Toluene

Major Uses and Sources of Emissions
Toluene occurs naturally as a component of crude oil and is a major aromatic constituent of petrol. It is used in household aerosols, nail polish, paints and paint thinners, lacquers, rust inhibitor, adhesives and solvent based cleaning agents. Sources of toluene emissions to air include: motor vehicles, aircraft, petroleum refineries and terminals, service stations, lawn mowers and other petrol-fuelled implements, chemical industry, rubber manufacturers, manufacture and use of paints, varnishes and lacquers, metal degreasing, printing and tobacco smoke.

Critical health endpoint.
Respiratory irritation and central nervous system depressant effects were considered to be the critical effects following short-term exposures of humans to toluene.

US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, September 2000 (ATSDR 2000)

Acute Exposures
The ATSDR report noted that several studies are available regarding the effects of single exposures to toluene, both in humans and animals (Andersen et al. 1983; Baelum et al. 1985; Echeverria et al. 1991). These studies identify the nervous system as well as nose and eye irritation as the critical toxicity targets of toluene, and describe dose-response relationships between end points and exposure levels. ATSDR considered that sufficient data for the inhalation route were available to derive an acute inhalation MRL based on a lack of neurological effects (NOAEL) in volunteers exposed to 40 ppm toluene for 6 hours (Andersen et al. 1983)

Key and supporting studies
Andersen, et al. (1983) reported the effects of toluene on 16 healthy young male subjects with no previous regular exposure to organic solvents were investigated. Groups of four subjects were in a chamber for 6 hours a day on 4 consecutive days. After 1 hour of exposure to clean air in the chamber, the concentration of toluene was steadily increased during 30 minutes to the concentration intended for the day. After hour of exposure, all subjects went through all physiological, discomfort, and performance measurements for the next 1.5 hours. After a 1 hour lunch, a similar series of measurements were made during the 5^{th} and 6^{th} hours of exposure. The concentration of toluene was 0, 10, 40, or 100 ppm with each group exposed to a different toluene concentration each day. Physiological measurements were performed, including nasal mucociliary flow, FVC, FEV, and FEF_{25-75}, and subjective measurements of discomfort. Eight different performance assessment tests were carried out.

There was a significant change in nasal mucus flow from control values during all of the toluene exposures. During the 100 ppm exposure, statistically significant increased irritation was experienced in the eyes and in the nose, but not in the throat or lower airways. There was also a statistically significant increase in the occurrence of headaches,

dizziness, and feelings of intoxication during the 100 ppm exposure, but not during the other concentrations. No statistically significant effects of toluene occurred in the eight performance tests. The subjects felt that the tests were more difficult and strenuous during the 100 ppm exposure, for which headache, dizziness, and feelings of intoxication were more often reported. No adverse effects were reported at the 10 and 40 ppm levels.

Baelum et al.(1985) reported a LOAEL of 100 ppm for neurological effects in humans. In this study, 43 occupationally-exposed subjects and 43 controls were exposed to either clean air or air containing 100 ppm toluene for 6.5 hours in a climate chamber. A battery of ten tests of visuomotor coordination, visual performance, and cortical function were administered during the 6.5 hour period. For both the controls and toluene exposed subjects, there were complaints of air quality, irritation of the nasal passages, and increased feelings of fatigue and sleepiness. Subjects also complained of headaches and dizziness. Toluene exposure decreased performance on four of the neurobehavioral tests; three of these were tests of visual perseverance. The fourth test affected was the simple peg board test of visuomotor function, where the effect was noted in toluene-exposed workers to a much greater extent than controls. Echeverria et al. (1991) reported a LOAEL of 75 ppm for neurological effects in humans. In this study, two groups of 42 students were exposed to 0, 75, and 150 ppm toluene for a 7 hour period. A complete battery of 12 tests was administered before and at the end of each exposure. Toluene caused a dose-related impairment of function on digit span pattern recognition, the one hole test, and pattern memory.

Rahill et al. (1996) also reported a LOAEL of 100 ppm for neurological effects in humans. In this study, six volunteers were exposed for 6 hours a day to either 100 ppm toluene or clean air. Three repetitions of two computerised neuropsychological tests were performed, with the composite score on the multitasking test being significantly lower with toluene exposure than with clean air.

Longer term exposures in humans

Studies of workers repeatedly exposed to toluene in workplace air at concentrations ranging from about 30 to 150 ppm have found evidence for increased incidence of self-reported neurological symptoms (Orbaek and Nise 1989; Yin et al. 1987), performance deficits in neurobehavioural tests (Boey et al. 1997; Foo et al. 1990; Orbaek and Nise 1989), hearing loss (Abbate et al. 1993; Morata et al. 1997), changes in visual-evoked brainstem potentials (Vrca et al. 1995, 1997a, 1997b), and colour vision impairment (Zavalic et al. 1998a, 1998b, 1998c). ATSDR considered that sufficient data for the inhalation route were available to derive a chronic MRL based on colour vision impairment in toluene exposed workers (Zavalic et al. 1998a).

Laboratory animal studies

Two animal studies have investigated the effects of toluene following chronic inhalation exposure (CIIT 1980; NTP 1990). Multiple end points of toxicity were investigated, including carcinogenicity, and the data indicate that toluene is not a carcinogen.

