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TOXICOLOGICAL REVIEW

OF

TOLUENE

(CAS No. 108-88-3)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

August 2002

NOTICE

This document is a **preliminary draft**. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for peer review on its technical accuracy and science policy implications.

U.S. Environmental Protection Agency
Washington D.C.

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to toluene. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of toluene.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

Chemical Manager
Lynn Flowers, Ph.D., D.A.B.T.
National Center for Environmental Assessment (NCEA)
USEPA Office of Research and Development
Washington, DC

Authors
Lynn Flowers, Ph.D., D.A.B.T.
NCEA
USEPA Office of Research and Development
Washington, DC

Mark Osier, Ph.D.
Syracuse Research Corporation
North Syracuse, NY

Peter McClure, Ph.D., D.A.B.T.
Syracuse Research Corporation
North Syracuse, NY

Contributing Author
Karen Hogan
NCEA
USEPA Office of Research and Development
Washington, DC

Reviewers

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

Internal EPA Reviewers
William Boyes, Ph.D.
Neurotoxicology Division
National Health and Environmental Effects Research Laboratory (NHEERL)
Office of Research and Development

Joyce Donohue, Ph.D.
Health Effects Criteria Division
Office of Water

Karen Hogan
NCEA
Office of Research and Development

Elaina Kenyon, Ph.D.
Pharmacokinetics Branch
Experimental Toxicology Division
NHEERL
Office of Research and Development

External Peer Reviewers

Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for toluene has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S.

EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Toluene is also known as toluol, phenylmethane, methylbenzol, methyl-benzene, monomethyl benzene, and methacide. Some relevant physical and chemical properties of toluene are listed below (ATSDR, 2000; NTP, 2001):

CAS Registry number: 108-88-3
Structural formula: $C_6H_5CH_3$
Molecular weight: 92.14
Density: 0.867 g/mL
Vapor pressure: 28.4 mm Hg at 25°C
Water solubility: 0.59 mg/mL at 25°C
Conversion factor: 1 ppm = 3.77 mg/m^3, 1 mg/m^3 = 0.265 ppm (25°C, 760 mmHg)

At room temperature, toluene is a clear-to-amber colorless liquid with a pungent, benzene-like odor. Although it is a liquid at room temperature, toluene's low vapor pressure results in extensive amounts of toluene volatilizing into the air. It is flammable, with a flash point of 4.4°C. Toluene is strongly reactive with a number of chemical classes, particularly nitrogen-containing compounds, and may attack some plastics. ACGIH (2000) has recommended an 8-hour time-weighted average (TWA) of 50 ppm (189 mg/m^3) for toluene, to protect against effects on the central nervous system. OSHA has promulgated an 8-hour PEL of 200 ppm, which corresponds to 754 mg/m^3 (OSHA, 1993).

Toluene is used as part of an additive to gasoline mixtures (BTX) to increase octane ratings, in benzene production, and as a solvent in paints, coatings, inks, adhesives, and cleaners. Additionally, toluene is used in the production of nylon, plastics, and polyurethanes. Toluene was once used as an anthelminthic agent against roundworms and hookworms.

Large amounts of toluene are released to the environment each year, mostly to the atmosphere. The largest source of toluene release is during the production, transport, and use of gasoline. In the atmosphere, toluene is degraded by reaction with hydroxyl radicals, with a typical half-life of approximately 13 hours. Toluene has been detected in drinking water supplies, in particular in locations with leaking underground fuel storage tanks. Toluene has

been found in soil and water in the vicinity of some hazardous waste sites. Toluene is also found in smoke from both wood and cigarettes.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION/DEPOSITION

3.1.1. Oral Exposure

Studies quantifying oral absorption of toluene are limited, but have demonstrated nearly 100% absorption following a single oral exposure. In volunteers exposed to an infusion of 2 mg toluene/minute for 3 hours (~5 mg/kg) via a gastric tube, absorption of toluene, measured by monitoring exhaled air for toluene and urine for toluene metabolites, was found to be complete (Baelum et al., 1993). Turkall et al. (1991) reported that greater than 99% of a single gavage dose of radiolabeled toluene in rats was eliminated in the urine or expired air, indicating near-total absorption of the exposure.

3.1.2. Inhalation Exposure

Several studies have examined the absorption of toluene during and following a single inhalation exposure in humans. Benoit et al. (1985) reported an average retention of 83% in four subjects exposed to 50 ppm (189 mg/m^3) toluene for ~90 minutes. Carlsson (1982) reported an average uptake (percent of inspired air) of about 55% in male subjects exposed to 300 mg/m^3 for 2 hours at rest; this value dropped to 50% during the next 2 hours of exposure at rest. When the subjects exercised, the percent uptake declined with exercise time and exercise load; the absolute uptake (in mg toluene) increased with exercise time and exercise load (due to increased pulmonary ventilation). Löf et al. (1990) reported a similar absorption percentage (~50% absorbed) in groups of 10 males exposed to 3.25 $mmol/m^3$ (~300 mg/m^3) at rest for 4 hours. In a subsequent paper, Löf et al. (1993) reported a similar absorption percentage for nine male volunteers exposed to 194 mg/m^3 for 2 hours under a light workload; during the first 20 minutes, relative uptake averaged 55%, then slowly fell over time to a plateau of 46% after 80 minutes (mean value 49.2%). A study by Neubert et al. (2001) found a good correlation between measured air toluene concentrations and toluene levels in the blood of rotogravure printers at the end of a 6-hour shift, though absorption itself was not quantified.

3.1.3. Dermal Exposure

Toluene is absorbed through human skin slowly (Dutkiewicz and Tyras, 1968), with absorption rates ranging from 14 to 23 mg/cm^2/hour. A number of other studies have demonstrated that percutaneous absorption can occur, though they did not quantitate the absorption rate. Sato and Nakajima (1978) reported that 30-minute immersion of the hands of volunteers in pure toluene resulted in a peak level of ~2 μmol toluene/L of blood, which was less than 25% of the blood toluene level achieved by a 2-hour inhalation exposure to 100 ppm (377

mg/m^3). Similar blood concentrations were reported by Aitio et al. (1984) in three volunteers who soaked their hands in toluene for 5 minutes; however, there was considerable interindividual variability in toluene blood levels.

Exposure of nude mice, attached to respirators to prevent inhalation, to up to 3000 ppm of toluene vapor resulted in absorption through the skin (Tsuruta, 1989). Absorption varied linearly with exposure concentration and exposure time. Absorption through the shaved skin of guinea pigs (Boman et al., 1995) and rats (Morgan et al., 1991) has also been demonstrated, as evidenced by increased blood levels of toluene following dermal application. Where comparisons were made, dermal absorption was always considerably less than absorption following inhalation exposure.

3.2. DISTRIBUTION

Toluene that is absorbed into the blood is distributed throughout the body. Ameno et al. (1989) reported that in a 51-year-old man who died from accidental oral overdose, the highest toluene concentrations (per gram tissue) were in the liver, followed by pancreas, brain, heart, blood, fat, and cerebrospinal fluid. However, Paterson and Sarvesvaran (1983) reported that a 16-year-old male who was found dead, presumably due to inhalation overdose of toluene, had greater concentrations in the brain than the liver. Takeichi et al. (1986) reported similar findings in a 20-year-old male painter who fell from a great height while working with a toluene-based paint; the greatest concentrations upon autopsy, expressed as μg toluene/gram of tissue, were found in the brain, followed by the liver and blood. Within the brain of a 31-year-old man who was found dead in a room full of toluene vapor, the highest concentrations of toluene were found in the corpus callosum, with the lowest in the caudate-putamen (Ameno et al., 1992). Thus, the available human data suggest that more toluene accumulates in the brain than in the liver following inhalation exposure, whereas following oral exposure, the liver contains the greatest concentrations of toluene.

Pyykko et al. (1977) exposed groups of rats by both the oral and inhalation routes and reported greater toluene concentrations (per gram of wet tissue) in the liver than the brain by both exposure routes. Following inhalation exposure during which dogs were allowed to rebreathe toluene, the liver and brain contained the highest levels (both ~190 μg/g tissue), with lesser levels in the kidneys (Ikeda et al., 1990). Several studies have shown relationships between blood and tissue levels of toluene, particularly for the brain (Benignus et al., 1984; Harabuchi et al., 1993). Toluene is able to cross the placenta and enter the fetus (Ghantous and Danielsson, 1986), and can be found in breast milk (Pellizzari et al., 1982).

3.3. METABOLISM

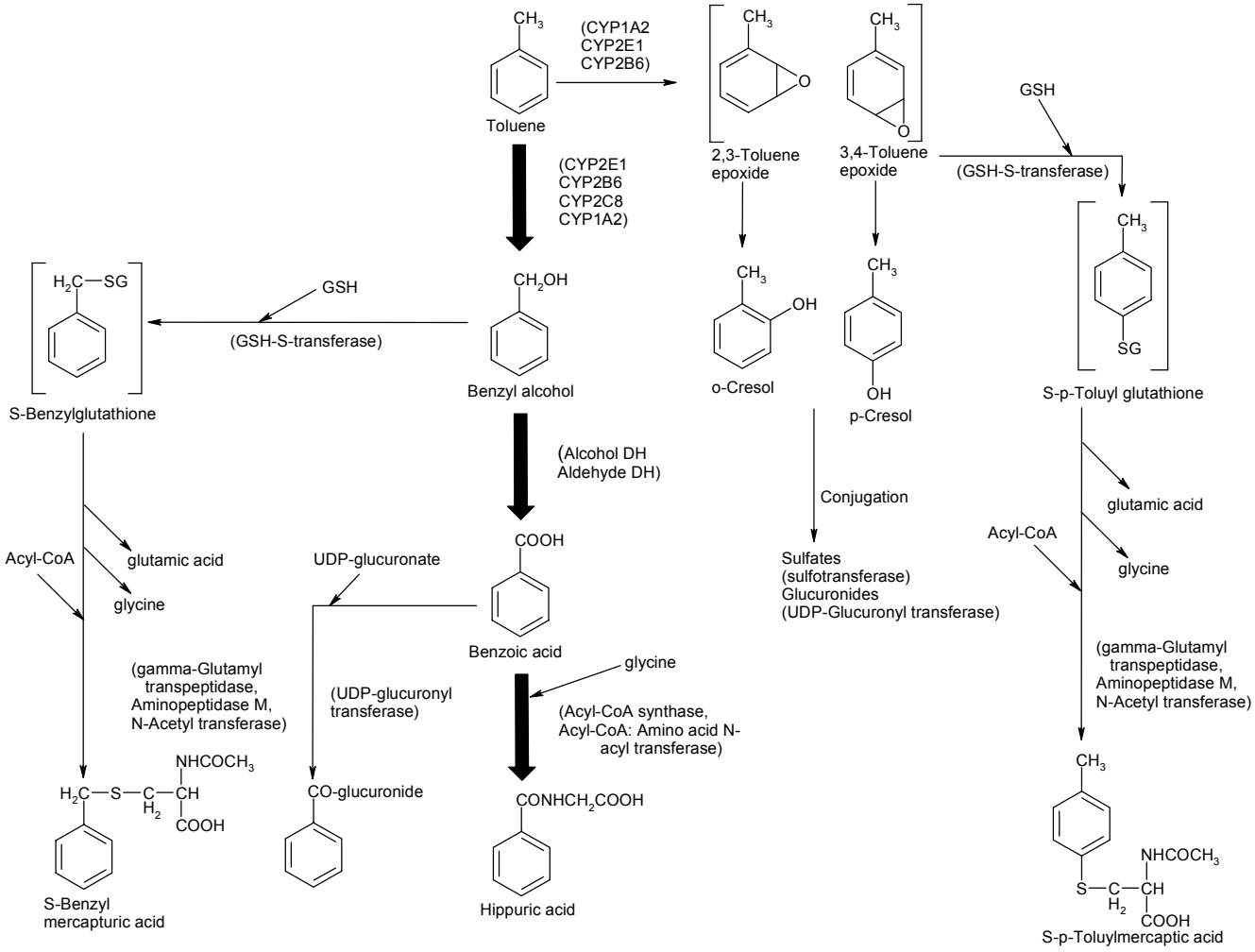
The main enzymatic pathways believed to be involved in toluene metabolism are shown in Figure 1 (Nakajima and Wang, 1994; Tassaneeyakul et al., 1996; Nakajima et al., 1997; Angerer et al., 1998; IARC, 1999). The initial step in toluene metabolism is transformation by

cytochrome P450 enzymes, mainly in the liver. The most prominent of these transformations is hydroxylation of the methyl group, forming benzyl alcohol. Benzyl alcohol is primarily converted to benzoic acid, then conjugated with glycine to form hippuric acid. A minor P450-related pathway, believed to be potentially toxicologically relevant at higher exposure levels, involves a transient epoxidation of the aromatic ring to form either *ortho*- or *para*-cresol. The cresols may undergo a variety of conjugation reactions, forming mainly sulfates and glucuronides. Glutathione conjugation may also occur, resulting in S-benzylglutathione, and eventually S-benzyl mercapturic acid (conjugation to benzyl alcohol) or S-*p*-toluyl glutathione, eventually resulting in S-*p*-toluylmercaptic acid (conjugation to the epoxidated ring). Because of its abundance of toluene-metabolizing enzymes, including cytochromes P450, the liver is expected to be the primary site of toluene metabolism.

Studies of urinary metabolites in toluene-exposed humans have identified hippuric acid as the major metabolite of toluene (Andersen et al., 1983; Angerer, 1979; Angerer et al., 1998; Baelum et al., 1987, 1993; Dossing et al., 1983; Inoue et al., 1986; Jonai and Sato, 1988; Kawai et al., 1992a, 1992b, 1996; Löf et al., 1990, 1993; Maestri et al., 1997; Ng et al., 1990). Minor urinary metabolites (in approximate order of decreasing abundance) include the glucuronyl conjugate of benzoic acid, the sulfate and glucuronide conjugates of *ortho*- and *para*-cresol, S-benzylmercapturic acid, and S-*p*-toluylmercapturic acid (Angerer et al., 1998; Nakajima and Wang, 1994; Nakajima et al., 1997; Tassaneeyakul et al., 1996).

Toluene metabolism is believed to result in a decrease in toluene-induced neurologic effects. Evidence supporting this includes a study by Ikeda and Ohtsuji (1971), who reported that increasing the rate of *in vivo* toluene metabolism by pretreatment of rats with phenobarbital (thereby inducing CYP enzymes) resulted in decreased recovery time from toluene-induced narcosis. Similarly, inhibition of toluene metabolism resulted in a potentiation of toluene-induced hearing loss in rats (Campo et al., 1998). The influence of metabolic processes on the effects of toluene on other organs or systems, including the liver, is less clear. Mattson et al. (1989) have reported similar neuroexcitatory effects between toluene and the metabolite o-cresol, suggesting that metabolites might contribute to some of the neuroactive properties of toluene.

Figure 1. Proposed Pathways for Toluene Metabolism



Adapted from ATSDR (2000)
Proposed enzymes are noted in parentheses.

Sources: Angerer et al., 1998; IARC, 1999; Nakajima and Wang, 1994; Nakajima et al., 1997; Tassaneeyakul et al., 1996
CoA = coenzyme A; CYP = cytochrome P-450; DH = dehydrogenase; GSH = glutathione; UPD = uridine 5'-diphosphate

3.4. ELIMINATION AND EXCRETION

Studies in both humans and animals have shown that the majority of toluene in the body is eliminated in the urine, mainly as metabolites (Löf et al., 1990, 1993; Turkall et al., 1991; Tardif et al., 1992, 1998). As discussed above, the primary urinary metabolite of toluene is hippuric acid, with additional metabolites (see Figure 1) resulting from minor metabolic pathways. Elimination from the blood is rapid (Sato and Nakajima, 1978; Carlsson, 1982; Löf et al., 1990, 1993), with three-phase elimination half times of 3, 40, and 738 minutes following a single inhalation exposure in humans (Löf et al., 1993). A lesser, but still significant, amount of inhaled toluene is removed in the expired air (Pellizzari et al., 1992; Monster et al., 1993). Elimination of toluene in the expired air is greatest at time points during or immediately after exposure, and decreases rapidly thereafter (Benoit et al., 1985). In rats, Turkall et al. (1991) estimated that ~22% of a single oral dose is eliminated in the expired air, with the remainder being mainly eliminated in the urine.

