16 ppm - according to the data of Guillemin *et al.* (1982)), the proportion of 12/18 lymphocyte subsets and the prevalence of abnormal values of immunologic phenotypes for 11/18 subsets were statistically different from the controls ($p < 0.001$ to < 0.05). When the workers were placed into three exposure groups (0, < 25 ppm, and > 25 ppm styrene), dose-response relationships were observed for prevalences of abnormal responses for four lymphocyte subsets and, in the case of two subsets, abnormal responses were observed in the group exposed to < 25 ppm styrene. Natural killer cell activity (a lymphocyte function), measured in a different group of workers in the same study, was decreased compared to unexposed worker controls. The median exposure, given in terms of urinary metabolites, was calculated as 21 ppm based on the data of Guillemin *et al.* (1982). The data show that exposure of these workers to air styrene levels below 50 ppm, and probably at levels near 25 ppm, resulted in alterations of the immune system.

Governa *et al.* (1994) observed reduced chemotactic responses of polymorphonuclear lymphocytes (PMNs) obtained from 21 styrene-exposed workers. However, the lack of exposure data prevents a quantitative assessment. In the same study, 0.1 - 0.6 mM styrene inhibited the chemotaxis of isolated healthy PMNs.

V. Effects of Animal Exposure

In a subchronic study, carried out under the auspices of NTP (NTP, 1992), mice and rats were exposed by inhalation to styrene vapors to establish a maximum tolerated dose for chronic studies. Mice were exposed to 0, 62.5, 125, 250, or 500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). Among males early deaths occurred in the 250 ppm group. Body weights among all exposed mice were lower than controls, and the difference was about 9 percent. Lung, olfactory epithelial, and forestomach lesions were observed in females and males. In females, degeneration of the adrenal gland cortex was observed. An effect not discussed in the chairperson's report, but recorded in the original laboratory report, was an increased estrous cycle length among the female mice at all styrene doses. A LOAEL of 62.5 ppm is indicated by the olfactory epithelial, forestomach and respiratory tract lesions in mice of both sexes and for lesions in the adrenal cortex in the female mice. Rats were exposed to 0, 125, 250, 500, 1000, or

1500 ppm styrene (6 hr/d, 5 d/wk, 13 wks). No deaths occurred, but reduced body weights were observed at the two highest doses. Lesions of the respiratory tract were observed at all dose levels. A LOAEL of 125 ppm is therefore indicated for the rats.

Rats were exposed by ingestion for 2-years to styrene in drinking water (0, 125, and 250 ppm). (The water solubility of styrene is 310 ppm.) The only effect was a styrene-related reduction in water consumption (Beliles *et al.*, 1985).

Kishi *et al.* (1995) carried out a developmental study on rat pups born to dams exposed by inhalation to styrene (0, 50, 300 ppm; 6-hr/d; gestation days 7-21). Although the small number of litters (n=2) at the 50 ppm dose prevented detailed statistical analysis, the data suggest that exposure of the dams to 50 ppm styrene resulted in deficits and delays in some motor and coordination abilities among the pups. Pups born to dams exposed to 300 ppm exhibited statistically significant increases in spontaneous activity and in the delay of some neurobehavioral functions. Many of the effects became diminished as the pups aged. Measurements of reproductive toxicity (maternal weight gain, length of gestation, number of live births) did not change. Postnatal body weights were lower among the styrene exposed pups, but the differences became less as the pups aged to 125-days.

Mice, exposed acutely (14 days) by inhalation to 125 - 500 ppm styrene, exhibited decreased spleen / body weight, splenic hypocellularity, altered lymphocyte proportions among subsets, and increased proliferative response to mitogens (Corsini *et al.*, 1994). Mice and rats, exposed by gavage to high levels of styrene (18, 27, 45 mg/kg - mouse; 118, 177, 294 mg/kg - rat) for 5 days/week for 4 weeks, exhibited decreased resistance to encephalomyocarditis virus, *Plasmodium berghie* (a malaria parasite), and *Nippostrongylus brasileinisi* (a parasitic worm) (Dogra *et al.*, 1992).

VI. Derivation of the U.S. EPA Reference Concentration (RfC) (U.S. EPA, 1996)

<i>Study</i>	Mutti <i>et al.</i> (1984b)
<i>Study populations</i>	Human (occupational)
<i>Exposure method</i>	Inhalation
<i>Critical effects</i>	Central nervous system
<i>LOAEL</i>	
<i>NOAEL</i>	22 ppm
<i>Exposure continuity</i>	8 hr/d (10 m ³ /day occupational inhalation rate), 5 d/wk
<i>Exposure duration</i>	8.6 years (average years at work)
<i>Average occupational exposure</i>	7.8 ppm for NOAEL group
<i>Human equivalent concentration</i>	7.8 ppm for NOAEL group
<i>LOAEL uncertainty factor</i>	1
<i>Subchronic uncertainty factor</i>	3
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	3
<i>Modifying factor</i>	3
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.3 ppm (300 ppb; 1 mg/m ³ ; 1,000 µg/m ³)

The study on which the U.S. EPA (1996) reference concentration (RfC) is based is well designed and executed. Careful attention was paid to include eligibility criteria for the control group that correct for confounders unique for this population, e.g., limited ethanol intake, a work-force not exposed to neurotoxic substances, and a test to allow a match for general intelligence. The use of urinary metabolites to measure exposure dose is based on the observation that the next-morning urinary MA+PGA is directly related to the air level of styrene. The Guillemin *et al.* (1982) reinforced plastics industry investigation provides the basis for the Mutti *et al.*, (1984b) conversion from urinary MA+PGA to styrene. For the calculation of the RfC, U.S. EPA (1996) used the 95 percent lower bound. The next-morning urinary metabolite level also emphasizes the chronic component of the exposure since styrene and MA+PGA are not completely cleared between work shifts (Guillemin and Bauer, 1979; Perbellini *et al.*, 1988).

In the Mutti *et al.* (1984b) study, the results were presented as continuous and quantal data. The derivation of the U.S. EPA RfC for styrene (U.S. EPA, 1996) is based on the continuous data presented for each of the eight neuropsychological tests.

A confounder in the analysis of CNS disturbance among styrene-exposed humans is ethanol consumption. Although difficult to control in epidemiologic investigations, most studies either attempt to control for ethanol consumption or analyze the contribution of such behavior to the results. Also, the worker populations used for these studies represent many geographical locations, each with its own pattern of drinking behavior. The results of the aggregate of the studies reported in this summary show that exposure to styrene can result in CNS disturbance after accounting for ethanol consumption.

A major source of exposure uncertainty is the lack of exposure data on individual workers in the reinforced plastics industry. At the present time, a system does not exist to obtain such information, although a recent report suggests a methodology is being developed (Jensen *et al.*, 1995). This industry, from which the workers in the Mutti *et al.* (1984) study were taken, is characterized by a large turnover of highly exposed workers (Wong, 1990; Kogevinas *et al.*, 1993). Ethanol consumption may also interfere with exposure assessment based on biomonitoring due to the ethanol-related decreased levels of MA (Berode *et al.*, 1986). This effect is reversible, and should therefore be minimal if morning after samples are utilized.

The rat and mouse inhalation study (NTP, 1992) also contains data that may be used to develop a chronic exposure level for styrene. The mice were more sensitive to the styrene vapors than were rats and a LOAEL of 62.5 ppm was identified based on lesions in various organs in both sexes. The adjustment factor for discontinuous exposure is $(6/24 \times 5/7) = 0.18$. The uncertainty factors are: 10 each for intraspecies variability, interspecies sensitivity, subchronic exposure, and adjustment for a NOAEL. The resultant exposure level is $(62.5 \text{ ppm} \times 0.18) / 10,000$ which equals 0.0011 ppm or 1.1 ppb ($4.7 \mu\text{g} / \text{m}^3$). While the exposure level based on the mouse data (NTP, 1992) is appropriate methodology, the RfC based on the well designed human study of Mutti *et al.* (1984) is preferable because it does not introduce the uncertainties associated with interspecies and subchronic to chronic extrapolations.

The Agency for Toxic Substances and Disease Registry (ATSDR) calculated a chronic inhalation minimal risk level (MRL) for styrene (ATSDR, 1992). The calculation was based on the same Mutti *et al.* (1984b) worker study used by U.S. EPA to calculate the RfC. ATSDR (1992) identified the lowest exposure group as a LOAEL and assigned an air styrene level of 25 ppm. To derive the MRL, ATSDR corrected the LOAEL for discontinuous exposure and applied uncertainty factors (UFs) for the use of a LOAEL and for intraspecies variability. The MRL was calculated as: $25 \times (8/24 \times 5/7) / 10 \times 10$ equal 0.06 ppm (ATSDR, 1992).

