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NCEA-S-1264

TOXICOLOGICAL REVIEW

OF

TOLUENE

(CAS No. 108-88-3)

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

December 2003

NOTICE

This document is an **external review 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 202-566-1676.

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This document and the accompanying IRIS Summary have previously undergone external peer review in August 2002. Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

Acknowledgments: The authors appreciate the contributions of Stiven Foster (NCEA) and Martin Gehlhaus (ASPH Fellow-EPA).

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 $µg/m^3$ 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 the hazard identification and dose-response assessment 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: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *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), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council*

Handbook: Peer Review (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA 2000d).

The literature search strategy 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, DART/ETIC, TOXLINE, CANCERLIT and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through July 2003.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Toluene is also known as toluol, phenylmethane, methylbenzol, methylbenzene, 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 volatilization. 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 react with 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 (1993) has promulgated an 8-hour PEL of 200 ppm (754 mg/m^3).

Toluene is used as an additive to gasoline mixtures 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.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

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 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.

The uptake by the lungs of experimental animals has been studied. The uptake ranged from 26 to 93% with a mean of 60% (Egle, 1976; Hobara et al., 1984; Bergman, 1979). Gospe and Al-Bayati (1994) compared oral and inhalation exposures to toluene in the rat. Male Fischer 344 rats were exposed to ^{14}C-toluene by gavage or inhalation. Oral doses of 110, 336, 741, and 911 mg/kg were administered to 82 rats and blood toluene levels were followed for six hours. For the 120 rats in the inhalation group, three-hour exposures were given at 10, 99, 549, or 1145 ppm. Blood toluene levels were measured during the uptake (exposure) phase and for a four-hour elimination period. The data from the two exposure methods were fitted to parametric kinetic models, and the resulting curves integrated. The authors concluded that oral dosing produces blood toluene levels that are similar to those produced by inhalation; however, the shape of the time-concentration profile differed for the two methods. Inhalation curves of concentration versus time reached asymptotic levels by one to two hours. Oral blood toluene curves reached

asymptotic levels from 1.6 to 6.3 hours post exposure. This suggests a slower absorption via the oral route as the concentration increased.

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 ~2μmol 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 while working with a toluene-based paint; the greatest concentrations upon autopsy 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 concentration of toluene was 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

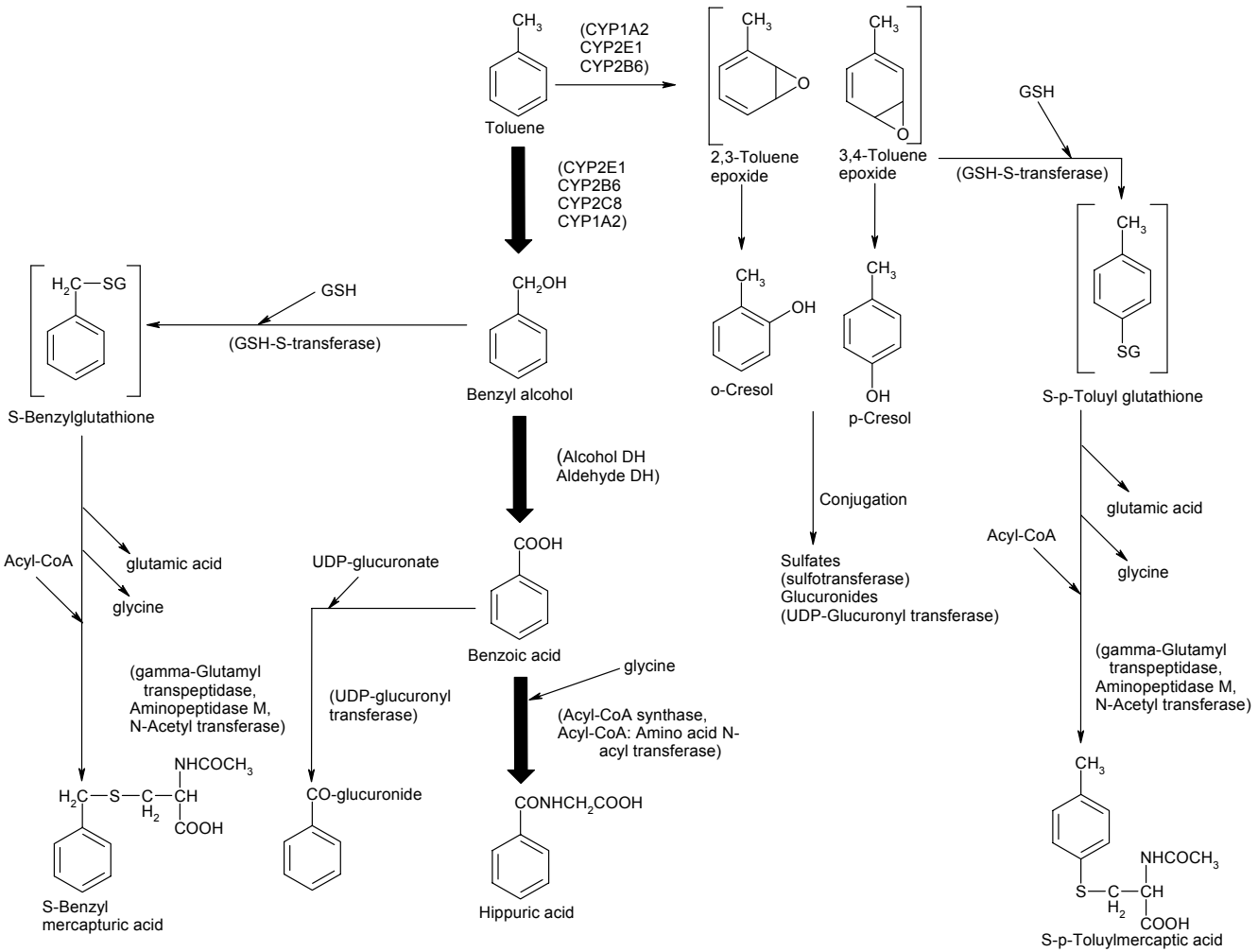
between blood and tissue levels of toluene, particularly for the brain (Benignus et al., 1984; Harabuchi et al., 1993). Toluene is able to cross the placenta and enter the fetus (Ghantous and Danielsson, 1986) and can be found in breast milk (Pellizzari et al., 1982).

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 liver is expected to be the primary site of toluene metabolism. Toluene is metabolized by sequential hydroxylation and oxidation to benzoic acid. The conjugation of glycine with benzoic acid to form hippuric acid constitutes the major route of toluene detoxification and elimination. The initial step in toluene metabolism is transformation by CYP enzymes which occurs mainly in the liver. The most prominent of these transformations is hydroxylation of the methyl group forming benzyl alcohol. Benzyl alcohol is primarily oxidized to benzoic acid, then conjugated with glycine to form hippuric acid. A minor CYP-related pathway 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
S-benzyl mercapturic acid (conjugation to benzyl alcohol), or S-*p*-toluyl glutathione and S-*p*-toluylmercaptic acid (conjugation to the epoxidated ring).

Studies of urinary metabolites in toluene-exposed humans have identified hippuric acid as the major metabolite (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).

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

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). Turkall et al. (1991) estimated that ~22% of a single oral dose is eliminated in the expired air in rats with the remainder being mainly eliminated in the urine.

Kostrzewski and Piotrowski (1991) have shown that initial elimination of toluene from blood upon termination of exposure is rapid in the range of a few minutes. At an exposure level of 34 ppm, the blood level at 16 hr postexposure was 2% of the maximum blood level reached. Blood levels immediately after exposure reflect TWA exposure during the preceding 8 to 10 hr (Foo et al., 1988). Because of the slow release of toluene from adipose tissue (half-life of 80 hrs), blood toluene levels on Monday mornings before work or near the end of the work week were observed to correlate with exposures during the preceding week (Nise et al., 1989). This slow decline in blood toluene has been demonstrated to result in detectable levels in workers who ceased exposure 2 weeks previous to sampling (Nise and Orbaek, 1988).

Urinary hippuric acid levels have generally been used as a biomarker of exposure to toluene. However, because of its short half-life (Lowry, 1987), hippuric acid levels correlate best to acute exposure situations. Blood concentration of toluene have been reported as the most reliable measure of toluene exposure (Kawai et al., 1993; Brugnone et al., 1995). Mizunuma et al. (1994) found blood toluene to be highly correlated to toluene in air at levels approximating 1 ppm where hippuric acid measurements as a marker of exposure are no longer useful.

3.5. PHYSIOLOGICALLY-BASED TOXICOKINETIC (PBTK) MODELS

PBTK 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 PBTK 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 PBTK model developed by DeJongh and Blaauboer (1996) is similar in design to the Tardif et al. (1993) rat PBTK 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 PBTK 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). This model is an adaptation of the Ramsey and Andersen (1984) design with the addition of a fifth compartment, a nonmetabolizing milk compartment with a varying volume. PBTK models for the oral route of exposure and for species other than the rat and human are not presently available.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS

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

Kiyokawa et al. (1999) conducted an electrophysiological evaluation of the visual function of patients with toxic neuropathy caused by toluene abuse. Fifteen patients (mean age 25.6 years, eight men and seven women) were diagnosed with bilateral optic neuropathy. Pattern visual evoked cortical potentials (PVECPs) and clinical symptoms were investigated. Visual acuities at the initial visit were less than 0.1 in 5 cases and 0.1-1.0 in 10 cases. PVECPs were followed up in the 15 cases. At the first recording, PVECP were nonrecordable in both eyes of 11 cases, the P100 peak latency was prolonged in both eyes of 3 cases, and only 1 case showed a normal P100 peak latency. After treatment, visual acuities improved more than 2 lines in 6 cases, 3 of whom showed normal P100 peak latency in the PVECPs. Visual prognosis and PVECP changes were identical in both eyes of all patients. In patients with toluene optic neuropathy, the P100 peak latency of PVECP shortened as visual acuity improved. The authors concluded that VECP abnormalities in these patients suggest that there is a severe effect on the optic nerve after prolonged exposure to toluene.

Baelum et al. (1985) investigated the effects of acute toluene exposure to 43 printers with 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 acutely 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 may develop 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.

Muttray et al. (1999) investigated the acute effects of high doses of toluene on color vision. Eight male printshop workers who had been working in the printing industry for 9.8 years (SD 5.4) were examined before and after cleaning printing containers with pure toluene. After cleaning, concentrations of toluene in blood were between 3.61 and 7.37 mg/l. Color vision was tested with the Farnsworth panel D-15 test, the Lanthony desaturated panel D-15 test, and the Standard Pseudoisochromatic Plates part 2. Eight workers of a metal-working factory without any neurotoxic exposure were tested according to the same procedure and served as controls. Acute exposure to toluene did not cause impairment of color vision. As noted by the authors, statistical power is limited due to the small number of exposed subjects.

4.1.2.2. Prechronic And Chronic Studies

A number of subchronic and chronic human studies are available. The majority of these studies are occupational studies. The studies described below do not necessarily constitute a complete treatise of the available studies but provide a representative selection of the low-dose studies available.

Hunnewell and Miller (1998) reported a case study where a 36-year old chronic toluene abuser exhibited slurred speech, progressive ataxia, blurred vision, and oscillopsia. Examinations showed dysconjugate torsional nystagmus and bilateral internuclear ophthalmoplegia (INO). Magnetic resonance imaging analysis showed generalized atrophic changes of the brainstem and cerebellum and diffuse atrophy of the corpus callosum.

Antti-Poika et al. (1985) examined the neurotoxic effects of toluene in 43 male rotogravure printers exposed to toluene. The mean age of workers was 41 years and the mean duration of exposure was 21.7 years (range of 11-40). A control group of 31 male offset printers of the same age with slight exposure to aliphatic hydrocarbons (mineral oils and isopropyl alcohol) was studied for comparison. A neurological examination, tests for autonomic nervous function, electroencephalography, psychological tests and computerized tomography of the brain were carried out in addition to a standardized interview. Exposure levels were evaluated for each person separately on the basis of his work history and the results of an earlier study on exposure levels at the same printing shops. Besides a thorough history of alcohol consumption, information about the printers' drinking habits was obtained from the occupational health care centers of the printing shops. The examinations found only slight abnormalities, and there were no statistically significant group differences in the prevalences of abnormalities. No correlations between the abnormalities and the exposure indices were found. This study detected no clinically significant abnormalities attributable to toluene alone among workers exposed to 68-185 ppm (mean 117) of toluene.

Yin et al. (1987) reported on a cohort of over 300 Chinese 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. Serum lactate dehydrogenase activity was statistically significantly decreased in males and females and leucine aminopeptidase activity was statistically significantly decreased in females only 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. (1987) study 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, were not identified by this study.

Ørbaek and Nise (1989) examined 30 Swedish 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 (11 ppm; 9 workers) and 157 mg/m^3 (42 ppm; 11 workers) toluene. The groups of workers were pooled for analysis. 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 controls, the printers complained 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 were identified for this study.

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 were reported as an 8-hour time-weighted average (TWA), although the number of samples taken and the actual sampling period were not given. The exposed and control cohorts (n=30) 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 including tests measuring manual dexterity (grooved peg board), visual scanning (trail making, visual reproduction, Benton visual retention and digit symbol) and verbal memory (digit span). When individual test results were linearly regressed against personal exposure concentrations, the slopes of the regression lines were all significantly nonzero indicating performance was related to toluene exposure. 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.

Nakatsuka et al. (1992) investigated color vision impairment in 111 women and 63 men employed in paint production or paint application plants. The reference population included 72 women and 48 men with no known occupational exposure to solvents. Solvent exposure was characterized as being more than 90% toluene with a geometric mean concentration of 44.1 $\pm$3.35 (mean $\pm$SD) ppm and 47.5 $\pm$3.93 ppm for men and women, respectively. The chemical identity of the remaining 10% solvent exposure was not determined. Exposure duration was not reported but mean age of exposed workers was 31.4 for men and 33.1 for men and women, respectively. Effects on color vision (dyschromatopsia) were examined using Lanthony's new color test and Ishihara' color vision test. No statistical significance in the prevalence of color vision impairment in workers was found in comparison to controls. No NOAEL or LOAEL was identified but the low dose (44 ppm) was without effect.

Abbate et al. (1993) conducted examinations of neurologic effects of toluene exposure by brainstem response audiometry on 40 rotogravure printing workers who had been exposed to an average of 97 ppm (366 mg/m^3) toluene, measured in the individual workplaces on the day of testing, for 12-14 years and 40 matched controls. All workers had normal hearing capacity. Examinations taken at random addressing one ear of each subject determined the brainstem auditory evoked potential (BEAP) with 11 and 90 second stimulus repetitions per second. Statistical analysis was performed on the latencies of waves I, III, and V generated. The mean latencies in BEAP were significantly higher in the exposed group relative to controls for each latency interval. This study identified a LOAEL of 97 ppm for increased wave latencies for auditory-evoked brain potentials; no NOAEL was identified.

Murata et al. (1993) examined 10 rotogravure printers from Saitama and 10 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 a mean exposure duration of 11 years (range of 1-36). Exposed workers were matched for age but not alcohol consumption. Blood samples for toluene analysis were taken 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.

Vrca et al. (1995, 1996, 1997) examined a group of 49 Croatian rotogravure printing workers relative to 59 controls for alterations in visual evoked potentials [VEP] (Vrca et al., 1995) changes in brainstem auditory evoked potentials [BAEP] (Vrca et al., 1996, 1997) as measured with a brain imager. Average length of work service for the printers and controls was 21.4 years (range 4-30, SD 7.4) and 20.6 years (range 4-32, SD 7.7), respectively. Exposure concentrations in air were not measured. 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. Parameters of exposure were measured in the morning in the middle of the work week and are thought to give the best approximation for the whole work week (WHO, 1985) because, in the case of exposure to low levels of toluene, low body accumulation occurs. The exposure levels were estimated to range from 40 to 60 ppm (151-226 mg/m^3) based on the average concentration of hippuric acid in urine after the workshift. 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). Effects on BAEP waves P1 through P5 were also examined and reported in Vrca et al. (1996, 1997). A significant decrease in wave amplitude and prolongation of P1 wave latency and an increased interval of interpeak latencies (P3-P5) was found in exposed workers. There was a statistically significant correlation between latency of the waves and the length of exposure for all waves except the P2 wave (Vrca et al.,

1997). Wave latency was significantly longer in exposed subjects which, according to the authors, could account for the reduced conduction in certain segments of the visual pathway. 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.