3.5. PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

PBPK models are available that describe the kinetics of toluene after inhalation exposure; two for humans (Fisher et al., 1997; Pierce et al., 1996, 1999) and two for rats (DeJongh and Blaauboer, 1996, 1997; Tardif et al., 1993). These models are all modifications of the standard four-compartment PBPK model developed for styrene (Ramsey and Andersen, 1984) in which:

(1) absorption into the lung blood is assumed to be dependent on the inhaled concentration of toxicant, the concentration of toxicant in alveolar air, blood flow to the lung, the blood/air partition coefficient, and alveolar ventilation rates;
(2) exchange of toxicant between arterial blood and tissue compartments is flow-limited;
(3) changes in the amount of toxicant in three nonmetabolizing tissue compartments (adipose tissue, slowly perfused tissues, and rapidly perfused tissues) are described by mass transfer differential equations with tissue volume, blood flow through the tissue (i.e., tissue perfusion rate), arterial blood toxicant concentration, and tissue/blood partition coefficients as explanatory variables; and
(4) changes in toxicant amount in the liver (the fourth compartment) are described by similar differential equations that additionally include a Michaelis-Menten term for overall rates of toxicant metabolism.

The five-compartment human model for toluene developed by Pierce et al. (1996) includes an additional equation describing mass balance across the lung that has a Michaelis-Menten metabolic term. A five-compartment rat PBPK model developed by DeJongh and Blaauboer (1996) is similar in design to the Tardif et al. (1993) rat PBPK model except that it contains an additional nonmetabolizing compartment representing the brain. The above models have all been partially- or fully-validated using *in vivo* pharmacokinetic data in the appropriate species. Another human PBPK model has been developed for volatile organic compounds that models transfer of toxicant via lactation from a mother to a nursing infant, but *in vivo*

pharmacokinetic data for toluene in breast milk were not available to validate this model (Fisher et al., 1997). PBPK models for species other than the rat and human are not presently available.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS - EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. Oral Exposure

Reports of oral exposure to toluene in humans are limited to case reports of accidental acute ingestions. Ameno et al. (1989) reported 15 deaths by accidental oral ingestion of paint thinner containing toluene over the period from 1977 to 1986. A case report of a 51-year old man who died approximately 30 minutes after he had ingested a large quantity of toluene was presented; the probable cause of death was severe central nervous system depression. Caravati and Bjerk (1997) reported on a case of a 46-year-old man who had ingested approximately one quart of paint thinner containing toluene. The patient presented with severe central nervous system depression, severe abdominal pain, diarrhea, and hemorrhagic gastritis. The patient recovered after 36 hours of supportive care. No reports of chronic oral exposure to toluene in humans were located.

4.1.2. Inhalation Exposure

4.1.2.1. Acute Studies

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 to 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 acclimatization 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 odor 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 color discrimination test; this

difference was seen in both exposed vs. nonexposed printers and exposed vs. nonexposed controls. Because the printers, who had been previously exposed to toluene, did not differ from naive subjects in their response to toluene, this study indicates that little tolerance develops to the acute irritative and central effects in exposed humans.

In a later study, Baelum et al. (1990) exposed 32 males and 39 females to clean air, 100 ppm (377 mg/m^3) toluene, or a varying exposure with a TWA value of 100 ppm, but which contained peaks of 300 ppm (1131 mg/m^3) every 30 minutes for a total of 7 hours. Toluene exposure led to significantly increased complaints about poor air quality, altered noise perception, increased irritation of the nose and lower airways, and a feeling of intoxication, as well as lower scores on a vigilance test. No differences were seen between subjects exposed to the 100 ppm exposure level compared to those who experienced peaks of 300 ppm.

Andersen et al. (1983) exposed 16 young healthy subjects to a single exposure of 0, 10, 40, or 100 ppm of toluene (0, 38, 151, or 377 mg/m^3) for 6 hours under controlled conditions. Toluene exposures did not affect nasal mucus flow or lung function. At 100 ppm, but not at 10 or 40 ppm, subjects reported a subjective irritation of the eyes and nose, as well as headache, dizziness, and feelings of intoxication. In eight tests measuring visual perception, vigilance, psychomotor function, and higher cortical functions, no statistically significant differences were found as a result of toluene exposure.

Forty-two college students (21 female and 21 male) were exposed to 0, 74 ppm (279 mg/m^3), or 151 ppm (569 mg/m^3) toluene for 7 hours over 3 days (Echeverria et al., 1989). Each subject received all three toluene exposure levels on different days. The odor 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.

4.1.2.2. Prechronic And Chronic Studies

Zavalic et al. (1998a) examined two groups of Croatian workers occupationally exposed to toluene for effects on color vision, relative to a group of unexposed controls. The first exposed group (group E1) consisted of 46 workers (3 men, 43 women) employed gluing shoe soles, while the second group (group E2) consisted of 37 workers (34 men, 3 women) employed in a rotogravure printing press. Mean exposure times were 16.21 ±6.1 (mean ±SD) years for group E1 and 18.34 ±6.03 years for group E2. The control group consisted of 90 workers (61 men, 29 women) who were not occupationally exposed to solvents. For all groups, smoking and alcohol consumption information was collected. Samples of air were collected at work stations

in both the shoe factory and printing press for analysis of airborne toluene concentrations; median 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. Urine samples were taken Wednesday after the work shift and analyzed for *ortho*-cresol and hippuric acid. Analysis of color vision was performed using the Lanthony 15 Hue desaturated panel, which is based on the ability to recombine a set of 15 desaturated color caps according to a definite chromatic sequence. Results are reported as the color confusion index (CCI) or age- and alcohol intake-adjusted color confusion index (AACCI). Color 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 (0.0042 μg/mg with a range of 0.0021 - 0.9422), *ortho*-cresol in urine (0.97 mg/g creatinine with a range of 0.26 - 4.01), and hippuric acid (1.872 g/g creatinine with a range of 0.322 - 2.875) in urine. 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 (1.29 ± 0.10 [mean ±SD] and 1.30 ± 0.11, respectively) relative to both controls (1.15 ± 0.10 and 1.14 ± 0.10, respectively) and to group E1 (1.17 ± 0.08 and 1.18 ± 0.10, respectively). 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 (1.17 ± 0.08 and 1.17 ± 0.08, respectively) than for non-consumers in the control group (1.13 ± 0.08 and 1.13 ± 0.09, respectively); 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. Actual data points (or mean ±SD) for AACCI scores were not reported. The results were presented graphically. 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 color vision in toluene-exposed workers based on AACCI scores.

Further analysis of color vision loss in the same groups of workers described above (Zavalic et al. 1998a) was carried out to compare loss in the blue-yellow and red-green ranges (Zavalic et al. 1998b). Both blue-yellow and red-green color confusion were significantly increased in printers, but there was no significant difference in the prevalence of either type of color confusion between exposed and unexposed workers.

Color vision impairment was also evaluated in another group of 45 male workers exposed to mean concentrations of about 120 ppm toluene (Zavalic et al. 1998c). Color vision, assessed by the age and alcohol intake adjusted color confusion score (AACDS), was significantly impaired in exposed workers compared with unexposed controls. Statistically significant correlations were noted between impaired color vision and air toluene, blood toluene, and urinary hippuric acid concentrations. A comparison of color vision assessments made on Monday and Wednesday mornings showed no significant difference. This suggests that color vision impairment results from chronic rather than acute exposure to toluene.

In another study, Cavalleri et al. (2000) examined a cohort of 33 rubber workers (mean exposure duration, 117 months) and 16 referents for changes in color vision, as evaluated by the Lanthony D-15 desaturated panel. Urine samples were taken at the end of the day and analyzed for unmetabolized toluene. Exposed workers showed significant impairments in color vision, as evidenced by increases in CCI or total confusion index (TOTCI) scores, relative to control workers. However, while the indices of color vision showed linear correlations with the product of the urinary toluene and total exposure duration, airborne levels of toluene cannot be determined from the data presented in the manuscript. Because this study did not identify exposure levels of toluene, instead correlating response with urinary toluene levels, no NOAEL or LOAEL values could be identified.

Foo et al. (1990) 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 time-weighted average (TWA), although the number of samples taken and the actual sampling period were not given. No historical exposure values were given. Co-exposure to other solvents was not addressed in the study. The exposed and control cohorts were matched for age, ethnicity, and use of medications. Members of these cohorts did not use alcohol and were nonsmokers. Medical histories were taken to eliminate any histories of central or peripheral nervous system disorders. 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. Personal air samplers indicated that exposed workers breathed mean toluene air levels of 88 ppm (332 mg/m^3) as a TWA and control workers breathed a mean of 13 ppm (49 mg/m^3) (TWA). A battery of eight neurobehavioral 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. When individual test results were linearly regressed against personal exposure concentrations, the slopes of the regression lines were all significantly nonzero. However, there was considerable scatter among the data sets, with correlation coefficients ranging from 0.44 to 0.30. Irritation effects were not evaluated in this study, and no clinical signs or symptoms were reported. This study identified a LOAEL of 88 ppm of toluene (332 mg/m^3) for neurobehavioral changes from chronic exposure to toluene.

From a group of 300 rotogravure printing workers, and 300 matched controls, Abbate et al. (1993) selected 40 workers who had been exposed to an average of 97 ppm (366 mg/m^3), measured in the individual workplaces on the day of testing, for 12-14 years and 40 controls for examination of neurologic effects of toluene exposure by brainstem response audiometry. For each latency interval, the mean latencies in brainstem auditory evoked potentials were significantly higher in the exposed group relative to controls. This study identified a LOAEL of 97 ppm for increased wave latencies for auditory-evoked brain potentials; no NOAEL was identified.

Boey et al. (1997) examined a group of 29 electronics workers occupationally exposed to 90.9 ppm (343 mg/m^3) toluene, as assessed by personal exposure monitors on the day of testing, for an average of 4.9 years for neurobehavioral changes relative to a group of 29 controls. The controls were found to have been exposed to 12.2 ppm of toluene (46 mg/m^3). Measured tests included logical memory, digit span, visual reproduction, Benton visual retention test, trail making test, symbol digit modality test, grooved pegboard test, and finger tapping tests. Performance of the exposed workers were found to be significantly impaired relative to controls for the digit span, visual reproduction, trail making, symbol digit modality, and grooved pegboard tests. This study identified a LOAEL of 90.9 ppm for neurobehavioral alterations; no NOAEL was identified.

Murata et al. (1993) examined 10 rotogravure printers and 10 age-matched controls for differences in electrocardiographic R-R intervals (CV_{RR} and C-CV_{HF}), the distribution of nerve conduction velocities (DCV), and the maximal motor and sensory nerve conduction velocities (MCV and SCV) in the median nerve. Toluene exposure was estimated to be 83 ppm (313 mg/m^3), with exposure durations of 1-36 years. Blood samples for toluene analysis were taken from the workers before electrophysiological testing during normal working hours, while urine samples for hippuric acid analysis were taken at 5:00 pm the day following electrophysiological analysis. CV_{RR} and C-CV_{HF} were significantly reduced in the toluene-exposed workers, as were MCV in the forearm and SCV in the palm. MCV in the palm and SCV in the forearm were not significantly different from controls. The electrophysiological data were not significantly correlated with blood toluene or urinary hippuric acid levels, or with exposure duration. This study identified a LOAEL of 83 ppm for alterations in electrophysiological parameters; no NOAEL was identified.

Morata et al. (1997) examined 124 workers at a rotogravure printing facility for changes in hearing; no control subjects were reported. Toluene levels in the air ranged from 0.14 to 919 mg/m^3; co-exposure to ethanol and ethyl acetate was common. Exposure times ranged from 1 to 25 years, with a mean of 7 years. Hippuric acid in urine was utilized to assess total toluene exposure in 109 of the workers. While a number of other variables were considered, only the age of the subject and hippuric acid content of the urine showed significant correlations with hearing loss. This study failed to identify a NOAEL or LOAEL.

Ørbaek and Nise (1989) examined 30 rotogravure printers, compared to 72 controls, for reported neurologic symptoms and alterations in psychometric test performance. The exposed workers were in two plants, with mean time-weighted exposure levels of 43 mg/m^3 (19 workers) and 157 mg/m^3 (11 workers) of toluene; for analysis in the study, the groups of workers were pooled. Prior to 1980, the exposure levels had exceeded 300 mg/m^3. Employment times ranged from 4 to 43 years, with a median of 29 years. Compared to the referents, the printers complained significantly of most of the neurasthenic symptoms evaluated, including fatigue, memory loss, depression, concentration difficulty, headache, dizziness, and paresthesia. Age-adjusted test comparisons to referent performance showed significantly lower scores for the printers in the Synonyms, Benton (correct and errors), and Digit Symbol tests. However, present toluene exposure level was only weakly associated with the test results. Because pooling the workers of differing exposure levels for analysis adds uncertainty as to the appropriate exposure levels associated with the responses, no NOAEL or LOAEL values can be identified for this study.

Vrca et al. (1995, 1997) examined a group of 49 rotogravure printing workers relative to 59 controls for alterations in visual evoked potentials (VEP); a second study examined the 49 workers for changes in brain stem evoked potentials (BEAP), as measured with a brain imager. Average length of work service for the printers was 21.4 years, and exposure concentrations ranged from 40 to 60 ppm (151-226 mg/m^3). Toluene in peripheral blood was measured Wednesday morning before entering the work area, while urinary levels of hippuric acid and *ortho*-cresol were determined both before and after the Wednesday work shift. Of the three VEP waves examined (N75, N100, and N145), significant increases in amplitude were seen for all three, but no differences in time of wave onset, time of wave offset, total duration of each wave, or total duration of all waves combined were noted between the exposed and control groups (Vrca et al., 1995). In the second study, BEAP waves P1 through P5 were examined, and there was a significant correlation between latency of the waves and the length of exposure for all waves except the P2 wave (Vrca et al., 1997). No correlation between wave amplitude and exposure length was seen. Combined, these studies identify a LOAEL of 40-60 ppm for alterations in visual- and auditory-evoked brain potentials; no NOAEL was identified.

Yin et al. (1987) reported on a cohort of over 300 solvent workers, 94 of whom (38 men, 65 women) were exposed primarily to toluene at a mean concentration of 42.8 ppm (161 mg/m^3), relative to 129 controls. Workers were co-exposed to 1.3 ppm benzene. A small but significant decrease in hemoglobin was seen in exposed men, while a small but significant increase was seen in exposed women. In both sexes, increased numbers of eosinophils were found in the exposed workers relative to controls, and the values of both lactate dehydrogenase and leucine aminopeptidase activity in the serum were decreased relative to controls. Levels of inorganic phosphorus in the serum of exposed male workers, but not female workers, were also significantly lower than controls. In considering the prevalence of subjective symptoms (sore throat, headaches, and dizziness) workers were subgrouped into low (6-39 ppm, n = 28) and high (40-123 ppm, n = 29) exposure categories. Although the prevalence of subjective symptoms was significantly higher in the exposed workers compared with the control cohort ($p<0.01$), a

concentration-response relationship was not discernable among the groups. No other treatment-related effects were reported. The study was limited because the exposed and unexposed groups were not matched to control for confounding effects (e.g., age, smoking, alcohol consumption, exposure duration). This study identified a LOAEL of 43 ppm for increases in subjective symptoms in exposed workers; no NOAEL was identified.