Analysis of the U.S. EPA RfC for Styrene

The U.S. EPA (1996) calculated an RfC of 0.3 ppm ($1 \text{ mg}/\text{m}^3$), which OEHHA recommends as the chronic REL. Two aspects of the RfC require additional analysis. They are (1) the choice of an effective air styrene level (i.e., LOAEL or NOAEL and the assigned exposure level) and (2) the application of UFs.

Effective exposure level

The data obtained by Mutti *et al.* (1984b) are presented in two forms. One form is continuous data in which the deviation from a normal response is measured as a function of exposure for each of eight individual tests. The other form is quantal data in which the proportions of subjects responding abnormally to ≥ 1 , ≥ 2 , and ≥ 3 tests are measured as a function of exposure. U.S. EPA (1996) used the continuous data and chose the short term logical memory tests that individually exhibited a dose-response effect. According to the data, no effect was observed at the lowest exposure level, so this level was taken as the NOAEL. An abnormal response was observed for short-term verbal memory, but a dose-response was not apparent from the data. OEHHA staff also evaluated the quantal data and the Binomial Cumulative Function (Kirby,

1993) and determined the probabilities of abnormal responses among the exposed subjects based on the unexposed subjects whose response was assumed to be normal. The probability of the proportion of subjects responding abnormally to the tests was $p \leq 0.001$.

NOAEL/LOAEL. The deviation from normal responses for each of the individual tests yields important information on the effect of styrene on a specific expression of central nervous system (CNS) function. However, individuals may express changes in altered CNS function in different ways. Such differential expression is expected given the complexity of the mechanism(s) of styrene-induced CNS toxicity that may involve styrene, styrene oxide, or phenylglyoxylic acid (Savolainen, 1977; Mutti *et al.*, 1988; Costa, 1996). In a population of 50 subjects, individual test-specific effects that occur at low doses may not have been observed. If the criterion for abnormality is expressed in terms of CNS dysfunction, defined by all tests, the sensitivity of the testing procedure is increased and the low dose effects are more easily observed. The quantal data of Mutti *et al.* (1984b), i.e., the proportion of subjects responding abnormally to the tests, therefore provides a more sensitive approach to detecting low dose effects. At the most restrictive criterion, i.e., the proportion of subjects responding abnormally (compared to matched controls) to ≥ 3 tests, the probability of a chance occurrence was ≤ 0.001 for the lowest exposure group.

Exposure level. The exposure data in the Mutti *et al.* (1984b) study were expressed quantitatively as the next-morning level of urinary metabolites (mandelic acid (MA) + phenylglyoxylic acid (PGA)). The numerical values represent mmoles MA+PGA / mole creatinine and these units will be understood in the discussion that follows. To assign the test subjects to each of four exposure levels, Mutti *et al.* (1984b) set boundaries such that level 1 = <150 units, level 2 = 150-299 units, level 3 = 300-450 units, and level 4 = >450 units (see Table 3 (Mutti *et al.*, 1984b)). In the analysis of individual test scores, U.S. EPA (1996) identified the lowest exposure group as the NOAEL and assigned an exposure level of 150 units, specifically: “Workers with metabolite concentrations of *up to* 150 mmoles/mole appeared to have no significant effects, and this level is therefore designated as the NOAEL in this study.” However, according to the data presented in Mutti *et al.* (1984b), the lowest exposure group presented urinary metabolites *less than* 150 units. The only information on the specific level of exposure to this group is summarized in Table 3 of Mutti *et al.* (1984b) and is presented as a mean exposure level, in this case 75. The mean level of urinary MA+PGA in the level 2 exposure group was 216. If the corrected exposure level is applied to the U.S. EPA analysis, the effective dose level will be decreased by half.

Uncertainty factors (UFs)

U.S. EPA (1996) applied three UFs: 3 for intraspecies variability, 3 to adjust a subchronic 8.6 year occupational exposure to a lifetime environmental exposure, and 3 to account for an inadequate data base. The choice of the UF for intraspecies variability of 3 depends on the interpretation of the use of the biological exposure index for styrene, i.e., urinary MA+PGA. A relationship between the next-morning urinary MA+PGA and air styrene level was developed for a worker population (Guillemin *et al.*, 1982), wherein the extrapolation was presented as a central value, an upper 95 percent- and a lower 95 percent confidence limit. U.S. EPA (1996) used the lower 95 percent limit (about 88 percent of the central value) and assumed that because

this value takes into account differences in metabolism and toxicokinetic properties, only an intermediate UF of 3 is necessary to adjust for intraspecies variability.

Important issues of intraspecies variability may not be taken into account by this analysis. The lower 95% limit appears to be a measure of standard error of the mean rather than the standard deviation of the population and so therefore does not capture much of the population variability given the large study population (N= 90). The exposure in the Guillemin *et al.* (1982) study, like the Mutti *et al.* (1984b) study, is an occupational exposure. Inherent in the use of occupational cohorts are two phenomena that could impact the extrapolation of data from the styrene workers to the general population. They are (1) the selection of a healthy worker population at the time of entry into the industry, and (2) the survival of healthier workers after long periods of employment in the industry (Fox and Collier, 1976). Hence, the Guillemin *et al.* (1982) extrapolation does not take into account a population whose health status may be less than that of the studied worker population. A malnourished population may also be subject to a synergistic action of styrene on CNS function, as suggested by the effects of ingested styrene on rats fed low protein diets (Khanna *et al.*, 1994). For these reasons, a UF of 10 to account for intraspecies variability may be justified. If the U.S. EPA RfC derivation were adjusted to reflect the use of an intraspecies variability UF of 10, the resulting RfC would be one-third of the current value, i.e., $0.26 / 3 = 0.089 = 0.09$ ppm. If the adjustments to the effective exposure level were also made, the resulting RfC would be 0.035 ppm.

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CHRONIC TOXICITY SUMMARY

TOLUENE

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

CAS Registry Number: 108-88-3

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	400 µg/m³ (U.S. EPA RfC) This document summarizes the evaluation of non-cancer health effects by U.S. EPA for the RfC.
<i>Critical effect(s)</i>	Neurological disturbances in human workers
<i>Hazard index target(s)</i>	Nervous system; alimentary system; teratogenicity

II. Physical and Chemical Properties (HSDB, 1995 except as noted)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₇ H ₈
<i>Molecular weight</i>	92.13 g/mol
<i>Density</i>	0.861 g/cm ³ @ 25°C (Low <i>et al.</i> , 1988)
<i>Boiling point</i>	111° C
<i>Vapor pressure</i>	28.1 mm Hg @ 25°C (U.S. EPA, 1984)
<i>Solubility</i>	miscible in most organic solvents
<i>Conversion factor</i>	1 ppm = 3.77 mg/m ³ @ 25°C

III. Major Uses or Sources

Toluene occurs naturally as a component of crude oil and is produced in petroleum refining and coke oven operations (HSDB, 1995). It is used in household aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, adhesives and solvent based cleaning agents. Toluene is also utilized in printing operations, leather tanning and chemical processes. Benzene and other polycyclic aromatic hydrocarbons are common contaminants of toluene. Toluene is considered a sentinel chemical for benzene in the context of air and water sample monitoring.

IV. Effects of Human Exposures

Case studies of solvent abusers have shown that high doses of toluene (e.g., 425 mg/day) can cause neurobehavioral changes and degeneration of cerebellar, cortical, and brainstem functions.

A battery of neurobehavioral tests was performed in 30 female workers exposed to toluene vapors in an electronic assembly plant (Foo *et al.*, 1990). The average number of years worked was 5.7 ± 3.2 for the exposed group and 2.5 ± 2.7 years for the controls. The exposed group of workers inhaled a time-weighted average of 88 ppm (330 mg/m^3) toluene while the control workers inhaled 13 ppm (49 mg/m^3). A significant decrease in neurobehavioral performance was observed in the exposed workers in 6 out of 8 tests. Irritant effects were not examined, and concurrent exposures to other chemicals were not addressed. In this study, 88 ppm was considered a LOAEL for central nervous system effects.

Workers exposed to lesser concentrations have shown some impairment of CNS endpoints, however, controls and exposed individuals have not been well matched in many of these studies (Hanninen *et al.*, 1987; Iregren, 1982; Cherry *et al.*, 1985).

Solvent workers were exposed to 42.8 ppm toluene (estimated as a time-weighted average) for an average duration of 6.8 years (Yin *et al.*, 1987). No significant differences from controls were noted in treated individuals in questionnaires, hematology, or urinalyses. This study did not account for confounding variables, such as smoking and alcohol consumption.