Results from several inhalation exposure studies of animals indicate that exposure to levels of toluene that begin to produce maternal toxicity can cause foetal effects, including reduced foetal survival and retardation of growth and skeletal development. No-effect levels in animals for toluene on standard developmental end points range from about 133 ppm for a 24 hour/day exposure protocol (Ungvary and Tatrai 1985) to 133–750 ppm with 3–6 hours/day protocols (Huntingdon Research Centre 1992; Thiel and Chahoud 1997).

Californian Environmental Protection Agency, 1999 (CEPA 1999), Office of Environmental Health Hazard Assessment (OEHHA)

Acute Reference Exposure Level (REL)

OEHHA has also used the Andersen et al (1983) study on healthy volunteers exposed to toluene for a 6 hour duration for the derivation of an acute REL. They considered that the critical effects were impaired reaction time and symptoms of headache, dizziness, a feeling of slight intoxication (CNS depressant effects), and slight eye and nose irritation. The study has shown a LOAEL at 100ppm, and a NOAEL at 40ppm for both CNS and irritant effects of toluene.

Chronic REL

OEHHA has stated that if both human and animal adverse effect data on a chemical are available, they prefer to use the human data to develop a Chronic Reference Exposure Level (REL) when possible. The critical animal study (Hillefors-Berglund *et a*l., 1995) was used to derive an REL for toluene describes adverse neurological effects in rats after a well characterised inhalation exposure to toluene. The study results contain both a LOAEL and a NOAEL. Decreased brain (subcortical limbic area) weight and altered dopamine receptor binding (which the OEHHA consider to be specific and sensitive measures of neurotoxicity that would not be obtainable in human studies) compared to controls were noted at the NOAEL, but the changes were not statistically significant. This suggests that if a threshold for adverse neurological effects exists in this study, it would be at or below the observed NOAEL.

In contrast, the psychometric tests used to generate the neurotoxicity data in the human occupational exposure studies described above tend to be less sensitive and suffer from greater measurement uncertainty. Additionally, the Hillefors-Berglund *et a*l. (1995) study has better exposure characterisation than the human occupational exposure studies. Nonetheless, the human studies are useful in supporting the derivation of the Chronic REL for toluene.

Orbaek and Nise (1989)used pooled psychometric data from two rotogravure printing plants with different toluene concentrations (11.2 and 41 ppm) to determine significant neurotoxic effects by The range of RELs derived from that study lists the upper and lower bounds for risk associated with the pooled population exposures.

Yin *et al*., (1987) studied solvent workers exposed to 42.8 ppm toluene (estimated as a time-weighted average) for an average duration of 6.8 years reported a significantly greater incidence of sore throat, dizziness and headache than controls. Foo *et al*., (1990) found significant decrease in neurobehavioural performance in toluene exposed female workers in 6 out of 8 tests.. Boey *et al.* (1997) found similar effects in a follow-up study. Abbate *et al.* (1993) evaluated alterations induced in the auditory nervous system by exposure to toluene in a group of rotogravure workers and found statistically significant alteration in the BAER results in the toluene-exposed workers.

Zavalic *et al.* (1998a, b, c) examined the effects of chronic occupational toluene exposure on colour vision found significant impairment in toluene-exposed workers compared to controls.

Chronic solvent abusers (spray lacquer; ≈60% toluene, 10% dichloromethane) were examined by Rosenberg et al.,(1988) and neurological abnormalities were seen in four of 11 subjects and included brainstem, cerebellar, cognitive and pyramidal findings. Brain MRIs were abnormal in three of 11 subjects and indicated the occurrence of diffuse cerebral, cerebellar, and brainstem atrophy and loss of differentiation between the grey and white matter throughout the CNS.

Six chronic toluene abusers were examined using MRI by Caldemeyer et al. (1996). All patients examined demonstrated white matter atrophy The authors noted a correlation between the severity of white matter degeneration and degree of neurological dysfunction. However, there was no correlation between the severity of imaged white matter changes and the presence. Additionally, no definite clinical evidence of damage to the basal ganglia and thalami was found.

It should be stated however that the above human data on CNS structural alterations claimed to be by OEHHA, in similar brain areas as noted for rats (Hillefors-Berglund et al 1995), has been obtained from MRI studies of solvent abusers and thus the veracity of effects noted cannot be unequivocally said to be due to toluene alone as these subjects have been abusing other solvents and mixtures of solvents.

Air Quality Guidelines for Europe, 2nd edition -WHO Regional Office for Europe, 2000 (WHO 2000)

WHO has stated that the acute and chronic effects of toluene on the central nervous system are the effects of most concern. The lowest level of chronic occupational toluene exposure unequivocally associated with neurobehavioural functional decrements is 332 mg/m^3 (88 ppm) (Foo et al 1990, 1993). Effects on the central nervous system in humans are supported by findings in exposed animals (Forkman et al 1991).
Both the human and animal data (Johnson et al 1988) indicate that toluene is ototoxic at elevated exposures. Toluene has minimal effects on the liver and kidney, except in cases of solvent (toluene) abuse.