In the occupational study by Lee et al. (1988), prevalence of subjective symptoms in shoe-makers exposed to toluene was categorized with respect to exposure levels. The study population (193 women and 65 controls) completed a questionnaire. The exposures were reported as 8-hour TWAs, and workers were grouped in exposure categories of nonexposed, 1-50 ppm, 51-100 ppm, 101-150 ppm, and more than 151 ppm (duration of exposures was not reported). A concentration-dependent increase in prevalence was reported for 25/67 symptoms with increases in complaints over controls occurring at around 100 ppm (377 mg/m^3). Similar to the Yin et al. study (1987) described above, reported symptoms included headaches, sore throats, and dizziness. Although an effect level in humans of around 100 ppm is indicated by this study, no objective measures of toxicity were examined. NOAEL or LOAEL levels therefore cannot be identified by this study.

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) or workers exposed for greater than 12 years (Group 2; n=49); the control group consisted of 19 workers not exposed to toluene. Workers exposed for 12 years or under were exposed 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. Workers exposed for greater than 12 years may have been exposed to levels exceeding 100 ppm (377 mg/m^3) for up to 27 years. 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; the tests which were significantly altered were left hand finger tapping, retention times in the number learning test, and total time in the Bourdon-Wiersma 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 neurologic tests.

Wiebelt and Becker (1999) examined a cohort of 6830 German men from 11 rotogravure printing plants who were exposed to toluene between 1960 and 1992. Because of an incomplete availability of death certificates, a newly developed method was applied for the calculation of standardized mortality ratios (SMR). Individual exposure measurements were not taken. Of the three main work areas, two had concentrations generally lower than 30 ppm, and one was, in general, lower than the exposure limit of 100 ppm (200 ppm before 1985). For the total cohort, only the SMR for mental disorders, primarily alcoholism, was significantly elevated (SMR 303, 95% CI 184-541). No significant increases in cancer mortality or cause-specific cancer mortality

were reported for the entire cohort. If the workers from the work areas with the highest exposure are analyzed separately, significant increases in mortality from cancers of the bone (SMR 814, 95% CI 139-3243) and connective tissue (SMR 631, 95%CI 123-2595) were found. Due to a lack of exposure characterization, including potential co-exposures, the significance of these results is unclear. No NOAEL or LOAEL values were identified by this study.

Svensson et al. (1990) examined the rates of cancer formation in 1020 past and present rotogravure printers occupationally exposed to toluene for at least 3 months in one of eight printing establishments between 1925 and 1985. Exposure levels were estimated based on current exposure, past workplace measurements, and interviews with employees. Exposure levels were estimated to range from 350-450 ppm until 1960, after which they steadily fell, with a median level of ~50 ppm in 1985. Exposed workers showed no significant increase in general mortality or from dying of malignant disease. Statistically significant excesses in tumor incidences were seen in the gastrointestinal tract and stomach, organs that displayed SMRs of 2.06 (95% CI 1.13-3.45) and 2.72 (95% CI 1.09-5.61), respectively, when compared to those of unexposed controls. In addition, taking all cancer formation into account, there appeared to be a marginal excess of respiratory-tract cancers, with an SMR of 1.76 (95% CI 1.03-2.91). However, when the latter subset was limited to those employees with greater than 5 years of potential exposure and/or greater than 10 years latency, the resulting SMR of 1.26 (95% CI 0.57-2.38) failed to confirm an association between exposure and response.

Anttila et al. (1998) carried out a retrospective cohort analysis of 5301 workers (3922 male and 1379 female) monitored for biological markers of occupational exposure to styrene, toluene, or xylene over the period of 1973-1992. Exposure was monitored from 1978 to 1983 by analysis of toluene levels in the blood. No significantly increased incidence rates of cancer could be associated with toluene exposure.

A large number of other studies exist that examined humans exposed chronically to toluene, mainly in an occupational setting. The majority of those studies reported concentrations higher than those described above, and reported similar findings. Several other cohort mortality studies also exist (Gericke et al., 2001; Gerin et al., 1998; Walker et al., 1993), but were excluded from further analysis due to inability to account for confounding factors (i.e., smoking, age, co-exposure to other solvents).

The primary effects described in case reports of chronic toluene abusers are on the central nervous system, including abnormal electroencephalogram (EEG) activity, ataxia, tremors, temporal lobe epilepsy, decreased intelligence quotient, paranoid psychosis, hallucinations, nystagmus (involuntary eye movement), cerebral atrophy, and impaired speech, hearing, and vision (Byrne et al., 1991; Devathasan et al., 1984; Hunnewell and Miller, 1998; King et al., 1981; Maas et al., 1991; Meulenbelt et al., 1990; Miyagi et al., 1999; Ryu et al., 1998; Suzuki et al., 1983). Additionally, toluene abuse during pregnancy can have dramatic effects on the developing fetus (see section 4.3). Other known effects include effects on the kidneys, including acidosis, necrosis, proteinuria, and tubule dysfunction (Gerkin and LoVecchio, 1998; Goodwin,

1988; Jone and Wu, 1988; Kamijima et al., 1994; Kamijo et al., 1998; Kaneko et al., 1992; Meulenbelt et al., 1990; Patel and Benjamin, 1986; Taverner et al., 1988), and the heart, primarily cardiac arrhythmias (Anderson et al., 1982). However, these case reports are generally limited in exposure characterization, description of response, discussion of potential confounding factors, and are most often the result of excessively high exposure concentrations.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS - ORAL AND INHALATION

4.2.1. Oral Exposure

4.2.1.1. Prechronic Studies

The oral toxicity of toluene was investigated in a subchronic gavage study in F344 rats (NTP, 1990a). Groups of 10 rats/sex/group were administered toluene in corn oil at dosage levels of 0, 312, 625, 1250, 2500, or 5000 mg/kg, 5 days/week for 13 weeks. All animals receiving 5000 mg/kg died within the first week. One female and 8 males in the 2500 mg/kg group died, but two of these deaths were due to gavage errors. No deaths occurred at lower doses. Several toxic effects were noted at doses greater than or equal to 2500 mg/kg, including prostration, hypoactivity, ataxia, piloerection, lacrimation, excessive salivation, and body tremors. No signs of biologic significance were seen in groups receiving less than or equal to 1250 mg/kg. The only significant change in body weight was a decrease ($p<0.05$) for males in the 2500 mg/kg group. There were no toxicologically significant changes in hematology or urinalysis for any group of animals. Biochemical changes, including a significant increase ($p<0.05$) in SGOT in 2500 mg/kg males and a dose-related increase in cholinesterase in females receiving 2500 and 5000 mg/kg, were not considered to be biologically significant. There were several pathologic findings and organ weight changes in the liver, kidney, brain, and urinary bladder. In males, absolute and relative weights of both the liver and kidney were significantly increased ($p<0.05$) at doses greater than or equal to 625 mg/kg. In females, absolute and relative weights of the liver, kidney, and heart were all significantly increased at doses greater than or equal to 1250 mg/kg ($p<0.01$ for all comparisons except $p<0.05$ for absolute kidney and heart weights at 1250 mg/kg). Histopathologic lesions in the liver consisted of hepatocellular hypertrophy, occurring at greater than or equal to 2500 mg/kg. Nephrosis was observed in rats that died, and damage to the tubular epithelia of the kidney occurred in terminally sacrificed rats. Histopathologic changes were also noted in the brain and urinary bladder. In the brain, mineralized foci and necrosis of neuronal cells were observed in males and females at 2500 mg/kg and males at 1250 mg/kg. In the bladder, hemorrhage of the muscularis was seen in males and females at 5000 mg/kg and males at 2500 mg/kg. The NOAEL in rats for this study is 223 mg/kg-day (312 mg/kg) based on liver and kidney weight changes in male rats at 446 mg/kg-day (625 mg/kg). The toxicologic significance of these organ weight changes is strengthened by the occurrence of histopathologic changes in both the liver and kidney at higher doses. Because the exposure was for 5 days/week, the dose is adjusted (e.g., 312 x 5/7 = 223 mg/kg-day).

NTP (1990) also conducted a 13-week gavage study in B6C3F1 mice, following the same regimen described above. All mice receiving 5000 mg/kg died and 8/20 (4 males, 4 females) receiving 2500 mg/kg also died. Clinical signs seen in animals receiving greater than or equal to 2500 mg/kg included subconvulsive jerking, prostration, impaired grasping reflex, bradypnea, hypothermia, ataxia, and hypoactivity. By week 13, the mean body weight of 2500 mg/kg males was significantly ($p<0.05$) lower than controls; no significant changes in body weights were seen in female mice. In male mice, absolute kidney weight, but not relative kidney weight, was decreased in the 2500 mg/kg group. Relative brain and liver weights were increased and relative right testis weight was decreased in animals exposed to 1250 mg/kg-day or greater; the absolute weights for these organs were not significantly different from controls. In female mice, absolute liver weights were increased in the 312 and 2500 mg/kg groups, but not in the other treated groups; relative liver weights were increased in all treated groups. No other changes in organ weights were seen in female mice. Several small but statistically significant changes occurred in hematologic parameters, but did not appear to be related to toluene exposure. No histologic changes in the liver, brain, kidneys, or bladder of any group were reported.

Hsieh et al. (1989) exposed groups of male CD-1 mice (five animals/group) to 0, 17, 80, or 405 mg toluene/liter of drinking water for 4 weeks. Based on body weight and water consumption data, the authors calculated average daily toluene doses of 0, 5, 22, or 105 mg/kg-day, respectively. Animals were weighed once per week, and food and water consumption were monitored continuously. Water toluene concentration was determined daily, and fresh solutions were made every three days. After 28 days, the animals were sacrificed, and body, spleen, thymus, liver, and kidney weights were determined. Gross pathological examinations were performed on all mice. Total erythrocytes and leukocytes were determined, and differential leukocyte counts were measured. Splenocyte suspensions were prepared, and the lymphoproliferative responses to the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A), the B-cell mitogen *E. coli* lipopolysaccharide (LPS), and the combined mitogen pokeweed mitogen (PWM) were measured. Separate groups of animals were similarly exposed, and were sensitized by intraperitoneal injection of sheep red blood cells (SRBC) 4 days before the end of toluene exposure. The titer of anti-SRBC antibody in the serum collected was used in the plaque-forming colony (PFC) assay. Toluene exposure did not result in increased mortality or clinical signs in any exposed group. No significant changes in food or water consumption were noted, and no gross lesions of the liver, kidney, spleen, heart, thymus, lung, or brain were seen in any treatment group. No changes in body weight (mean ±SE) were seen as a result of toluene exposure. Relative liver weights of toluene-exposed rats were significantly increased (5.67 ±0.07, 6.09 ±0.11, 6.32 ±0.17, and 6.73 ±0.14 g/100 g body weight for 0, 5, 22, and 105 mg/kg-day treatment groups, respectively) and relative thymus weights (mean ±SE) were significantly decreased (0.019 ±0.02, 0.18 ±0.01, 0.18 ±0.02, and 0.13 ±0.02 g /100 g body weight for 0, 5, 22, and 105 mg/kg-day treatment groups, respectively) at 105 mg/kg-day compared to controls, but not at lower doses. The changes in organ weights at the highest dose correspond to a 19% increase in liver weight and a 32% decrease in thymus weight relative to controls. No changes were found in relative spleen and kidney weights at any dose. No significant changes in hematological parameters or spleen cellularity were reported. Splenocyte

cultures from animals in all treated groups showed significant reductions in proliferative response, measured by [^{3}H]TdR uptake, when cultured in the absence of mitogen, or in response to PWM. At the highest two dose levels, the proliferative response was also significantly decreased in response to LPS, PHA, and Con A. The biological significance of these effects is unknown. PFC response (both PFC/10^6 spleen cells and PFC/spleen) to SRBC was significantly reduced (46% and 63%, respectively) following exposure to 105 mg/kg-day. The 105 mg/kg-day dose group represents a LOAEL for this study for increased relative liver weight, decreased relative thymus weight, and immunological effects [reduced PFC response (>40%) to SRBC]; the NOAEL is 22 mg/kg-day.

In a later study, Hsieh et al. (1990) exposed groups of male CD-1 mice for 28 days as described above. At the end of the exposure, six discrete brain sections of the animals were tested for endogenous levels of norepinephrine (NE), dopamine (DA), and serotonin (5-HT), as well as their primary metabolites. No changes in body weight or clinical signs were observed. Toluene exposure induced increases in all of the biogenic amines examined at all dose levels, with the response generally peaking in the mid-dose group and decreasing in the high-dose group. Significant increases of norepinephrine and its metabolite, 3-methoxy-4-hydroxymandelic acid, were found in the midbrain of all dose groups. Significant increases in serotonin levels, but not its metabolite (5-hydroxyindoleacetic acid), were also seen in the midbrain of all dose groups. The unknown implications of changes in neurotransmitter levels, differences in the effects seen in various brain sections, and the biphasic dose-response make determinations of biological significance difficult. This study did not identify a NOAEL or LOAEL.

4.2.1.2. Chronic Studies

Wolf et al. (1956) administered groups of 10 female Wistar rats gavage doses of 0, 118, 354, or 590 mg/kg toluene dissolved in olive oil. A total of 138 doses were administered over 193 days, resulting in average doses of approximately 0, 84, 253, or 422 mg/kg-day. Hematologic, behavioral, gross, and histopathologic examinations were conducted with no toxic effects being reported at any dose. This study did not identify a NOAEL or LOAEL.

Maltoni et al. (1997) conducted carcinogenicity evaluations on a series of gasoline-related chemicals, including toluene. Groups of male and female Sprague-Dawley rats (40-50/sex/group) were exposed to 0 or 800 mg/kg by gavage four times/week for 104 weeks. In a separate experiment, animals were similarly exposed to 0 or 500 mg/kg. Duration-adjusted doses were 286 and 457 mg/kg-day for the 500 and 800 mg/kg groups, respectively. Mean daily food and drinking water consumption, as well as animal body weights, were determined weekly for 13 weeks, and once every 2 weeks thereafter. Animals were permitted to live their entire life span. Upon death, the animals were examined for gross and histologic carcinogenic organ changes. 800-mg/kg animals, but not 500-mg/kg animals, showed slightly reduced survival relative to controls. An increase in total tumor-bearing animals was reported, but was greater in the 500-mg/kg animals than in the 800-mg/kg animals; malignant tumor frequencies for both sexes combined were 24, 69, and 44% for the 0, 500, and 800 mg/kg groups, respectively. Mammary

cancers and lymphomas/leukemias in female rats were elevated in the 500 mg/kg animals, but not in the 800-mg/kg animals. A small but significant increase was seen in oral cavity tumors in 800-mg/kg male rats only. A lack of dose-response for the majority of the tumors reported and lack of details of noncarcinogenic endpoints limits the interpretability of this study.

4.2.2. Inhalation Exposure

4.2.2.1. Prechronic Studies

Fischer 344/N rats (10/sex/group) were exposed to toluene vapors at 0, 100, 625, 1250, 2500, and 3000 ppm (0, 377, 2355, 4711, 9422, and 11,307 mg/m^3, respectively) 6.5 hours/day, 5 days/week (duration-adjusted to 0, 73, 455, 911, 1823, and 2187 mg/m^3, respectively) for 15 weeks (NTP, 1990a). Organ weights were measured and histological examinations were performed only on controls, 2500- and 3000-ppm groups, and animals that died before the end of the study. Eight of 10 males exposed to 3000 ppm died, all during the second exposure week. No females died at any exposure level. Compared to the controls, final body weights were 15 and 25% lower in the males and 15 and 14% lower in the females of the 2500- and 3000-ppm groups, respectively. There was a concentration-related increase in the relative liver weight, significant at 1250, 2500, and 3000 ppm in males and at 2500 and 3000 ppm in females. The relative weights of the heart, lung, kidney, and right testis were also significantly elevated in the 2500- and 3000-ppm animals compared to those of the controls, although no histopathology was observed in any exposure group. Toxic effects noted in a concurrently conducted gavage study (urinary bladder hemorrhages in the two highest exposure groups) were not noted in this subchronic inhalation study. A subsequent report on the neurologic effects of these exposures did not indicate any neurobehavioral changes as a result of toluene exposure (Tilson, 1990), but details of the test results are lacking. This study identified a NOAEL of 625 ppm and a LOAEL of 1250 ppm for changes in relative liver weight in male rats.