Subjective symptoms of headache, sore throat and dizziness were reported by workers exposed to approximately 100 ppm toluene (time-weighted average, duration unspecified) (Lee *et al.*, 1988). The prevalence of these symptoms was concentration-dependent. A similar study examined the psychomotor, manual dexterity, and visual perception abilities of college students exposed to 0, 74, or 151 ppm (0, 278, or 566 mg/m^3) toluene 7 hours/day for 3 days (Echeverria *et al.*, 1989). In addition to the above objective measures, subjective symptoms of eye irritation, headache, and somnolence were noted at 151 ppm. Visual perception and manual dexterity performances both decreased, while reported symptoms increased in the 151 ppm group. No effects of toluene on psychomotor tests were observed. In this study, 74 ppm was a NOAEL.

Baelum *et al.* (1985) found that subjects exposed to 0 or 100 ppm (375 mg/m^3) toluene for 6.5 hours experienced a loss of color discrimination, regardless of their prior solvent exposure history. Other signs of toxicity included visual perception and visual motor function, although these signs were only observed in the occupationally-exposed individuals.

Liver toxicity has been documented in toluene solvent abusers (Fornazzari *et al.*, 1983). In a cross-sectional study of 289 printing workers exposed to an estimated 53 ppm (200 mg/m^3) for 8 hours/day, 8 workers had significantly elevated serum enzymes (ALT/AST ratio) indicative of liver damage (Guzelian *et al.*, 1988). However, another cross-sectional study by Boewer *et al.* (1988) showed no significant effects on serum enzymes in 181 printing workers exposed to concentrations below 53 ppm (200 mg/m^3).

Toluene was identified as the principal solvent associated with an increased incidence of urinary tract birth defects in a retrospective cohort study of 301 cases compared with 301 referent controls (McDonald *et al.*, 1987). The cohort was matched for age, employment, date of delivery, and educational level.

V. Effects of Animal Exposures

Rats (20 per group) exposed for 2 years to 0, 600, or 1200 ppm (0, 2261, or 4523 mg/m³) toluene 6.5 hours/day, 5 days/week for 103 weeks were examined for hematological and histopathological effects in addition to gross observations of toxicity (NTP, 1990). Significant erosion of the olfactory epithelium was observed in male rats while degeneration of the respiratory and nasal epithelium was observed in both sexes at 600 ppm.

A study of the chronic effects of toluene in rats (5-20 animals per group) exposed for 106 weeks to 0, 30, 100, or 300 ppm (0, 113, 375, or 1125 mg/m³) toluene showed no treatment-related effects on histopathology of major organs, including the nasal turbinates (CIIT, 1980). In this study, the samples taken for nasal histopathology examination may have been inadequate to substantiate the nasal lesions reported by the NTP (1990).

Reproductive toxicity to maternal rats was observed during exposure to 1500 ppm toluene, 24 hours/day on days 9 to 14 of gestation (Hudak and Ungvary, 1978). Two dams out of 19 died during exposure. Fetuses from the 1500 ppm group showed increased incidence of sternebral alterations, extra ribs and missing tails. The same concentration given on days 1 through 8 of gestation resulted in 5 deaths out of 14 dams. Fetuses in this regimen showed increased incidence of hydrocephaly and growth retardation compared to controls. A third regimen that exposed maternal rats to 1000 ppm on days 1 through 21 of gestation resulted in no maternal deaths or toxicity, and an increase in the incidence of skeletal variations in the fetuses. When exposed to 1500 ppm continuously, maternal mice died within 24 hours of exposure whereas exposure to 500 ppm had no apparent effect. Examination of the fetal mice showed significant growth retardation in the 500 ppm group.

Inhalation of 0 or 800 mg/m³ toluene for 6 hours/day on gestation days 14-20 (rats), or days 6-11 (hamsters) showed significant exposure-related decrease in birth weight of the rats compared with controls (Da Silva *et al.*, 1990). In addition to low birth weight, the numbers of live pups was significantly lower in the 800 ppm group. No deficits in any parameter were noted in the hamsters. In this study, no neurobehavioral effects were noted in the offspring.

A 2-generation study of the effects of 0, 100, 500, or 2000 ppm (0, 377, 1885, or 7538 mg/m³) toluene in rats (males, 10-40 per group; females, 20-80 per group) (API, 1985). Rats were exposed for 6 hours/day, 7 days/week for 80 days and a 15 day mating period. The mated females were then exposed to the same concentrations during days 1-20 of gestation and days 5-20 of lactation. After weaning, the F1 pups were exposed 80 times to the appropriate exposure level and then randomly mated to members of the same exposure group. The F1 generation showed significantly decreased body weight which remained throughout lactation. No effects were observed on histopathology. No data were presented for the F2 generation.

Mice exposed chronically to 0, 120, 600, or 1200 ppm (0, 452, 2261, or 4523 mg/m³) toluene 6.5 hours/day, 5 days/week, for 2 years (NTP, 1990). The only treatment-related effect was a significant increase in the number of animals with hyperplasia of the bronchial epithelium in the 1200 ppm exposure group.

Determination of Noncancer Chronic Reference Exposure Levels
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No significant effects of 1481 ppm toluene exposure were noted in rats (15/sex/group) after 26 weeks exposure (API, 1985). Examined in this study were neurohistopathological responses, hematology, serum enzymes and urinalyses.

Ototoxicity in the form of hearing loss was observed in rats exposed to 1000 ppm 14 hours/day for 2 weeks (Pryor *et al.*, 1984). In this study, the auditory brainstem reponse and behavioral changes both indicated hearing loss. A lower concentration of 700 ppm for 14 hours/day for 16 weeks did not result in any significant hearing loss.

VI. Derivation of U.S. EPA RfC

<i>Study</i>	Foo <i>et al.</i> , 1990; NTP, 1990; U.S. EPA, 1994
<i>Study population</i>	30 Female workers in an electronic assembly plant
<i>Exposure method</i>	Occupational inhalation
<i>Critical effects</i>	Neurobehavioral deficits in 6 out of 8 tests
<i>LOAEL</i>	88 ppm
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	10 m ³ /day occupational inhalation rate, 5 days/week
<i>Average occupational exposure</i>	31.4 ppm (88 x 10/20 x 5/7)
<i>Exposure duration</i>	5.7 ± 3.2 years (exposed group); 2.5 ± 2.7 years (controls)
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Modifying factor</i>	3 (database deficiencies including the lack of animal neurotoxicity and irritation studies)
<i>Cumulative uncertainty factor</i>	300
<i>Inhalation reference exposure level</i>	0.1 ppm (100 ppb; 0.4 mg/m ³ ; 400 µg/m ³)

The major strength of the U.S. EPA RfC is the use of human exposure data from workers exposed over a period of years. The major weaknesses are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of a NOAEL observation and of dose-response information.

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Determination of Noncancer Chronic Reference Exposure Levels
Do Not Cite or Quote. SRP Draft – May 1999

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CHRONIC TOXICITY SUMMARY

TRICHLOROETHYLENE

(trichloroethylene; 1,1-2-trichloroethylene, 1,1-dichloro-2-chloroethylene, acetylene trichloride, and ethylene trichloride)

CAS Registry Number: 79-01-6

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	600 µg/m³
<i>Critical effect(s)</i>	Neurotoxicological effects (drowsiness, fatigue, headache) and eye irritation in workers.
<i>Hazard index target(s)</i>	Nervous system; eyes

II. Physical and Chemical Properties (Fan, 1988)

<i>Description</i>	Colorless vapor; sweetish, chloroform-like odor
<i>Molecular formula</i>	C ₂ HCl ₃
<i>Molecular weight</i>	131.4
<i>Density</i>	1.47 (water = 1)
<i>Boiling point</i>	87.7°C
<i>Vapor pressure</i>	77 mm Hg @ 25°C
<i>Vapor density</i>	4.5 (air = 1)
<i>Solubility</i>	Soluble in alcohol, ethers, petroleum distillates and other halogenated solvents
<i>Conversion factor</i>	1 ppm = 5.37 mg/m ³ @25° C

III. Major Uses or Sources

Trichloroethylene was once used as an extractant in food processing and has been used as an anesthetic and analgesic for medical purposes (Waters *et al.* 1977). Currently, it is widely used as a solvent in the industrial degreasing of metals, with secondary solvent uses in adhesive paint and polyvinyl chloride production (U.S. EPA, 1985). Trichloroethylene is used as a solvent in the textile industry, as a solvent for adhesives and lubricants, and as a low-temperature heat transfer fluid (IARC, 1979). Trichloroethylene is also implemented in the manufacturing of pesticides and other chemicals (Feldman, 1979).