Findings of studies on animals have shown foetal development retardation, low birth weight, skeletal anomalies, and developmental neurotoxicity (Hudak and Ungvary 1978, Shigeta et al 1986). There has been no indication that toluene is carcinogenic in bioassays conducted to date (Svensson et al 1990), and the weight of available evidence indicates that it is not genotoxic.

US EPA , *Integrated Risk Information System summary for Toluene* - update for Reference Concentration for Chronic Inhalation Exposure (RfC) (IRIS 2002, and 1992)

The USEPA in their 2002 IRIS update consider that two studies, Zavalic et al. (1998a) and Eller et al. (1999) have identified the no observe adverse-effect level, and a lowest adverse effect level for impairment of colour vision in workers exposed to toluene in occupational settings.

The study of Zavalic et al.(1998a)was selected as the principal study for derivation of an inhalation RfC. It was considered to be an adequate cross-sectional study of chronically-exposed humans which identified both a NOAEL (32 ppm) and LOAEL (132 ppm)for neurological effects (impaired colour vision).Impaired colour vision is the critical effect. Effects were correlated with both airborne and blood toluene concentrations.

The study of Eller et al.(1999) defined a similar NOAEL (25 to 32 ppm)for decreased performance in neurobehavioural and neuropsychological tests in rotogravure printing workers, but the effect levels in this study were less clearly characterised. Both of these NOAEL values lie slightly below the 40 to 100 ppm range where available data suggest that persistent neurological effects in humans chronically-exposed to toluene begin to manifest.

The USEPA in 1992 stated in the IRIS assessment that the study by Foo, et al (1990), was the principal human study to determine the chronic inhalation reference concentration. However, it did not provide NOAEL concentrations for the CNS depressant effects, therefore the LOAEL was needed to be considered in developing the RfC. Further, this effect is supported by a number of other occupational studies that show effects around 100 ppm, (Baelum et al., 1985; Echeverria et al., 1989).

In conclusion, the available studies each have a number of limitations. However, when considered jointly, these studies indicate that humans repeatedly exposed to toluene concentrations ranging from 40 to 132 ppm have an increased risk of developing neurological effects.

Government of Canada, Health and Welfare Canada, Environment Canada: Priority Substances List Assessment Report No. 4 Toluene (1992)

The report states that available data on the effects of exposure to toluene in humans are derived from studies in volunteers, effects of its use as a solvent of abuse and epidemiological studies of exposed workers.

Andersen *et al.,* (1983) study that reported decreases in neurological function and irritation of the respiratory tract at 375 mg/m^3 and no adverse effects at 150 mg/m ^{3} was also judged by the Canadians to be an adequate clinical study in human volunteers;

The study by Baelum et al., (1985) on neurological function was stated to be less reliable owing to limitations of study design.

In cases of intentional abuse, exposures have been extremely high (up to 112,500 mg/m^3). Moreover, reported cases of intentional abuse and epidemiological studies of occupationally-exposed populations have generally involved exposures to complex mixtures with toluene as the principal constituent, and the power of most of the epidemiological studies has been limited. These investigations are, therefore, of less value than clinical studies in human volunteers in assessing exposure-response relationships for toluene.

They state that available epidemiological data were considered inadequate to assess the carcinogenicity and clastogenicity of toluene in humans. Toluene has not been found to be carcinogenic following inhalation in rats and mice in a well-conducted bioassay (NTP, 1990) and in rats in a less sensitive bioassay (CIIT, 1980).

Repeated short-term exposure of animal species to moderate to high concentrations of toluene causes central nervous system depression and adverse effects on the liver, kidney and lungs. The lowest concentration at which effects have been reported in well-documented and adequate subchronic bioassays following inhalation is 100 ppm (375 mg/m^3) which induced a decrease in body weight (7.5 and 12% reduction in final weight relative to controls in males and females, respectively) in a 14-week study in mice conducted by the NTP (1990).

Toluene does not appear to be teratogenic in mice, rats, or rabbits, on the basis of limited data. It is foetotoxic at high concentrations (1 000 mg/m^3) which are not toxic to the dam, causing a reduction in foetal weight in mice and rats, and retarded ossification with some increase in minor skeletal anomalies (Ungvary and Tatrai, 1985).

Summaries of Pivotal Studies Identified in the above reviews.

Effects from Short term exposure to toluene

Echeverria et al., (1989) studied forty-two college students (21 female and 21 male) who were exposed to 0, 74 ppm (279 mg/m^3), or 151 ppm (569 mg/m^3) toluene for 7 hours over 3 days. This exposure sequence was repeated for a total of 42 exposures over a 3-month period. The odour of toluene was masked. A battery of performance tests was administered to each participant prior to starting the exposures and again at 4 and 7 hours during the exposure; the initial test served as a control for those tests performed during the exposure. A 5-10% decrement in performance was considered significant if consistent with a linear trend. Test results for visual perception differed from control values for both exposure levels. Results of a manual dexterity test differed from control values at the higher but not the lower exposure level. Psychomotor test results were unaffected by toluene exposure. Subjective symptomatology increased with exposure with increasing numbers of complaints of eye irritation, headache, and somnolence. A NOAEL of 74 ppm (279 mg/m^3) is indicated for these results. The duration-adjusted value is 122 mg/m^3 for these acute effects.