Poon et al. (1994) exposed groups of Sprague-Dawley rats of both sexes to 0, 30, or 300 ppm toluene for 6 hours/day, 5 days/week for 4 weeks. Slightly increased relative liver weights, but not absolute liver weights, were seen in 30 ppm males, but not in the 300 ppm males or at any concentration in females. Females exposed to 30 ppm, but not to 300 ppm, had a mild reduction in thyroid follicle size. A slight epithelial degeneration in the nasal conchae was noted in 30-ppm males, but not in the higher-dose males or in either exposure group of female rats. Because the responses occurred in the low exposure group but not the high exposure group, no NOAEL or LOAEL could be identified from this study.

von Euler et al. (2000) exposed 30 male Sprague-Dawley rats to 80 ppm (302 mg/m^3) of toluene for 6 hours/day, 4 days/week for 4 weeks. Control animals (n=30) were similarly exposed, but to air only. Four weeks after the last exposure, the animals were evaluated for neurobehavioral alterations using tests for spatial learning and memory (Morris water maze), open-field activity (open field test), and beam-walk performance. Following the conclusion of the neurobehavioral tests, animals were examined for changes in brain morphology using

magnetic resonance imaging. Animals were sacrificed, and the brains were examined for effects on the dopamine D_3 receptor. No effects on body weight were seen as a result of exposure. Exposed rats did not differ significantly from controls in the results of the open field test; however, toluene exposure resulted in significant changes in the water maze test (increased time in the correct quadrant) and significantly reduced performance in the beam walk test. MRI analysis revealed a selective decrease of approximately 6% in the area of the parietal cortex, but whole brain volumes, also assessed by MRI, were not significantly different between exposed and control rats. Autoradiographic analysis revealed a 7-10% decrease of the cerebrocortical area. Autoradiography did not reveal differences in binding to the dopamine D_3 receptor as a result of toluene exposure. This study identified a LOAEL of 80 ppm (302 mg/m^3) for neurobehavioral alterations 4 weeks after cessation of a 4-week exposure to toluene; no NOAEL was identified.

Inhalation exposure to toluene has been shown to result in irreversible high-frequency hearing loss in rats. Pryor et al. (1984) exposed young male Fischer 344 rats to a variety of exposure concentrations and durations. Hearing loss was evaluated by a behavioral technique (avoidance response elicited to an auditory signal) or brainstem auditory-evoked responses (elicited by tone pips of differing loudness and frequency and detected by subdural scalp electrodes). Hearing loss, as measured by both techniques, was observed after as few as 2 weeks of exposure to 1000 ppm toluene for 14 hours/day. Lower concentrations of 700 ppm for 14 hours/day were without effect after 16 weeks of exposure. Intermittent exposure to 3000 ppm for 30 minutes/hour for 8 hours/day caused hearing loss within 2 weeks, whereas a similar exposure schedule for only 4 hours/day was without effect after 9 weeks. Hearing loss was irreversible, as evidenced by a failure to return to normal response after 3 months of recovery. This study identified a LOAEL of 1000 ppm for hearing loss in rats; no NOAEL was identified. It should be noted that later reports indicated the "high-frequency" hearing loss was most likely an atypical mid-frequency hearing loss because the earlier studies did not assess the auditory frequency range of rats which can hear much higher frequencies than humans (Crofton et al., 1994).

McWilliams et al. (2000) exposed groups of 8 guinea pigs to 0, 250, 500, or 1000 ppm (0, 943, 1885, or 3770 mg/m^3) of toluene for 8 hours/day, 5 days/week for 1 week or 500 ppm (1885 mg/m^3) for 4 weeks. At 1 and 4 weeks, animals were examined for changes in hearing by the cubic distortion-product otoacoustic emission (CDP) technique, while after 4 weeks of exposure, selected animals were examined histologically for changes in the cochlea. After 1 week of exposure, a dose-related decrease in CDP amplitudes was seen, with complete recovery evident after a 3 day rest period. A 4 week exposure to 500 ppm of toluene resulted in more severe disturbances in hearing than were seen after 1 week, but the effects were still reversible. After 4 weeks of exposure, the cochlear cells located near the base (and high frequency) showed a loss of succinate dehydrogenase (SDH) staining. This study identified a NOAEL of 250 ppm (943 mg/m^3) and a LOAEL of 500 ppm (1885 mg/m^3) for diminished startle response and histologic alterations of the cochlea in exposed guinea pigs.

The effects of inhalation exposure to toluene on pulmonary host defenses were evaluated by Aranyi et al. (1985). CD1 mice were exposed (3 h/day) to approximately 0, 1, 2.5, 5, 10, 25, 50, 100, 250, and 500 ppm toluene for 1 day 0, 1, 50, 100, 250, and 500 ppm for 5 consecutive days, or 0 and 1 ppm for 20 days (5 days/week). Pulmonary bacteriocidal activity towards *Streptococcus zooepidemicus* was significantly decreased at 500, 250, 100 and 2.5 ppm following a single exposure. No significant effects were observed for single exposures at or below 50 ppm with the exception of anomalous effects seen at 2.5 ppm. Five days (3 hr/day) exposure to 1 ppm toluene caused a decrease in bacteriocidal activity, although 1 and 20 days of exposure did not. The results indicate the immunological effects may be transient but the interpretation is confounded by the lack of a clear dose-response relationship.

4.2.2.2. Chronic Studies

In a 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 vapors, 6.5 hours/day, 5 days/week (duration-adjusted to 0, 437, and 875 mg/m^3, respectively) for 103 weeks (NTP, 1990a). To generate toluene vapor, the liquid material was heated, and the vapor was diluted with nitrogen and mixed with the chamber ventilation air. An interim sacrifice was carried out at 15 months on control and 1200 ppm groups (10/sex/group) to conduct hematology and histopathology of the brain, liver, and kidney. Body weights were measured throughout the study. Gross necropsy and micropathology examinations were performed at the end of the study on all major organs including the nasal passage tissues (three sections), lungs, and mainstem bronchi. 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 interim sacrifice, there was a mild- to- moderate degeneration in the olfactory and respiratory epithelium of the nasal cavity in 39/40 rats of the 600 and 1200 ppm groups compared with 7/20 controls. 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). No other increases in the incidence of neoplastic or non-neoplastic lesions were reported in exposed rats. A LOAEL of 600 ppm toluene was identified 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 identified from this study.

B6C3F1 mice (60/sex/group) were exposed to 0, 120, 600, or 1200 ppm (0, 452, 2261, or 4523 mg/m^3, respectively) toluene 6.5 hours/day, 5 days/week (duration-adjusted to 0, 87, 47, and 875 mg/m^3, respectively) for 2 years (NTP, 1990a). Mean body weights were not significantly different among groups and no treatment-related clinical signs were observed.

Deaths (moribund and natural) occurred in all exposure groups but were not related to exposure and were not greater than the control rates. At the 15-month interim sacrifice, minimal hyperplasia in the bronchial epithelium was observed in 4/10 females exposed to 1200 ppm. At the end of the study, there was a concentration-dependent increase in the incidence of splenic pigmentation in the exposed males (9/60, 11/60, and 18/59 at 120, 600, and 1200 ppm, respectively) compared to controls (4/60). In the females, the incidence was 37/50, 33/50, 34/49, and 28/47 at 0, 120, 600, and 1200 ppm, respectively. The occurrence of endometrial hyperplasia was present in 14% of the animals exposed to the highest concentration, but only in 4% in the low-exposure groups and controls. No differences were noted between the exposed and control mice of either sex in the incidence of degeneration of either the olfactory or respiratory epithelium. No other changes in the incidences of non-neoplastic or neoplastic lesions were observed in exposed mice.

Fischer 344 rats (120/sex/group) inhaled 0, 30, 100, or 300 ppm (0, 113, 377, or 1130 mg/m^3, respectively) toluene (99.9% purity), 6 hours/day, 5 days/week (duration-adjusted to 0, 20, 67, or 202 mg/m^3, respectively) for 106 weeks (CIIT, 1980; Gibson and Hardisty, 1983). Vapor, generated by bubbling clean air through toluene, was passed through the air supply duct and mixed with air by turbulent flow to produce the desired concentration. Hematology, blood chemistry, and urinalysis were conducted in all groups at 6 (5/sex), 17 (5/sex), 18 (10-20/sex), and 24 months (10/sex). Histopathology was evaluated only in the control and 300 ppm groups at 6 (5/sex), 12 (5/sex), and 18 months (20/sex). At 24 months, histopathological examinations were conducted in organs of all surviving animals, including the respiratory system and sections through the nasal turbinates (number not indicated). No treatment-related non-neoplastic effects were observed in the exposed animals. Although the male rats exposed to 300 ppm had a significant increase in body weight compared to controls, no concentration-response was evident. At the end of the exposure period, the female rats exposed to 100 or 300 ppm exhibited a slight but significant reduction in hematocrit; an increase in the mean corpuscular hemoglobin concentration was also noted but only in the females exposed to 300 ppm. Gross and microscopic examination of tissues and organs identified no increase in neoplastic tissue or tumor masses among treated rats when compared with controls, though because the study was conducted at exposure levels below the MTD, the significance of this finding is less clear. The highest concentration examined in this study, 300 ppm, is designated as a NOAEL for toxicity remote from the respiratory tract in rats. CIIT (1980) reported that the technical and raw data were not audited by their quality assurance group during the study period, although CIIT did conduct a quality assessment procedure to review the data. The available pathology reports containing these data indicate that at least the lower respiratory tract was examined. Communication with the testing sponsor has provided information indicating that only one section was examined from the nasal cavity of these test animals. It is not clear whether this single section would have been sufficient to elucidate the areas of lesions noted in the NTP (1990) study. Consequently, the designation of the 300 ppm exposure level as a NOAEL for respiratory lesions (see NTP, 1990a) is problematic.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Studies In Humans

Toluene is believed to cause congenital defects in infants born to mothers who abused toluene during pregnancy, though exposure levels in the available studies, if reported at all, were very high. A detailed discussion of the high-dose effects of toluene on reproduction and development is beyond the scope of this document; a discussion of a representative sample of the available studies is included. In a case report study, Hersh et al. (1985) describes clinical and morphometric characteristics common to three children whose mothers had abused toluene (but apparently not alcohol or any other substance) for a period of 4-5 years including during their pregnancies with the affected children. Clinical findings common to these three children included microcephaly, CNS dysfunction, attention deficits, and developmental delay/mental deficiency. Phenotypic similarities included a small midface, deep-set eyes, micrognathia (smallness of the jaws), and blunting of the fingertips.

Studies examining the effects of toluene in humans following long-term low-level exposure are less common. Plenge-Bönig and Karmaus (1999) examined the influence of toluene on the fertility of 150 male and 90 female rotogravure printing workers. The men were, in general, exposed to higher concentrations than the women; the women worked exclusively in the stacking and bookbinding areas, and received low levels of toluene exposure. Quantitative exposure levels were not reported. After adjustment for age and smoking of the partner, no association between exposure of men to toluene and fertility could be identified. In female workers, however, a significant association between toluene exposure and reduced fertility was found.

A case-referent study was conducted by McDonald et al. (1987) who examined the history of exposure to chemicals of 301 women who had recently given birth to an infant with an important congenital defect. An identical number of women (referents) who had given birth to normal children were matched with respect to age, employment (hours/week), date of delivery, and educational level. In initial matched-pair analysis, chemical exposure was higher in the cases than in the referents (63 cases:47 referents) due to excess cardiac and miscellaneous defects. In further analysis by chemical categories, only exposure to aromatic solvents showed a clear excess of defects, mostly in the urinary tract. Details of these cases (n = 19) showed that toluene was identified as the solvent in 11 of these cases.

4.3.2. Studies in Animals

4.3.2.1. Oral Exposure

Kostas and Hotchin (1981) exposed NYLAR mice pre- and postnatally to toluene provided in the drinking water at concentrations of 0, 16, 80, or 400 ppm. Effects were noted in all dosed groups on rotorod performance, measured at 45 to 55 days of age, but there was an

inverse dose-response relationship. No effects of toluene exposure were seen on maternal fluid consumption, offspring mortality rate, development of eye or ear openings, or surface-righting response. The interpretation of this study is limited because only 6 to 9 pregnancies/dose group were obtained, and because the dose-response relationship was inverse.

Gospe et al. (1994, 1996; Gospe and Zhou, 1998, 2000) conducted a series of studies examining the effects of oral prenatal toluene exposure on the development of rats. In the first experiment (Gospe et al., 1994), pregnant rats received 520 mg/kg in corn oil by gavage on gestational days 6-19, and offspring were examined on gestational day 19. Toluene exposure did not result in maternal deaths, but did result in a significant decrease in weight gain (24% decrease) and a 12% reduction in food consumption, although this difference was not statistically significant. No differences in number of implantations or resorptions were found between control and exposed groups, but fetal body weights, organ weights (liver and kidney), and placenta weights were significantly decreased in toluene-exposed animals. No gross fetal malformations were reported, and histologic examination of the brain revealed no treatment-related changes. In the second experiment (Gospe et al., 1996), pregnant rats received 650 mg toluene/kg in corn oil by gavage on gestation days 6-19; both control (corn oil only) and pair-fed controls were also examined. Fetuses were delivered and examined on day 19 of gestation. Toluene exposure resulted in significantly decreased fetal weight, decreased organ weights (brain, liver, heart, and kidney), and a delay in skeletal ossification. No differences were seen between pair-fed animals and control animals, and toluene-induced changes were significantly different from the pair-fed group as well. In the third experiment (Gospe and Zhou, 1998), groups of pregnant rats were exposed by gavage to 650 mg toluene/kg in corn oil on gestation days 6-19, and offspring were examined on gestation day 19, or on postnatal days 10 or 21. Toluene exposure did not result in changes in litter size, maternal death, or maternal liver weight. At gestational day 19, fetal body weights, as well as the weights of the heart, liver, kidney, and brain, were significantly reduced in toluene-exposed animals. At postnatal day 10, the body, heart, and kidney weights of prenatally-exposed weights remained significantly lower than controls, while by postnatal day 21, no differences between control and treated animals were seen for body or organ weights. While prenatal exposure to 650 mg toluene/kg on gestational days 6-21 did not result in decreased organ or body weights on postnatal day 21, histologic analysis of the brain revealed decreased neuronal packing and alterations in the patterns of staining with bromodeoxyuridine (Gospe and Zhou, 2000), indicating compound-induced alterations in neurogenesis and neuronal migration.

4.3.2.2. Inhalation Exposure

Pregnant Wistar rats and hamsters (group size not indicated) inhaled 0 or 800 mg/m^3 (212 ppm) toluene vapors 6 hours/day on gestational days 14-20 (rats) or gestational days 6-11 (hamsters) (DaSilva et al., 1990). In the exposed rats, there was a significant ($p<0.05$) increase in the number of litters with one or more low birth weight pups (less than 4.9 g), from 10% in the controls to 54% in the exposed dams. A decrease ($p<0.05$) in the number of live pups at birth was also noted in the litters of exposed dams. No evaluation of malformations or anomalies was

performed. The neurobehavioral development of the offspring of the exposed rats was assessed using tests of spontaneous alternation, rim escape, and avoidance responses. The only effect noted in the rats, a shortened first trial latency in choosing one side of a maze, was minimal and its significance unclear. No comparable reproductive deficits occurred in the exposed hamsters. The only effect noted in the neurobehavioral tests of the hamster offspring was an equivocal effect in rotarod performance. No neurobehavioral effect levels were designated from this study, although it appears that the rat developmental processes are more sensitive than those of the hamster, exhibiting adverse effects at 800 mg/m^3.