IV. Effects of Human Exposure

An occupational study of trichloroethylene (TCE) vapor emissions in a pump room was conducted by Vandervort and Polnkoff (1973). Workers were an average age of 40 and had been employed for an average of 8 years. For 11-day shift workers, individual 8 hour time weighted average (TWA) TCE exposure concentrations were extrapolated from two area samples; and these averages ranged from 170-420 mg/m³ (32-78 ppm). Nineteen workers (including the 11 workers whose work areas were sampled) completed a questionnaire and reported the following symptoms: 73% eye irritation, 70% drowsiness, 58% heart palpitations, 58% cough, 53% weakness and 52% dizziness. About half of the 19 exposed workers reported that consumption of small amounts of alcohol outside of work resulted in changes of skin color and severe intoxication. One worker of the 19 reported no adverse effects from the occupational exposure. Nine control workers experienced none of the above symptoms. Urine samples from the 19 exposed and 9 unexposed workers were collected before and after the work shift and examined for the TCE metabolites trichloroacetic acid (TCA) and trichloroethanol (TRI). TRI levels ranged from 4-260 mg/l and TCA levels ranged from 4-197 mg/l. Results of the urine assays showed a range of TCE metabolite concentrations and therefore, confirmed that the workers were exposed to a variety of concentrations in their environments.

Nomiyama *et al.* (1977) examined 36 trichloroethylene workers, of which 9 males and 12 females were occupationally exposed to a constant concentration of trichloroethylene (TCE) and 18 males were exposed to variable concentrations (duration of exposure unspecified). The control group consisted of 6 males and 10 females who were of similar educational, sociologic and economic status to the trichloroethylene workers. Researchers used urinary excretion of TCE metabolites as an indicator of the level of TCE exposure in the working environment; total excreted trichloro-compounds of 100 mg in 4 hours corresponded to 100 ppm TCE present in the working environment (Bardodej, 1958; Medek, 1958). Of the 36 exposed workers, 5 were exposed to 0-25 ppm; 14 were exposed to 25-50 ppm; 6 were exposed to 50-100 ppm; 8 were exposed to 100-150 ppm; and 3 were exposed to 150-200 ppm TCE. In the low exposure group, workers experienced mucous membrane irritation in the eyes, nose and throat, in addition to drowsiness, fatigue and headache. These symptoms were persistent through the higher concentration exposures with an increase in eye irritation, headache, fatigue, and nasal obstruction above 100 ppm TCE. Increases in rhinorrhea and drowsiness were seen above 50 ppm TCE exposure.

Kimmerle and Eben (1973) exposed 4 human subjects (3 males and 1 female) to a subacute regimen of 48 ± 3 ppm trichloroethylene (TCE) for 4 hours a day over a period of 5 days. Levels of TCE and the metabolites trichloroethanol (TRI) and trichloroacetic acid (TCA) were determined. Trichloroethanol-blood levels were elevated immediately after exposure, and detection of trichloroethanol occurred up to 7 days after the last exposure. TCE-blood concentration increased slightly over the 5 days. Levels of urinary excreted trichloroethanol, as well as the TCA-concentration, increased throughout the study, with the female showing a significantly higher excretion of TCA. Levels of TCA were detected up to 12 days after the final exposure.

Okawa and Bodner (1973) studied the occupational exposure of 24 electrical plant workers to trichloroethylene (TCE). The plant worker group consisted of 22 males and 2 females ranging in age from 21-52 years old. Environmental samples of TCE were collected over three days and yielded varying concentrations of TCE related to the task performed in certain areas (duration of exposure unspecified). Spray booth operators were exposed to an average of 25.3 ppm TCE (13-40 ppm range) in addition to averages of 15.2 ppm n-propyl acetate (NPA) and 6 ppm toluene (TOL). Workers involved in washing board units were exposed to an average value of 39 ppm (6-82 ppm range) TCE. Although the workers wore respiratory protection during the washing procedure, the overall average of airborne TCE in this area was 48.3 ppm. In the testing area of the plant, researchers report that the amounts of toluene and n-propyl acetate were insignificant. Here, TCE levels were an average of 24.4 ppm (range = 8-44 ppm). The solder machine operators were exposed to an average of 44.0 ppm TCE (range = 23-87 ppm) with no NPA or TOL present. During the cleaning of the soldering machines, TCE levels rose to an average of 70.5 ppm (range = 30-106 ppm). Concentrations were only at these elevated levels for 20-30 minutes a day. Researchers note that although other agents were used in the work area, TCE was the only chemical found in significant amounts throughout the work area and that the levels of NPA and TOL were insignificant. An analysis of urinary TCE metabolites indicated that the workers were exposed to a time weighted average concentration of <50 ppm TCE. Three of the 24 workers reported that they were unaffected by their working conditions, but the most prominent complaints consisted of 70.8% workers experiencing nausea, 54.2% headache, 33.3% dizziness, 25.0% fatigue, 25% nose and throat irritation, and 20.8% eye irritation. Workers reported that these symptoms were alleviated hours after leaving the work environment. Researchers collected 8 hour urine samples from 20 of the workers and from 9 controls and analyzed them for TCE metabolites. Results of urinary analysis showed that the controls had exposure to an unspecified amount of TCE. TCA levels in exposed workers were elevated from that of the controls and correlated to the different exposures in specific work areas.

Phoon *et al.* (1984) reported on 5 cases of Stevens-Johnson syndrome (erythema multiforme major) with liver involvement which followed exposure to TCE. In two cases, reactions to the exposure began with a fever followed by an itchy rash on the face spreading over the body. Lesions were observed on the face, arms and in the mouth. Liver function tests were abnormal. One of the two developed jaundice with hepatomegaly. Case #3 developed a similar reaction after 5 weeks of exposure to 216-912 mg/m³ TCE (40-170 ppm) as did case #5 after two weeks of exposure to 370 mg/m³ TCE (69 ppm). Case #4 involved a 39 year old man exposed to <50 mg/m³ TCE (< 9.3 ppm) for three weeks who developed the characteristic rash, lesions and jaundice with slight hepatomegaly. Upon returning to work over the next three weeks, he developed generalized erythrodermia and facial oedema, hepatosplenomegaly and liver failure with septicemia from which he died 14 days later.

Stewart *et al.* (1974) studied the effects of subacute trichloroethylene (TCE) exposure in combination with alcohol consumption. Seven men exposed to 200 ppm TCE ingested 1 quart of beer or 90 ml of 100-proof vodka and developed red blotches on their faces 30-40 minutes later. These lesions enlarged with time until they reached a peak intensity, whereupon they faded. One subject experienced facial flush with the consumption of alcohol for three weeks after the last TCE exposure, while another showed flushing six weeks after the last exposure.

V. Effects of Animal Exposure

Kjellstrand *et al.* (1983) studied the effects of both intermittent and continuous exposures of various concentrations of trichloroethylene on male and female mice over a period of 30 days. The concentrations used range from 37-3600 ppm, and 7 of the 14 groups were continuously or intermittently exposed to lower concentrations of 37, 75, 150, 225 and 300 ppm TCE. Continuous exposure studies were conducted over a period of 30 days for exposure groups of 37, 75, 150 and 300 ppm TCE. All groups consisted of 10 males and 10 females (except the 37 ppm group, consisting of 20 males and 20 females) and were compared to identical groups of air-exposed controls. Liver weights increased in a non-linear fashion as the concentration level of TCE increased. All groups exhibited statistically significant increases in liver weights as compared to the controls. In both the 37 and the 75 ppm groups, the increase in females was less than in males. No increase in spleen weight was detected at either the 37 or 75 ppm exposure level. At the 37 ppm level, a slight increase in plasma butyrylcholinesterase (BuChE) activity (not statistically significant) was also detected. A significant increase in kidney weight was seen in the male 75 ppm group and was more pronounced with increasing concentration. Male mice in the 75 ppm group also showed statistically significant increases in BuChE activity. In the 150 ppm group, male and female liver weight increases were statistically significant and of equal magnitude. A statistically significant increase was seen in the BuChE activity of the 150 ppm male mice. It was not until female mice were exposed to 300 ppm, that they showed slight increase in BuChE activity, while the males increased 3.5 times the controls. Liver weight increases for the 300 ppm group were close to the maximum with females showing greater increase than the males. Ten male and 10 female mice were continuously exposed to 150 ppm TCE for 30 days, but then allowed a 120 day rehabilitation period. Following rehabilitation, liver weights returned to levels comparable to the controls. The elevated BuChE activity returned to a normal level. No significant effects were seen after the period of rehabilitation. A continuous study was performed on 10 male and 10 female mice for 120 days at an exposure level of 150 ppm TCE. No further increase in liver weight occurred beyond the level reached in the 30 day study. Body weight gain was slightly decreased, and the same level of BuChE activity was seen as in the 30 day exposure. The intermittent study consisted of 30 days exposure to 225 ppm TCE for 16 hours a day, 7 days a week. A significant increase was seen in the BuChE activity of male mice, while females did not exhibit an increase in BuChE activity. Both males and females showed statistically significant increases in liver weight. Kidney weight increased in the same manner as in the continuous exposures. The authors noted that “extrapolation of the concentration-effect curve suggests that both liver weight and BuChE activities are influenced at still lower concentration.”