The acute study by Baelum et al. (1990) evaluated 32 males and 39 females exposed to 0 or 100 ppm (0 or 377 mg/ mg/m^3), or to varying exposures of 50-300 ppm (188-1131 mg/m^3) (TWA = 102 ppm), for 7 hours. Volunteers exercised on an ergometer cycle for 3 periods of 15 minutes each during the exposure. No significant differences were found in the performances between the exposed and control groups in a battery of tests for performance, visual attention, and reaction times. Exposed subjects reported an increase over nonexposed subjects ($p<0.1$) in nose and lower respiratory irritation, feelings of intoxication, dizziness, increased coughing, and headaches. Differences were not noted between the group exposed to a constant level (100 ppm) and the group exposed to the same TWA, but with peaks of up to 300 ppm.

Baelum et al. (1985) investigated the effects of a 6.5-hour toluene exposure to 43 printers with a long-term occupational exposure to a mixture of solvents including toluene and 43 controls with no history of exposure to solvents or other chemicals. The duration of employment for the workers ranged from 9-25 years. Each individual was exposed only once to either 0 or 100 ppm (0 or 377 mg/m^3) toluene during a 6.5-hour exposure period, preceded by a 1-hour acclimatisation period. These subjects were then subgrouped into printers exposed to toluene (n = 20), printers exposed to air (n = 23), controls exposed to toluene (n = 21), and controls exposed to air (n = 22). All subjects carried out a battery of tests for psychometric performance, visual perception, and vigilance evaluation. Both printers and controls complained of nasal and eye irritation, unacceptable air quality, and unacceptable odour level during the toluene exposure. Signs of neurotoxicity, including moderate fatigue, sleepiness, headaches, and a feeling of intoxication, were likewise similarly reported for both groups. A significant decrease in performance was found for the pegboard visual motor function test in the exposed printers, but not in the controls exposed to 100 ppm toluene. A decrease in psychometric performance, primarily in

visual perception and accuracy, was observed in toluene-exposed individuals. Acute exposure to toluene resulted in a lower performance in 4/10 tests conducted, 3 of these 4 evaluated visual perception. The most profound difference between subjects exposed to 100 ppm toluene and those exposed to clean air was observed in the colour discrimination test; this difference was seen in both exposed vs. nonexposed printers and exposed vs. nonexposed controls. This study indicates that little tolerance develops to the irritative and central effects in humans exposed to toluene and that 100 ppm (377 mg/m^3) is the effect level for these symptoms.

Effects of long term exposure to toluene

Visual Impairment

Zavalic et al.(1998a) examined two groups of Croatian workers occupationally exposedto toluene for effects on colour vision, relative to a group of 90 unexposed controls. The first exposed group (group E1) consisted of 46 shoe gluing workers, while the second group (group E2) consisted of 37 rotogravure printing workers. Mean exposure times were 16.21 ±6.1 (mean ±SD) years for group E1 and 18.34 ±6.03 years for group E2. For all groups, smoking and alcohol consumption information was collected. Measured toluene (medians) concentrations were 32 ppm (121 mg/m ^{3}); range of 11.3-49.3 ppm for group E1 and 132 ppm (498 mg/m ^{3}), range of 66-250 ppm for group E2. Samples of venous blood were taken in all three groups on Wednesday before the work shift, and toluene concentrations were determined. Analysis of colour vision was performed using the Lanthony 15 Hue desaturated panel, which is based on the ability to recombine a set of 15 desaturated colour caps according to a definite chromatic sequence. Results are reported as the colour confusion index (CCI) or age-and alcohol intake-adjusted colour confusion index (AACCI).Colour vision was tested on Wednesday morning before the work shift, at least 16 hours after the last exposure to toluene, and on Monday, at least 64 hours after the last exposure to toluene. In the high-exposure group (group E2), there were significant correlations between toluene in air (132 ppm with a range of 66 -250 ppm) and toluene in blood. Correlation between toluene in air and blood for group E1 was positive, but was not statistically significant. CCI scores on both Wednesday and Monday were significantly higher in group E2 relative to both controls and to group E1. CCI scores for group E1 were not significantly different from controls at any time examined. In all groups, including controls, a significant correlation between CCI and both age and alcohol consumption was reported. CCI scores for those workers who consumed no alcoholic beverages at all were significantly greater for group E1 than for non- consumers in the control group, however, age- matching of these two subgroups was not reported. Given the dependence on age and alcohol intake, the AACCI scores are considered more relevant indicators of toluene exposure than CCI scores. AACCI scores for group E2 were significantly correlated with toluene in blood, toluene in air, *ortho* - cresol in urine, and hippuric acid in urine. No statistically significant correlation was established between AACCI scores and any marker of toluene exposure for group E1. The AACCI scores were significantly higher ($p<0.05$) group E2, but not group E1, compared to controls. This study identified a NOAEL of 32 ppm (121 mg/m ^{3}, group E1)

and a LOAEL of 132 ppm (498 mg/m^3, group E2) for alterations in colour vision in toluene- exposed workers based on AACCI scores.