Thiel and Chahoud (1997) exposed groups of pregnant Wistar rats to 0, 300, 600, 1000, or 1200 ppm (0, 1131, 2262, 3370, or 4524 mg/m^3) toluene for 6 hours/day from day 9 to day 21 of pregnancy. At birth, the number of live and dead pups was determined, as well as mean pup weight per litter. Postnatal weight gain was recorded weekly, and signs of physical development, including eruption of incisors, fur development, eye opening, testes descent, and vaginal opening, were monitored. Prior to weaning, reflex testing was performed on the offspring. After weaning, offspring were tested for locomotor activity and discrimination learning. Toluene exposure did not result in changes in duration of pregnancy or litter size. At the two highest doses, toluene produced a significant decrease in maternal body weight gain and mean pup weight. High-dose offspring had a significantly increased mortality during the suckling period (postnatal days 2-21). Postnatal development (time of testes descent or vaginal opening) was accelerated at 600 ppm, but was delayed at 1000 ppm of toluene or greater. No changes in neurobehavioral parameters of the exposed offspring were noted relative to controls. With the exception of an increased mean fertility in the 600 ppm group, the fertility of the offspring was not different from that of controls.

Dalgaard et al. (2001) exposed groups of pregnant Wistar rats to airborne concentrations of 1200 ppm of toluene for 6 hours/day on gestational days 7 to 18, and examined male offspring on postnatal day 110 for alterations in semen quality. No effect of toluene exposure on semen quality was seen. In a subsequent study (published in the same manuscript), groups of pregnant rats were exposed to 1800 ppm of toluene from gestational days 7 to 20, and the male offspring were examined on postnatal days 11, 21, or 90. Mean body weights in exposed pups were lower than controls on day 11, but were not significantly different on days 21 or 90. Absolute and relative testes weights were decreased in all age groups, but the differences were not statistically significant. Histologic analysis of the testes revealed no effects of toluene exposure in any age group. Microscopic examination of the hippocampus revealed no changes in apoptotic neurodegeneration in any group, whereas toluene induced a statistically significant increase in apoptosis in the cerebellar granule layer on postnatal day 21, but not day 11 or 90.

In another study with similar exposure conditions, Hougaard et al. (1999) exposed groups of pregnant Wistar rats to airborne levels of 1800 ppm of toluene for 6 hours/day from gestational days 7 to 20. Body weights of exposed offspring were lowered until postnatal day 10, after which no significant differences were noted. Neurobehavioral evaluation of the pups revealed no effects on motor function, activity level, acoustic startle, and prepulse inhibition. Measurement of hearing function revealed small but significant changes in male offspring.

Significant effects on cognitive function, assessed by Morris water maze, were reported for both sexes of offspring, but were most pronounced in female offspring.

A subsequent study by the same investigators (Hass et al., 1999) exposed groups of pregnant Wistar rats to 1200 ppm of toluene for 6 hours/day on gestational days 7 to 18. The exposure did not cause maternal toxicity or decreased offspring viability. As was the case with the previous study, offspring body weights were significantly reduced through postnatal day 10, and were not significantly different thereafter. Alterations in Morris water maze performance were evident in female offspring at 3.5 months of age; no other changes in neurobehavioral parameters were reported.

Ungvary and Tatrai (1985) exposed New Zealand rabbits (8-10/group) to 0, 500, or 1000 mg/m^3 toluene, 24 hours/day, on gestational days 7-20, and CFLP mice (15 females/group) to 0, 500, 1000, or 1500 mg/m^3 toluene, also continuously, on gestational days 6-15. The control groups consisted of 115 mice and 60 rabbits. All of the female mice that were exposed to 1500 mg/m^3 died. In the mice exposed to 1000 mg/m^3, there was an increase in fetuses with retarded weight (29%, level of retardation not indicated) and in fetuses with skeletal retardation (12%) compared to 7 and 5%, respectively, in the controls, which did not differ from the animals exposed to 500 mg/m^3. Of the 8 pregnant rabbits exposed to 1000 mg/m^3, 2 died, 4 had spontaneous abortions, and the remaining 2 had total litter resorption. No deaths occurred in the 10 rabbits exposed to 500 mg/m^3, but 1/10 rabbits had a spontaneous abortion (as compared to 0/60 reported for the controls). No effects were seen on fetal development in rabbits exposed to 500 mg/m^3.

Pregnant Charles River CD-1 mice (15-16 females/group) inhaled filtered air or 200 or 400 ppm (754 and 1508 mg/m^3) toluene 7 hours/day on gestational days 7-16 (Courtney et al., 1986). The relative liver weight in the exposed dams was reported to be significantly lower in the two exposed groups compared to the controls, although no data were presented. A statistically significant increase in lactate dehydrogenase activity in the brain of the dams exposed to 400 ppm was also reported. The exposed pregnant mice did not exhibit any significant differences in the number of implantation sites, number of live fetuses, fetal deaths, or fetal body weight compared to the control values. A statistically significant increase over controls in the incidence (both per litter and per fetus) of enlarged renal pelves was noted in dams exposed to 200 ppm, but not to 400 ppm. A statistically significant alteration from controls in the rib profile (percentage of fetuses with 1 or 2 additional/fewer ribs) was reported for fetuses from dams exposed to 400 ppm, but not to 200 ppm. The toxicological significance of this finding is not clear.

Ono et al. (1995) exposed groups of pregnant Sprague-Dawley rats to 0, 600, or 2000 ppm (0, 2262, or 7540 mg/m^3) of toluene for 6 hours/day from days 7 to 17 of gestation, and examined the offspring for malformations and alterations in behavioral parameters. Preweaning tests included surface righting and negative geotaxis, while postweaning tests included an open field test (postnatal week 4), the Biel water maze (postnatal week 6), and rotorod tests (postnatal

week 7). At the conclusion of the study, animals were sacrificed and examined histologically. Serum biochemistry and hematologic parameters were also evaluated. No biochemical, teratogenic, or histologic changes attributable to toluene exposure were reported in either parental rats or the offspring in the 600 ppm group. Exposure to 2000 ppm resulted in significant maternal toxicity, as well as decreased body weight of offspring, increased fetal mortality, and decreased offspring weight gain. However, no differences in external, internal, or skeletal anomalies were reported for any exposure group. Similarly, no differences were found in the results of preweaning or postweaning behavioral testing at any exposure level.

In a later study, Ono et al. (1996) exposed groups of male and female Sprague-Dawley rats to 0, 600, or 2000 ppm (0, 2262, or 7540 mg/m^3) of toluene for examination of effects on fertility. Females were exposed from 14 days before mating to day 7 of gestation, while males were exposed for 90 days, beginning at 60 days before pairing. In females exposed to 2000 ppm, increased salivation and lacrimation were noted starting 20 days after exposure. No changes were noted in mating behavior or fertility at either exposure level. Fetal mortality and the number of dams with dead fetuses were both increased in the 2000 ppm animals, but these differences were not statistically significant. In males exposed to 2000 ppm for 90 days, increased kidney weight and decreased thymus weights were observed. Additionally, high-dose males showed a decreased epididymal weight, though no abnormalities of the testes or epididymis were noted histologically. Sperm counts were significantly reduced in the 2000 ppm animals; the sperm count of the 600 ppm group was slightly decreased, but did not attain statistical significance.

A 2-generation inhalation reproductive study was conducted in CD rats (10-40 males/group, 20-80 females/group) (API, 1985). Animals were exposed by whole-body inhalation to toluene at 0, 100, 500, or 2000 ppm (0, 377, 1885, or 7538 mg/m^3, respectively) 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 pups in this generation (F1) were exposed for a minimum 80-day pre-mating period. The animals were then randomly mated with members of the same exposure group (2 females/1 male) for 15 days, during which exposure was continued, to produce the second generation (F2). Mean male body weights were slightly reduced (maximum of 10%) in the first 2 weeks of the exposure in the animals exposed to 500 and 2000 ppm, although the size of the reduction was not related to exposure. No differences were observed in male or female fertility indices, length of gestation, mean numbers of viable and nonviable pups at birth, or pup survival indices during lactation in either the F0 or F1 generation. No abnormal histopathology was noted in organs examined. A significant decrease ($p<0.05$) in weight relative to controls was observed in the first generation offspring during study weeks 19 through 36. The decrease was maintained throughout the lactation period in the F1 pups from F0 dams exposed to the highest exposure and in those from the ancillary group in which F0 females exposed to the 2000 ppm concentration were mated with males having no exposure. No additional data were available in the report about the F2 generation.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Data

4.4.1.1. Oral Exposure

Mehta et al. (1998) exposed groups of male and female Sprague-Dawley rats to a single gavage dose of 0, 3, 4.5, or 6 ml toluene/kg (0, 2600, 3900, or 5200 mg/kg, respectively). On days 1 (2-3 hours after exposure), 7, and 14 post-exposure, the animal body weights were recorded, and a functional observation battery (FOB) was conducted to detect neurobehavioral changes. A significant, dose-dependent decrease in body weight occurred at day 7 for male rats. Decreases in body weight gain were noted in male rats at 14 days and female rats at 7 days, but the differences were not statistically significant. On day 1, but not on days 7 and 14, toluene-treated rats of both sexes exhibited a dose-dependent increase in abnormal gait. The open-field rearing scores were lower for all groups of both sexes at day 1 only, though only achieved statistical significance in high-dose females. Horizontal motor activities were significantly lower in both sexes at all dose levels on day 1; the values remained lower in all treated female groups and the 3 and 4.5 ml/kg male rats on day 7, and in 3 and 4.5 ml/kg female rats on day 14. Rats of both sexes showed increased incidences of lacrimation and/or salivation on day 1 only; the effect was more pronounced in females.

Dyer et al. (1988) exposed groups of male Long-Evans rats to a single gavage dose of 0, 250, 500, or 1000 mg toluene/kg in corn oil. Flash-evoked potential (FEP) tests were administered 45 minutes later as a test of the ability of the nervous system to process visual information. Toluene exposure resulted in a significant decrease of the N3 peak of the FEP in all dose groups, though the decrease was not dose-related. In an additional study, presented in the same manuscript, rats were exposed to 500 mg/kg and FEP was examined at 4, 8, 16, and 30 hours post-exposure. Depression of the N3 peak remained at 8 hours post-exposure, but by 16 hours recovery appeared complete.

4.4.1.2. Inhalation Exposure

A number of acute animal studies have examined the neurological effects of inhaled toluene; these studies generally reported impaired response in neurologic examinations. For example, Rebert et al. (1989a,b) reported abnormal flash-evoked potentials in rats exposed to a single inhalation exposure of 500-16,000 ppm toluene. Wood et al. (1983) exposed rats to toluene levels up to 3000 ppm for 4 hours prior to behavioral evaluation, and reported that toluene reduced performance in behavioral tests, particularly at the 1780 and 3000 ppm exposure levels. Wood and Colotla (1990) reported a biphasic response in mice exposed to toluene for 1 hour. An increase in activity was seen at concentrations up to 1000 ppm, beyond which decreased activity was seen. Similar results were reported by Wood and Cox (1995), with rats exposed at concentrations up to 1000 ppm showing progressive increases in activity, with decreasing activities at higher concentrations, up to and including 3000 ppm.

4.4.2. Genotoxicity

Toluene has tested negative for reverse mutation in *Salmonella typhimurium*, both with and without S-9 activating mixture (Mortelmans and Riccio, 1980; Nestmann et al., 1980; Bos et al., 1981; Litton Bionetics, Inc., 1981; Connor et al., 1985; NTP, 1990a). Toluene also tested negative in the *umu* test in *S. typhimurium* (Nakamura et al., 1987), and was negative for reverse mutation in *E. coli* (Fluck et al., 1976). NTP (1990) reported no increase in sister-chromatid exchanges (SCE) or chromosomal aberrations in Chinese hamster ovary cells exposed to toluene. Available studies (Gerner-Smidt and Friedrich, 1978; Richer et al., 1993) have reported no increase in SCE in human lymphocytes exposed *in vitro* to toluene, even at concentrations that inhibited cellular growth.

Dobrokhotov and Einkeev (1977) exposed male rats (strain not specified) to 610 mg/m^3 toluene for 4 hours/day for 4 months, reporting a reversible increase in chromosomal gaps and breaks in isolated bone marrow cells. Mice exposed to toluene at concentrations of 100 or 400 ppm for 6 hours/day, 5 days/week for 8 weeks showed no increase in dominant lethal mutations (measured as pre- or post-implantation embryo loss) relative to controls (API, 1981a). BDF_1 mice exposed to 500 ppm toluene for 6 hours/day, 5 days/week for up to 8 weeks showed no increase in DNA damage, assessed by starch gel electrophoresis, relative to controls (Plappert et al., 1994).

The majority of studies in toluene-exposed workers (Forni et al., 1971; Funes-Craviota et al., 1977; Maki-Paakkanen et al., 1980) have reported no differences in chromosomal aberrations between control subjects and toluene-exposed workers. Similarly, humans exposed to toluene have not generally demonstrated increases in SCE (Funes-Craviota et al., 1977; Haglund et al., 1980; Maki-Paakkanen et al., 1980; Richer et al., 1993), cell cycle delay (Richer et al., 1993), or DNA damage as indicated by Comet assay (Pitarque et al., 1999). However, a few studies of exposed workers (Bauchinger et al., 1982; Nise et al., 1991) have found increases in chromosomal breaks, exchanges, and/or gaps relative to controls. Other studies (Schmid et al., 1985; Pelclová et al., 1990) have reported genotoxic changes in toluene-exposed workers, but the changes have either been reversible or unable to be directly attributed to toluene exposure due to confounding factors.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION – ORAL AND INHALATION

4.5.1. Oral Exposure

Published toxicity studies of oral exposure to toluene in humans are limited to case reports of acute oral overdoses (Ameno et al., 1989; Caravati and Bjerk, 1997). Clinical effects in these cases have included central nervous system depression, severe abdominal pain, diarrhea, and hemorrhagic gastritis. Chronic toxicity studies of oral toluene exposure in animals are not available. Maltoni et al. (1997) conducted a 2-year gavage study of toluene in rats; however,

only carcinogenic endpoints were reported. NTP (1990) conducted a 13-week corn oil gavage study of toluene in F344 rats and B6C3F1 mice. In rats, which were more sensitive to toluene than mice, the most sensitive effects reported were in the liver, with organ weight changes at low levels accompanied at higher levels by histologic changes; a NOAEL of 223 mg/kg-day and a LOAEL of 446 mg/kg-day in rats were identified. At higher exposure levels (893 mg/kg-day for males and 1786 mg/kg-day for females), an increased incidence of rats with mineralized foci and necrosis of normal brain cells was observed; this effect was not noted in mice at any exposure level (NTP, 1990a). Hsieh et al. (1989, 1990) exposed CD-1 mice to toluene in the drinking water for 28 days. At 5 mg/kg-day, significant changes in brain neurotransmitter levels were reported (Hsieh et al., 1990), as well as significant changes in the *ex vivo* response to mitogens with no significant change in antibody response at this dose (Hsieh et al., 1989). Neither of these effects were correlated with impaired neurological or immunological outcomes; their toxicological significance is unclear. However, additional effects on immunological parameters, e.g., a significant decrease in antibody response, was noted at higher concentrations and is considered a co-critical effect. At 105 mg/kg-day, statistically significant changes in liver and spleen weights and plaque-forming colony response to sheep red blood cells were reported.