Briving *et al.* (1986) examined neurotoxicity as a result of chronic trichloroethylene (TCE) inhalation exposure. Two groups of gerbils (6 in each group) were exposed to 50 or 150 ppm TCE for a period of 12 months. Two equivalent groups were used as controls. Two areas of the brain were specifically observed, the hippocampus and the posterior part of the cerebellar vermis. These discrete brain areas were previously shown to be sensitive towards chlorinated aliphatic solvents (Haglid *et al.*, 1981). Following exposure, gerbils were decapitated and measurements were made of total free tissue amino acids as well as high-affinity uptake and release of ³H-aminobutyric acid (GABA) and ¹⁴C-glutamate. A significant increase in

glutathione was seen in the hippocampus of the 150 ppm gerbils, but amino acid levels were not significantly affected. In the posterior part of the cerebellar vermis, glutamate and GABA accumulation levels increased in a dose-dependent manner, with significant increases seen at both 50 and 150 ppm TCE. Evaluation of the hippocampus revealed no significant changes. Authors suggest that the stimulation of transport functions for GABA and glutamate may be triggered by the presence of the TCE metabolite, trichloroethanol. Therefore, the levels of GABA and glutamate are indicative of the amount of trichloroethanol from TCE in the brain.

Kligerman *et al.* (1994) exposed 20 male CD rats to 0, 5, 50, or 500 ppm trichloroethylene (TCE) for 6 hours a day, over a period of 4 days. Groups at each concentration consisted of 5 rats. One of the cytogenetic effects measured was peripheral blood lymphocytes (PBLs), abnormal with regard to sister chromatid exchanges. Also analyzed, were the cell cycle, bone marrow micronuclei in polychromatic erythrocytes (MN-PCEs/1000) and micronuclei in cytochalasin B-blocked binucleated cells (MN-BN/1000). The 5 ppm and 500 ppm exposure groups showed a decrease (not statistically significant) in cell cycle. In addition, the 50 ppm group exhibited a statistically significant decrease in cell cycle. For all concentrations, there was an overall increase in the PCE percentage. The number of PCEs with micronuclei also rose with the increasing concentrations of 50 ppm and 500 ppm TCE (not statistically significant due to high control values). The researchers conclude that the resulting increase of MN in exposed rats is indicative of aneuploidy induction as opposed to chromosomal breakage, and that the lack of chromosome aberrations corresponds to spindle effects such as aneuploid induction. Concurrent results of increased levels of leukocyte aneuploidy were also found by Konietzko *et al.* (1978) in degreasing workers occupationally exposed to TCE.

Haglid *et al.* (1981) continuously exposed gerbils to 60 ppm or 320 ppm trichloroethylene (TCE) for 3 months. Following the exposure period, gerbils were maintained for 4 months in TCE-free conditions in order to observe any restoration of neuronal function. Both of the exposed groups as well as the control group consisted of six pairs of males and females. Brain samples were collected from the gerbils after the 4 month non-exposure period and used for determination of DNA and proteins. In order to determine areas of the brain that were sensitive to TCE, researchers examined biochemical and morphological changes in the hippocampus, the posterior part of the cerebellar vermis, and the brain stem. In addition to the biochemical tests, the cerebellum, brain stem, and cerebrum of two gerbils from each group, including the control, were used for neuropathological examination. Brain tissue from 2 gerbils in the control group and the 320 ppm group were examined under the electron microscope. No difference was seen in the body and brain weights of the exposed gerbils compared with controls. A slight but significant increase in soluble proteins was detected in the frontal cerebral cortex of the 60 ppm group, and a more significant elevation was seen in the visual cerebral cortex of both the 60 ppm and 320 ppm groups. In the 60 ppm group, a slight but significant decrease was seen in the soluble proteins of the sensory-motor cortex. Both groups exhibited significant decreases in levels of soluble proteins in the hippocampus, the brain stem, and in the posterior part of the cerebellar vermis. Soluble protein levels in the cerebellar hemisphere and anterior part of the vermis of gerbils in both exposed groups did not differ from the controls. The 320 ppm group showed significantly increased DNA levels in the posterior part of the sensory motor cortex and cerebellar vermis. The glial cytoplasmic protein (S 100 fraction) level of the 60 ppm group was decreased in the frontal and visual cerebral cortex, but increased in the posterior part of the

cerebellar hemisphere and the sensory-motor cortex. However, only a slight decrease of S 100 protein was observed in the visual cerebral cortex of 320 ppm exposed gerbils. The most notable S 100 increase occurred in the hippocampus, brain stem and the posterior part of the cerebellar vermis, indicating that either the glial cells were directly affected or that damage to surrounding neuronal cells caused an indirect response. There was an increase in DNA in the posterior part of the cerebellar vermis in the exposed gerbils, suggesting that TCE induced astroglial cell mitosis. Light microscopy revealed shrinkage of cell bodies and axon swelling occurred in various parts of the brain. The electron microscopy performed on control and 320 ppm brain tissues revealed increased levels of filament bundles in the cytoplasm of some Purkinje and Golgi cell perikarya, lysosomes, myelin bodies and lipid containing lysosomal structures in the exposed gerbils. Unique arrangements of filament bundles were seen in Purkinje and Golgi cell dendrites of the exposed group. A significant decrease in the number of microtubules was observed as well as a decrease in the number of synaptic vesicles in the granular layer. Also, the granular layer had decreased maximal nerve cell surface area. Nerve cells were affected by the exposure as several types were reduced in size with fewer organelles and more lysosomes and myelin bodies. Many axons and dendrites had reduced numbers of microtubules, and there were filament bundles observed that were not present in the controls. Lysosomal structures were increased in the synaptic terminals.

Kimmerle and Eben (1973) performed a subchronic study on 20 male rats for a period of 14 weeks. Rats were exposed to a mean concentration of 55.0 ± 4 ppm trichloroethylene (TCE) for 8 hours a day, 5 days a week. The control group consisted of 20 rats who were housed in similar inhalation chambers under similar conditions to that of the exposed rats. Ten exposed rats were analyzed for TCE metabolite excretion on a daily basis. Blood levels of trichloroacetic acid (TCA), trichloroethanol (TRI) and chloral hydrate (CH) were measured during the 2nd, 3rd, 4th, 6th, 9th and 14th weeks. Weekly measurements of body weights were recorded. Macroscopic examinations were performed on the thyroid gland, heart, lungs, liver, kidneys, testes and adrenal glands. Hematological evaluations, liver function tests, and renal function tests were also conducted following exposure. Urinary levels of TRI varied individually among the rats, but a continuous increase in TRI was observed through the 10th week. TCA levels remained fairly constant throughout the duration of the experiment. TCE was not detectable in the blood or the tissues of exposed rats. Although liver and renal function tests did not reveal abnormalities, there was an increase in the liver weights of the exposed rats. The weights of the other organs examined were similar to the controls.

Norpoth *et al.* (1974) observed an increase in liver cytochrome P450 activity in 9 rats exposed to 50 ppm trichloroethylene for 28 days, compared with 9 control rats.

VI. Derivation of Chronic Reference Exposure Level

<i>Study</i>	Vandervort and Polnkoff (1973)
<i>Study population</i>	28 workers
<i>Exposure method</i>	Discontinuous occupational inhalation exposure
<i>Critical effects</i>	Drowsiness, fatigue, headache, and eye irritation
<i>LOAEL</i>	32 ppm (170 mg/m ³)
<i>NOAEL</i>	Not observed
<i>Exposure continuity</i>	8 hours a day (10 m ³ /day occupational inhalation rate), 5 days a week
<i>Exposure duration</i>	8 years
<i>Average occupational exposure</i>	11.4 ppm for LOAEL group (32 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	11.4 ppm for LOAEL group
<i>LOAEL uncertainty factor</i>	10
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Inhalation reference exposure level</i>	0.1 ppm (100 ppb; 0.6 mg/m ³ ; 600 µg/m ³)

The Vandervort and Polnkoff (1973) study accounted for 8 years of human occupational exposure to TCE vapors. Sensitive, non-specific neurotoxicological endpoints were exhibited by a majority of those workers exposed. Although the time-weighted averages (TWAs) included a wide range of concentrations, the TWA of 32 ppm (170 mg/m³) was shown to contribute to the high incidence (52 - 73%) of adverse effects experienced by the workers. Many of the symptoms reported by the workers may have been due to short-term fluctuations in the concentrations in the workplace. The symptoms were not reported separately for the various TWAs, therefore, the lowest TWA (32 ppm) was chosen as a LOAEL. Uncertainty includes the small number of workers studied, the limited extent of the effects mentioned, and the lack of a NOAEL. Strengths include the use of human data, the demonstration of a dose-response relationship, and exposure estimates correlated with urinary excretion measurements.