Other studies of human colour vision impairment suggest that vision impairment results from chronic, rather than acute, exposure to toluene (Muttray et al. 1999; Zavalic et al. 1998 a, b, c). The mechanism by which toluene exposure influences colour vision is not known. Vrca et al. (1995, 1997a, 1997b) showed that visual evoked potentials are affected in chronically exposed individuals and show exposure-related changes in amplitude and latency. However, it was not clear whether the impairment of colour vision produced by toluene exposure is due solely to neurological damage or also involves damage to the eyes.

Neurological effects

Eller et al.(1999)reported on the neurological effects of 98 male rotogravure printers chronically exposed to toluene. Exposed workers were divided into workers exposed for 1-12 years, Group 1(n=30) to levels estimated at 25-32 ppm (94-121 mg/m^3), though some procedures still involve higher exposure levels for short periods of time. And Group 2(n=49), workers exposed for greater than 12 years who may have been exposed to levels exceeding 100 ppm (377 mg/m^3)for up to 27 years. The control group consisted of 19 workers not exposed to toluene. For the scores of self-reported symptoms, the controls and Group 1 were found to be similar, while Group 2 showed a statistically significantly higher incidence of symptoms relative to controls, even after correction for age and alcohol consumption. In neurological tests, no differences
between Group 1 and controls were noted. Group 2 showed a statistically significantly poorer performance, relative to the other groups, on 1 of 7 neurological tests and 2 of 5 sets of neuropsychological tests; amongst the tests which were significantly altered were left hand finger tapping and retention times in the number learning test. This study identified a NOAEL of 25-32 ppm and a LOAEL of 100 ppm for increases in subjective symptoms and decreased performance in neurological tests.

Foo et al. (1990,1993) conducted a cross-sectional study involving 30 exposed female workers employed at an electronic assembly plant where toluene was emitted from glue. Toluene levels reported in the study were from personal sample monitoring and reported as an 8-hour TWA. Co-exposure to other solvents was not addressed in the study. The average number of years (± SD) worked by the exposed population was 5.7 ± 3.2 and by the controls was 2.5 ± 2.7. Exposed workers breathed toluene air levels of 88 ppm (332 mg/m^3) as a TWA and control workers 13 ppm (49 mg/m^3) (TWA. A battery of eight neurobehavioural tests were administered to all exposed and control workers. The tests were performed midweek, before the workers reported to their stations for the day. Group means revealed statistically significant differences in 6/8 tests; all tests showed that the exposed workers performed poorly compared with the control cohort

The incomplete exposure information, coupled with the small size of the cohort, limits the interpretation of this study, although the results were essentially confirmed in a clinical study in which the toluene concentrations were carefully controlled (Echeverria

et al., 1989) at levels bracketing 88 ppm. Although the data in Echeverria et al. (1989) were generated from short- term exposures (3-7 hours over a period of 142 days), the results may be considered relevant to longer-term exposures as several studies indicate the absence of a duration-response relationship in toluene-induced symptomatology. Another group of 29 exposed workers in Singapore (average TWA toluene exposure of 90.9 ppm) performed more poorly than a control group (average TWA exposure of 12.2 ppm) on 8 neurobehavioral tests. The exposed group performed significantly more poorly in verbal and nonverbal memory as measured by the digit span and visual reproduction tests (Boey et al. 1997).

Yin et al. 1987, reported that a group of 95 workers exposed to TWA of 41–46 ppm toluene were evaluated for symptoms and signs of exposure when compared to 130 control subjects. The incidence of health-related complaints among the toluene exposed workers was 2–3 times that of the controls. Dizziness was reported by about two-thirds of the toluene exposed respondents. These subjects also complained of headaches, sore throats, eye irritation, and difficulty with sleep. When the exposed subjects were divided into 2 groups, one with TWA exposures of less than 40 ppm and the other with exposures greater than or equal to 40 ppm, the incidence of headache and sore throat, but not dizziness, showed a concentration-response pattern.

Orbaek and Nise 1989 studied thirty rotogravure printers from two plants and 72 unexposed workers completed a questionnaire designed to record their neurasthenic complaints and were given a series of tests designed to evaluate psychometric function. At the time of the study (1985), 19 of the printers were exposed to TWA toluene levels of 11.6 ppm, and the remainder were exposed to 42.4 ppm. However, the printers had been exposed to solvents for at least 10 years (employment ranged 4–43 years), and estimated air concentrations at earlier times were much higher (as high as 453 ppm prior to 1970). Taking the midpoints in the ranges of concentration estimates for 1970–1985 for the two factories and calculating their mean yields, a representative exposure concentration of 140 ppm was determined. Significantly more printers reported neurasthenic symptoms than controls, but no significant differences were found between printers and controls for 10 of 11 psychometric tests and in the remaining test (Cylinder Board test of motor skill), printers performed better than controls.

Developmental Effects - Most of the information concerning the adverse developmental effects of toluene in humans comes from case reports among children of deliberate toluene "sniffers." Children whose mothers had inhaled large quantities of toluene during pregnancy were found to have microcephaly, facial and limb abnormalities, attention deficits, hyperactivity, developmental delay with greater language impairment, and growth retardation. Multiple solvent and/or other substance abuse may have contributed to the observed abnormalities and the lack of exposure data limit the conclusions that can be drawn (Hersh 1988; Hersh et al. 1985; Pearson et al. 1994).