While the most sensitive effects of inhalation exposure to toluene involve neurologic endpoints, detailed reports of similar effects following prolonged oral exposure are lacking. A report of a case study of an accidental oral overdose of toluene did report central nervous system depression (Caravati and Bjerk, 1997), but no quantitative measure of these effects exists. Animal studies have identified effects on the liver, and perhaps immunologic effects, as the most sensitive effects of known toxicologic relevance. Brain lesions have been observed in orally-exposed rats (NTP, 1990a), but these effects occurred only at exposure levels ($\geq$ 893 mg/kg-day) higher than those affecting liver endpoints, and were not observed in mice at any exposure level. Other effects (changes in brain neurotransmitter levels, decreased response to mitogenic stimulation) have been reported (Hsieh et al., 1989, 1990), but their toxicological significance is not presently clear. Liver effects have been reported to be the most sensitive following oral toluene exposure in animals, possibly because the liver contains large amounts of the enzymes for toluene metabolism, and the fact that orally-absorbed toluene will pass through the liver before being distributed to the rest of the body. This observation is further supported by the fact that studies examining the distribution of toluene following oral exposure have generally found greater amounts of toluene per gram of tissue in the liver than in the brain (Ameno et al., 1989; Pyykko et al., 1977). The generation of arene oxide intermediates formed in the metabolic pathway from toluene to *ortho*- or *para*-cresol (see Figure 1) could hypothetically result in increased toxicity at the site of metabolism, particularly at high exposure levels when other pathways may achieve saturation. However, no data are available directly evaluating this potential mode of action.

In summary, data on the effects of toluene in humans following oral exposure are limited to case reports of accidental oral ingestions. Both a 4 week study in mice (Hsieh et al., 1989, 1990) and a 13 week study in rats (NTP, 1990a) identified increases in liver weight as the most

sensitive toxicologically relevant endpoint; the 4 week study also reported decreased thymus weights and immunological effects at the same exposure level.

4.5.2. Inhalation Exposure

Many studies have been published examining neurological endpoints resulting from repeated low-level toluene exposure in occupationally-exposed workers. Results from these studies suggest that subtle neurologic effects are the most sensitive endpoints following repeated inhalation exposure to toluene. The NOAEL/LOAEL values of several chronic human studies are shown in Table 1. As can be seen, a number of studies of occupationally-exposed humans have reported effect levels ranging from 40 to 132 ppm, with two studies (Zavalic et al., 1998a; Eller et al., 1999) identifying NOAEL values as well.

There is evidence that exposure to toluene results in both transient and persistent effects on neurologic endpoints, but the available data often do not clearly discern persistent effects from transient effects, even on the same endpoint. For example, Baelum et al. (1985) reported that the neurologic responses, including altered color vision, of rotogravure printers (average toluene exposure of 9 to25 years) exposed to a single 6.5 hour exposure of 100 ppm toluene did not differ from a control group who had not been previously exposed to toluene, suggesting that the acute effects of toluene on color vision were transient, rather than being dependent on previous exposure history. In contrast, Zavalic et al. (1998c) reported that analysis of color vision scores in toluene-exposed workers on Wednesday did not differ from the scores in the same workers on Monday, after at least 48 hours without exposure, suggesting that the effect was persistent. Similarly, McWilliams et al. (2000) reported that guinea pigs exposed to 500 ppm toluene for up to 4 weeks showed a reversible hearing loss, while Pryor et al. (1984) reported that hearing loss in male rats exposed to 1000 ppm toluene or greater for up to 2 weeks was still present after a 3-month recovery period.

The most compelling evidence for the ability of repeated toluene inhalation exposure to produce persistent neurologic effects comes from case reports of toluene abusers, who are generally exposed to concentrations exceeding 1000-10,000 ppm. MRI examinations of the brain of solvent abusers (Filley et al., 1990; Rosenberg et al., 1988a, 1988b) suggest a preferential atrophy in lipid-rich regions of the brain. Rosenberg et al. (1988a,1988b) found MRI evidence of diffuse central nervous system demyelination in 6 toluene abusers with clinically obvious neurological impairment, whereas Filley et al. (1990) noted that the degree of MRI-detected white matter abnormality in 14 solvent abusers was correlated with neurological impairment. The observed changes in MRI signals may be related to lipid compositional changes in the white matter, since these regions are more lipid-rich than gray matter (Ameno et al., 1992).

Table 1 - Selected Human Studies of Chronic Toluene Inhalation

Reference	NOAEL (ppm)	LOAEL (ppm)	Effect
Abbate et al., 1993	None*	97	Increased wave latencies for auditory-evoked brain potentials
Boey et al., 1997	None	90.9	Performance deficits in several neurobehavioral tests
Eller et al., 1999	25-32	>100	Increased incidence of subjective symptoms, poorer performance in neurological testing
Foo et al., 1990	None	88	Performance deficits in neurobehavioral tests in exposed workers
Murata et al., 1993	None	83	Reductions in electrophysiological parameters (ECG and nerve conduction)
Vrca et al., 1995, 1997	None	40-60	Alterations in visual- and auditory-evoked brain potentials
Yin et al., 1987	None	43	Increased prevalence of subjective symptoms (headache, dizziness) in exposed workers
Zavalic et al., 1998a	32	132	Significantly increased age- and alcohol-adjusted color confusion index

* No NOAEL identified by the study

Animal data have also suggested that respiratory tract irritation, particularly in the nasal cavity, is a sensitive effect of toluene. However, the primary study that reported this effect (NTP, 1990a) only examined concentrations of 600 ppm and greater. A lifetime chronic (CIIT, 1980) and 28-day subchronic (Poon et al., 1994) study have each examined exposed rats for changes in the nasal epithelium following exposure to 300 ppm toluene, and both failed to report a treatment-related effect. Available data from acutely-exposed humans demonstrates that nasal and/or ocular irritation is not reported by subjects until the airborne toluene concentration reaches 100 ppm (Echeverria et al., 1989; Andersen et al., 1983), while subtle neurologic changes can be noted at lower concentrations. Thus, it appears that neurologic effects are the most sensitive effect observed in humans exposed to airborne toluene. Only one study in animals has reported persistent neurobehavioral effects at concentrations known to cause similar effects in humans; von Euler et al. (2000) reported diminished performance in the water maze test in rats 4 weeks after exposure to 80 ppm toluene for 6 hours/day, 4 days/week for 4 weeks. Possible reasons for the lack of animal neurologic data at low toluene concentrations include the limitations on sample size in animal studies and the ability of human neurologic examinations to reveal more subtle changes in neurologic endpoints relative to animal neurotoxicity assays. Benignus et al. (1998) address the issue of apparent rat-human sensitivity differences to toluene exposure. After

using a PBPK model to estimate blood toluene concentrations at the time of behavioral assessment, their review of the literature showed that behavioral effects in humans are reported at lower blood concentrations than in rats. This might be attributed either to different behavioral assessment techniques used in testing the two species, or to a variety of biological factors.

A number of developmental effects, particularly neurodevelopmental changes, have been reported in children of women who abused toluene during pregnancy. Effects reported in children exposed *in utero* to toluene include microcephaly, CNS dysfunction, attention deficits, developmental delay/mental deficiency, small midface, deep-set eyes, micrognathia (smallness of the jaws), and blunting of the fingertips (Byrne et al., 1991; Devathasan et al., 1984; Hunnewell and Miller, 1998; King et al., 1981; Maas et al., 1991; Meulenbelt et al., 1990; Miyagi et al., 1999; Ryu et al., 1998; Suzuki et al., 1983). Several studies in rats have reported altered neurobehavioral parameters in offspring following exposure of pregnant dams to high ($\geq$ 800 ppm) concentrations of toluene (DaSilva et al., 1990; Hass et al., 1999; Hougaard et al., 1999). Significant changes in other developmental endpoints have also been reported in animal studies, including increases in spontaneous abortions, resorptions, altered pup body and organ weights, and altered pup development, but generally only at doses ($\geq$ 1000 ppm) where significant maternal toxicity was also reported (Dalgaard et al., 2001; Ono et al., 1995, 1996; Thiel and Chahoud, 1997; Ungvary and Tatrai, 1985). A 2-generation inhalation reproduction study in rats did not report alterations in any indices of fertility, though a decreased pup weight in the F1 generation exposed to 2000 ppm toluene was reported during the first 15 weeks of life, after which weights did not significantly differ from controls (API, 1985).

Despite an abundance of data characterizing the toxic effects of toluene, understanding of the mechanisms by which toluene may exert its toxic effects is limited. The neurologic effects of toluene are subtle, as evidenced by the lack of histologic evidence for degenerative changes of the brain reported in the 2-year inhalation bioassay conducted by NTP (1990). For these effects, the parent compound, rather than a metabolite, is believed to be responsible. Support of parent-material involvement comes from the observation that pretreatment of rats with phenobarbital, thereby increasing the levels of cytochrome P450, increased the rate of *in vivo* toluene metabolism and shortened the time of recovery from narcosis from single intraperitoneal doses of toluene (Ikeda and Ohtsuji, 1971). Also, inhibition of toluene metabolism by pretreatment with ethanol resulted in a potentiation of toluene-induced hearing loss in rats (Campo et al., 1998).

On a molecular scale, little is known about mechanisms by which toluene produces acute or residual CNS effects. The Meyer-Overton theory of partitioning of the parent compound into membrane lipids has been widely accepted for a century (Franks and Lieb, 1985, 1987). Recently, it has been proposed that the presence of solvent molecules in cholesterol-filled intersticies between phospholipids and sphingolipids changes membrane fluidity, thereby altering intercellular communication and normal ion movements (Engelke et al., 1996). It is not known if this mechanism is involved in the chronic effects of inhaled toluene, but the observed neural demyeliniazation in toluene abusers (Rosenberg et al., 1988a,b) would be suggestive evidence of such a role. An alternative hypothesis is that toluene partitions into hydrophobic regions of

proteins and interacts with them, thereby altering membrane-bound enzyme activity and/or receptor specificity (Balster, 1998). Other evidence suggests that toluene and other VOCs may act by enhancing $GABA_A$ receptor function (Mihic et al., 1994), attenuating NMDA receptor-stimulated calcium flux (Cruz et al., 1998), and/or activating dopaminergic systems (von Euler, 1994). Other neurologic effects may involve a number of neurochemical alterations including: changed whole-brain concentrations of dopamine, norepinephrine and 5-hydroxytryptamine in rats exposed for 8 hours to 100, 300, or 1000 ppm toluene (Rea et al., 1984); changed dopamine D2 receptor binding in rats exposed to 80 ppm toluene, 6 hours/day, 5 days/week for 4 weeks (von Euler et al., 1993, 1994); and increased cerebellar concentrations of glial cell protein markers (α-enolase, creatine kinase-B, and β-S100 protein) in rats exposed to 100, 300, or 1000 ppm toluene, 8 hours/day for 16 weeks (Huang et al., 1992). However, the persistence of these effects, which would implicate these mechanisms in the effects of chronic toluene exposure, has not been established.

The mechanism and pathogenesis of color vision impairment (dyschromatopsia) associated with occupational exposure to toluene and other organic solvents are not clearly understood (Muttray et al., 1997, 1999; Zavalic et al., 1998a,b). Although potential mechanisms of action for the pathogenesis have been proposed, there is currently no data to support any of the postulates.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), toluene is classified in Group D (*Not Classifiable as to Human Carcinogenicity*), based on inadequate data on the carcinogenicity of toluene in humans and inadequate evidence of carcinogenicity in animals. This classification has not changed from the previous IRIS entry (9/7/1988). Under the draft revised cancer guidelines (U.S. EPA, 1999), *data are inadequate for an assessment of human carcinogenic potential* of toluene, because studies of humans chronically-exposed to toluene are inconclusive, toluene was not carcinogenic in adequate inhalation cancer bioassays of rats and mice exposed for life (CIIT, 1980; NTP, 1990a), but increased incidences of mammary cancer and leukemia were reported in a lifetime rat oral bioassay at a dose level of 500 mg/kg-day, but not at 800 mg/kg-day (Maltoni et al., 1997). Toluene has generally not been found to be genotoxic in short-term testing.

According to the National Toxicology Program (NTP, 1990b), there is no evidence of carcinogenic activity for male or female F344/N rats exposed to toluene during two-year inhalation studies at concentrations of 600 ppm or 1200 ppm. There is no evidence of carcinogenic activity for male or female B6C3F1 mice exposed by inhalation to toluene at concentrations of 120, 600, or 1200 ppm for 2 years. IARC states that toluene is not classifiable as to its carcinogenicity to humans (group 3); there is inadequate evidence in humans and evidence suggesting a lack of carcinogenicity of toluene in experimental animals (IARC, 1999).

Available studies in toluene-exposed workers have reported very limited or no evidence suggesting carcinogenic effects of toluene exposure (Antilla et al., 1998; Svennson et al., 1990; Wiebelt and Becker, 1999). A cohort mortality study in toluene-exposed workers (Wiebelt and Becker, 1999) did not report an increase in cancer-specific mortality for the entire cohort. A subcohort of highly-exposed workers demonstrated statistically significant increases in mortality from cancers of the bone and connective tissue, but lack of exposure characterization, co-exposure information, and categorization of and adjustment for other confounding factors (age, smoking, etc.) within the subcohort precludes drawing conclusions from these results as to the possible association between toluene exposure and cancer risk. Svennson et al. (1990) similarly did not report increased cancer-specific mortality among rotogravure printers. While an increase in tumors of the respiratory tract was reported, this increase was not statistically significant when only subjects with exposure periods of five years or more were examined, and no dose-response relationships were present for tumor incidence. Antilla et al. (1998) carried out a retrospective cohort analysis of 5301 workers monitored for biological markers of occupational exposure to styrene, toluene, or xylene; no significantly increased incidence rates of cancer could be associated with toluene exposure. Other studies examining the carcinogenicity of toluene in occupationally-exposed humans have failed to adequately account for co-exposure to other compounds.

NTP (1990) has conducted a 2-year inhalation carcinogenicity study in F344 rats and B6C3F1 mice, and found no evidence for carcinogenicity in either sex of either species at exposure levels up to 1200 ppm. Another inhalation carcinogenicity study in F344 rats (CIIT, 1980; Gibson and Hardisty, 1983) likewise reported no evidence for carcinogenic effects of toluene at exposure levels up to 300 ppm. A lifetime carcinogenicity study in Sprague-Dawley rats by the oral route (Maltoni et al., 1997) was suggestive of potential carcinogenic effects of toluene, but the dose-response relationships were not well defined (i.e., the 500-mg/kg animals had considerably more tumors than those in the 800-mg/kg group) and study details were inadequately reported.

Available studies examining the genotoxic effects of toluene have generally reported negative results. Toluene was found to be nonmutagenic in reverse mutation assays with *S. typhimurium* (Mortelmans and Riccio, 1980; Nestmann et al., 1980; Bos et al., 1981; Litton Bionetics, Inc., 1981; Snow et al., 1981; Connor et al., 1985; Nakamura et al., 1987; NTP, 1990a) and *E. coli* (Fluck et al., 1976; Mortelmans and Riccio, 1980), with and without metabolic activation. Toluene did not induce mitotic gene conversion (Litton Bionetics, Inc., 1981; Mortelmans and Riccio, 1980) or mitotic crossing over (Mortelmans and Riccio, 1980) in *S. cerevisiae.* Although Litton Bionetics, Inc. (1981) reported that toluene did not cause increased chromosomal aberrations in bone marrow cells, several Russian studies (Lyapkalo, 1973; Dobrokhotov and Einkeev, 1977) report toluene as effective in causing chromosomal damage in bone marrow cells of rats. There was no evidence of chromosomal aberrations in blood lymphocytes of workers exposed to toluene only (Forni et al., 1971; Maki-Paakkanen et al., 1980), although a slight increase was noted in workers co-exposed to toluene and benzene (Forni et al., 1971; Funes-Craviota et al., 1977). This finding is supported by studies of cultured

human lymphocytes exposed to toluene *in vitro*; no elevation of chromosomal aberrations or sister chromatid exchanges was observed (Gerner-Smidt and Friedrich, 1978).