This study was the best chronic account of the non-carcinogenic effects of TCE on humans, but several other studies show similar results. Nomiya *et al.* (1977) found similar endpoints of drowsiness, fatigue and eye irritation in 36 workers occupationally exposed to trichloroethylene. Okawa *et al.* (1973) also saw non-specific neurological endpoints in 24 electrical plant workers who were similarly exposed to TCE.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the lack of reproductive and developmental toxicity studies and the lack of observation of a NOAEL.

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CHRONIC TOXICITY SUMMARY

XYLENES

(Xylol or commercial xylenes (mixture of 60-70% m- and remaining percentage is mix of o- and p- xylenes), technical grade xylenes or mixed xylenes (20% o-xylene, 40% m-xylene, 20% p-xylene, 20% ethyl benzene, and traces of toluene and C9 aromatics), o-xylene (1,2-dimethylbenzene or 2-xylene), m-xylene (1,3-dimethylbenzene or 3-xylene), p-xylene (1,4-dimethylbenzene or 4-xylene), also noted as methyltoluene, benzene-dimethyl, dimethylbenzene)

**CAS Registry Numbers.: 1330-20-7 (technical mixture of o-, p-, and m-xylene);
95-47-6 (o-xylene); 108-38-3 (m-xylene); 106-42-3 (p-xylene)**

I. Chronic Toxicity Summary

<i>Inhalation reference exposure level</i>	700 µg/m³ (for technical or mixed xylenes or sum of individual isomers of xylene)
<i>Critical effect(s)</i>	CNS effects in humans; irritation of the eyes, nose, and throat
<i>Hazard index target(s)</i>	Nervous system; respiratory system

II. Physical and Chemical Properties (ATSDR, 1995; HSDB, 1995)

<i>Description</i>	Colorless liquid
<i>Molecular formula</i>	C ₈ H ₁₀
<i>Molecular weight</i>	106.16 g/mol
<i>Density</i>	0.864 g/cm ³ @ 20°C(technical mixture); 0.881 (o-); 0.860 (m-); 0.861 (p-)
<i>Boiling point</i>	137-140°C @ 760 mm Hg (technical mixture); 144.4°C (o-); 139.1°C (m-); 138.4°C (p-)
<i>Vapor pressure</i>	6.6 mm Hg (o-); 8.39 mm Hg (m-); 8.87 mm Hg (p-) all @ 25°C.
<i>Solubility</i>	Practically insoluble in water; miscible with absolute alcohol, ether and many other organic solvents
<i>Conversion factor</i>	1 ppb = 4.34 µg/m ³

III. Major Uses or Sources

Mixtures of o-, p-, and m-xylenes are extensively used in the chemical industry as solvents for products including paints, inks, dyes, adhesives, pharmaceuticals, and detergents (HSDB, 1995). In the petroleum industry xylenes are used as antiknock agents in gasoline, and as an intermediate in synthetic reactions. Of the three isomers, p-xylene is produced in the highest

quantities in the U.S. for use in the synthesis of phthalic, isophthalic, and terephthalic acid used in manufacture of plastics and polymer fibers including mylar and dacron.

IV. Effects of Human Exposure

Information on the toxicity of xylenes to humans is almost exclusively limited to case reports of acute exposures and studies of occupational exposures in which persons often inhaled a mixture of hydrocarbon solvents 8 hours per day, 5-6 days per week. These studies often have incomplete information on the airborne concentrations of xylene and other hydrocarbons. One study examining chronic effects in humans from inhalation of predominantly mixed xylenes was identified (Uchida *et al.*, 1993) and one study examining subchronic effects of p-xylene exposure was identified (Hake *et al.*, 1981). No studies examining the chronic effects of oral or dermal xylene exposure in humans were identified.

Pharmacokinetic studies have documented the absorption of xylene in humans through inhalation, oral, and dermal routes of exposure. Approximately 60% of inspired xylene is retained systemically (Sedivec and Flek, 1979). The majority of ingested xylene (~90%) is absorbed into the systemic circulation (ATSDR, 1995). Xylene is also absorbed dermally; the rate of absorption of xylene vapor is estimated as 0.1-0.2% of that by inhalation (Riihimaki and Pfaffli, 1978). Measurement of the rate of absorption through direct contact with the skin produced variable results ranging from 2 $\mu\text{g}/\text{cm}^2/\text{min}$ (Engstrom *et al.*, 1977) to 75-160 $\mu\text{g}/\text{cm}^2/\text{min}$ (Dutkiewicz and Tyras, 1968).

Xylene exposure has been associated with effects in a number of organ systems including the lungs, skin and eyes; neurological system; heart and gastrointestinal system; kidney; and possibly the reproductive system.

Pulmonary effects have been documented in occupational exposures to undetermined concentrations of mixed xylenes (and other solvents) and include labored breathing and impaired pulmonary function (Hipolito 1980; Roberts *et al.*, 1988). High levels of xylene exposure for short periods are associated with irritation of the skin, eyes, nose and throat (ATSDR, 1995). Chronic exposure to xylenes has been associated with eye and nasal irritation (Uchida *et al.*, 1993).

The central nervous system is affected by both short term and long term exposure to high concentrations of xylene with: 100-200 ppm associated with nausea and headache; 200-500 ppm with dizziness, irritability, weakness, vomiting, and slowed reaction time; 800-10,000 ppm with lack of muscle coordination, giddiness, confusion, ringing in the ears, and changes in sense of balance; and >10,000 ppm with loss of consciousness (HESIS, 1986). Other documented, neurological effects include impaired short term memory, impaired reaction time, performance decrements in numerical ability, and impaired equilibrium (dizziness) and balance (Carpenter *et al.*, 1975; Dudek *et al.*, 1990; Gamberale *et al.*, 1978; Riihimaki and Savolainen, 1980; Savolainen and Linnavuo, 1979; Savolainen and Riihimaki 1981; Savolainen *et al.*, 1979; 1984; 1985).

Chronic exposure to xylenes (with other hydrocarbons) has been associated with cardiovascular and gastrointestinal effects. Heart palpitations, chest pain, and abnormal electrocardiogram were noted (Hipolito, 1980; Kilburn *et al.*, 1985) as were effects on the gastrointestinal system producing nausea, vomiting and gastric discomfort in exposed workers (Goldie, 1960; Hipolito, 1980; Uchida *et al.*, 1993; Klaucke *et al.*, 1982; Nersesian *et al.*, 1985).

Results of studies of renal effects of xylene are mixed and come from case reports and occupational studies where multiple chemical exposures are common. The effects from subchronic exposure documented by Hake *et al.* (1981) and from chronic exposure documented by Uchida *et al.* (1993) did not include renal effects. However, Morley *et al.* (1970) found increased BUN and decreased creatinine clearance; Martinez *et al.* (1989) found distal renal tubular acidemia; Franchini *et al.* (1983) found increased levels of urinary β -glucuronidase; and Askergren (1981, 1982) found increased urinary excretion of albumin, erythrocytes, and leukocytes.

Reproductive effects were documented by Taskinen *et al.* (1994) who found increased incidence of spontaneous abortions in 37 pathology and histology workers exposed to xylene and formaldehyde in the work place. The multiple chemical exposures and the small number of subjects in this study limit the conclusions that can be drawn as to reproductive effects of xylene in humans.

No hematological effects have been identified in studies where exposure was to xylene only. Previous studies identifying hematological effects included known or suspected exposure to benzene (ATSDR, 1995; ECETOC, 1986). One series of case reports identified lowered white cell counts in two women with chronic occupational exposure to xylene (Hipolito, 1980; Moszczynsky and Lisiewicz, 1983; 1984), although they may also have had multiple chemical exposures.