Boey et al. (1997) examined a group of 29 electronics workers from Singapore who were occupationally exposed to toluene for neurobehavioral changes relative to a group of 29 controls. The TWA level of toluene in air was 90.9 ppm (343 mg/m^3) as assessed by passive absorption monitors on the day of testing. Mean blood concentrations of toluene at the end of the work shift were 1.25 μg/L (SD = 0.37 μg/L). Occupational exposure in this group was for an average of 4.9 (range of 1 to 13 years; SD = 3.5 years). 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 was found to be decreased in a statistically significant manner 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.

Morata et al. (1997) examined 124 workers at a rotogravure printing facility in Brazil for changes in hearing. No control subjects were reported. Toluene levels in the air ranged from 0.14 to 919 mg/m^3 (0.04 to 243 ppm). Workers were exposed to varying levels of noise and an organic solvent mixture of toluene, ethyl acetate and ethanol. 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. The workers underwent pure-tone audiometry and immittance audiometry testing. Forty-nine percent of the workers had hearing loss. 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. The odds ratio estimates for hearing loss were 1.07 times greater for each increment of 1 year of age [95% confidence interval (95% CI) 1.03-1.11] and 1.76 times greater fro each gram of hippuric acid per gram of creatinine (95% CI 1.00-2.98). No NOAEL or LOAEL was identified.

Stengel et al. (1998) assessed immunologic and early renal effects of chronic toluene exposure in a longitudinal study of 92 printers and 74 control subjects. Pre- and poststudy samples of blood and urine were taken for the following measurements: immunoglobulin E (IgE), antiglomerular basement membrane (anti-GBM) and antilaminin (anti-LAM) antibodies in blood; creatinine and beta2-microglobulin in blood and urine; and microalbumin, N-acetyl-b-D-glucosaminidase (NAG) and alanine-aminopeptidase in urine. Creatinine clearance was calculated according to the Cockroft-Gault formula. Eight-hour personal air samples were collected twice to assess present exposure to toluene. The mean exposure concentration was 50 ppm (187 mg/m^3; range of 26 to 62 ppm). A job-exposure matrix was developed to estimate past cumulative exposure. The mean duration of exposure was 16.3 years (SD 13.1) and 16.9 years (SD 12.2) for exposed and control workers, respectively. Information about potential confounders was recorded by questionnaire. Multiple regression analysis was performed to study dose-effect relations adjusted for age and smoking. No relationship was observed between the markers studied and present exposure to toluene except that creatinine clearance was higher

among the exposed subjects than among the controls. A dose-response relationship was observed between cumulative toluene exposure and both IgE and NAG excretion. No relationship was observed between hypertension and exposure, but the relationship with NAG did not persist when subjects with hypertension were excluded. According to the results of this study, toluene at 50 ppm is not related to detectable renal dysfunction. A NOAEL of 50 ppm was identified.

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. Air sampling tubes were fixed onto the work tables or machines at nose height. Air was collected continually throughout the workday. 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. Samples were taken in the middle of the work week because WHO (1985) determined that, in the case of toluene exposure where low body accumulation occurs, this sampling period gives the best approximation of exposure.

Analysis of color vision was performed using the Lanthony D-15 desaturated panel, which is based on the ability to recombine a set of 15 desaturated color caps according to a definite chromatic sequence. The Lanthony D-15 test was designed to measure small changes in color discrimination and has been used to monitor color vision changes which accompany disease progression or environmental chemical exposure (Iregren et al., 2002; Gobba and Cavalleri, 2003). This test differs from other color vision tests by its hue saturation and brightness. The colors are pastel (chromal saturation 2 on a scale of 0-14) and bright (value 8 on a scale of 0-10). Its sensitivity for acquired dyschromatopsia is explained by the observation that the first phases of color vision impairment manifest as paling of colors (Lanthony, 1984). The use of pale colors in the panel, contrary to the saturated version, permits the detection of early phases of trichromatic abnormalities.

Subjects who wore spectacles or contact lenses used them. The test was applied for each eye separately. The score index for both eyes was calculated according to a formula developed by Bowman (1982). 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, both in natural sunlight. Results are reported as the color confusion index (CCI) or age- and alcohol intake-adjusted color confusion index (AACCI). The CCI was calculated by dividing the results by an ideal or correct score. AACCI was calculated on the basis of age and alcohol intake influence on CCI in the nonexposed group. Deviation from the ideal score depended on the order in which each subject arranged the caps.

In the high-exposure group (group E2), there were statistically 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 statistically 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 ($p<0.05$) for 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. However, the mean ±SD AACCI scores were obtained from the author and are included in Appendix C (Table C-1). 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., 1996; Zavalic et al. 1998b, c). Zavalic et al. (1996) evaluated qualitative color vision impairment in the E1 group and controls using the Lanthony D-15 desaturated panel according to Verriest's classification: type I, loss in the red-green range; type II, loss in the blue-yellow and red-green ranges; and type III, loss in the blue-yellow range. Subjects were classified as dyschromates if specific acquired loss was determined in at least one eye. Color vision was described as normal, yellow-blue, red-green range or complex impairment. In the control group nine (31%) group members were found to have a blue-yellow range impairment and 20 (69%) had normal color vision. In the exposed group the blue-yellow range was impaired in 14 (34%) group members, one (2%) had a complex impairment, and 26 (63%) had normal color vision. There was no significant difference in the prevalence of impairment in the blue-yellow range between the examined groups, although the impairment in the exposed group was higher than in the control.

Zavalic et al. (1998b) evaluated qualitative color vision impairment in the E2 group and controls using the Lanthony D-15 desaturated panel. As described above color vision was described as normal, yellow-blue, red-green range or complex impairment. Both blue-yellow and red-green color confusion were significantly increased in the exposed group compared to controls, but there was no significant difference in the prevalence of either type of color confusion.

Zavalic et al. (1998c) further evaluated qualitative color vision impairment in groups E1, E2 and controls. Type III dyschromatopsia (see definition in study description above) was detected in all groups examined: 26.6% of the workers in the nonexposed group, 31.7% of the workers in group E1, and 50% of those in group E2. As many as 15.6% of the workers in group E2, 4.8% of those in group E1, and 1.2% of those in the nonexposed group had type II impairment. A statistically significant difference in the prevalence of total dyschromatopsia (type II + type III) was established among the three groups together ($P < 0.01$), between group E2 and E1 ($P < 0.05$), and between group E2 and the nonexposed group ($P < 0.005$), whereas no significant decrease was found between group E1 and the nonexposed group. Type III impairment was significantly correlated with age in the nonexposed group ($P < 0.01$) and group E1 ($P < 0.005$). In group E2, both type II ($P < 0.05$) and type III impairment correlated with toluene in ambient air and with duration of exposure to toluene (both $P < 0.005$). In group E2, total dyschromatopsia correlated with toluene in ambient air and in blood (both $P < 0.05$) as well as with hippuric acid in urine ($P < 0.001$).

Muttray et al. (1999) investigated the chronic effects of toluene on color vision in eight male printshop workers who had been working in the industry for 9.8 years (SD 5.4). Eight workers from a data bank served as controls. Workers were matched for age, alcohol consumption, gender and smoking habits and were tested on a Monday prior to the work shift. Color vision was tested with the Farnsworth panel D-15 test, the Lanthony desaturated panel D-15 test, and the Standard Pseudoisochromatic Plates part 2. Exposure concentrations were not determined. The color confusion index of three workers was slightly elevated. The errors were of the blue-yellow type but were not statistically significant.

Eller et al. (1999) reported on the neurological effects of 98 male Danish photogravure printers chronically exposed to toluene. The study population consisted of 10 lithographers, 42 typographers, 23 printing workers and 23 employees with other work functions including blacksmiths, electricians and executive staff/foremen. Workers were divided into 3 Groups: no exposure to organic solvents (Group 0; n=19); those exposed to <20 ppm toluene for 1-12 years (Group 1; n=30); or those for greater than 12 years (Group 2; n=49). Workers exposed for 12 years or less were exposed to levels estimated at 25-32 ppm (94-121 mg/m^3), though some work conditions were described as involving 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. Exposure levels were estimated from multiple historical measurements of ambient air, personal air, etc. The workers were examined neuropsychologically using a Cognitive Function Scanner, and neurologically by computerized methods measuring coordination ability, tremor and position stability. For the scores of self-reported symptoms, Group 0 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.

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. Exposure was estimated as cumulative exposure since no changes in production technology had been introduced into the factory during the past few years and no significant variation in occupational exposure to toluene was expected. An index value was calculated that was representative of the total cumulative exposure to toluene: cumulative exposure = unmetabolized toluene (μg/L) x exposure duration (months). The mean value of unmetabolized toluene was 63 μg/L (SD = 27 μg/L). On the basis of previous data (Ghittori et al., 1987), this value was calculated to correspond to an environmental level of toluene of about 42 ppm. Exposure to other solvents (i.e., n-hexane, xylene, methyl isobutyl ketone, and ethyl acetate) was monitored on several occasions and levels were well below 1/100 of the occupational threshold limit. Thus, the authors considered exposure to these solvents as nonrelevant. 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. This study did not identify exposure levels of toluene, instead correlating response with urinary toluene levels. No NOAEL or LOAEL values were identified but statistically significant effects on color vision were observed at an estimated exposure level of 42 ppm.

Campagna et al. (2001) examined the relationship between acquired color vision loss and exposure to toluene and total hydrocarbons among 125 male workers in France. Seventy-two toluene-exposed printers were compared with 34 workers from the same photogravure plant with ambient background exposure, and with 19 workers from a bookbinding plant located in the same town (nonexposed). Duration of employment was 8 years (range of 1-35), 19 years (range of 2 to 37), and 18 years (range of 1-36) for the control, ambient exposure and exposed groups, respectively. The mean toluene exposure level at each individual workstation was estimated from 8 hr sampling on two separate occasions. No blood or urine sampling for toluene or metabolites was conducted. The mean current toluene exposure was 36 ppm (136 mg/m^3; range of 5^{th} and 95^{th} percentiles of 13 to 79) and 8 ppm (32 mg/m^3; range of 5^{th} and 95^{th} percentiles 4 to 20) for the exposed group and ambient exposure group, respectively. Historic exposure data from the last 30 years were used to construct two cumulative exposure indices, one for toluene and one for total hydrocarbons. The testing of visual function was performed within the first 3 hours of a day workshift. Color vision was assessed by the Lanthony D-15 desaturated panel. Color vision loss was quantitatively established by the Color Confusion Index (CCI) and classified by type of acquired dyschromatopsia according to Verriest's classification. A higher proportion of participants was affected with acquired dyschromatopsia (type I, II, or III) in the exposed worker group (52% among exposed participants and 56% among the group with an ambient exposure) than in the nonexposed group (21%). CCI was positively related to current airborne toluene levels, and cumulative exposure indices for toluene and total hydrocarbons ($0.18 \leq r \leq 0.35$). The CCI values for the mean (range) of both eyes were 1.08 (1.00-1.36), 1.19 (1.00-1.72), and 1.23 (1.00-1.81). Adjustment for age, daily alcohol consumption and duration of employment did not modify these relationships. Odds ratios of acquired dyschromatopsia were statistically significant for current airborne toluene, and toluene and total hydrocarbon past exposure (1.27

[1.02-1.58], 1.21 [1.04-1.39], 1.15 [1.02-1.31], respectively). No NOAEL or LOAEL was identified by the authors; however, statistically significant effects on color vision were noted at 8 ppm.

Neubert et al. (2001) and Gericke et al. (2001) reported on the acute and chronic health effects, respectively, of toluene exposure in a controlled, multicenter, blinded field trial in German rotagravure workers. For the acute studies, medical examinations (inquiries on subjective symptoms, and standard tests of psycho-physiological and psychomotor functions) were performed on almost 1500 volunteers of whom 1290 were toluene-exposed (1178 men and 112 women) and about 200 served as controls (157 men and 37 women). The psycho-physiologic and psychomotor tests used included digit span (verbal memory span), digit symbol (visuomotor performance), visual reproduction test (immediate visual memory), scales of self-feeling (self-rating of feeling), Wiener reaction test (auditory and visual vigilance), critical flicker fusion frequency test and personality dispositions. All volunteers were from the morning shift (6 hours exposure). Both individual ambient air concentrations (TWA) during the workshift, as well as blood toluene concentrations after the work shift were measured. For the endpoints evaluated, neither blood toluene levels of 850 to 1700 μg/L (high exposure group) nor ambient air concentrations (between 50 and 100 ppm or 188-375 mg/m^3) were associated with alterations in subjective symptoms or performance on medical examinations.

Adverse health effects associated with chronic exposure were evaluated by Gericke et al. (2001). Male volunteers (n=1226) were recruited and information on exposure and medical data was compiled for 1077 men in total. Evaluations included a physical examination, standard tests of psycho-physiological and psychomotor performance (identified above), self-reporting of subjective symptoms, and data on a variety of laboratory blood tests. The medical data were correlated with the length of toluene exposure and an estimate of the extent of exposure (i.e., highly exposed printers vs. other workers with negligible exposure). An examination of the influence of duration of exposure found no significant correlation to any effect that did not demonstrate a similar correlation with age, a covariable for length of employment. Volunteers reported a significant increased incidence of insomnia, dry mucus membranes, and allergies when compared to a reference population. Neither the exposure classification nor duration of exposure for the individuals reporting these symptoms were presented.

Chouaniere et al. (2002) conducted a cross-sectional study in two printing plants on 129 workers who were exposed to low levels of toluene. Ambient air sampling indicated toluene concentrations of 1 to 18 ppm in an offset printing plant and from 2 to 27 ppm in a heliogravure plant. Workers answered a self-administered questionnaire on neurotoxic symptoms and performed six psychometric tests on a computer-assisted version of battery studies. After adjustment for confounders, statistically significant changes were found in performance in Digit Span Forwards tests (decrement is 1 digit for 40 ppm; $P<0.04$) and Digit Span Backwards tests (decrement is 1 digit for 25 ppm; $P<0.01$). Neurotoxic self-reported symptoms were not statistically significantly correlated with current exposure. In addition, cumulative exposure was estimated. No association was found between estimated cumulative exposure and either psychometric performance or neurotoxic symptoms. No NOAEL or LOAEL was identified by the authors but statistically significant effects on psychomotor performance were observed for doses that would be equivalent to 25 and 40 ppm.

Zupanic et al. (2002) studied psychomotor performance and subjective symptoms in 278 male workers from 14 German rotogravure printing plants. The workers were divided into two exposure groups. Printers or print helpers from the rotogravure printing area with moderate exposure formed the exposed group (n=154). Workers with low exposure to toluene from the endprocessing area of the same plants were considered to be controls (n=124). The mean duration of employment was 15.3 years (SD=9.7; range of 7.5 to 23.3) for the exposed workers and 14.5 years (SD=8.6; range of 7.5 to 19.8) for the controls. Individual exposure to toluene was measured by two variables reflecting long term and current exposure. Long term exposure was calculated as lifetime weighted average exposure (LWAE). The calculation was based on individual job exposure matrices which were based on interviews related to job contact with toluene. These data were combined with historical measurements of toluene measurements in the air from 5 printing plants over the last three decades. The product of the different concentrations of toluene for different jobs (cumulative lifetime exposure) was weighted by the exposure time of a worker's life (LWAE). The current exposure to toluene was calculated as the mean of two to four measurements during normal working days with active sampling in the breathing zone. A mean LWAE of 45.1 ppm (SD=16.4; range of 34.2-57.9) toluene in air for exposed workers with a mean current exposure of 24.7 ppm (SD=17.6; range of 11.1 to 34.5) was found. Likewise, a mean LWAE of 9.3 ppm (SD=7.6; range of 5.8 to 10.6) toluene in air for control workers with a mean current exposure of 3.3 ppm (SD=4.8; range of 1.3 to 2.9) was found.