Cancer - Studies indicate that cancers of most sites are not significantly associated with toluene exposure. The information from these studies is inadequate to assess the carcinogenic potential of toluene, predominantly because of the lack of consistent

findings across the studies and the likelihood that many of the studied groups were exposed to multiple chemicals (Svensson et al. 1990; Gérin et al. 1998).

Effects of exposure in laboratory animals

CNS effects:

Dose-dependent decreases in behavioural performance activity depression and central nervous system depression were observed in mice and rats exposed by inhalation to toluene at concentrations ranging from 100 to 12,000 ppm (Forkman et al. 1991). Younger animals were more susceptible to toluene toxicity and mice were more sensitive than rats of the same age.

Changes in the levels of brain neurotransmitters in rodents exposed to toluene have been observed. Significant localized changes in dopamine (DA) or noradrenaline (NA) brain levels were noted in rats exposed to 400 ppm toluene 24 hours/day for 30 days (Ikeda et al. 1986) and in newborn male rats 7 weeks after a 10-day exposure to 80 ppm toluene for 6 hours per day (von Euler et al. 1989). Neurotransmitter levels in some areas of the brain were increased, in some areas were decreased, and in other areas remained the same. Because of the variability in response, these data are difficult to evaluate.

Hillefors-Berglund et al. (1995) exposed male rats to toluene (0, 40, 80, 160 or 320 ppm, 4 weeks, 6 hours/day, 5 days/week) , followed by a post exposure period of 29-40 days, reported that the rats had decreased caudate-putamen ($p < 0.05$) and subcortical limbic area brain wet weights ($p < 0.001$) compared to controls at concentrations of 80 ppm and higher (with trend test for dose-response significant at $p <0.01$). Toluene exposure did not significantly affect the wet weights of the whole brain.

They reported in the same study that toluene exposure at 80 ppm also significantly altered dopamine receptor activity (trend test for dose-response) as indicated by decreased inhibition constant ($p < 0.05$) for high-affinity receptor sites and for low-affinity receptor sites, and high-affinity receptor site specific binding values for dopamine competitive inhibition of [^{3}H]raclopride binding in the caudate-putamen. Exposure to xylene or styrene (80 and 40 ppm, respectively; 4 weeks, 6 h/day, 5 days/week) followed by a post exposure period of 26-32 days had no effect on the parameters described above.

The authors concluded that long-term exposure to low concentrations of toluene (≥ 80 ppm), but not xylene (80 ppm) or styrene (40 ppm), leads to persistent increases in the affinity of dopamine D2 agonist binding in the rat caudate-putamen.

Long-term Exposure

Respiratory Effects
A CIIT, (1980) study of the chronic effects of toluene in rats exposed to toluene up to 300 ppm showed no treatment-related effects, however, in a NTP (1990) study, exposures 600

ppm and above was noted to cause significant erosion of the nasal epithelium and degeneration of the respiratory and nasal epithelium.

Carcinogenic potential:
In a NTP (1990), 2-year bioassay, Fischer 344 rats (60/sex/group) were exposed to 0, 600, or 1200 ppm (0, 2261, or 4523 mg/m^3, respectively) toluene vapours, 6.5 hours/day, 5 days/week (duration-adjusted to 0, 437, and 875 mg/m^3, respectively) for 103 weeks. No carcinogenic responses in rats were found. Mean body weights in both exposed groups were not different from controls for either sex. No exposure-related clinical signs were reported, and survival rate was similar for all groups. At the end of 2 years, there was a significant ($p<0.05$) increase in the incidence of erosion of the olfactory epithelium (males: 0/50, 3/50, and 8/49; females: 2/49, 11/50, and 10/50; at 0, 600, and 1200 ppm, respectively) and of degeneration of the respiratory epithelium (males: 15/50, 37/50, and 31/49; females: 29/49, 45/50, and 39/50; at 0, 600, and 1200 ppm, respectively) in the exposed animals. The females exposed to 600 and 1200 ppm also exhibited a significant increase in inflammation of the nasal mucosa (27/49, 42/50, and 41/50 at 0, 600, and 1200 ppm, respectively) and respiratory metaplasia of the olfactory epithelium (0/49, 2/50, and 6/50 at 0, 600, and 1200 ppm, respectively). A LOAEL of 600 ppm toluene was determined for the concentration-dependent increase in erosion of the olfactory epithelium in male rats and the degeneration of the respiratory epithelium in both sexes. No NOAEL could be derived from this study.

Reproductive and Developmental Toxicity in Laboratory Animals
An IRDC 1985, study relating toluene exposure and retardation of development was conducted in rats where 2 generations were exposed for 6 hours per day up to 2,000 ppm toluene during an 80 day premating period and a 15 day mating period. Adult females of both generations were also exposed on days 1-20 of gestation and on days 5-21 of lactation. The mean body weights of foetuses of both generations of dams exposed to 2,000 ppm were significantly decreased compared to controls. No maternal toxicity was reported. Exposure at this level only to the male parent did not result in any adverse effects. The NOAEL for foetotoxic effects in this study was 500 ppm.