The Uchida *et al.* (1993) study included a relatively large number of workers studied, exposure for an average of 7 years to xylenes predominately and a comprehensive set of medical examinations to document potential effects. A survey of 994 Chinese workers involved in the production of rubber boots, plastic coated wire and printing processes employing xylene solvents was carried out. The survey consisted of fitting individual workers with diffusive samplers for an 8 hour shift. At the end of the 8 hour shift the samplers were recovered for analysis of solvent exposure, and urine samples were collected for analysis of xylene metabolites. The following day workers answered a questionnaire concerning subjective symptoms, and blood and urine were collected for analysis. Out of this group of xylene-exposed workers, 175 individuals (107 men and 68 women) were selected for further study and analysis based on completion of their health examinations and on results from diffusive samplers showing that xylene constituted 70% or more of that individual's exposure to solvents in the workplace. The control population consisted of 241 (116 men and 125 women) unexposed workers from the same factories or other factories in the same region, of similar age distribution, of similar time in this occupation (average of 7 years), and having a similar incidence of alcohol consumption and cigarette usage. The xylene-exposed and unexposed groups were given health examinations which evaluated hematology (red, white, and platelet cell counts, and hemoglobin concentration), serum biochemistry (albumin concentration, total bilirubin concentration, aspartate aminotransferase,

alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, leucine aminopeptidase, lactate dehydrogenase, amylase, blood urea nitrogen, creatinine), and subjective symptoms (survey of symptoms occurring during work and in the previous three months).

Results of analysis of the diffusive samplers showed that workers were exposed to a geometric mean of 14.2 ± 2.6 ppm xylene (arithmetic mean of 21.3 ± 21.6 ppm). This was broken down into geometric means of 1.2 ppm o-xylene, 7.3 ppm m-xylene, 3.8 ppm p-xylene, 3.4 ppm ethyl benzene, and 1.2 ppm toluene. n-Hexane was rarely present and no benzene was detected. Analysis of data from the health examinations found no statistically significant difference ($p < 0.10$) between hematology and serum biochemistry values for xylene-exposed and unexposed populations. The frequency of an elevated ratio of aspartate aminotransferase to alanine transferase and of elevated ratio of alkaline phosphatase to leucine aminopeptidase was significantly ($p < 0.01$) higher in exposed men than in the control population of men. Results of the survey of subjective symptoms found differences in symptoms occurring during work and over the previous three months, apparently related to effects on the central nervous system and to local effects on the eyes, nose and throat. The frequency of five symptoms experienced during work were significantly ($p < 0.01$) elevated in either xylene-exposed men or women including: dimmed vision, unusual taste, dizziness, heavy feeling in the head, and headache. The frequency of four symptoms experienced during work were significantly ($p < 0.01$) elevated in both men and women including irritation in the eyes, nasal irritation, sore throat, and floating sensation. Ten subjective symptoms occurring in the previous three months were significantly ($p < 0.01$) elevated in exposed men and women including nausea, nightmare, anxiety, forgetfulness, inability to concentrate, fainting after suddenly standing up, poor appetite, reduced grasping power, reduced muscle power in the extremities, and rough skin. Dose dependency appeared to exist for 3 subjective symptoms noted during work: irritation in the eyes, sore throat, floating sensation, and one symptom occurring in the last three months, poor appetite.

V. Effects of Animal Exposure

A limited number of chronic toxicity studies are available for xylene including two inhalation studies with o-xylene (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970) and one oral chronic study with mixed xylenes (NTP, 1986). No chronic dermal studies could be identified. A spectrum of adverse effects has been documented in shorter term studies which potentially could occur with chronic exposure. These studies are presented here along with a brief description of the three chronic studies identified. Xylene affects a number of organ systems including the pulmonary system, the cardiovascular system, the gastrointestinal system, the hepatic system, the renal system, the dermis, and the eye, and it has numerous neurological effects and developmental effects.

Animal data are consistent with human data in documenting respiratory effects from xylene exposure. Acute and subacute exposures in mice, rats, and guinea pigs have been associated with decreased metabolic capacity of the lungs; decreased respiratory rate; labored breathing; irritation of the respiratory tract; pulmonary edema; and pulmonary inflammation (Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1981; Elovaara *et al.*, 1987; 1989; Furnas and Hine, 1958; Korsak *et al.*, 1988; 1990; Patel *et al.*, 1978; Silverman and Schatz, 1991; Toftgard and Nilsen, 1982).

Limited evidence is available in animal studies for cardiovascular effects resulting from xylene exposure. Morvai *et al.* (1976; 1987) conducted two studies. The first study observed rats following acute and intermediate duration inhalation exposure to very high (unspecified) levels of xylene and recorded ventricular repolarization disturbances, atrial fibrillation, arrhythmias, occasional cardiac arrest and changes in electrocardiogram (Morvai *et al.*, 1976). In a subsequent study morphological changes in coronary microvessels were seen in rats exposed to 230 ppm xylene (isomer composition unspecified) (Morvai *et al.*, 1987). However the chronic toxicity studies conducted by the National Toxicology Program (NTP, 1986) and by Jenkins *et al.* (1970), as well as other shorter term studies (Carpenter *et al.*, 1975; Wolfe, 1988), have not identified histopathological lesions of the heart.

Studies identifying adverse gastrointestinal effects, hematological effects, or musculoskeletal effects in animals were not identified. Studies reporting no hematological effects include Carpenter *et al.* (1975) (rats exposed to 810 ppm of mixed xylenes for 10 weeks, 5 days/week, 6 hours/day and dogs exposed for 13 weeks to 810 ppm mixed xylenes, 5 days/week, 6 hours/day) and Jenkins *et al.* (1970) (rats, guinea pigs and dogs exposed for 6 weeks to 780 ppm o-xylene, 5 days/week, 8 hours per day). Carpenter *et al.* (1975) and the NTP (1986) reported no effects on the musculoskeletal system.

Hepatic effects have been documented after acute exposure to high concentrations of xylene (2,000 ppm) or subacute exposure to lower concentrations (345-800 ppm) of mixed xylene or individual isomers. These effects include increased cytochrome P-450 and b5 content, increased hepatic weight, increased liver to body weight ratios, decreased hepatic glycogen, proliferation of endoplasmic reticulum, changes in distribution of hepatocellular nuclei, and liver degeneration (Bowers *et al.*, 1982; Condie *et al.*, 1988; Elovaara, 1982; Elovaara *et al.*, 1980; Muralidhara and Krishnakumari 1980; Patel *et al.*, 1979; Pyykko 1980; Tatrai and Ungvary, 1980; Tatrai *et al.*, 1981; Toftgard and Nilsen, 1981; 1982; Toftgard *et al.*, 1981; Ungvary *et al.*, 1980).

Renal effects have been identified in studies with rats, guinea pigs, dogs, and monkeys exposed to 50-2,000 ppm of xylenes. These effects include increased cytochrome P-450 content and increased kidney to body weight ratios (Condie *et al.*, 1988; Elovaara 1982; Toftgard and Nilsen, 1982). Condie *et al.* (1988) also noted tubular dilation, atrophy, and increased hyaline droplets in the kidney of Sprague-Dawley rats administered 150 mg/kg/day orally of mixed xylenes. This response is consistent with early nephropathy.

Xylene has been found to affect the dermis and eyes of animals. Hine and Zuidema (1970) found skin erythema and edema, epidermal thickening, and eschar formation in response to xylene exposure. Direct instillation of xylenes into the eyes of rabbits produces eye irritation (Hine and Zuidema, 1970; Smyth *et al.*, 1962)

Numerous neurological effects have been documented in response to acute and subchronic xylene exposures ranging between 160 to 2,000 ppm. This is consistent with effects on neurofunction documented in humans. These effects include narcosis, prostration, incoordination, tremors, muscular spasms, labored respiration, behavioral changes, hyperactivity, elevated auditory thresholds, hearing loss, and changes in brain biochemistry (Andersson *et al.*,

1981; Carpenter *et al.*, 1975; De Ceaurriz *et al.*, 1983; Furnas and Hine, 1958; Ghosh *et al.*, 1987; Kyrklund *et al.*, 1987; Molnar *et al.*, 1986; NTP, 1986; Pryor *et al.*, 1987; Rank 1985; Rosengren *et al.*, 1986; Savolainen and Seppalainen, 1979; Savolainen *et al.*, 1978; 1979a; Wimolwattanapun *et al.*, 1987).

Developmental effects have been documented in pregnant animals exposed to xylenes. ATSDR (1995) concluded that the body of information available for developmental effects is consistent with the hypothesis that xylene is fetotoxic and many of the fetotoxic responses are secondary to maternal toxicity. However, the ATSDR also observed that there was a large variation in the concentrations of xylene producing developmental effects and of those producing no developmental effects. The ATSDR thought that these differences were influenced by a number of factors (strain and species of animal, purity of xylene, method of exposure, exposure pattern and duration, etc.). The two most common test species have been the rat and the mouse.