Psychomotor performance was determined by five subtests of the computer administered test battery motor performance series. The subtests included measures of steadiness, line tracing, aiming, tapping, and peg board. The tests were performed in sequence to examine dynamic and static elements of psychomotor performance of the upper limbs. Subjective symptoms were measured with the psychological-neurological questionnaire which includes information on psycho- and neurovegetative lability, neurological symptoms, lack of activation and motivation, excitability, lack of concentration and memory difficulties, and special symptoms that appear in subjects exposed to neurotoxicants (alcohol intolerance, and unpleasant taste and smell). No statistically significant differences were found between the two exposure groups. The results indicate no dose-response relationship for psychomotor functions and subjective symptoms among workers exposed to mean current concentrations of toluene of 3.3 and 24.7 ppm in air or mean LWAE concentrations of 9.3 and 45.1 ppm.

Schaper et al. (2003) studied the ototoxicity of occupational exposure to toluene in a longitudinal study over 5 years with four repeated examinations of 333 male workers from rotogravure printing plants. Past lifetime weighted average exposures (LWAE) to toluene and noise were determined from individual work histories and recent individual exposures were measured 10 times during the study by active sampling. The auditory thresholds were measured with pure tone audiometry. The mean LWAE exposures to toluene were 45 ppm (SD 17) for printers (high toluene exposure) and 10 ppm (SD 7.1) for end-processors (low toluene exposure). The mean current exposures to toluene during the study were 26 ppm (SD 20) for printers and 3 ppm (SD 3) for end-processors. Repeated measurement analyses (grouping factors: toluene intensity, exposure duration and noise intensity) and logistic regressions did not reveal statistically significant effects of toluene intensity, of exposure duration and of interactions between toluene intensity and noise intensity. A NOAEL of 45 ppm was identified for LWAE and 26 ppm for current exposure.

4.1.2.3. Cancer Studies

Svensson et al. (1990) examined the rates of cancer formation in 1020 past and present Swedish rotogravure printers occupationally exposed to toluene for at least 3 months between 1925 and 1985 in one of eight printing establishments. 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 time 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 increases 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 incidence into account, there was 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 Finnish 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. The authors computed the indirectly standardized incidence ratios (SIR) with 95% confidence interval (CI) with regard to age-, gender-, and period-specific incidence rates of cancer in the Finnish general population. The overall rate of cancer incidence for the total cohort was fairly similar to that of the general population. The risk for nervous system tumors was increased at 10 years after the first personal measurement (SIR 2.80, CI 1.03-6.08). No significantly increased incidence rates of cancer were associated with toluene exposure.

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 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. No NOAEL or LOAEL values were identified by this study.

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, 1990). 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. The only significant change in body weight was a decrease ($p<0.05$) for males in the 2500 mg/kg group. There were no significant changes in hematology or urinalysis for any group of animals. Some biochemical changes, including a significant increase ($p<0.05$) in SGOT in 2500 mg/kg males and a dose-related increase in cholinesterase activity in females receiving 2500 and 5000 mg/kg, were noted. 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. Absolute liver weights (mean $\pm$SE) in males were 10,490 $\pm$360 (100%), 11,310 $\pm$300 (108%), 11,850 $\pm$390 (113%), 14,440 $\pm$480 (138%), and 14,130 $\pm$1220 (135%) grams for 0, 312, 625, 1250, and 2500 mg/kg doses, respectively. Relative liver weights (mean $\pm$SE) in males were 33.3 $\pm$0.81 (100%), 34.5 $\pm$0.68 (104%), 35.9 $\pm$0.68 (108%), 45.0 $\pm$1.69 (135%), and 59.4 $\pm$3.28 (178%) grams/100 g body weight for 0, 312, 625, 1250, and 2500 mg/kg doses, respectively. Absolute kidney weights (mean $\pm$SE) in males were 1,084 $\pm$14 (100%), 1,159 $\pm$34 (107%), 1,213 $\pm$39 (112%), 1,292 $\pm$34 (119%), and 1,227 $\pm$114 (113%) grams for 0, 312, 625, 1250, and 2500 mg/kg doses, respectively. Relative kidney weights (mean $\pm$SE) in males were 3.5 $\pm$0.06 (100%), 3.5 $\pm$0.07 (100%), 3.7 $\pm$0.06 (106%), 4.0 $\pm$0.06 (114%), and 5.1 $\pm$0.32 (146%) grams/100 g body weight for 0, 312, 625, 1250, and 2500 mg/kg doses, respectively. In females, absolute and relative weights of the liver, kidney, and heart were all significantly increased at doses greater than or equal to 1250 mg/kg ($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 doses greater than 2500 mg/kg. Nephrosis was observed in rats that died, and damage to the tubular epithelia of the kidney occurred in terminally sacrificed rats. Kidney sections were examined in particular for the occurrence of hyaline droplets in the proximal tubules with negative findings. Histopathologic changes were also noted in the brain and urinary bladder (hemorrhages in the two highest dose groups). 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). Because the exposure was for 5 days/week, the dose is adjusted (e.g., 312 mg/kg 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 as no dose response was observed. 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/L 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. Antibody production was measured as anti-SRBC antibody and expressed as a titer. The anti-SRBC antibody in the serum collected was used in the plaque-forming colony (PFC) assay. Interleukin-2 (IL-2) production was determined in splenic lymphocytes from treated and control animals with or without Con A (to stimulate IL-2 production). Supernatants were assayed for IL-2 content by the ability to enhance proliferation of an IL-2-dependent murine T-helper cell line (HT-2 cells).

Toluene exposure did not result in increased mortality or clinical signs of toxicity 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 mice 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 (0.19 ±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 relative liver weight and a 32% decrease in relative thymus weight compared 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 statistically 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 two highest dose levels, the proliferative response was also statistically significantly decreased in response to LPS, PHA, and Con A. PFC response (i.e., the number of antibody producing cells measured as both PFC/10^6 spleen cells and PFC/spleen) to SRBC was statistically significantly reduced (46% and 63% of controls, respectively), as was IL-2 synthesis (45% of controls) following exposure to 105 mg/kg-day toluene. Based on a weight of evidence 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 [e.g., reduced PFC response (>40%) to SRBC]; the NOAEL is 22 mg/kg-day.

In a later study, Hsieh et al. (1990a) 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. This study did not identify a NOAEL or LOAEL.

Hsieh et al. (1990b) exposed CD-1 mice (5/group) for 28 days to 0, 80, or 325 mg/L toluene (purity 99.7%) in a comparison study with benzene immunotoxicity. Based on previous calculations of daily intake rates from this laboratory the doses are estimated at 0, 22 and 85 mg/kg-day, respectively. No differences were observed in kidney, liver, spleen, thymus, or whole body weights. Erythrocyte, leukocyte, and lymphocyte counts were normal. No effect was observed on the response of lymphocytes to stimulation with Con A, LPS, PHA, and PWM. Both treatment groups demonstrated a significant decrease in the incorporation of [^{3}H]TdR in cultures of mixed lymphocytes that were stimulated with allogenic yeast artificial chromosome (YAC-1) lymphoma cells. Uptake of [^{3}H]TdR was decreased by more than 50% for responder-to-stimulator ratios of 2:1 and 4:1 for both treatment groups. The report was unclear about the effect of toluene on the capacity of cytotoxic T lymphocytes to respond to YAC-1 cells. No difference in the synthesis of IL-2 was observed by measuring the uptake of [^{3}H]TdR in Con A-stimulated T-lymphocytes. The number of antibody plaque-forming cells (PFC) produced in response to SRBC was reduced in mice that received the highest dose. This reduction was observed when the results were expressed as the number of antibody PFC per million splenocytes, but was not apparent when expressed as the total number of PFC in the spleen. Toluene exposure had no effect on the production of SRBC antibodies. This study identified a LOAEL of 22 mg/kg-day for mixed lymphocyte culture response to YAC-1 cells.

Hsieh et al. (1990c) reported that under the same experimental conditions as above (Hsieh et al., 1990b), exposure to toluene did not alter tissue weights of whole brains or regional sections. Increased concentrations of biogenic amines [norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT)] and their major metabolites (vanillylmandelic acid (VMA), DOPAC, HVA, 5-HIAA) were observed in several regions of the brain following exposure to toluene. The increase in biogenic amines was generally not dose-dependent. The dose-response was biphasic. No LOAEL or NOAEL was established.

Hsieh et al. (1991) exposed groups of male CD-1 mice (5/group) to concentrations of 0, 20, 100, or 500 mg/L toluene (purity 99.7%) for 28 days. The authors calculated the equivalent doses from measured concentrations of toluene and water intake rates to be 0, 5, 22, and 105 mg/kg-day. Increased concentrations of NE and its major metabolite, VMA, were observed in all treatment groups. The highest level of NE was observed at the medium dose (22 mg/kg). Concentrations of NE were increased by 35-63% and concentrations of VMA by 66-150%. A 2.1- to 3.8-fold increase in the measured amounts of adrenocorticotropic hormone (ACTH) was observed with increasing dose. Corticosterone levels were elevated by more than 100% in the highest dose group at days 14 and 28. The production of IL-2 was measured by the uptake of [^{3}H]TdR in Con A-stimulated T-lymphocytes. Splenocytes from the high dose group exhibited a 25% decrease in IL-2 production. The toxic effect of increased activity in the hypothalamic-pituitary-adrenocortical axis is uncertain. For this reason, the decreased production of IL-2 in the 105 mg/kg-day dose group represents a LOAEL for this study; the NOAEL is 22 mg/kg-day.

Burns et al. (1994) used toluene as a comparative control in an investigation of the immunotoxicity of mono-nitrotoluenes. B6C3F1 mice (4 females/group) were treated with 0 or 600 mg/kg-day toluene by oral gavage for 14 days. No differences were observed in brain, liver, lung, spleen, thymus or body weights. No gross or histopathological lesions were identified. The mean number of leukocytes was 30% lower in treated animals while the mean number of circulating reticulocytes was almost twice the mean value for the control group. The impact on DNA synthesis in bone marrow cells was inconsistent. In an initial experiment synthesis was elevated by approximately 30%, but the control group value was lower than expected. The experiment was repeated (details not provided), and no increase in bone marrow DNA synthesis was observed. Toluene did not affect the proportion of T cells in the spleen, or the number of IgM antibody forming cells produced in response to sRBC. The number of IgG antibody forming cells produced in response to sRBC was not reported to be significantly different for either number of cells per 10^6 spleen cells or number of cells per spleen, but in both cases the mean for treated animals was approximately 40% lower than control group mean. There was no effect on the proliferative response of spleen cells to the B cell mitogen LPS or the T cell mitogen Con A. However, an increased proliferative response was observed with 5 μg/ml of the T cell mitogen PHA. Response of mixed leukocytes to DBA/2 allogenic cells was not affected by toluene exposure.

The delayed hypersensitivity response to keyhole limpet hemocyanin was not altered by toluene exposure. Serum complement levels, expressed as the number of cells necessary to lyse 50% of target cells (CH50), were depressed by approximately 50%, but the study's authors questioned the biological significance of this change. No alterations were observed in the

differential counts of peritoneal cells. There was no change in the percentage of fluorescent-labeled Covaspheres or chicken erythrocytes that were phagocytized by peritoneal adherent cells or exudate cells, respectively. A similar macrophage enzyme profile was observed in peritoneal cells for both treated and control animals. Toluene treatment did not affect the clearance of sheep erythrocytes by the reticuloendothelial system. Natural killer cell activity was decreased slightly at the smallest effector to target cell ratio (25/1), but this effect was not seen when the mice were stimulated with poly rI:rC. Toluene treatment did not alter the poly rI:rC inducible interferon levels. Host resistance to microbial and tumor challenge was measured by administration of the infectious agent on day 15. Host resistance to challenges with *Listeria monocyogenes*, *Streptococcus pneumoniae*, *Plasmodium yoelii*, or B16F10 melanoma were not affected. A reduced incidence of tumors was observed in mice that were challenged with PYB6 fibrosarcoma. The only dose of toluene used in this study, 600 mg/kg-day, was the LOAEL. A NOAEL was not defined.

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 effects 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 the incidence of oral cavity tumors in 800-mg/kg male rats only.

4.2.2. Inhalation Exposure

4.2.2.1. Prechronic Studies

Fischer 344 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. Concentration-dependent increases in the severity of nephropathy was observed. No evidence was found of an increase in hyaline droplets in the proximal tubules. Other changes that usually accompany an increase in hyaline droplet formation, such as granular casts at the junction of the inner and outer stripe of the outer medulla in short term studies, were not found. 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 did not indicate any neurobehavioral changes as a result of toluene exposure at these levels (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) 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. Magnetic resonance imaging (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). CD-1 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, 1990; Huff,

2003). 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. 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). Concentration-dependent increases in the severity of nephropathy was noted. No evidence was found of an increase in hyaline droplets in the proximal tubules. In addition, other changes that usually accompany an increase in hyaline droplets, such as linear mineralization of the medulla in long-term studies, were not found. No other increases in the incidence of non-neoplastic lesions were reported in exposed rats. No neoplasms were noted in male rats, and one nasal, two kidney, and two forestomach neoplasms observed in female rats were considered not to be assocaited with toluene exposure. 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, 1990; Huff, 2003). 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 later conduct a quality assessment procedure to review the data. The available pathology reports containing these data indicate that at a minimum 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. The 300 ppm exposure level is identified as a NOAEL for respiratory lesions but it is uncertain whether other respiratory effects may have occurred at lower doses..

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES - ORAL AND INHALATION

4.3.1. Studies In Humans

Toluene is believed to cause congenital defects in infants born to mothers who abused toluene during pregnancy. Exposure levels in the available studies, if reported at all, are very high. A detailed discussion of the high-dose effects of toluene on reproduction and development is beyond the scope of this document. As an example, 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 48 female Nya:NYLAR mice pre- and postnatally to toluene provided in the drinking water at concentrations of 0, 16, 80, or 400 ppm (estimated at 0, 7.2, 14.4 and 72 mg/kg-day, respectively). In this experiment mice were mated and assigned 12/group to one of the 4 exposure conditions. Exposure was begun on the first hour of the 60 hr mating period and continuing throughout pregnancy and lactation. The offspring were maintained on the same drinking water solution from weaning at 21 days of age through behavioral testing. Only 6 to 9 pregnancies/dose group were obtained. 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.

NIOSH (1983) conducted a study to determine the maximum tolerated dose (MTD) for toluene in adult female CD-1 mice and then used the MTD to determine adverse reproductive effects in timed-pregnant (5-days) mice. Doses of 0, 735, 1470, 2945, 5890, and 8700 mg/kg toluene were administered by gavage to groups of ten female mice once per day for eight consecutive days. Animals were observed for clinical signs of toxicity (including mortality) during the first hour after each dosing, with at least one additional post-dosing observation on the day of dosing. The mice were then observed once daily until the termination of the study. Animals were weighed on the first and last day of dosing, and also on days four and eight post-dosing. Decreases in body weight were less than 10% when compared to controls. All mice in the two highest dose groups (5890 and 8700 mg/kg toluene) died by dosing day three, while two animals receiving 2945 mg/kg died on dosing day 4. None of the animals in the two lowest groups (735 and 1470 mg/kg) died. The dose selected for the reproductive study was 2350

mg/kg. Toluene (2350 mg/kg) was administered daily to fifty timed-pregnant female mice by gavage between gestation days seven and fourteen. Females were weighed on day eighteen of gestation and day three postpartum. Beginning eighteen days after mating, cages containing pregnant females were inspected daily for the presence of a litter. The number of living and dead pups was counted within twelve hours of birth and then forty-eight hours later. Total litter weights were also measured at these time points. Females that were mated but did not deliver a litter by the twenty-third day after mating were sacrificed and necropsied, and the status of pregnancy was determined during necropsy. During the experiment only one test animal died. There were no statistically significant differences between test and control groups in any of the categories for evaluation of reproductive toxicity. A non-statistically significant trend towards lower weight gain in pregnancy was noted.