Hudak and Ungvary (1978) exposed three groups of pregnant CFY rats to toluene during different periods of gestation and for different durations of exposure. The first of these was exposed to 1500 mg/m^3 for 24 hours/day during gestational days 9 to 14. Two dams died during these exposures. Foetotoxicity was noted as sternebral alterations (6% vs. 1% in controls), extra ribs (22% vs. 0% in controls), and the presence of foetuses with missing tails (2/213, none observed in 315 controls). Under these exposure conditions, 1500 mg/m^3 was the LOAEL for foetotoxicity and the level for maternal toxicity. The second group received this same concentration continuously but on days 1-8 of gestation. Five dams died under these exposure. Slight hydrocephaly was noted in 4 foetuses (all from the same litter), and 17% growth retardation was noted vs. 7% in the controls. Thus these exposure conditions are a LOAEL for foetotoxicity. A third group was exposed to 1000 mg/m^3 for 8 hours/day from the 1st to the 21st day of gestation. No maternal deaths or toxicity occurred. Minor skeletal retardation was present in the exposed foetuses 25%, compared to concurrent controls, 0% These results indicate that 1000 mg/m^3 is a LOAEL

for developmental effects under these exposure conditions. This concentration is also a NOAEL for maternal effects.

In the same study, groups of pregnant CFLP mice were exposed to either air or 1500 or 500 mg/m^3 toluene continuously during days 6-13 of pregnancy. All mice exposed to the high concentration died within 24 hours of the beginning of exposure. No dams died in the lower exposure group. In this group, the average foetal weight decreased to 0.96 g from the average control weight of 1.07 g, and the percentage of weight-retarded foetuses (less than 0.9 g) increased to 27.6% from 6.5% in the controls. No difference in incidence of skeletal malformations or anomalies was noted between these and control foetuses. For mice, 500 mg/m^3 is the LOAEL for developmental effects.

Courtney et al., (1986), exposed pregnant Charles River CD-1 mice to filtered air or 200 or 400 ppm (754 and 1508 mg/m^3) toluene 7 hours/day on gestational days 7-16. The exposed pregnant mice did not exhibit embryotoxicity, or foetal body weight differences compared to the control values. A statistically significant increase over controls in the incidence (both per litter and per foetus) of enlarged renal pelvis was noted in dams exposed to 200 ppm but not 400 ppm. A statistically significant alteration from controls in the rib profile (percentage of foetuses with 1 or 2 additional/fewer ribs) was reported for foetuses from dams exposed to 400 ppm but not 200 ppm which is a NOAEL for reproductive and developmental effects in mice.

Toluene References

Abbate C, Giorgianni C, Munao F, et al. 1993. Neurotoxicity induced by exposure to toluene. An electrophysiologic study. Int Arch Occup Environ Health 64:389-392.

Andersen I, Lundqvist GR, Molhave L, et al. 1983. Human response to controlled levels of toluene in six-hour exposures. Scand J Work Environ Health 9:405-418.

Baelum J, Andersen I, Lundqvist GR, et al. 1985. Response of solvent-exposed printers and unexposed controls to six-hour toluene exposure. Scand J Work Environ Health 11:271-280.

Boey KW, Foo SC, Jeyaratnam J. 1997. Effects of occupational exposure to toluene: a neuropsychological study on workers in Singapore. Ann Acad Med Singapore 26(2):84-7.

Caldemeyer KS, Armstrong SW, George KK, et al. 1996. The spectrum of neuroimaging abnormalities in solvent abuse and their clinical correlation. J Neuroimaging 6(3):167-73.

CIIT. 1980. Chemical Industry Institute of Toxicology. A 24 month inhalation toxicology study in Fischer-344 rat exposed to atmospheric toluene. Executive Summary and Data

Tables. Conducted by Industrial Bio-Test Laboratories, Inc., Decatur, IL, and Experimental Pathology Laboratories, Inc., Raleigh, NC for CIIT, Research Triangle Park, NC. October 15, 1980.

Courtney KD, Andrews JE, Springer J, Menache M, Williams T, Dalley L, *et a*l. 1986. A perinatal study of toluene in CD-1 mice. Fundam Appl Toxicol;6:145-154.

Echeverria D, Fine L, Langolf G. Schork A. Sampaio C., 1989, Acute neurobehavioral effects of toluene. Br J Ind Med 46:483-495.

Echeverria D, Fine L, Langolf G, et al. 1991. Acute behavioural comparisons of toluene and ethanol in human subjects. Br J Ind Med 48:750-761.

Eller,N.,B.Netterstrom and P.Laursen.1999.Risk of chronic effects on the central nervous system at low toluene exposure.Occup.Med.49(6):389-395.

Foo SC, Jeyaratnam J, Koh D. 1990. Chronic neurobehavioural effects of toluene. Br J Ind Med 47:480-484.

Foo,S.C.,Ngim, C.H., Salleh I., Jeyaratnam J,. Boey KW. 1993 Neurobehavioural effects in occupational chemical exposure.Environmental research,60 :267 –273

Forkman,B.A., BA. Ljungberg T., Johnson AC., Nylen P., Stahle L., Hoglund G., Ungerstedt U., 1991, Long-term effects of toluene inhalation on rat behavior. Neurotoxicology & Teratology. 13(5):475-81

Hersh JH. 1988. Toluene embryopathy: Two new cases. J Med Genet 26:333-337.