With respect to rats, Mirkova *et al.* (1983) exposed groups of pregnant rats (unspecified strain of white rats) to clean air or 2.3, 12, or 120 ppm of xylene (unspecified composition) for 6 h/day on days 1-21 of gestation. They reported increased postimplantation losses and fetotoxicity (reduced fetal weights) as well as a statistically increased incidence of visceral abnormalities (including ossification defects in bones of the skull) at xylene air concentrations of 12 ppm and above. The ATSDR has suggested that the Mirkova *et al.* (1983) study results may have been influenced by poor animal husbandry as indicated by the low conception rates and the high incidence of fetal hemorrhages seen in the controls. Hass *et al.* (1993) attempted to replicate the findings of Mirkova *et al.* (1983). Hass *et al.* (1993) exposed groups of 36 pregnant Wistar rats to clean air or 200 ppm of xylene for 6 h/day on days 4-20 of gestation. Unlike Mirkova *et al.* (1983), there was no sign of maternal toxicity and no decrease in fetal weights and no increase in soft-tissue or skeletal malformations. A large increase in the incidence of delayed ossification of the *os maxillare* of the skull, however, was observed (53% of experimental fetuses as opposed to 2% of the controls). Potential neurological/muscular changes measured as performance on a rotorod were also noted upon testing of 2-day-old rat pups.

Ungvary *et al.* (1985) exposed CFY rats by inhalation to air concentrations of xylene (60 ppm, 440 ppm, 800 ppm) for 24 h/day on days 7-15 of gestation. Maternal toxicity was described as moderate and dose-dependent. They observed weight retarded fetuses at all air concentrations. However, there was no increase in malformations, and an increase in minor anomalies and resorbed fetuses occurred only at the highest concentration. In a separate study investigating the interactions between solvents and other agents, Ungvary (1985) exposed CFY rats to either 140 ppm or 440 ppm of xylene on days 10-13 of gestation and also reported increases for either condition in weight retarded and skeletal retarded fetuses without any increase in malformations. Hudak and Ungvary (1978) had earlier examined the effect of 230 ppm xylene (24 h/day, days 9-14 of pregnancy) in the CFY rat and reported effects on skeletal development (e.g., fused sternebrae). In contrast to the other Ungvary findings, no effect on fetal weight was observed. Bio/dynamics (1983) conducted an inhalation exposure study in the rat (CrL-CD (SD) BR strain). Rats were exposed 6 h/day during pre-mating, mating, gestation and lactation. Exposure concentrations were 0, 60, 250, and 500 ppm. Most measures for adverse effects on fetal development were not significantly increased. Mean fetal weights at the highest exposure level were lower than controls, but this difference was significant only for the female fetuses. These

depressed weights were, however, still significant on day 21 of lactation. Other adverse effects (such as increased soft tissue and skeletal abnormalities, increased fetal resorptions) were not increased significantly at any of the test concentrations.

Ungvary *et al.* (1980a) tested by inhalation the individual ortho, meta, and para isomers of xylene in the CFY rat. Pregnant rats were exposed 24 h/day on days 7–14 of pregnancy to 35, 350, or 700 ppm of each isomer. An increased incidence of weight retarded fetuses was observed for each isomer at the 700 ppm level, and for the ortho isomer at the 350 ppm level. Post implantation losses were increased only at the 700 ppm level in the para-xylene exposed group. Skeletal anomalies were increased only at the 700 ppm level for the meta and para isomers of xylene. Rosen *et al.* (1986) evaluated the effects of prenatal exposure to para-xylene in the rat. They exposed pregnant Sprague-Dawley rats by inhalation to either 800 ppm or 1600 ppm of p-xylene from days 7-16 of gestation. Despite the high concentrations, no effects were seen on litter size or weight at birth or on the subsequent growth rates of the pups.

With respect to mice, Ungvary *et al.* (1985) exposed CFLP mice by inhalation to air concentrations of xylene (120 ppm, 230 ppm) for 24 h/day on days 7-15 of gestation. In the mouse, they observed increased incidences of weight-retarded fetuses and increased skeletal retarded fetuses at 230 ppm. Shigeta *et al.* (1983) exposed pregnant ICR mice to approximately 0, 120, 230, 460, and 920 ppm of xylene in an exposure chamber for 6 h/day on days 6-12 of gestation. Shigeta *et al.* (1983) reported significant decreases in fetal weight in the 460 ppm and 920 ppm dose groups only. There was no difference in the number of live or dead fetuses. Decreased weight gains and delayed development of body hair and teeth were observed at the 920 ppm exposure level. Dose-response relations were reported for delayed ossification of the sternebrae. Marks *et al.* (1982) noted that 2060 mg/kg/day of mixed xylene administered orally is associated with cleft palate and decreased fetal weight in the mouse.

Ungvary *et al.* (1985) also tested the individual ortho, meta, and para isomers of xylene at 120 ppm in the CFLP mouse. Each isomer of xylene also increased the incidence of weight-retarded fetuses and skeletal retarded fetuses at 120 ppm. There was no increase in malformations.

Of the three chronic studies available (Tatrai *et al.*, 1981; Jenkins *et al.*, 1970; NTP 1986) none comprehensively examined systemic effects. The study by Tatrai *et al.* (1981) exposed rats for one year, 7 days/week, 8 hours per day to 1096 ppm o-xylene. This exposure was a LOAEL for body weight gain in males and a NOAEL for hepatic effects in male rats. Jenkins *et al.* (1970) exposed rats, guinea pigs, squirrel monkeys, and beagle dogs for 90-127 days continuously to 78 ppm of o-xylene. The study examined body weight gain; hematological parameters including white cell counts, red blood cell counts, and hematocrit; serum biochemistry including bromosulfalein retention, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatinine and liver function including alkaline phosphatase, tyrosine aminotransferase, and total lipids. No effects were observed in any of the parameters examined in this study. This study found a NOAEL for all effects examined of 78 ppm o-xylene. The NTP (1986) study administered 0, 250, or 500 mg/kg/day doses of mixed xylene in corn oil by gavage 5 days/week for 103 weeks to groups of F344/N rats of both sexes, 50 animals per group. B6C3F1 mice were treated in a similar manner but given 0, 500 or 1000 mg/kg/day of mixed xylenes in corn oil by gavage. A complete histopathological examination of

all tissues was made as well as determination of body weight gain. Based on histopathology of all organ systems, a NOAEL of 500 mg/kg/day was observed for rats and a NOAEL of 1000 mg/kg/day was observed for mice.

VI. Derivation of Chronic Reference Exposure Level (REL)

<i>Study</i>	Uchida <i>et al.</i> (1993)
<i>Study population</i>	175 xylene-exposed factory workers and control population of 241 factory workers
<i>Exposure method</i>	Inhalation
<i>Critical Effects</i>	Dose related increase in the prevalence of eye irritation, sore throat, floating sensation, and poor appetite.
<i>LOAEL</i>	14.2 ppm (geometric mean of exposure concentrations)
<i>NOAEL</i>	Not applicable
<i>Exposure continuity</i>	Occupational exposure for an average of 7 years
<i>Average exposure concentration</i>	5.1 ppm for LOAEL group (14.2 x 10/20 x 5/7)
<i>Human equivalent concentration</i>	5.1 ppm for LOAEL group
<i>LOAEL uncertainty factor</i>	3
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	1
<i>Intraspecies uncertainty factor</i>	10
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation reference exposure level</i>	0.17 ppm (170 ppb; 0.7 mg/m ³ ; 700 µg/m ³) for mixed xylenes or for total of individual isomers

A number of issues are important in considering the uncertainty associated with this REL. ATSDR (1995) concludes that animal and human toxicity data suggest that mixed xylenes and the different xylene isomers produce similar effects, although different isomers are not equal in potency for producing a given effect. Therefore exposure of workers to a mix of xylenes in the Uchida *et al.* (1993) study would be expected to generate a similar spectrum of responses as exposure to single isomers, however the intensity of particular effects could be different. The use of a neurological endpoint for derivation of a REL is supported by the large number of inhalation and oral studies associating neurological effects with xylene exposure. ATSDR (1995) indicates that neurological effects are a sensitive endpoint. The observation that floating sensation is apparently related to dose further supports the concept that this subjective symptom related to neurological effects was due to xylene exposure. The use of a factor of 3 for using a LOAEL as the basis for the REL should serve to protect populations from neurological effects as should the use of a factor of 10 for sensitive individuals within the population. Another issue is the use of diffusive samplers in the Uchida *et al.* (1993) study. These samplers provide a time weighted average concentration of hydrocarbon and cannot indicate the maximum concentrations a worker is exposed to. It is unknown whether peak concentrations alter the response to xylenes in humans.

The strengths of the inhalation REL include the use of human exposure data from workers exposed over a period of years. Major areas of uncertainty are the uncertainty in estimating exposure, the potential variability in exposure concentration, and the lack of observation of a NOAEL.

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Determination of Noncancer Chronic Reference Exposure Levels
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