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. 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 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 the same study, groups of pregnant rats were exposed to 1800 ppm 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 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 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. 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.

Ono et al. (1995) exposed groups of pregnant Sprague-Dawley rats to 0, 600, or 2000 ppm (0, 2262, or 7540 mg/m^3) 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) 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 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 2600 and 3900 mg/kg male rats on day 7 and 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 the same study 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, 1990; Huff, 2003). 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 they could not be directly attributed to toluene exposure due to confounding factors.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS – 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 gavage study of toluene in F344 rats and B6C3F1 mice. In rats, which were more sensitive to toluene than mice, effects reported were in the kidney and liver, with organ weight changes at the low doses accompanied by nephrosis at higher doses with no evidence of hyaline droplet formation. At higher exposure levels an increased incidence of rats with mineralized foci and necrosis of normal brain cells was also observed; this effect was not noted in mice at any exposure level (NTP, 1990). Hsieh et al. (1990a, c) exposed CD-1 mice to toluene in drinking water for 28 days and at 5 mg/kg-day, significant changes in brain neurotransmitter levels were reported. Neurotoxicity studies from oral exposure to toluene have not been performed.

The immunotoxicity of toluene has been studied by several laboratories (Hsieh et al. 1989, 1990b, 1991; Burns et al., 1994) for purposes of comparison to benzene and nitrotoluenes (known immunotoxicants). Immunosuppressant effects from toluene exposure have been demonstrated in *in vivo* and *ex vivo* assays. However, the host resistance assays by Burns et al. (1994) indicate a lack of immunotoxic response when animals treated with toluene are

challenged. Host resistance to challenges with *Listeria monocyogenes*, *Streptococcus pneumoniae*, *Plasmodium yoelii*, or B16F10 melanoma were not affected at a dose of 600 mg/kg-day for 14 days. In addition, a reduced incidence of tumors was observed in mice that were challenged with PYB6 fibrosarcoma.

4.5.2. Inhalation Exposure

There are numerous occupational studies and case reports from inhalation exposure to toluene available in the literature. Many studies have been published examining neurological endpoints resulting from repeated exposure in occupationally-exposed workers. Results from these studies suggest that neurologic effects are the most sensitive endpoint following inhalation exposure to toluene. Two of the most studied endpoints at lower exposure levels include color vision deficits and hearing loss. A summary of selected subchronic and chronic occupational studies is shown in Table 1.

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 in the range of 1000-10,000 ppm. Magnetic resonance imaging (MRI) examinations of the brain of solvent abusers (Filley et al., 1990; Rosenberg et al., 1988a, b) suggest a preferential atrophy in lipid-rich regions of the brain. Rosenberg et al. (1988a, b) 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).

There is evidence that exposure to toluene results in both transient and persistent effects on neurologic endpoints. For example, Baelum et al. (1985) reported that the neurologic responses, including altered color vision, of rotogravure printers (average long-term toluene exposure of 9 to 25 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. (1998b) 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.

Table 1 - Selected Occupational Studies of Subchronic and Chronic Toluene Inhalation

Reference	Number of workers	Duration of exposure (mean years)	NOAEL (ppm)	LOAEL (ppm)	Effect
Abbate et al., 1993	exposed (n=40) control (n=40)	12-14	None*	97	Increased wave latencies for auditory-evoked brain potentials
Antti-Poika et al., 1985	exposed (n=43) control (n=31)	21.7	68-185	None	No neurotoxic effects
Boey et al., 1997	exposed (n=29) control (n=29)	4.9	None	90.9	Performance deficits in several neurobehavioral tests
Cavalleri et al., 2000	exposed (n=33) control (n=16)	9	42 ppm	None	No changes in color vision
Campagna et al., 2001	exposed (n=74) ambient exposed (n=34) control (n=19)	18 (exposed) 19 (ambient exposed) 8 (control)	8	36	Statistically significant changes in color vision
Eller et al., 1999	high exposed (n=49) low exposed (n=30) control (n=19)	>12 (high exposed) 1-12 (low exposed)	25-32	>100	Increased incidence of subjective symptoms, poorer performance in neurological testing
Foo et al., 1990	exposed (n=30) control (n=30)	5.7	None	88	Performance deficits in neurobehavioral tests in exposed workers
Murata et al., 1993	exposed (n=10) control (n=10)	11	None	83	Reductions in electrophysiological parameters (ECG and nerve conduction)
Nakatsuka et al., 1992	exposed (n=174) control (n=120)	__	44	None	No effects on color vision

Schaper et al., 2003	high exposed (n=155) low exposed (n=178)	—	26 (45 LWAE)	None	No effect on hearing parameters
Stengel et al., 1998	exposed (n=92) control (n=74)	16.3	50	None	No effects on measures of immune function and early renal dysfunction
Vrca et al., 1995, 1997	exposed (n=49) control (n=59)	21	None	40-60	Alterations in visual- and auditory-evoked brain potentials
Yin et al., 1987	exposed (n=94) control (n=129)	—	None	43	Increased prevalence of subjective symptoms (headache, dizziness) in exposed workers
Zavalic et al., 1998a	high exposed (n=37) low exposed (n=46) control (n=90)	16.2 (high exposed) 18.3 (low exposed)	32	132	Statistically significant increase in age- and alcohol-adjusted color confusion index
Zupanic et al., 2002	exposed (n=154) low exposed/controls (n=124)	15.3 (exposed) 14.5 (low exposed/control)	24.7 (45.1 LWAE)	None	No effects on psychomotor functions or subjective symptoms

* 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, 1990) only examined concentrations of 600 ppm and greater. Lifetime chronic (CIIT, 1980) and 28-day subchronic (Poon et al., 1994) studies have 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 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 may be noted at lower concentrations. 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 Benignus et al. (1998) address the issue of apparent rat-human sensitivity differences to toluene exposure. After using a PBTK 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 high doses ($\geq$ 1000 ppm) (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).

4.5.3. Mode of Action

Understanding of the mechanisms by which toluene may exert its toxic effects is limited. 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 CYP enzymes, 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 the other hand, 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.

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 a 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 toluene, but the observed neural demyelinization 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 mode of action of color vision loss induced by solvents is not known. In general, the blue-yellow range of color vision is affected but in some cases workers also have a red-green range deficit. According to "Koller's rule" this suggests a retinal location of the effect (Hart, 1992). One possible mechanism may be related to a direct effect of solvents (or metabolites) on cone function (e.g., membrane metabolism), or to an interference with neurotransmitters (like dopamine). Alternatively, color vision loss may be the result of a distal axonopathy of the optic pathway, as suggested by Shaumberg and Spencer (1978) for n-hexane.

Toluene causes alterations of the c-wave and the standing potential (Skoog et al., 1981) in monkeys which are due to changes of the potentials of the receptor-pigment epithelial complex (Griff, 1991; Steinberg et al., 1983). The retinal pigment epithelium has three major functions: vitamin A transport, phagocytotis of the upper tenth of the photoreceptor outer segment, and potassium buffering, in addition to transport and metabolism of different substances. However, patients suffering form chronic abuse of toluene and resultant visual disturbances have been shown to have optic neuropathies and changes in the electroretinograms which were different from those observed in the monkeys (Skoog et al., 1981; Toyonaga et al., 1989). Further research in this area is needed.

Little is known about the mode of action of renal toxicity following toluene exposure. Al-Ghamdi et al. (2003) found decreased cell viability, lactate dehydrogenase release, increased levels of malondialdehyde, increased CYP2E1 activity, but no DNA fragmentation when LLC-PK1 cells (proximal tubule cells) were exposed to 5 mM toluene for 48 hr suggesting necrosis as the predominant mode of cell death. The results of this study also suggest a pivotal role of CYP2E1 in the induction of oxidative stress and necrosis as the effects were inhibited by co-exposure to disulfuram (an inhibitor of CYP2E1). Some studies have shown that renal failure in toluene abusers was accompanied by myoglobinuria, which might be attributed to rhabdomyolysis and not to primary kidney toxicity (O'Brien et al., 1971; Reisin et al., 1975).

Other studies of organic solvents suggest that the mechanism of lesion formation in the kidney may be due to the induction of damage to the alveolar basement membrane leading to a production of alveolar basement membrane antibodies, which cross react with the glomerular basement membrane and initiate glomerular disease (Carlier et al., 1980). It should be noted, however, that ingestion of organic solvents is an established cause of acute renal injury (Drayer and Reidenberg, 1973; Crisp et al., 1979).

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall-Weight-of-Evidence

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, 1990; Huff, 2003), and 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). In the NTP (1990; Huff, 2003) studies no neoplasms were noted in male rats, and one nasal, two kidney, and two forestomach neoplasms observed in female rats were considered not to be assocaited with toluene exposure. No increase in the incidence of neoplasms was observed in mice. Toluene has generally not been genotoxic in short-term testing protocols. The previous IRIS assessment classified toluene as Group D (*not classifiable as to human carcinogenicity*) under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986c) based on inadequate data on the carcinogenicity of toluene in humans and inadequate evidence of carcinogenicity in animals. IARC has classified toluene as Group 3 (*not classifiable as to its carcinogenicity in humans*) with a supporting statement that there is inadequate evidence in humans and evidence suggesting a lack of carcinogenicity of toluene in experimental animals (IARC, 1999).

4.6.2. Synthesis of Human, Animal and Other Supporting Evidence

Available studies in toluene-exposed workers have reported very limited or no evidence of 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 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 but 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, 1990) 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 AND LIFE STAGES

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 shown 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 with respect to toluene 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 PBTK 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) PBTK 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 not reported sex-related differences in effects, with the exception of the study of Plenge-Bönig and Karmaus (1999), which reported decreased fertility in occupationally-exposed women, but not in occupationally-exposed men. In rats and mice exposed to toluene orally for 13 weeks (NTP, 1990), males of both rats and mice showed toxic effects at lower doses than females. Similarly, in 15-week inhalation studies (NTP, 1990), 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, 1990). 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

Color vision has been shown to decrease 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). These populations may be more susceptible than the general population to any decrements in color vision. 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.

Toluene is initially metabolized to benzyl alcohol by the microsomal mixed-function oxidase system. Subsequent oxidation to benzaldehyde and then to benzoic acid is carried out by alcohol and aldehyde dehydrogenase, respectively. There are two forms of the dehydrogenases (low K_m and high K_m). Japanese and possibly other populations of Asian origin and Native Americans have a defective gene for the low K_m dehydrogenase. When toluene exposed Japanese workers (both male and female) were evaluated for the defective gene, it was found that those

possessing the defective gene had lower levels of urinary hippuric acid and *o*-cresol than those with the normal of heterozygous gene (Kawamotot et al., 1994). Thus, these individuals may be at a higher risk of toluene-induced CNS impairment due to a decreased rate of metabolism, assuming that the parent chemical is responsible for adverse effects.

5. DOSE RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

No studies examining the chronic or subchronic 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 principal study for derivation of an RfD. One subchronic study examining oral exposure to toluene in rodents (rats and mice) is available. This study (NTP, 1990) was chosen as the principal study. The critical effect is increased kidney weight. NTP (1990) exposed both sexes of F344 rats and both sexes of B6C3F1 mice to toluene by gavage for 13 weeks. In male rats, absolute and relative weights of both the liver and kidney were significantly increased ($p<0.05$) at doses greater than or equal to 446 mg/kg-day. Absolute kidney weights were 100, 107, 112, 119, and 113 percent of controls; relative kidney weights were 100, 100, 106, 114, and 146 percent of controls for 0, 223, 446, 900, or 1800 mg/kg-day. 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 of 446 mg/kg-day. Histopathologic lesions in the liver consisted of hepatocellular hypertrophy, occurring at doses greater than 2500 mg/kg. Nephrosis was observed in rats that died, and damage to the tubular epithelia of the kidney occurred in terminally sacrificed rats. Kidney sections were examined in particular for the occurrence of hyaline droplets in the proximal tubules with negative findings. A concentration-dependent nephropathy was also seen in chronic inhalation cancer bioassays (NTP, 1990; Huff, 2003).

The choice of increased kidney weight as a critical effect is supported by several acute oral and inhalation toxicity human studies indicating renal tubule toxicity. One case report following lethal oral exposure to 625 mg/kg (Ameno et al., 1989) noted acute tubular necrosis and acidosis was reported in a nonlethal case report of thinner ingestion (Cavarti and Bjerk, 1997). Inhalation of high doses of toluene has caused distal renal tubular acidosis (Taher et al., 1974; Fischman and Oster, 1979) among drug users, sometimes with tubular proteinuria (Kamijima et al., 1994). A case of focal segmental glomerulosclerosis was noted for a leather worker exposed to toluene for 40 years (Bosch et al., 1988). Toluene sniffing has been associated with the formation of renal stones (Kroege et al., 1980), proteinuria (Streicher et al., 1981), and hepato-renal damage (O'Brien et al., 1971). In addition, a case of anti-glomerular basement membrane antibody-mediated glomerularnephritis has also been reported in a woman who sniffed glue for several weeks (Bonzel et al., 1987). It should be noted that several studies involving painters (Askergren, 1982; Franchini et al., 1983) or printers (Gericke et al., 2001) with toluene exposure have reported no effect on renal function. Askergren (1982) and Franchini

et al. (1983) found no effect on excretion of beta-2-microglobulin and Gericke et al. (2001) found no effect on serum creatinine levels or glomerular filtration rate.

A number of immunotoxicity studies are available (Hsieh et al., 1989, 1990b, 1991; Burns et al., 1994) and were considered for use as the principal study. Changes in thymus weight in the Hsieh et al. (1989) study was not considered an adverse effect since no change was observed in a later studies by Hsieh et al. (1990) and Burns et al. (1994). In addition, Luster et al. (1992) have indicated that thymus weights may not be predictive of immunotoxic potential based on an evaluation of studies conducted by NTP.

Additional effects on immunological endpoints were considered as potential critical effects. For example, statistically significant and dose-related decreases in antibody response were noted by Hsieh et al. (1989, 1990b, 1991). There is evidence that the antibody-forming cell assay (PFC) is among the most predictive tests available for immunotoxicity (Luster et al., 1992) and that suppression of the antibody response is predictive of decreased resistance to challenge with infectious agents or tumor cells (Luster et al., 1993). An important objective of the use of the PFC assay and anti-SRBC ELISA in immunotoxicity testing is to determine the ability of the immune system to respond to an antigenic challenge. As such, it tests the ability of three primary immune system cells (i.e., macrophages [phagocytosis and processing of SRBCs], T lymphocytes [assist B lymphocytes] and B lymphocytes [production and release of anti-SRBC specific antibody]) to respond to this antigen in a coordinated manner leading to the production of antibodies to SRBC. However, the host resistance assays by Burns et al. (1994) indicate a lack of immunotoxic response when animals treated with toluene are challenged. Host resistance to challenges with *Listeria monocyogenes*, *Streptococcus pneumoniae*, *Plasmodium yoelii*, or B16F10 melanoma were not affected at a dose of 600 mg/kg-day for 14 days. In addition, a reduced incidence of tumors was observed in mice that were challenged with PYB6 fibrosarcoma. For these reasons, immunotoxic endpoints are not considered critical effects.

Additional studies by Hsieh et al. (1990a, c, 1991) found statistically significant increases in brain neurotransmitter levels at exposure levels as low as 5 mg/kg-day. The study authors measured levels at one time point immediately at the termination of toluene treatment; it cannot be determined if the effects observed were persistent. Neurotoxicity studies from oral exposure to toluene have not been performed, therefore, the changes in neurotransmitter levels have not been correlated with behavioral, neuropsychological, or neuroanatomical changes. Available reproductive studies (Gospe et al., 1994, 1996; Gospe and Zhou, 1998, 2000) were conducted at higher doses than those used in the studies described above with minimal effects on dams and offspring.