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

Only limited data exist that examine the potential differences in susceptibility to toluene between children and adults. Children have been demonstrated to have differences in levels of CYP enzymes and several phase II detoxification enzymes (e.g., N-acetyl transferases, UDP-glucuronyl transferases, and sulfotransferases) relative to adults (Leeder and Kearns, 1997; Nakajima et al., 1992; Vieira et al., 1996), as well as other physiological differences (e.g., children have higher brain mass per unit of body weight, higher cerebral blood flow per unit of brain weight, and higher breathing rates per unit of body weight: see Snodgrass [1992]). However, data on the possible contributions of these differences to potential age-related differences are lacking.

Transfer of toluene to nursing infants from breast milk of currently exposed mothers is expected to be a possibility because of the lipophilicity of toluene and the relatively high lipid content of breast milk. Elimination kinetics data for nonpregnant or nonlactating humans and rats following toluene exposure, however, indicate that most absorbed toluene is rapidly eliminated from the body and that a much smaller portion (that which gets into adipose tissues) is slowly eliminated (Leung and Paustenbach, 1988; Löf et al., 1993; Pierce et al., 1996, 1999; Pellizzari et al., 1992; Rees et al., 1985). Thus, mobilization during pregnancy or lactation of stored toluene from preconception exposure may not be a major concern.

Fisher et al. (1997) developed a human PBPK model that predicts transfer of toxicant via lactation from a mother to a nursing infant and used the model to estimate the amount of toluene that an infant would ingest via milk if the mother was occupationally exposed to toluene at the ACGIH (2000) Threshold Limit Value (TLV = 50 ppm) throughout a workday. The model predicted that such an infant would have a daily oral intake of 0.46 mg toluene/day. It should be noted, however, that no human (or animal) studies are available regarding *in vivo* distribution of toluene into breast milk or elimination kinetics from breast milk, and the Fisher et al. (1997) PBPK model has not been validated with *in vivo* data.

4.7.2. Possible Gender Differences

Available studies in humans and animals have not definitively demonstrated whether sex-related differences in the toxicity of toluene exist. Human occupational studies have failed to report sex-related differences in effects, with the exception of the study of Plenge-Bönig and Karmaus (1999), which reported decreased fertility in the occupationally-exposed women, but not in occupationally-exposed men. In rats and mice exposed to toluene orally for 13 weeks (NTP, 1990a), males of both rats and mice showed toxic effects at lower doses than females.

Similarly, in 15-week inhalation studies (NTP, 1990a), males were demonstrated to be more sensitive to the effects of toluene than females; however, no differences were noted between males and females in a 2-year inhalation bioassay (NTP, 1990a). Another chronic inhalation study in rats (CIIT, 1980) failed to show significant differences between males and females with regard to toxicity, but females appeared to be more sensitive with regard to changes in hematocrit.

4.7.3. Other Possible Susceptibility

It has been stated that the effects of toluene exposure on color vision may be potentiated in the elderly and consumers of alcohol (Zavalic et al., 1998a, b). It is not known if this effect is related to the possible differences in metabolism rates under these conditions or some other inherent property related to age or alcohol consumption. It should be noted that color vision decreases with age (Ruddock, 1965; Bowman et al., 1984), diabetes (Matyjavri, 1992; Utku and Atmaca, 1992) and alcohol intake (Russell et al, 1980; Mergler et al., 1988).

5. DOSE RESPONSE ASSESSMENTS

5.1. ORAL RfD

5.1.1. Choice of Principal Study and Critical Effect

No studies examining the subchronic or chronic effects of oral exposure to toluene in humans are available. A lifetime gavage study in rats (Maltoni et al., 1997) reported only carcinogenic endpoints, and is, therefore, not suitable for use as the key study for an RfD. Two subchronic studies examining oral exposure to toluene in rodents exist. NTP (1990) exposed both sexes of F344 rats and both sexes of B6C3F1 mice to gavage doses of 0, 223, 446, 900, 1800, or 3600 mg/kg-day (duaration adjusted) for 5 days/week for 13 weeks. The study in rats established a NOAEL of 223 mg/kg-day for increases in liver and kidney weights of male rats, with a LOAEL value of 446 mg/kg-day. Liver effects in B6C3F1 mice were less clear, with relative liver weights significantly increased in all treated groups of female mice, but significant increases in absolute weight were evident only at the 223 and 1800 mg/kg-day dose groups. No other significant changes were seen in mice exposed to less than 1250 mg/kg. Hsieh et al. (1989) exposed groups of male CD-1 mice to 5, 22, or 105 mg toluene/kg/day in the drinking water for 28 days. Food and water consumption were monitored. Toluene concentration in water was determined daily. At the two highest doses, a number of immunological parameters (*ex vivo* response to stimulation with T- and B-cell mitogens) were significantly different from those of unexposed animals; however, the toxicological significance of these effects is not clear. Mice exposed to 105 mg/kg-day showed significantly increased relative liver weights and significantly decreased relative thymus weights; absolute organ weights were not reported. Also at 105 mg/kg-day, a significant reduction in plaque-forming colony (PFC) formation (>40%) following immunization with sheep red blood cells (SRBC) was observed. A followup study by the same authors (Hsieh et al., 1990) found significant increases in brain neurotransmitter levels at

exposure levels as low as 5 mg/kg-day. The study authors only looked at one time point immediately at the termination of toluene treatment; it cannot be determined if the effects observed were persistent. In addition, no data were provided to correlate the neurochemical changes with behavioral, neuropsychological, or neuroanatomical changes.

An RfD of 0.2 mg/kg-day was previously entered on the IRIS data base in 1990. This value was based on the NOAEL of 223 mg/kg-day for increased relative liver and kidney weights in rats identified by the 13 week NTP gavage study (NTP, 1990a). A total uncertainty factor of 1000 was used to account for inter- and intraspecies extrapolations, for subchronic-to-chronic extrapolation and for limited reproductive and developmental toxicity data. Individual uncertainty factors were not identified. The two studies by Hsieh et al. (1989, 1990) were not discussed in the previous assessment of the RfD for toluene.

Despite its shorter duration and examination of only one sex of animals, the Hsieh et al. (1989) study was selected as the principal study for derivation of the RfD. Decreased thymus weight (32% decrease compared to controls) and decreased antibody response (>40%) were observed in the Hsieh et al. (1989) study at a dose of 105 mg/kg-day. The biological relevance of immunological effects bioassays is difficult to gauge and is the subject of some debate (Luster et al. 1992). However, given that thymus weight and antibody response was significantly decreased, coupled with the observed effects on host defenses following inhalation exposure (Aranyi et al., 1985), immunological effects were identified as the primary effect with decreased thymus weight as the critical effect. Decreased antibody response was not chosen as the critical effect due to a lack of confidence that the dose-response relationship is representative of chemical-induced immunological effects. Both the NTP (1990) and Hsieh et al. (1989) studies identified NOAEL and LOAEL values for altered relative liver weight, but the LOAEL of 105 mg/kg-day and NOAEL of 22 mg/kg-day identified by Hsieh et al. (1989) is lower than the NOAEL of 223 mg/kg-day defined by NTP (1990). However, the changes in liver weight were not correlated with any histological or anatomical alterations, thus this endpoint is not considered a critical effect.

An additional factor that supports the use of the Hsieh et al. (1989) study is that the method of oral exposure, i.e., ingestion via drinking water, is considered preferable to exposure via gavage that was utilized in the NTP (1990) study. For these reasons, and because data are inadequate to determine which species (rat or mouse) or which mouse strain (B6C3F1 or CD-1) is a more appropriate model for oral toluene toxicity in humans, the study with the most conservative (i.e., health protective) NOAEL, that from Hsieh et al. (1989), was selected for derivation of the RfD.

A route-to-route extrapolation from inhalation to oral exposure was considered inappropriate. Neurological effects are identified in numerous inhalation studies as consistent adverse effects following exposure to toluene. Potential neurological effects were identified in one oral study (Hsieh et al., 1990); however, the observed altered neurotransmitter levels cannot be defined as a clear adverse effect. No other studies identified neurological effects by the oral

pathway. Thus, sufficient evidence of a similar critical effect via the oral and inhalation pathways is lacking.

5.1.2. Methods of Analysis

The RfD was derived by the benchmark dose approach (BDS. Version 1.3). The benchmark response (BMR) was defined as the default of a change of one standard deviation (U.S. EPA, 2000d). Benchmark analysis was performed for thymus weight changes as an indicator of potential immunological effects. A BMDL of 41 mg/kg-day was derived and used as the point of departure. Details of the model results are presented in Appendix B.

Several well-documented PBPK models for toluene exist for rats and humans. However, to date they have not been applied to mice, nor have comparisons to *in vivo* pharmacokinetic data in mice been utilized to validate the applicability of these models in that species. The absence of an empirical PBPK model for mice precludes utilization of such a model in extrapolating from mice to humans.

5.1.3. RfD Derivation - Including Application of Uncertainty Factors and Modifying Factors

The BDL of 41 mg/kg-day for decreased thymus weight from the Hsieh et al. (1989) study was utilized as the basis for the calculation of the RfD.

Total UP – 1000.

A total uncertainty factor of 1000 was applied to this effect level: 10 for extrapolation for interspecies differences (UF_A; animal to human), 10 for consideration of intraspecies variation ($_{UFA}$; human variability), and 10 for use of a subchronic study to estimate chronic effects (UF_S; duration of exposure). The total UF = 10 x 10 x 10 = 1000.

A 10-fold uncertainty factor was used to account for laboratory animal-to-human interspecies differences (UF_A). No information is available on differences or similarities in the toxicity of toluene between animals and humans; there is a lack of human oral exposure data.

A 10-fold uncertainty factor for intraspecies differences (UF_A) was used to account for potentially sensitive human subpopulations. This UF was not reduced because of the lack of human oral exposure information.

A 10-fold uncertainty factor was used to account for extrapolating from less than chronic results on experimental animals when there are no useful long-term human data (UHs).

An uncertainty factor was not needed to account for extrapolating from a LOAEL to a NOAEL because BMD modeling was used.

Because a number of studies by both the oral and inhalation routes have demonstrated that toluene does not elicit developmental or reproductive effects except at doses that are significantly higher than those that cause other systemic effects (see Section 4.3), an additional database uncertainty factor was not deemed necessary. In addition, a 2-generation inhalation toxicity study is available which lends support to the oral database in that effects are noted only at high concentrations.

An additional modifying factor was not necessary.

The RfD for toluene was calculated as follows:

$$RfD = NOAEL \div UF$$
$$= 41 mg/kg/day \div 1000$$
$$= 0.04\ mg/kg/day$$

Confidence in the principal study is low, because while the study examined what appears to be the most sensitive endpoint, the study was of only 28 day duration and in a single species and sex. Confidence in the data base is rated medium due to the lack of chronic animal data and uncertainties regarding potential discrepancies in the low dose effects between the NTP (1990) 13 week study and the Hsieh et al. (1989) 28-day study. There is low confidence in the resulting RfD.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

A substantial data base examining the effects of toluene in chronically-exposed humans exists. These studies have identified neurologic effects (i.e., impaired color vision, impaired hearing, decreased performance in neurobehavioral analysis, headache, dizziness) as the most sensitive endpoints, though in many cases, it cannot be determined whether these effects are transient, persistent, or contain both transient and persistent components. Animal studies (NTP, 1990a) have also suggested respiratory irritation as a sensitive effect, but this effect in humans appears to occur only at higher exposure concentrations than those resulting in neurologic effects.

Two studies (Zavalic et al., 1998a; Eller et al., 1999) have identified no-effect levels in occupationally-exposed humans as shown in Table 1 (Section 4.5.2). Eller et al. (1999) reported no neurobehavioral effects in workers exposed to 25 to 32 ppm toluene for 1 to 12 years, while workers exposed to greater concentrations ($\geq$ 100 ppm) showed statistically significant neurologic impairment. Zavalic et al. (1998a) reported a NOAEL of 32 ppm and a LOAEL of 132 ppm for alterations in color vision in exposed workers. A number of additional studies (see Table 1), however, have identified effect levels for neurologic endpoints in exposed humans at levels between 40 and 100 ppm. 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 neurologic effects.

The study of Zavalic et al. (1998a) was selected as the principal study for derivation of an inhalation RfC. It is an adequate cross-sectional study of chronically-exposed humans which identified both a NOAEL (32 ppm) and LOAEL (132 ppm) for neurologic effects (impaired color vision). Impaired color 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 neurobehavioral and neuropsychological tests, but the effect levels in this study were less clearly characterized. Both of these NOAEL values lie slightly below the 40 to100 ppm range where available data (see Section 4.5.2, Table 1) suggest that persistent neurological effects in humans chronically-exposed to toluene begin to manifest.

An RfC of 0.4 mg/m^3 was previously entered on the IRIS data base in 1992. This value was based on the LOAEL of 88 ppm (332 mg/m^3) for decreased performance in neurological tests identified by the study of Foo et al. (1990). This LOAEL was adjusted to a continuous exposure level of 119 mg/m^3, and a total uncertainty factor of 300 (10 for use of a LOAEL, 10 for intrahuman variability, and 3 for data base deficiencies, including the lack of data and well-characterized laboratory animal exposures evaluating neurotoxicity and respiratory irritation) was applied. While the Foo et al. (1990) study was selected as the key study for the previous RfC derivation, a number of other studies provided evidence that neurological alterations would occur at toluene concentrations at or near the 88 ppm LOAEL. In addition, the Foo et al. (1990) study does not address co-exposure to other solvents and workers were exposed for a shorter period of time (i.e., 5.7 ±3.2 years).

In addition to neurologic effects in humans, the previous RfC was also based on irritation of the upper respiratory tract, specifically the nasal epithelium, as reported in the chronic NTP (1990) study in rats. However, these effects occurred in rats exposed to high concentrations (600 ppm or greater) of toluene, and did not show an appreciable increase with increasing concentration (i.e., the incidence of the lesions was greater at 600 ppm than at 1200 ppm). Support that the nasal lesions are a high-exposure phenomenon comes from the results of a chronic inhalation study in rats performed by CIIT (1980), which reported no effects on the nasal epithelium of animals exposed to 300 ppm. A 28 day inhalation study in rats (30 and 300 ppm) likewise failed to demonstrate treatment-related lesions in the nasal epithelium (Poon et al., 1994). Acute studies in humans have demonstrated that subjective reports of irritation of the nose and/or eyes occurs at exposure levels of 100 ppm or greater (Baelum et al., 1985, 1990; Echeverria et al., 1989; Andersen et al., 1983), but not at exposures below 100 ppm (Echeverria et al., 1989; Andersen et al., 1983). Because neurologic endpoints are a more sensitive endpoint for exposed humans, they alone were selected as the critical endpoint in the previous assessment for derivation of the inhalation RfC.

There is a growing body of literature indicating that chronic exposure to a variety of volatile organic solvents including toluene, styrene, perchloroethylene and mixed solvents is

associated with subtle deficits in visual perception measured either as deficits in color vision or deficits in visual contrast sensitivity. In several studies these two measures have been altered in concert. Other studies have measured or reported changes in only one parameter or the other. The growing body of evidence indicates a special susceptibility of visual perceptual measures to organic solvent exposures. These changes are considered adverse in their own right, but may also be indicators of additional neurological effects. For review articles see Geller and Hudnell (1997), and Fox and Boyes (2001) . The changes in color and/or contrast perception gives confidence that the changes in color vision reported by Zavalic (1998a) are a valid outcome of toluene exposure and, therefore, a legitimate basis of the RfC.