Hersh JH, Podruch PE, Rogers G, et al. 1985. Toluene embryopathy. J Pediatr 106:922-927.

Hillefors-Berglund M, Liu Y, von Euler G. 1995. Persistent, specific and dose-dependent effects of toluene exposure on dopamine D2 agonist binding in the rat caudate-putamen. Toxicology 100:185-94.

Hudak A and Ungvary G. 1978. Embryotoxic effects of benzene and its methyl derivatives: Toluene, xylene. Toxicology 11:55-63.

Huntingdon Research Centre. 1992. Toluene: Effect on pregnancy of the rat by inhalation (status report). Huntindon Research Centre, Ltd., Huntingdon, Cambridgeshire, England. APT 2/91279.

Ikeda M, Koizumi A, Kasahara M, et al. 1986. Combined effects of n-hexane and toluene on norepinephrine and dopamine levels in rat brain tissues after long-term exposure. Bull Environ Contam Toxicol 36:510-517.

IRDC (International Research and Development Corporation), 1985, Two-generation inhalation reproduction/fertility study on a petroleum derived hydrocarbon with toluene. API Medical Research Publication no. 32-32854. Washington (DC): American Petroleum Institute.

Johnson AC, Juntunen L, Nylen P, et al. 1988. Effect of interaction between noise and toluene on auditory function in the rat. Acta Otolaryngol 101:56-63.

Morata TC, Fiorini AC, Fischer FM, et al. 1997. Toluene-induced hearing loss among rotogravure printing workers. Scand J Work Environ Health 23(4):289-98.

NTP. 1990. National Toxicology Program technical report series toxicology and carcinogenesis studies of toluene (CAS No. 108-88-3) in F344/N rats and 86C3F mice (inhalation studies). Research Triangle Park, NC: U.S. Environmental Protection Agency, Department of Health and Human Services. No. 371. PB90-256371.

Orbaek P and Nise G. 1989. Neurasthenic complaints and psychometric function of toluene-exposed rotogravure printers. Am J Ind Med 16:67-77.

Pearson MA, Hoyme HE, Seaver LH, et al. 1994. Toluene embryopathy: delineation of the phenotype and comparison with fetal alcohol syndrome. Pediatrics 93(2):211-5.

Rosenberg NL. Spitz MC. Filley CM. Davis KA. Schaumburg HH. 1988, Central nervous system effects of chronic toluene abuse--clinical, brainstem evoked response and magnetic resonance imaging studies. [Journal Article] Neurotoxicology & Teratology. 10(5):489-95.

Shigeta S., Aikawa H., Misawa T., Yoshida T., Momotani H., Suzuki K., 1986. Learning impairment in rats following low-level toluene exposure during brain development--a comparative study of high avoidance rats and Wistar rats. Industrial Health. 24(4):203-11.

Svensson B-G, Nise G, Englander V, et al. 1990. Deaths and tumours among rotogravure printers exposed to toluene. Br J Ind Med 47:372-379.

Thiel R and Chahoud I. 1997. Postnatal development and behaviour of Wistar rats after prenatal toluene exposure. Arch Toxicol 71(4):258-65.

Ungvary G. and Tatrai E. 1985. On the embryotoxic effects of benzene and its alkyl derivatives in mice,rats and rabbits. Arch Toxicol (Suppl)8:425-430.

von Euler G, Fuxe K, Hansson T, et al. 1989. Persistent effects of neonatal toluene exposure on regional brain catecholamine levels and turnover in the adult male rat. Toxicology 54:1-16.

Vrca A, Bozicevic D, Karacic V, et al. 1995. Visual evoked potentials in individuals exposed to long-term low concentrations of toluene. Arch Toxicol 69(5):337-40.

Vrca A, Bozicevic D, Bozikov V, et al. 1997a. Brain stem evoked potentials and visual evoked potentials in relation to the length of occupational exposure to low levels of toluene. Acta Medica Croatica 51:215-219.

Vrca A, Karacic V, Bozicevic D, et al. 1997b. Cognitive evoked potentials VEP P300 in persons occupationally exposed to low concentrations of toluene. Arh Hig Rada Toksikol 48:277-285.

WHO. (2000). *Air Quality Guidelines for Europe*, *2nd edition* -Copenhagen, WHO Regional Office for Europe, Publication No. 91.

Yin S, Li G, Hu Y, et al. 1987. Symptoms and signs of workers exposed to benzene, toluene or the combination. Ind Health 25:113-130.

Zavalic M, Mandic Z, Turk R, et al. 1998a. Quantitative assessment of color vision impairment in workers exposed to toluene. Am J Ind Med 33(3):297-304.

Zavalic M, Mandic Z, Turk R, et al. 1998b. Assessment of colour vision impairment in male workers exposed to toluene generally above occupational exposure limits. Occup Med 48(3):175-180.

Zavalic M, Mandic Z, Turk R, et al. 1998c. Qualitative color vision impairment in toluene-exposed workers. Int Arch Occup Environ Health 71:194-200.