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, 2000). Benchmark analysis was performed for absolute kidney weight changes in male rats (NTP, 1990). Male rat kidney data were chosen for BMD modeling as these data exhibited a greater response than that seen in female rats (see study description in Section 4.2.1.1). A BMDL of 238 mg/kg-day was derived and used as the point of departure. This corresponds to a 9% response level (i.e., increase in kidney weight from control). Details of the model results are presented in Appendix B.

PBTK models are available that describe the kinetics of toluene after inhalation exposure (Fisher et al., 1997; Pierce et al., 1996, 1999; DeJongh and Blaauboer, 1996, 1997; Tardif et al., 1993). When appropriate human and rat PBTK models are developed for the oral route of exposure they could be used to estimate human oral exposure levels associated with an appropriate internal dose. Alternatively, the available toluene PBTK models could be utilized to extrapolate the risks of neurotoxic outcomes from inhalation exposure to oral exposure. Route-to route extrapolation would be of particular interest for color vision deficits, which are the proposed critical effects from inhalation exposure. However, in the case of toluene, unpublished data from the laboratory of Dr. Philip Bushnell (memorandum dated October 29, 2003, from Dr. William Boyes, U.S. EPA, to Dr. Lynn Flowers, U.S. EPA "Potential use of PBTK modeling to support route-to-route extrapolation or duration adjustments for chronic exposure to toluene") suggests that behavioral deficits observed in rats exposed to toluene by inhalation exposure are not observed in rats given toluene by oral gavage at doses expected to produce the same concentrations of toluene in the brain. The mechanism for this apparent difference in the effect of toluene by the oral and inhalation routes is not understood at this time.

5.1.3. RfD Derivation - Including Application of Uncertainty Factors

The BMDL of 238 mg/kg-day for increased kidney weight from the NTP (1990) study was utilized as the basis for the calculation of the RfD.

Total UF – 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 (UF_H; 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.

A 10-fold uncertainty factor for intraspecies differences (UF_H) 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 (UF_s).

An uncertainty factor was not needed to account for extrapolating from a LOAEL to a NOAEL because NOAEL/LOAEL methodology was not used to identify the point of departure.

An oral subchronic study in two species and several immunotoxicity studies are available. 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 for details). The available toxicokinetic information indicates that the absorption kinetics of toluene is similar and extensive following both oral and inhalation exposure. For example, Gospe and Al-Bayati (1994) compared oral and inhalation exposures to toluene in the rat and concluded that oral dosing produces blood toluene levels that are similar to those produced by inhalation (see Section 3.1.2). Finally, a 2-generation inhalation toxicity study is available which lends support to the oral database in that effects are noted only at high concentrations. For these reasons a data base UF was not needed.

The RfD for toluene was calculated as follows:

$$RfD = NOAEL \div UF$$
$$= 238 mg/kg - day \div 1000$$
$$= 0.2 mg / kg - day$$

5.1.4. Previous Oral Assessment

The previous IRIS assessment utilized the NTP (1990) 13-week rat gavage study as the principal study and changes in liver and kidney weights as the critical effect for derivation of the RfD (0.2 mg/kg-day). The NOAEL was identified as 223 mg/kg-day. A composite UF of 1000 was applied to account for interspecies and intraspecies extrapolations, for subchronic-to-chronic extrapolation and for limited reproductive and developmental toxicity data.

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 subchronic and chronic occupationally-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 (see Table 1, Section 4.5.2). Animal studies (NTP, 1990) 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.

A number of occupational studies were considered for the principal study upon which to base the derivation of an RfC. Numerous studies have identified NOAELs in the range of 25-50 ppm toluene (Cavalleri et al, 2000; Eller et al., 1999; Nakatsuka et al., 1992; Schaper et al., 2003; Stengel et al., 1998; Zavalic et al., 1998a; Zupanic et al., 1992). These studies were designed to measure effects on subjective symptoms (e.g., headache, dizziness), color vision, neurological and psychomotor functioning, hearing, and immune function. Other studies have shown statistically significant effects in workers on subjective symptoms, auditory evoked brain potentials, neurobehavioral parameters, neurological functioning, electrophysiological cardiac parameters, and color vision in the range of 83-132 ppm toluene (Abbate et al., 1993; Boey et al., 1997; Eller et al., 1999; Foo et al., 1990; Murata et al., 1993; Zavalic et al., 1998a). Conversely, one study found no neurotoxic effects at toluene levels of 68-185 ppm (Antti-Poika et al., 1985), whereas Campagna et al. (2001) found statistically significant effects on color vision in workers exposed to 8 and 36 ppm toluene. In addition, Vrca et al., (1995, 1997) and Yin et al. (1987) found subjective symptoms and alterations in visual- and auditory-evoked brain potentials in workers exposed to 40-60 ppm toluene.

Some of the available studies are confounded by known co-exposure to other contaminants (Nakatsuka et al., 1992; Yin et al., 1987) or inadequate exposure information (i.e., no duration of exposure reported or no direct air sampling) (Antti-Poika et al., 1985; Murata et al., 1993; Nakatsuka et al., 1992; Vrca et al., 1995, 1997) and were not considered further for the principal study. Several studies (Boey et al., 1997; Cavalleri at al., 2000) were of short exposure (5-9 years) duration and were not considered further since there are other studies available of chronic duration which would be more suitable for the derivation of an RfC. A number of studies did not identify an adverse effect (Antti-Poika et al., 1985; Cavalleri et al., 2000; Nakatsuka et al., 1992; Schaper et al., 2003; Stengel et al., 1998; Zupanic et al., 2002), thus making them unsuitable for the principal study when other more suitable studies are available.

The Zavalic et al. (1998) study is an adequate cross-sectional study of chronically-exposed humans. The study utilized two exposed groups and conducted measurements of color vision on Mondays and Wednesdays providing some evidence that the effects were persistent at least over the 64 hour weekend period. Impaired color vision is the critical effect in this study. 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) as that found in the Zavalic et al. (1998) study for decreased performance in neurobehavioral and neuropsychological tests, but the effect levels, exposure durations and exposure levels in this study were less clearly characterized. The Campagna et al. (2001) study shows statistically significant effects on color vision at estimated exposure levels of 8 and 36 ppm toluene. Similar studies of color vision impairment (Cavalleri et al., 2000; Nakatsuka et al., 1992; Zavalic et al., 1998) all found no statistically significant effects at higher doses (30-40 ppm). In addition, the Campagna et al. (2001) study did not measure exposure via blood levels of toluene, whereas the Zavalic et al. (1998a) study found correlations between between color vision impairment, airborne toluene levels and blood toluene levels. While the Foo et al. (1990) study was selected as the principal study for the previous RfC derivation, it

contains a single exposure group and a shorter exposure period (i.e., 5.7 ±3.2 years) making it less suitable than the Zavalic et al. (1998a) study which contains two exposure groups and a longer exposure duration. Thus, given the weight of evidence of exposure ranges, observed effects among the available studies and the confounders discussed above, the Zavalic et al. (1998a) study was chosen as the principal study.

There is a growing body of literature indicating that chronic exposure to a variety of volatile organic solvents including toluene, styrene, perchloroethylene and carbon disulfide is associated with subtle deficits in visual perception measured either as deficits in color vision or deficits in visual contrast sensitivity (for reviews see Gobba, 2000; Iregren et al., 2002). Occupation-related color vision impairment, like other acquired dyschromatopsias, usually results in impairment of blue-yellow color discrimination or, less frequently, in a combination of blue-yellow and red-green loss. Congenital dyschromatopsias more frequently result in red-green deficits (Hart, 1987, 1992; Mergler et al., 1987, Gobba and Cavalleri, 2003). The mode of action of color vision loss induced by solvents is not known. However, according to "Koller's rule" the impairment of blue-yellow discrimination suggests a retinal location of the effect (Hart, 1992). One possible mechanism may be related to a direct effect of solvents (or metabolites) on cone function (e.g., membrane metabolism), or to an interference with neurotransmitters (like dopamine) (see Gellar and Hudnell, 1997 for a review). Alternatively, color vision loss may be the result of a distal axonopathy of the optic pathway, as suggested by Shaumberg and Spencer (1978) for n-hexane.

Additional ocular effects from toluene exposure have been noted. Visual-evoked potentials have been shown to be altered in printing press workers chronically exposed to high levels (Urban and Lukas, 1990) as well as lower concentrations of toluene (Vrca et al., 1995,1997). Optic neuropathies with dyschromatopsia, blindness, changes in pattern visual-evoked potentials, pendular nystagmus, ocular flutter, opsoclonus, bilateral internuclear ophthalmoplegia and retinal impairment have also been reported in participants who chronically sniffed toluene or toluene-based glue (Hormes et al., 1986; Hunnewell and Miller, 1998; Kiyokawa et al., 1999; Lazar et al., 1983; Poblano et al., 1996; Sasa et al., 1978; Toyonaga et al., 1989; Ehyai and Freemon, 1983).

Impairment of color vision may also be an indicator of additional neurological effects [see Dick et al., 2000; Mergler et al., 1987]. Toluene abusers who have been exposed for long periods of time exhibit a variety of neurologic manifestations, including ataxia, tremor, anosmia, sensorineural hearing loss, dementia, corticospinal tract dysfunction, abnormal brainstem auditory-evoked potentials, and epileptic seizures (Hormes et al., 1986; Lazar et al., 1983; Sasa et al., 1978; Ron, 1986). Abnormal magnetic resonance imaging findings in toluene abusers include generalized cerebral, cerebellar, and brainstem atrophy, atrophy of the corpus callosum, loss of gray-white matter discrimination, multifocal high signal intensity in the cerebral white matter, and hypointensity of the thalami on T2-weighted images (Xiong et al., 1993; Rosenberg et al., 1988a, b). Dick et al. (2000) suggested that color vision loss may be part of a neurological syndrome related to organic solvent exposure, also including coarse tremor, impaired vibration sensation in the legs and cognitive impairment.

A number of 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. von Euler et al. (2000) exposed 30 rats to 80 ppm toluene for 4 weeks and found a selective decrease of approximately 6% in the area of the parietal cortex by magnetic resonance imaging. Autoradiographic analysis revealed a 7-10% decrease of the cerebrocortical area. Inhalation exposure to toluene has also been shown to result in irreversible high-frequency hearing loss in rats. Pryor et al. (1984) evaluated hearing loss 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. Hearing loss was irreversible, as evidenced by a failure to return to normal response after 3 months of recovery.

In addition to neurologic effects in humans, the previous RfC on the IRIS data base 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 effects are a more sensitive endpoint for exposed humans, impaired color vision alone was selected as the critical endpoint in this assessment.

5.2.2. Methods of Analysis

The RfC was derived by the benchmark dose approach (BDS. Version 1.3). Benchmark analysis was performed for decreases in the color confusion index as adjusted for age and alcohol consumption (Zavalic et al., 1998a). The benchmark response (BMR) was calculated for various increments of standard deviations from the control mean. The upper 98th percentile of the control distribution was taken to describe an upper limit of "normal" values. Details of the model results are presented in Appendix C. In the absence of information on the level of response to consider adverse, a change in the mean equal to one standard deviation from the control mean was used according to the U.S. EPA Benchmark Dose Guidance (U.S. EPA, 2000d). A BMCL of 99 ppm (374 mg/m^3) was derived and used as the point of departure. This concentration corresponds to an anticipated 13 percent extra population risk (see Appendix C). Thus, 13 percent of the population exposed at the BMCL would be expected to exceed the 98th percentile of the control distribution of adjusted scores, assuming the data are normally distributed and that the fitted model is plausible.

PBTK models are available that describe the kinetics of toluene after inhalation exposure (Fisher et al., 1997; Pierce et al., 1996, 1999; DeJongh and Blaauboer, 1996, 1997; Tardif et al., 1993). These models could theoretically be utilized for conducting a dose-based duration adjustment. It has been shown that neurotoxic effects of acute exposure to toluene and other volatile organic compounds can be predicted by the momentary target tissue concentration of those compounds (Boyes et al. 2003; see also memorandum dated October 29, 2003, from Dr. William Boyes, U.S. EPA, to Dr. Lynn Flowers, U.S. EPA "Potential use of PBTK modeling to support route-to-route extrapolation or duration adjustments for chronic exposure to toluene"). Studies have been limited to the evaluation of functional changes following acute exposure, which are reversible after termination of exposure, and subsequent clearance of the compound from tissues. A critical part of the acute research is the finding that the peak tissue concentration of trichloroethylene (a different VOC with similar acute neurotoxicity as toluene) predicted momentary changes in neurological function, and that the total amount of exposure (expressed either as air concentration x duration product, or area under the curve of the tissue dose level) did not predict the measured effect (on visual function in this case) (Boyes et al., 2003).

In the case of chronic exposure it is not clear that the peak tissue concentration is the appropriate measure of internal dose to use in conducting a dose-based duration adjustment. The standard use of the human equivalent concentration multiplied by the 5/7 ratio to adjust for the hours/day and days/week exposure differences is based on the logical premise that the total amount of exposure, rather than the momentary tissue concentration, is the appropriate predictor of chronic toxic effects. At this time data are not available to determine the proper dose metric for the chronic effects of toluene exposure on color vision, thus the standard default methodology for duration adjustment was used.

5.2.3. RfC Derivation - Including Application of Uncertainty Factors

The BMCL of 99 ppm (374 mg/m^3) from the Zavalic et al. (1998a) study was adjusted from an occupational exposure scenario to continuous exposure conditions as follows:

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

$$= 374\,mg/m^3 \times \frac{10m^3}{20m^3} \times \frac{5\,days}{7\,days}$$

$$= 130\,mg/m^3$$

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 susceptible human subpopulations. Pelekis et al. (2001) have developed a model employing pharmacokinetic information to derive a chemical-specific intraspecies UF for toluene. The result of the effort is an informed quantitation of "normal" human-to-human and adult-to-child variability. The Pelekis model is based solely on the pharmacokinetic differences between adults and children. In the case of intraspecies variability, the differences in humans may be due to lifestage (childhood versus advanced age), genetic polymorphisms, decreased renal clearance in disease states, unknown pharmacodynamic variations in response to toluene exposure, etc. It is not clear that the variability defined in the Pelekis model accounts for the differences in pharmacokinetics and pharmacodynamics of these various human states.

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

An uncertainty factor to account for extrapolating from less than chronic results was not necessary (UF_s). Workers were chronically exposed to toluene for a mean duration of 16 - 18 years in the Zavalic et al. (1998a) study.

An uncertainty factor was not needed to account for extrapolating from a LOAEL to a NOAEL because NOAEL/LOAEL methodology was not used in the derivation of the point of departure.

The data base for inhalation exposure to toluene is considered adequate. Numerous human and animal chronic and subchronic studies are available. Animal studies have demonstrated reproductive and developmental effects of toluene at exposure levels higher than those used for the determination of the point of departure. In addition, neurotoxicity and 2-generation inhalation toxicity studies are available.

The RfC for toluene is derived as follows:

$$RfC = BMCL_{(HEC)} \div UF$$
$$= 130\,mg/m^3 \div 10$$
$$= 13\,mg/m^3$$

5.2.4. Previous Inhalation Assessment

The previous IRIS assessment utilized the Foo et al. (1990) occupational study as the principal study and neurological effects as the critical effect for the derivation of the RfC (0.4 mg/m^3). The LOAEL was identified as 332 mg/m^3 (88 ppm) which was converted to a human equivalent concentration of 119 mg/m^3. A composite UF of 300 was used which consisted of a 10-fold UF for intraspecies variability, a 10-fold UF for the use of a LOAEL instead of a NOAEL and a 3-fold UF for data base deficiencies including a lack of animal exposure data evaluating neurotoxicity and respiratory irritation.