5.2.2. Methods of Analysis

The Zavalic et al. (1998a) study examined two exposure concentrations other than controls, and identified both a NOAEL of 32 ppm and a LOAEL of 132 ppm. Only one effect level was identified, i.e., 132 ppm, thus limiting the study's ability to describe the exposure-response relationship. Actual data points and statistics are not available; data are presented in graphical form only. Other studies of occupationally-exposed humans have identified adverse effect levels for neurologic effects ranging from 40 to 100 ppm, further supporting a NOAEL of 32 ppm. For these reasons, the data were not modeled and a benchmark concentration (BMC) approach was not used. A number of available PBPK models for toluene inhalation exist, but because the Zavalic et al. (1998a) study does not require extrapolation from animals to humans, from subchronic to chronic exposure duration, or from another route of exposure, applying these models is unlikely to reduce the uncertainty associated with the derivation of an RfC. The NOAEL value identified in the Zavalic et al. (1998a) study was, therefore, used for the derivation of the RfC.

5.2.3. RfC Derivation - Including Application of Uncertainty Factors and Modifying Factors

The NOAEL of 32 ppm (121 mg/m^3) from the Zavalic et al. (1998a) study was adjusted from an occupational exposure scenario to continuous exposure conditions as follows:

$$NOAEL_{(ADJ)} = NOAEL \times \frac{VEho}{VEh} \times \frac{5\,days}{7\,days}$$

$$= 121\,mg/m^3 \times \frac{10m^3}{20m^3} \times \frac{5\,days}{7\,days}$$

$$= 43\,mg/m^3$$

Where:
VEho = human occupational default minute volume (10 m^3 breathed during the workday)
VEh = human ambient default minute volume (20 m^3 breathed during the entire day)

Total UF–10

A total uncertainty factor of 10 was applied to this effect level, i.e., 10 for consideration of intraspecies variation (UF_H; human variability).

A 10-fold uncertainty factor for intraspecies differences (UF_H) was used to account for potentially sensitive human subpopulations.

An uncertainty factor to account for laboratory animal-to-human interspecies differences (UF_A) was not necessary because the NOAEL is based on human exposure data.

An uncertainty factor to account for extrapolating from less than chronic results was not necessary (UFs). Workers were exposed to toluene for a mean duration of 16 - 18 years.

An uncertainty factor was not needed to account for extrapolating from a LOAEL to a NOAEL because a NOAEL was available.

The data base for inhalation exposure to toluene is considered adequate. A single study of reproductive effects of humans occupationally exposed to toluene (Plenge-Bönig and Karmaus, 1999) reported no effects on male fertility, but a significant association between female exposure and reduced fertility was found. However, exposure levels for this study were not quantified, and confounding variables were not distributed evenly among the control and exposed groups, which hindered statistical adjustment. Numerous animal studies have demonstrated reproductive and developmental effects of toluene only at exposure levels which result in maternal toxicity (e.g., decreased maternal bodyweight). In addition, a 2-generation inhalation toxicity study is available.

An additional modifying factor was not necessary.

The RfC for toluene is derived as follows:

$$RfC = NOAEL_{(HEC)} \div UF$$
$$= 43.2\ mg/m^3 \div 10$$
$$= 4\ mg/m^3$$

Confidence in the principal study is medium. The Zavalic et al. (1998a) study is an adequate cross-sectional study in chronically-exposed humans that examined appropriate endpoints of concern at multiple exposure levels. However, only one effect level was identified, thus limiting the study's ability to describe the exposure-response relationship. Confidence in the database is high. Chronic studies in humans exist which identify subtle neurological alterations as a sensitive effect of long-term repeated toluene exposure at concentrations in the range of 40 to150 ppm. In addition, numerous animal studies of the reproductive and

developmental effects of toluene exist, which identify these effects only at exposure to much greater toluene concentrations. There is medium confidence in the resulting RfC.

5.3. CANCER ASSESSMENT

5.3.1. Oral Slope Factor

Not applicable.

5.3.2. Inhalation Unit Risk

Not applicable.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Toluene (CAS no.108-88-3) has the chemical formula C_7H_8 (structural formula $C_6H_5CH_3$) and a molecular weight of 92.14. At room temperature, toluene is a clear-to-amber colorless liquid with a pungent, benzene-like odor. Toluene has a low vapor pressure which can result in volatilization into the air. It is flammable, with a flash point of 4.4ºC. Toluene is strongly reactive with a number of chemicals, particularly nitrogen-containing compounds, and may react with some plastics. Toluene is used as part of an additive to gasoline mixtures (BTEX) to increase octane ratings, in benzene production, and as a solvent in paints, coatings, inks, adhesives, and cleaners. Additionally, toluene is used in the production of nylon, plastics, and polyurethanes. Toluene was once used as an anthelminthic agent against roundworms and hookworms.

Data on the effects of toluene in humans following oral exposure are limited to case reports of accidental oral ingestions. Both a 4 week study in mice (Hsieh et al., 1989, 1990) and a 13 week study in rats (NTP, 1990a) identified changes in liver weights as the most sensitive toxicologically relevant endpoints, with the 4 week study also reporting decreased thymus weight and decreased antibody response at the same exposure level. The 4 week studies in mice also noted changes in brain neurochemistry at lower exposure levels. However, these effects were not correlated with neurobehavioral of neuroanatomical changes, thus the relevance of these more sensitive changes is unclear.

A number of occupational studies have examined the effects of toluene exposure via inhalation. The most sensitive effects observed in humans following inhalation exposure are neurologic effects, including altered color vision, dizziness, fatigue, headache, and decreased performance in neurobehavioral tests. Exposure to higher levels in humans and animals have

resulted in respiratory tract irritation. Animal studies have also demonstrated effects on other organ systems, but only at high exposure levels (generally 600 ppm or greater).

In mothers who inhaled very high levels of toluene during pregnancy, the children showed a number of physical (small midface, deep-set eyes, micrognathia, and blunting of the fingertips) and clinical (microcephaly, CNS dysfunction, attention deficits, and developmental delay/mental deficiency) changes attributed to toluene. Animal studies of toluene inhalation have revealed delayed neurodevelopment and decreased offspring weight, though generally only at levels that also resulted in maternal toxicity. A series of studies in rats examining the developmental effects of oral toluene exposure found decreases in fetal body and organ weights, but only at levels equal to or greater than those that caused maternal toxicity. Gross malformations were not noted at any exposure level.

Toluene is classified as group D, *not classifiable as to human carcinogenicity*, based on the 1986 cancer guidelines (U.S. EPA, 1986a). Under the draft revised cancer guidelines (U.S. EPA, 1999), *data are considered inadequate for an assessment of the human carcinogenic potential* of toluene. Studies of humans who were chronically-exposed to toluene are inconclusive. Toluene was not carcinogenic in adequate inhalation cancer bioassays of rats and mice exposed for life (CIIT, 1980; NTP, 1990a). Increased incidences of mammary cancer and leukemia were reported in a lifetime rat oral bioassay at a dose level of 500 mg/kg-day, but not at 800 mg/kg-day (Maltoni et al., 1997). Toluene has generally not been found to be genotoxic in short-term testing.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

There are no chronic or subchronic oral dose-response data for toluene in humans. A single lifetime gavage study in rats (Maltoni et al., 1997) did not adequately examine noncancer endpoints, and was not suitable for use in derivation of an RfD. A 13 week gavage study by NTP (1990) identified a NOAEL of 223 mg/kg-day and a LOAEL of 446 mg/kg-day for increased relative liver and kidney weights in rats. The same study reported changes in relative liver weight at all doses of B6C3F1 mice, though changes in absolute liver weight occurred only in the 223 mg/kg-day (the lowest dose level examined) and 1786 mg/kg-day groups. Hsieh et al. (1989) conducted a 4 week drinking water study in CD-1 mice, which reported significant changes in relative liver and thymus weights compared to controls at 105 mg/kg-day, but not at 22 or 5 mg/kg-day; absolute liver weights were not reported. No histochemical effects on the liver was noted in either study. The Hsieh et al. (1989) study also reported changes in *ex vivo* immune response to mitogen stimulation at all exposure levels and significantly reduced antibody response at the highest concentration. A subsequent study by the same authors (Hsieh et al., 1990) reported neurochemical changes in the brains of CD-1 mice exposed to 5 mg/kg-day for 4 weeks. The biological significance of the neurochemical changes cannot be determined. Immunological effects were selected as the primary toxicological endpoint with decreased

thymus weight selected as the critical endpoint for RfD determination. The previous RfD of 0.2 mg/kg-day that was entered on IRIS in 1990 was based on changes in relative liver weights (NTP, 1990). The data of Hsieh et al. (1989, 1990) were apparently not considered in the previous derivation of the RfD. The Hsieh et al. (1989) study identifies effects at a lower dose than the NTP (1990) study and is a drinking water study as opposed to gavage. A BDL of 41 mg/kg-day was derived based on decreased thymus weight. A composite uncertainty factor of 1000 (10 for animal to human extrapolation, 10 for intrahuman variability, and 10 for use of a subchronic study) was applied to give a chronic RfD of 0.04 mg/kg-day. Confidence in the principal study is low, because the study was of only 28 day duration and in a single species and sex. Confidence in the data base is rated medium due to the lack of chronic data. There is low confidence in the resulting RfD.

6.2.2. Noncancer/Inhalation

A number of studies examining the toxicity of toluene following inhalation exposure in humans exist. The available data indicate that subtle neurobehavioral changes are the most sensitive effect of chronic inhalation exposure to toluene, with effects generally first being reported at concentrations between 40 and 132 ppm (see Table 1). The previous RfC of 0.4 mg/m^3 that was entered on IRIS in 1992 was based on a LOAEL of 88 ppm for neurobehavioral alterations in toluene-exposed workers identified by Foo et al. (1990); no NOAEL was identified by the study. Of the studies now available, the study of Zavalic et al. (1998a) was selected as the principal study for RfC derivation, as it established both a NOAEL of 32 ppm and a LOAEL of 132 ppm for impaired color vision in exposed workers. The NOAEL of 32 ppm (121 mg/m^3) was adjusted for continuous exposure, resulting in a $NOAEL_{(ADJ)}$ of 43.2 mg/m^3. To this, an uncertainty factor of 10 (for intrahuman variability) was applied to give a chronic RfC of 4 mg/m^3. Confidence in the principal study is medium, as the Zavalic et al. (1998a) study is an adequate cross-sectional study in chronically-exposed humans that examined appropriate endpoints of concern at multiple exposure levels. However, only one effect level was identified, thus limiting its ability to describe the exposure-response relationship. Confidence in the database is high; several chronic studies in humans exist that examine the most sensitive endpoint, and numerous studies of the reproductive and developmental effects, including the results of a 2-generation reproductive toxicity study, exist. There is medium confidence in the resulting RfC.

6.2.3. Cancer/Oral and Inhalation

Data in both humans and animals are inadequate to evaluate potential associations between toluene exposure and human cancer. Lifetime inhalation studies in both rats and mice (CIIT, 1980; NTP, 1990a) failed to report any increase in carcinogenicity as a result of toluene exposure. A lifetime gavage study in rats (Maltoni et al., 1997) reported an increase in toluene-induced tumors in both males and females, but the study was lacking in experimental detail and the dose-response relationship was inverse at the highest dose, making the interpretation of the study difficult. Toluene is, therefore, classified as group D, *not classifiable as to human*

carcinogenicity, based on the 1986 cancer guidelines (U.S. EPA, 1986a). Under the draft revised cancer guidelines (U.S. EPA, 1999), the *data are considered inadequate for an assessment of human carcinogenic potential*.

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Appendix A. Summary of External Peer Review and Public Comments and Disposition

Appendix B. Benchmark Dose Modeling Results and Output

Benchmark dose (BMD) modeling was performed to identify the critical effect level for derivation of the RfD for toluene. The modeling was conducted according to draft EPA guidelines (U.S. EPA, 2000d) using Benchmark Dose Software Version 1.3 (BMDS), available fromU.S. EPA (U.S. EPA, 2001). The BMD modeling results are summarized in Table B-1; the output is attached. A brief discussion of the modeling results is presented below.

Since the endpoint is a continuous variable, the continuous models available with BMDS (power, polynomial, and Hill models) were used. The hybrid model was not used, because the hybrid model software in BMDS is still undergoing Beta-testing, and was not considered sufficiently validated to use a BMDL from this model as the basis for the quantitative dose-response assessment. (The hybrid modeling approach defines the benchmark response [BMR] directly in terms of risk, as opposed to the standard approach, which defines the BMR in terms of a change in the mean.) For all of the modeling conducted, the BMR was defined as a 1.0 SD change in the mean, since this is the default measure recommended by the U.S. EPA (U.S. EPA, 2000d) in the absence of a clear biological rationale for selecting an alternative response level.

Table B-1: Benchmark modeling summary for relative thymus weight, Hsieh et al. (1989)

Concentration in drinking water (ppm)	Exposure, mg/kg-day	Number examined	Thymus weight, in g/100g body weight (± SE)
0	0	5	0.19 ± 0.02
20	5	5	0.18 ± 0.01
100	22	5	0.18 ± 0.02
500	105	5	0.13 ± 0.02

Continuous models	Goodness-of-fit p-value	AIC	Maximum χ^2 residual near POD	EC_S[a] (mg/kg/d)	LEC_S[a] (mg/kg/d)
Linear *	0.91	-109	0.14	66	41
Polynomial	0.70	-107	0.14	77	41
Power (power parameter restricted)	0.69	-105	0.14	76	45
Hill (power parameter restricted)	NA	-103	0.14	44	4.6

* Linear model selected as best fitting model, with lowest AIC among those with adequate goodness-of-fit test results (p>0.10). Linear model is given by:

$P(d) = b_0 + b_1 \times d$, where $b_0 = 0.19$

= -0.0005

[a] EC_S, LEC_S correspond to a benchmark response of 1 standard deviation from the control mean. For this endpoint, this was estimated at 0.036g/100g body weight, approximately a 20% change from the control mean.

```
 ================================================================
         Polynomial Model. $Revision: 2.1 $ $Date: 2000/10/11 17:51:39 $
         Input Data File: F:\USER\KHOGAN\_BMDS\TOLUENE_RFD.(d)
         Gnuplot Plotting File:  F:\USER\KHOGAN\_BMDS\TOLUENE_RFD.plt
                                                  Thu Jun 13 16:17:19 2002
 ================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = MEAN
   Independent variable = dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =   0.00163072
                            rho =            0   Specified
                         beta_0 =     0.188068
                         beta_1 = -0.000547506



                           Parameter Estimates

        Variable           Estimate            Std. Err.
           alpha          0.00131665         0.000416362
         beta_0            0.188068           0.0102851
         beta_1         -0.000547506         0.000191535


            Asymptotic Correlation Matrix of Parameter Estimates

                   alpha       beta_0       beta_1
     alpha             1    -9.7e-011     3.4e-011
    beta_0    -9.7e-011            1        -0.61
    beta_1     3.4e-011        -0.61            1


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean    Obs Std Dev   Est Mean   Est Std Dev   Chi^2 Res.
------     ---   --------    -----------   --------   -----------   ----------

    0       5       0.19       0.0448        0.188       0.0363         0.055
    5       5       0.18       0.0224        0.185       0.0363        -0.138
   22       5       0.18       0.0448        0.176       0.0363         0.110
  105       5       0.13       0.0448        0.131       0.0363        -0.028


  Model Descriptions for likelihoods calculated


 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2
```

```
 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                      Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
              A1           56.418772       5    -102.837544
              A2           57.808114       8     -99.616228
           fitted          56.326638       2    -108.653276
              R            52.388059       2    -100.776118

 Test 1:  Does response and/or variances differ among dose
levels
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous (A1 vs A2)
 Test 3:  Does the Model for the Mean Fit (A1 vs. fitted)

                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df     p-value

   Test 1              10.8401          6         0.01262
   Test 2              2.77868          3           0.427
   Test 3             0.184268          2           0.912

The p-value for Test 1 is less than .05.  There appears
to be a
difference between response and/or variances among the
dose levels.
It seems appropriate to model the data

The p-value for Test 2 is greater than .05.  A
homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .05.  The model
chosen appears
to adequately describe the data


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

 BMD =       66.2745


BMDL =       40.5754
```