5.3. CANCER ASSESSMENT

5.3.1. Choice of Study/Data

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, 1990; Huff, 2003), and 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 genotoxic in short-term testing protocols. A quantitative assessment of carcinogenic potential was not performed.

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 4.4°C. 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. One subchronic study examining oral exposure to toluene in rodents (rats and mice) is available. NTP (1990) exposed F344 rats and B6C3F1 mice to toluene by gavage for 13 weeks. In male rats, absolute and relative weights of both the liver and kidney were significantly increased ($p<0.05$) at doses greater than or equal to 446 mg/kg-day. Histopathologic lesions in the liver consisted of hepatocellular hypertrophy, occurring at doses greater than 2500 mg/kg. Nephrosis was observed in rats that died, and damage to the tubular epithelia of the kidney occurred in terminally sacrificed rats. Kidney sections were examined in particular for the occurrence of hyaline droplets in the proximal tubules with negative findings.

Toluene has been evaluated for immunosuppressive effects primarily in comparison studies with the known immunotoxicants, benzene and nitrotoluenes (Hsieh et al., 1989, 1990b, 1991; Burns et al., 1994). For example, statistically significant and dose-related decreases in antibody response were noted by Hsieh et al. (1989, 1990b, 1991). Indeed, there is evidence that the antibody-forming cell assay (PFC) is among the most predictive tests available for immunotoxicity (Luster et al., 1992) and that suppression of the antibody response is predictive of decreased resistance to challenge with infectious agents or tumor cells (Luster et al., 1993). However, the host resistance assays by Burns et al. (1994) indicate a lack of immunotoxic response when animals treated with toluene are challenged. Host resistance to challenges with *Listeria monocyogenes*, *Streptococcus pneumoniae*, *Plasmodium yoelii*, or B16F10 melanoma were not affected at a dose of 600 mg/kg-day for 14 days. In addition, a reduced incidence of tumors was observed in mice that were challenged with PYB6 fibrosarcoma.

Additional studies by Hsieh et al. (1990a, c, 1991) found statistically significant increases in brain neurotransmitter levels at exposure levels as low as 5 mg/kg-day but these changes have not been correlated with behavioral, neuropsychological, or neuroanatomical changes. Several reproductive studies (Gospe et al., 1994, 1996; Gospe and Zhou, 1998, 2000) have indicated minimal effects on dams and offspring at the doses tested.

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 as an addictive euphoric 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 at levels that also resulted in maternal toxicity. Gross malformations were not noted at any exposure level.

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 inhalation cancer bioassays of rats and mice exposed for life (CIIT, 1980; NTP, 1990; Huff, 2003). 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. One subchronic study examining oral exposure to toluene in rodents (rats and mice) is available. This study (NTP, 1990) was chosen as the principal study. The critical effect is increased kidney weight. NTP (1990) exposed F344 rats and B6C3F1 mice to toluene by gavage for 13 weeks. In male rats, absolute kidney weights were statistically significantly increased (100, 107, 112, 119, and 113 percent of controls; relative kidney weights were 100, 100, 106, 114, and 146 percent of controls for 0, 223, 446, 900, or 1800 mg/kg-day, respectively). Nephrosis was observed in rats that died, and damage to the tubular epithelia of the kidney occurred in terminally sacrificed rats. Kidney sections were examined in particular for the occurrence of hyaline droplets in the proximal tubules with negative findings. A BMDL of 238 mg/kg-day was derived based on increased kidney weight. This corresponds to a 9% response level (i.e., increase in kidney weight from control). 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 0.2 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 neurological changes are the most sensitive effect of chronic inhalation exposure to toluene. The study of Zavalic et al. (1998a) was selected as the principal study for RfC derivation with a critical effect of impaired color vision in exposed workers. A BMDL of 99 ppm (374 mg/m^3) was derived based on color confusion index measurements which were adjusted for age and alcohol consumption. This concentration corresponds to an anticipated 13 percent extra population risk (see Appendix C). Thus, 13 percent

of the population exposed at the BMCL would be expected to exceed the 98th percentile of the control distribution of adjusted scores. The BMDL was adjusted for continuous exposure, resulting in a $BMDL_{(ADJ)}$ of 130 mg/m^3. An uncertainty factor of 10 (for intrahuman variability) was applied to give a chronic RfC of 10 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 endpoints at multiple exposure levels. Confidence in the database is high; several chronic studies in humans are available that examine neurotoxicity and effects on color vision, and numerous reproductive and developmental studies, as well as a 2-generation reproductive toxicity study exist. There is medium confidence in the resulting RfC.

6.2.3. Cancer/Oral and Inhalation

Under the Draft Revised Cancer Guidelines (U.S. EPA, 1999), *data are considered inadequate for an assessment of human carcinogenic potential* for toluene. 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, 1990; Huff, 2003) 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.

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Appendix A. Summary of External Peer Review and Public Comments and Disposition (August 2002)

The support document and IRIS Summary for toluene have undergone both internal review by scientists within EPA and a more formal external peer review in August 2002. The external review was conducted in accordance with EPA guidance on peer review (U.S. EPA, 1998, 2000). Comments made by the internal reviewers were addressed prior to submitting the documents for external review and are not part of this appendix. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific charge questions addressing areas of scientific controversy or uncertainty. The charge questions are reproduced below. A summary of significant comments made by the external reviewers and EPA's response to these comments follows. EPA also received comments from the public. A summary of public comments and EPA's response are included in a separate section.

Charge to External Reviewers

1) **RfD Derivation**

a) *Principal Study, Section 5.1.1*: *Two subchronic animal studies are available (NTP, 1990; Hsieh et al., 1989). The current IRIS entry utilizes the 13-week oral gavage study (NTP, 1990) for the derivation of an RfD. The 28-day drinking water study (Hsieh et al., 1989) was not considered. This latter study has now been chosen as the principal study. Is this the correct choice for the principal study?*

b) Critical Effect, Section 5.1.1: The critical effect is identified as immunological effects: decreased thymus weight. Is this the correct critical effect and is it adequately described?

c) Methods of Analysis, Section 5.1.2: Is the point of departure determined appropriately, i.e., benchmark dose approach?

d) Uncertainty Factors, Section 5.1.3: Are the appropriate uncertainty factors applied? Is the explanation for each transparent?

*2) **RfC Derivation***

a) Principal Study, Section 5.2.1: Several human epidemiological studies are available. The study used in the previous IRIS assessment (Foo et al., 1990) is not used in the reassessment; the study by Zavalic et al. (1998) is chosen as the principal study. Is this the correct choice for the principal study? Are adequate explanations given to explain why this study was chosen over the other available studies? An attempt is made to explain the choice of principal study, critical effect and NOAEL by examining the entire data base. Was this attempt successful?

b) Critical Effect, Section 5.2.1: The critical effect is identified as impaired color vision. Is this the correct critical effect and is it adequately described?

c) Methods of Analysis, Section 5.2.2: Is the point of departure determined appropriately, i.e., NOAEL/LOAEL approach versus benchmark dose approach?

d) Uncertainty Factors, Section 5.2.3: Are the appropriate uncertainty factors applied? Is the explanation for each transparent?

3) ***Cancer Weight-of-Evidence Characterization***

The weight of evidence and cancer characterization are discussed in Section 4.6. Have appropriate criteria been applied from both the 1986 EPA Guidelines for Carcinogen Risk Assessment (Federal Register 51 (185):33992-34003) and the 1999 EPA draft revised Guidelines for Carcinogen Risk Assessment (Review Draft, NCEA-F-0644, July 1999. Risk Assessment Forum)?

Scientific Comments from External Peer Review

1) **RfD Derivation**

a) *Principal Study, Section 5.1.1*: *Two subchronic animal studies are available (NTP, 1990; Hsieh et al., 1989). The current IRIS entry utilizes the 13-week oral gavage study (NTP, 1990) for the derivation of an RfD. The 28-day drinking water study (Hsieh et al., 1989) was not considered. This latter study has now been chosen as the principal study in the draft reassessment. Is this the correct choice for the principal study?*

Comment: All four reviewers agreed with the choice of principal study.

Response: During the external review period of the draft assessment, it was discovered that several relevant *in vivo* oral exposure studies were omitted. Please see summary of public comments below. Consideration of these studies had a significant impact on the choice of principal study and critical effect for the RfD. The principal study has been changed to the 13-week gavage study (NTP, 1990) in rats. The critical effect has been changed to increased kidney weight. These changes are discussed in Sections 5.1.1.and 5.1.2.

The following oral *in vivo* studies have been added to the Toxicological Review:
Burns, L.A., S.G. Bradley, K.L. White, Jr., et al. 1994. Immunotoxicity of mono-nitrotoluenes in female B6C3F1 mice: I. para-nitrotoluene. Drug Chem. Toxicol. 17: 317-358.

Hsieh G.C., R.P. Sharma, R.D. Parker, et al. 1990a. Evaluation of toluene exposure via drinking water on levels of regional brain biogenic monoamines and their metabolites in CD-1 mice. Ecotoxicol. Environ. Saf. 20: 175-184.

Hsieh, G.C., R.D.R. Parker, R.P. Sharma, et al. 1990b. Subclinical effects of ground water contaminants. III. Effects of repeated oral exposure to combinations of benzene and toluene on immunologic responses in mice. Arch. Toxicol. 64: 320-328.

Hsieh G.C., R.P. Sharma and R.D. Parker. 1990c. Subclinical effects of groundwater contaminants. Effects of repeated oral exposure to combinations of benzene and toluene on regional brain monoamine metabolism in mice. Arch. Toxicol. 64: 669-676.

Hsieh G.C., R.P. Sharma, R.D. Parker. 1991. Hypothalmic-pituitary-adrenocortical axis activity and immune function after oral exposure to benzene and toluene. Immunopharmacol. 21:23-31.

Kostas, J. and J. Hotchin. 1981. Behavioral effects of low-level perinatal exposure to toluene in mice. Neurobehav. Toxicol. Teratol. 3: 467-469.

b) *Critical Effect, Section 5.1.1*: *The critical effect is identified as immunological effects: decreased thymus weight. Is this the correct critical effect and is it adequately described?*

Comment: There was general agreement among the reviewers on the use of immunological effects as the critical effect but discrepancies in immunotoxicity study descriptions were noted as well as a concern for the use of thymus weight as the true endpoint. It was noted that thymus weight is not generally considered a reliable indicator of immunosuppression.

Response: Upon consideration of the additional studies discussed above, the critical effect was changed to increased kidney weight. The rationale for this selection is discussed in Section 5.1.1.

c) *Methods of Analysis, Section 5.1.2*: *Is the point of departure determined appropriately, i.e., benchmark dose approach?*

Comment: Several reviewers agreed with the use of benchmark dose modeling for the derivation of the point of departure. One reviewer questioned the used of benchmark dose modeling for thymus weight changes since effects were seen primarily at the high doses.

Response: The critical effect has changed as discussed above to increased kidney weight. Benchmark dose modeling has been used to determine a point of departure for this endpoint and is described in Section 5.1.2.

d) *Uncertainty Factors, Section 5.1.3*: *Are the appropriate uncertainty factors applied? Is the explanation for each transparent?*

Comment: Three reviewers agreed with the choice of uncertainty factors. One reviewer commented that there was no scientific basis for the choice of individual uncertainty factors.

Response: No response needed.

2) **RfC Derivation**

a) *Principal Study, Section 5.2.1*: *Several human epidemiological studies are available. The study used in the previous IRIS file (Foo et al., 1990) is not used in the reassessment; the study by Zavalic et al. (1998) is chosen as the principal study. Is this the correct choice for the principal study? Are adequate explanations given to explain why this study was chosen over the other available studies? An attempt is made to explain the choice of principal study, critical effect and NOAEL by examining the entire data base. Was this attempt successful?*

Comment: Three of the four reviewers indicated that the selection of Zavalic et al. (1998a) was the correct choice for the principal study. One reviewer stated that the available data are inadequate to appropriately protect the public's health and that additional research is needed.

Response: No response required. Note that an updated literature search identified several additional inhalation exposure studies which have been included in Section 4.1.2.

Comment: One reviewer noted specific issues concerning the use of the Zavalic et al. (1998a) study as the principal study including the following:

1) Measurements of toluene exposure were not performed in accord with modern standards and were not individual exposure measurements.

Response: Zavalic et al. (1998a) referenced standard methods for measuring both ambient air concentrations (NIOSH, 1974) and individual blood toluene levels (Nise and Orback, 1988). Sampling tubes were fixed onto the work tables or machines at nose height and air was collected continually throughout the working day. Personal air monitors were not used in this study, however, individual blood samples were taken from all workers. Urine samples were taken from all workers in the high dose group.

2) Alcohol consumption, an important risk factor, was not adequately controlled. Alcohol consumption data obtained from questionnaires should not be considered reliable.

Response: Alcohol consumption is considered an important independent risk factor for impaired color vision (Russell et al., 1980). Zavalic et al. (1998a) were cognizant of this fact and utilized self-reported alcohol consumption data to adjust the color confusion index scores. Self-reported alcohol consumption data are generally not considered to be exceptionally accurate, but there is no reason to suspect that the data from the exposed group are any less accurate that the data from the control group. Moreover, when alcohol consumers were excluded from the data set, a significant correlation was still observed between exposure and CCI scores.

3) The effects reported by Zavalic et al. (1998) were not observed by Nakatsuka et al. (1992), a study that was not included in the Toxicological Review.

Response: The Nakatsuka et al. (1992) study has been added to the Toxicological Review in Section 4.1.2.2. Impaired color vision associated with exposure to toluene has been reported by several investigators (Campagna, et al., 2001, Cavelleri et al., 2000, Muttray, et al., 1999, Zavalic et al., 1998a, 1998b), but was not observed by Nakatsuka et al. (1992). The discrepancy may be due to the method of color vision assessment. For example, Nakatsuka et al. (1992) assessed color vision in a qualitative manner indicated by color vision loss. The other studies measured color confusion indicies directly. Nakatsuka et al. (192) also used the Lanthony "new test" which may also have contributed to the varied results.

4) Zavalic et al. (1998a) state that toluene was used for manually cleaning the rollers. This would result in an unusual exposure that would likely be underestimated by the exposure assessment procedures that were used.

Response: The use of toluene to manually rinse the rollers may have been a significant source of exposure for the printers who were examined by Zavalic et al. (1998a). The authors do not state whether the collected air samples would characterize this exposure scenario.

b) *Critical Effect, Section 5.2.1*: *The critical effect is identified as impaired color vision. Is this the correct critical effect and is it adequately described?*

Comment: Two reviewers felt that impaired color vision was the appropriate selection for a critical effect, but one of these reviewers felt that the documentation of this adverse effect was lacking. This opinion was shared by another reviewer, who questioned the biological significance of this endpoint.

Response: Additional text was added to Section 4.1.2.2 to better describe the Lanthony D-15 color vision test. Additional text was also added to Sections 4.5.3 and 5.2.1 to support the use of impaired color vision as a critical effect.

Comment: One reviewer noted the observed prevalence of dyschromatopsia in the control group (43%) would be considered high leading one to question the validity of the results.

Response: The prevalence of dyschromatopsia in the unexposed study population (28%), which was reported in a separate publication (Zavalic et al., 1998b), is consistent with other studies where the Lanthony D-15 desaturated panel (D-15d) has been employed (Campagna et al., 2002; Geller and Hudnell, 1997). The D-15d is considered a highly sensitive test for detecting mild to moderate dyschromatopsia, but can yield false positive results (Geller and Hudnell, 1997). Nevertheless, the D-15d has proven effective in detecting acquired color vision deficits associated with chemical exposure (Geller and Hundell, 1997; Gobba and Cavalleri, 2003).

Comment: One reviewer noted a statistically significant impact on color vision was only observed when the data were adjusted for age and alcohol intake which leads to questionable results.

Response: The reported mean CCI score for the high exposure population (E_2) was significantly different from both the low exposure (E_1) and unexposed (NE) populations ($P < 0.001$). To examine the relationship between CCI scores and exposure measurements the authors attempted to control for two potential risk factors, age and alcohol, by calculating age and alcohol intake adjusted color confusion index (AACCI) values. The authors state that the AACCI values were obtained using the residuals from a linear regression analysis of the unexposed population, but these data were not provided.

c) *Methods of Analysis, Section 5.2.2*: *Is the point of departure determined appropriately, i.e., NOAEL/LOAEL approach versus benchmark dose approach?*

Comment: Two reviewers stated that the point of departure was appropriately determined using the NOAEL/LOAEL approach. One of these reviewers stated that reports of other neurologic effects at concentrations ranging from 40 to 100 ppm support the use of a NOAEL in this case. Another reviewer was concerned that the low dose group might not provide an adequate basis for the determination of a NOAEL since the biomarker-based measures of exposure were not well correlated with the measured time-weighted-average (TWA) air concentration of toluene for the low dose exposure group $r = 0.47$, $p = 0.09$). This reviewer suggested the TWA air concentrations may not have been an accurate measure of true exposure either because of inaccurate measurement, or possibly because of dermal absorption of toluene. This reviewer also stated that the data should lend itself to benchmark dose modeling for the determination of the point of departure if tabular data were available and suggested contacting the authors to obtain the complete quantitative data. The fourth reviewer indicated the validity of extrapolation from an 8 hour/day exposure to continuous chronic exposure is debatable. The statement was made that large individual variations in inhalation are to be expected during various levels of strenuous activity and that it is doubtful that a two day longer exposure period (i.e., the weekend) would change the body burden by 5/7.

Response: The external review draft relied on a NOAEL/LOAEL approach for the determination of the point of departure for the RfC. Individual data points were not available at that time. The standard deviations of the AACCI means have now been obtained which allows for the use benchmark dose modeling to derive a BMDL as the point of departure. This methodology utilizes the entire data set which somewhat circumvents the issue surrounding the accuracy of the NOAEL. The NOAEL is no longer relied upon as the point of departure. The point of departure is now 99 ppm (374 mg/m^3).

The extrapolation from an 8 hour/day exposure to continuous chronic exposure is a default procedure outlined in the Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994). The default approach for human exposure scenarios is to adjust by the default occupational ventilation rate and for the intermittent work

week schedule as indicated in Section 5.2.3. PBTK models are available that describe the kinetics of toluene after inhalation exposure (Fisher et al., 1997; Pierce et al., 1996, 1999; DeJongh and Blaauboer, 1996, 1997; Tardif et al., 1993). These models could theoretically be utilized for conducting a dose-based duration adjustment. It has been shown that neurotoxic effects of acute exposure to toluene and other volatile organic compounds can be predicted by the momentary target tissue concentration of those compounds (Boyes et al. 2003; see also memorandum dated October 29, 2003, from Dr. William Boyes, U.S. EPA, to Dr. Lynn Flowers, U.S. EPA "Potential use of PBTK modeling to support route-to-route extrapolation or duration adjustments for chronic exposure to toluene"). However, studies have been limited to evaluation of functional changes following acute exposure, which are reversible after termination of exposure, and subsequent clearance of the compound from tissues. A critical part of this research was the finding that the peak tissue concentration of trichloroethylene (a different VOC with similar acute neurotoxicity) predicted momentary changes in neurological function, but that the total amount of exposure (expressed either as air concentration x duration product, or area under the curve of the tissue dose level) did not predict the measured effect (on visual function in this case) (Boyes et al. 2003).

In the case of chronic exposure, however, it is not clear that the peak tissue concentration is the appropriate measure of internal dose to use in conducting a dose-based duration adjustment. The standard use of the human equivalent concentration multiplied by the 5/7 ratio to adjust for the hours/day and days/week exposure differences is based on the logical premise that the total amount of exposure, rather than the momentary tissue concentration, is the appropriate predictor of chronic toxic effects. At this time data are not available to determine the proper dose metric for the chronic effects of toluene exposure on color vision, thus the standard default methodology for duration adjustment was used.

d) *Uncertainty Factors, Section 5.2.3*: *Are the appropriate uncertainty factors applied? Is the explanation for each transparent?*

Comments: Three of the reviewers stated that the appropriate uncertainty factors were applied and that the explanation for each was transparent. The fourth reviewer did not comment on the choice of uncertainty factors for the RfC.

Response: No response required.

e) *General Comments on the RfC Derivation*

Comment: One reviewer requested inclusion of where the human inhalation studies were conducted in study descriptions.

Response: The country of origin (if known) of the workers in the inhalation studies has been included in Section 4.1.2.2.

3) **Cancer Weight-of-Evidence Characterization**

The weight of evidence and cancer characterization are discussed in Section 4.6. Have appropriate criteria been applied from both the 1986 EPA Guidelines for Carcinogen Risk Assessment (Federal Register 51 (185):33992-34003) and the 1999 EPA draft revised Guidelines for Carcinogen Risk Assessment (Review Draft, NCEA-F-0644, July 1999. Risk Assessment Forum)?

Comment: Three reviewers agreed with the classification and descriptor used. Another reviewer agreed that data in humans and animals are barely sufficient for a reliable conclusion on the carcinogenic potency of toluene but that more emphasis should be placed on the negative human study by Wiebolt and Becker (1999).

Response: A description of the Wiebolt and Becker (1999) study is included in Section 4.1.2.2. This study is a cohort mortality study in toluene-exposed workers (Wiebelt and Becker, 1999) which reported no change in cancer-specific mortality when the entire cohort was taken into consideration. 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 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. The results of this study are taken into consideration in the weight-of-evidence evaluation and cancer characterization described in Section 4.6.2. This study is described first as it is the most informative human study available. A summary of the available human studies, chronic animals bioassays and genotoxic studies is the primary emphasis of this section. All three contribute to the cancer evaluation. It should be noted that since the external peer review, the IRIS Program has implemented sole use of the Draft Revised Cancer Guidelines (U.S. EPA, 1999) in accordance with the Agency practice (Federal Register 66 (230):59593-59594). In addition, note that the NTP (1990) cancer bioassy has been published (Huff, 2003) and is included as a citation.

Scientific Comments from the Public

Comment: One reviewer disagreed with the principal study and critical effect identified for the derivation of the RfD. This reviewer recommended the use of the NTP (1990) study used in the assessment currently on the IRIS data base. The rationale for not using the proposed study (Hsieh et al., 1989) and critical effect (immunological effects; decreased thymus weight) was several-fold. First, the reviewer stated that the relative differences in thymus weight are small and the significance of this effect is questionable given the lack of information about absolute thymus weights and thymus to brain ratios. This reviewer also questioned the reliability and significance of thymus weight differences and the PFC assays from the Hsieh et al. (1989) study given other research by Hsieh et al. (1990b) and Burns et al. (1994) which was not included in the Toxicological Review. Hsieh et al. (1990b) exposed CD-1 male mice to toluene for 28 days in drinking water at doses of 0, 22, and 90 mg/kg-day and found no effects or changes in thymus weights or liver weights at either dose. Hsieh et al. (1990b) also found no effects in the PFC assays except minimal changes in the PFC/10^6 splenocytes at the high dose. In addition, Burns et al. (1994) exposed B6C3F1 mice to significantly higher doses than either Hsieh (1989, 1990b)

study, i.e., 600 mg/day, and observed no effects on thymus weight and several immune response assays including several standard host resistance assays.

Response: The reviewer is correct in that several relevant *in vivo* oral exposure studies were omitted from the external review draft (August 2002). Consideration of these studies had a significant impact on the choice of principal study and critical effect for the RfD. The principal study has been changed to the 13-week gavage study (NTP, 1990) in rats. The critical effect has been changed to increased kidney weight. These changes are discussed in Sections 5.1.1.and 5.1.2.

The following oral *in vivo* studies have been added to the Toxicological Review:
Burns, L.A., S.G. Bradley, K.L. White, Jr., et al. 1994. Immunotoxicity of mono-nitrotoluenes in female B6C3F1 mice: I. para-nitrotoluene. Drug Chem. Toxicol. 17: 317-358.

Hsieh G.C., R.P. Sharma, R.D. Parker, et al. 1990a. Evaluation of toluene exposure via drinking water on levels of regional brain biogenic monoamines and their metabolites in CD-1 mice. Ecotoxicol. Environ. Saf. 20: 175-184.

Hsieh, G.C., R.D.R. Parker, R.P. Sharma, et al. 1990b. Subclinical effects of ground water contaminants. III. Effects of repeated oral exposure to combinations of benzene and toluene on immunologic responses in mice. Arch. Toxicol. 64: 320-328.

Hsieh G.C., R.P. Sharma and R.D. Parker. 1990c. Subclinical effects of groundwater contaminants. Effects of repeated oral exposure to combinations of benzene and toluene on regional brain monoamine metabolism in mice. Arch. Toxicol. 64: 669-676.

Hsieh G.C., R.P. Sharma, R.D. Parker. 1991. Hypothalmic-pituitary-adrenocortical axis activity and immune function after oral exposure to benzene and toluene. Immunopharmacol. 21:23-31.

Kostas, J. and J. Hotchin. 1981. Behavioral effects of low-level perinatal exposure to toluene in mice. Neurobehav. Toxicol. Teratol. 3: 467-469.

Comment: One reviewer indicated that the selection of Zavalic et al. (1998a) as the principal study for the derivation of the RfC is appropriate. This reviewer stated that the study contains data on atmospheric toluene level in the workplace, and uses an appropriate test for color vision confusion. Using this study minimizes the needs for extensive use of uncertainty factors since it was performed in humans exposed to toluene for long durations, and has both a NOAEL and LOAEL.

Response: The Zavalic et al. (1998a) study has been retained as the principal study for the derivation of the RfC. However, since the standard deviations of the means of the AACCI color vision scores have become available, the data have now been input into benchmark dose modeling software and a BMDL has been derived instead of relying on a single dose level, i.e., a NOAEL, as the point of departure. The uncertainty factors remain the same.

Appendix B. Benchmark Dose Modeling Results for the Derivation of the RfD

Benchmark dose (BMD) modeling was performed to identify the critical effect level for the derivation of the RfD for toluene. The modeling was conducted according to draft EPA guidelines (U.S. EPA, 2000) using Benchmark Dose Software Version 1.3 (BMDS) available from the U.S. EPA (U.S. EPA, 2003). The BMD modeling results are summarized in Table B-1, and the model outputs are attached. A brief discussion of the modeling results is presented below.

Changes in rat kidney weight were modeled from the NTP study (1990). Male rat kidney data were chosen for BMD modeling as these data exhibited a greater response than that seen in female rats. Data from the high dose group (2500 mg/kg) were eliminated since only 2/10 rats survived. Absolute kidney weights were used because the maximum tolerated dose was exceeded at the high dose. The endpoint selected is a continuous variable, therefore, the continuous models available with BMDS (linear, polynomial, power, and Hill) were used. The Hybrid model software in BMDS is still undergoing Beta-testing, and was not used because it was not considered sufficiently validated for use in quantitative dose-response assessment. (The hybrid modeling approach defines the benchmark response [BMR] in terms of change in the mean.) The modeling was conducted for BMR defined as a 1.0 standard deviation (SD) change in the mean. This BMR definition was selected because this is the default measure recommended by the U.S. EPA (U.S. EPA, 2000) in the absence of clear biological rationale for selecting an alternative response level.

The BMDL estimates for the endpoint of increased kidney weight are presented for the linear, quadratic, and power models in Table B-1. The Hill model, however, failed to give adequate Goodness-of-fit p-values for the model fits and did not provide any adequate BMDL estimates. The Goodness-of-fit P-values varied widely for the other models. The only values that were adequate (using a value of P=0.1) were for the models with the restrictions of rho=0. An analysis of the model fit (lowest Maximum Chi-square-residual) in the low dose region (region of the BMDLs) indicated that some models better fit the data in this region. The Akaike Information Criterion (AIC) was similar for the models.

The model selected for this endpoint was the quadratic model with rho=0, over the linear and power models with rho=0. The linear model with rho=0 has a larger Chi-square-residual (0.333) than the quadratic model with rho=0 (0.084) and the power model with rho=0 (0.019), and, subsequently, does not fit the data at the low dose range as well as the quadratic and power models. The AIC value for the quadratic model with rho=0 (410.5) is higher than that for the linear model with rho=0 (409.1) and lower than the power model with rho=0 (412.5), but these differences are not significant. Of the three models discussed, the linear model with rho=0 does not fit the data as well as the other two at the low dose range, and the power model with rho=0 has the highest AIC value of the models. Therefore, the quadratic model with rho=0 is the most useful model for the kidney weight data, and provides an estimated point of departure at 238 mg/kg-day at one standard deviation corresponding to a BMR of 9%.

Table B-1: Benchmark Modeling Summary for relative kidney weight, NTP TR 371.					
Toluene Dose	Number weighed	Kidney weight in male rats (grams) ± SE			
0 mg/kg	10	1,084 ± 14			
312 mg/kg	10	1,159 ± 34			
625 mg/kg	10	1,213 ± 39			
1250 mg/kg	10	1,292 ± 34			
Continuous Models	Restrictions	Goodness-of-fit p-value	AIC	Maximum chi-square residual near POD	BMDL (mg/kg), 1 Std. Dev.
Linear	model determined	<0.0001	410.9	0.428	320
	rho=0	0.734	409.1	0.333	428
Quadratic	model determined	<0.0001	412.9	**0.428**	320
	rho=0	0.912	410.5	0.084	238
Power	model determined	<0.0001	408.6	NA	NE
	rho=0	0.920	412.5	-0.019	107
Hill	model determined	NE	419.3	NA	0+
	rho=0	NE	414.5	0.000	140
	$n \geq 1$	NE	414.0	NA	NE
	rho=2, $n \geq 1$	<0.0001	410.8	0.009	193
	rho=2, n = 2	<0.0001	411.1	NA	NE

```
        Polynomial Model. Revision: 2.2  Date: 9/12/2002
        Input Data File: F:\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.(d)
        Gnuplot Plotting File:  F:\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.plt
                                              Wed Sep 03 14:48:35 2003
 ================================================================

 Male kidney quadratic std dev=1 parms rho=0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 1
   Maximum number of iterations = 1000
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                User Inputs Initial Parameter Values
                         alpha =       0.0001
                           rho =            1   Specified
                        beta_0 =         1100
                        beta_1 =            1
                        beta_2 =        0.001



                                Parameter Estimates

                                                                95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha          9068.31          2027.74             5094.01
13042.6
         beta_0          1084.95          28.8495             1028.41
1141.5
         beta_1         0.250443         0.117244           0.0206492
0.480236
         beta_2    -6.80453e-005     8.69974e-005        -0.000238557
0.000102466


           Asymptotic Correlation Matrix of Parameter Estimates

                   alpha       beta_0       beta_1       beta_2
     alpha             1      -6e-006     1.3e-007       2e-006
    beta_0      -6e-006            1        -0.74         0.59
    beta_1     1.3e-007        -0.74            1        -0.96
    beta_2       2e-006         0.59        -0.96            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    10  1.08e+003         44.3    1.08e+003         95.2        -0.0316
```

```
  312    10  1.16e+003          108    1.16e+003         95.2         0.0842
  625    10  1.21e+003          123    1.21e+003         95.2         -0.063
 1250    10  1.29e+003          108    1.29e+003         95.2         0.0105


  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         -202.244681      5     414.489362
              A2         -197.498037      8     410.996073
           fitted        -202.250763      3     410.501527
              R          -212.606673      2     429.213345

 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              30.2173          6          <.0001
   Test 2              9.49329          3          0.0234
   Test 3            0.0121647          1          0.9122

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 less than .05.  Consider
running a
non-homogeneous variance model

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

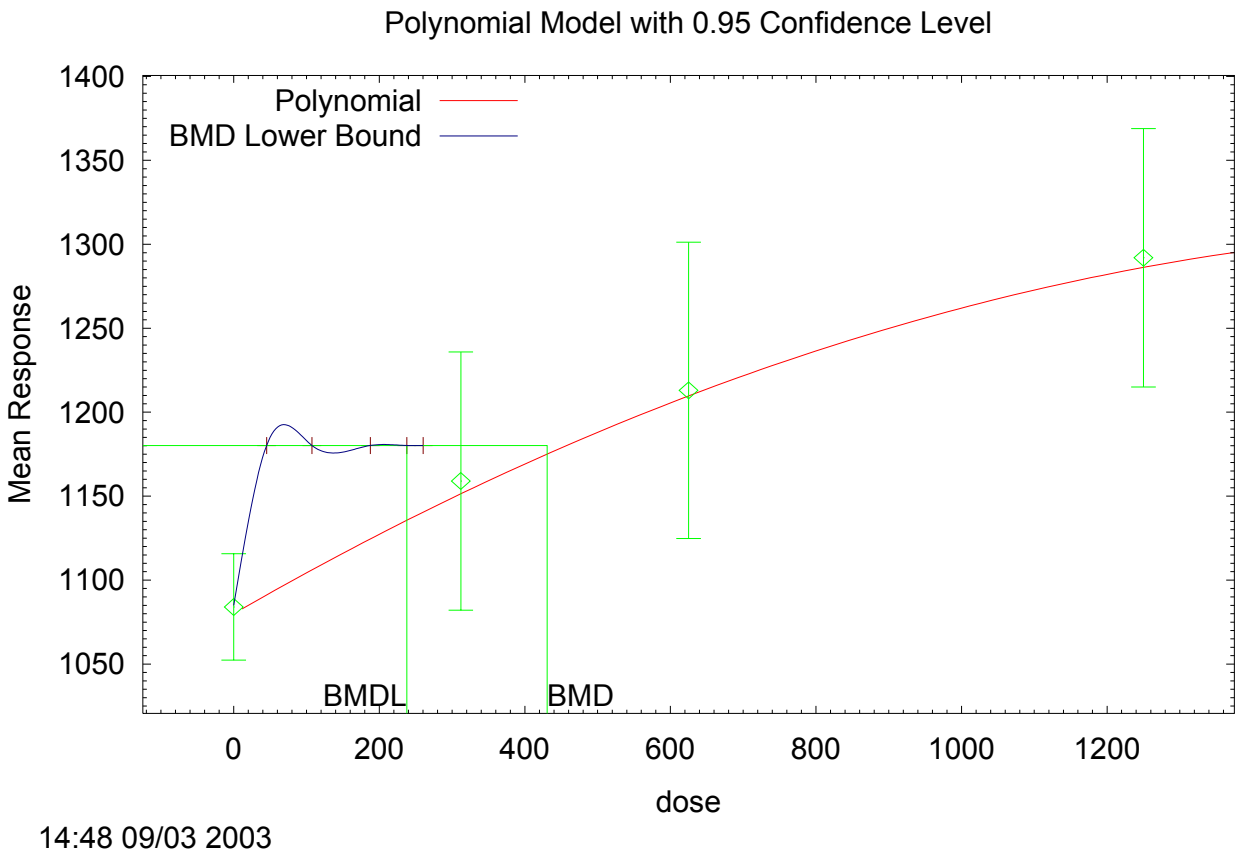

 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

             BMD =        430.62


            BMDL =       237.787
```


Polynomial Model with 0.95 Confidence Level
Polynomial
BMD Lower Bound
Mean Response
1400
1350
1300
1250
1200
1150
1100
1050
BMDL
BMD
0
200
400
600
800
1000
1200
dose
14:48 09/03 2003

```
 ===================================================================
         Polynomial Model. Revision: 2.2  Date: 9/12/2002
         Input Data File: F:\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.(d)
         Gnuplot Plotting File:  F:\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.plt
                                              Wed Sep 03 15:48:56 2003
 ===================================================================

Male kidney linear std dev=1  parms free
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 5
   Total number of records with missing values = 1
   Maximum number of iterations = 1000
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                User Inputs Initial Parameter Values
                         alpha =       0.0001
                           rho =            0
                        beta_0 =         1100
                        beta_1 =            1


                                  Parameter Estimates

                                                                    95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha           1e-008     6.48795e-008       -1.17161e-007
1.37161e-007
            rho          3.88492         0.915011              2.09153
5.67831
         beta_0          1093.41          20.6962              1052.84
1133.97
         beta_1         0.172212         0.033839             0.105888
0.238535


           Asymptotic Correlation Matrix of Parameter Estimates

                   alpha          rho       beta_0       beta_1
     alpha            -1            1       -0.028        0.045
       rho             1           -1        0.027       -0.044
    beta_0        -0.028        0.027            1        -0.71
    beta_1         0.045       -0.044        -0.71            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    10  1.08e+003         44.3    1.09e+003         79.9         -0.372
  312    10  1.16e+003          108    1.15e+003         87.7          0.428
  625    10  1.21e+003          123     1.2e+003         95.9          0.394
```

```
1250    10  1.29e+003          108    1.31e+003          113         -0.465


 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 A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = alpha*(Mu(i))^rho

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


 Warning: Likelihood for fitted model larger than the Likelihood for model A3.

                       Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
              A1         -202.244681       5     414.489362
              A2         -197.498037       8     410.996073
              A3         -792.622946       6    1597.245893
           fitted        -201.467874       4     410.935749
              R          -212.606673       2     429.213345


                    Explanation of Tests

 Test 1:  Does response and/or variances differ among Dose
levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)

                      Tests of Interest

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

   Test 1              30.2173          6          <.0001
   Test 2              9.49329          3          0.0234
   Test 3              1190.25          2          <.0001
   Test 4             -1182.31          2          <.0001

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 less than .05.  A
non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .05.  You may want
to consider a
different variance model

The p-value for Test 4 is less than .05.  You may want
to try a different
model
```

```
 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

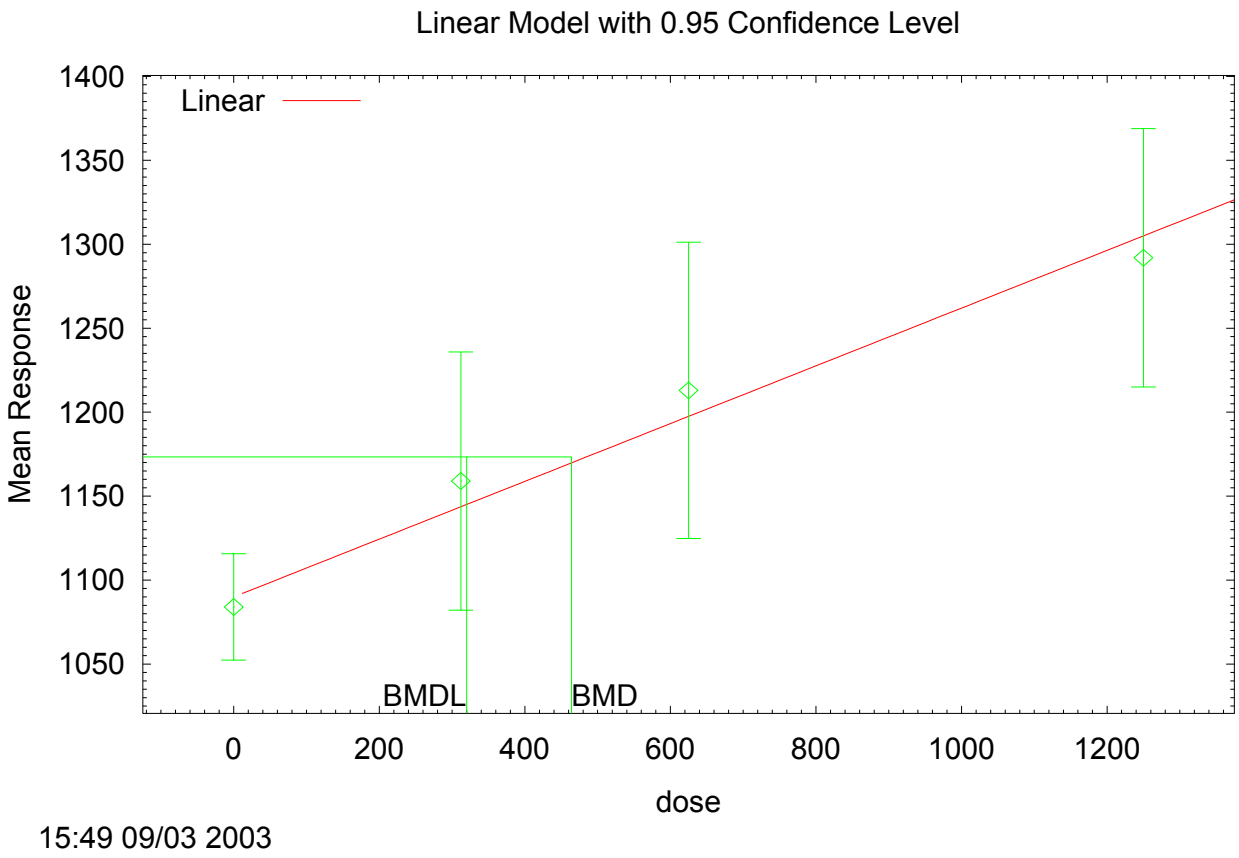

Confidence level =          0.95

             BMD =       464.141


            BMDL =        320.11

BMDL computation failed for one or more point on the BMDL curve.

                    The BMDL curve will not be plotted
```


Linear Model with 0.95 Confidence Level
Linear
Mean Response
1400
1350
1300
1250
1200
1150
1100
1050
BMDL
BMD
0
200
400
600
800
1000
1200
dose
15:49 09/03 2003

```
=====================================================================
        Polynomial Model. Revision: 2.2  Date: 9/12/2002
        Input Data File: C:\DOCUMENTS AND SETTINGS\AMARCUS\MY
DOCUMENTS\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.(d)
        Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\AMARCUS\MY
DOCUMENTS\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.plt
                                          Tue Sep 02 22:43:03 2003
 =====================================================================

 Male kidney linear model  std dev = 1 parms rho==0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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 positive
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 1
   Maximum number of iterations = 1000
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                User Inputs Initial Parameter Values
                         alpha =        10000
                           rho =            1   Specified
                        beta_0 =         1000
                        beta_1 =            1



                                  Parameter Estimates

                                                                  95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha          9206.98          2058.74             5171.92
13242
         beta_0          1098.24          23.4986             1052.18
1144.29
         beta_1         0.162349        0.0328205            0.098022
0.226676


           Asymptotic Correlation Matrix of Parameter Estimates

                    alpha       beta_0       beta_1
     alpha              1    -7.5e-007     8.4e-008
    beta_0    -7.5e-007            1        -0.76
    beta_1     8.4e-008        -0.76            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    10  1.08e+003         44.3     1.1e+003           96         -0.469
  312    10  1.16e+003          108    1.15e+003           96          0.333
  625    10  1.21e+003          123     1.2e+003           96          0.438
```

```
 1250    10  1.29e+003          108     1.3e+003           96         -0.302


  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         -202.244681       5     414.489362
              A2         -197.498037       8     410.996073
           fitted        -202.554337       2     409.108675
              R          -212.606673       2     429.213345

 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              30.2173          6          <.0001
   Test 2              9.49329          3          0.0234
   Test 3             0.619313          2          0.7337

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 less than .05.  Consider
running a
non-homogeneous variance model

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



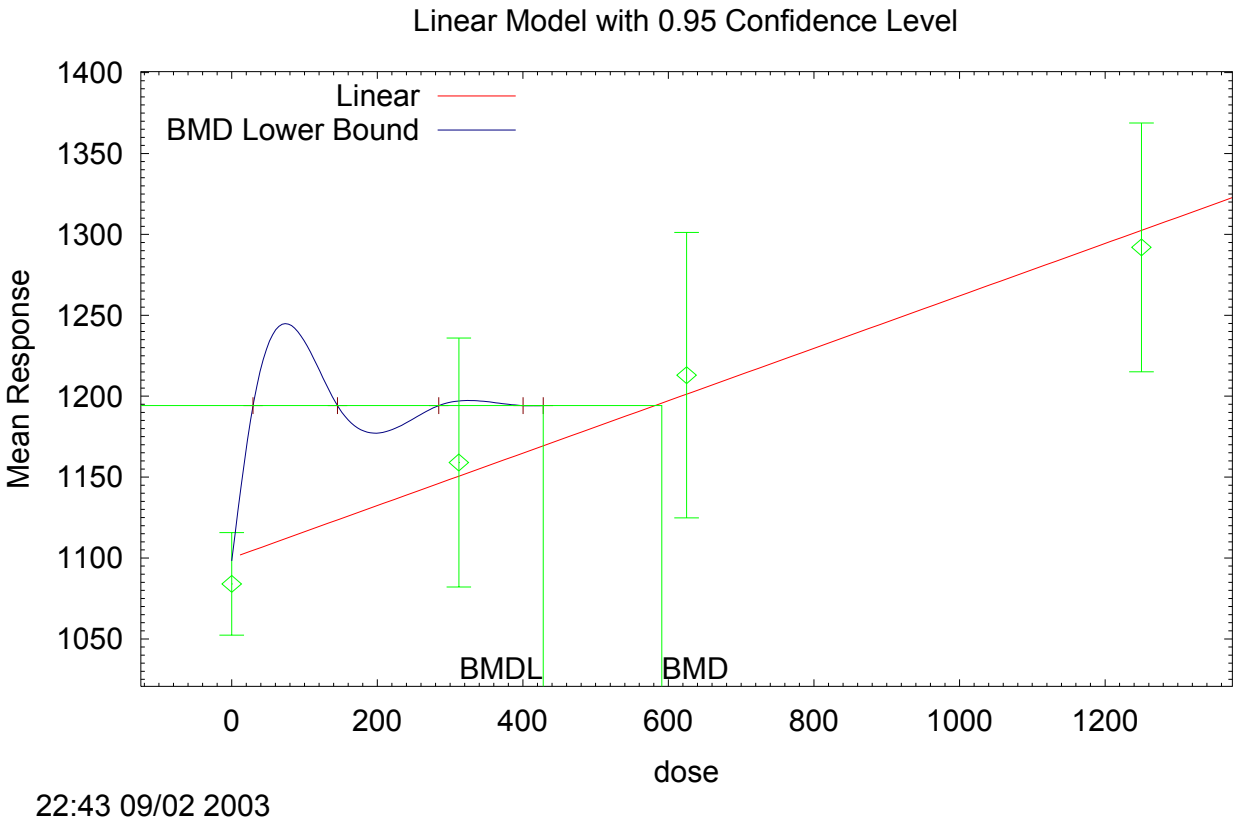
 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

             BMD =       591.029


            BMDL =        427.88
```


Linear Model with 0.95 Confidence Level
Linear
BMD Lower Bound
Mean Response
1400
1350
1300
1250
1200
1150
1100
1050
BMDL
BMD
0
200
400
600
800
1000
1200
dose
22:43 09/02 2003

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 ========================================================================
        Polynomial Model. Revision: 2.2  Date: 9/12/2002
        Input Data File: F:\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.(d)
        Gnuplot Plotting File:  F:\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.plt
                                              Wed Sep 03 15:18:44 2003
 ========================================================================

 Male kidney quadratic std dev=1  parms free
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

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


   Dependent variable = MEAN
   Independent variable = Dose
   Signs of the polynomial coefficients are not restricted
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 5
   Total number of records with missing values = 1
   Maximum number of iterations = 1000
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 User Inputs Initial Parameter Values
                          alpha =       0.0001
                            rho =            0
                         beta_0 =         1100
                         beta_1 =            1
                         beta_2 =        0.001


                                 Parameter Estimates

                                                                95.0% Wald Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha           1e-008     6.48758e-008       -1.17154e-007
1.37154e-007
            rho          3.88492         0.914924              2.0917
5.67814
         beta_0           1093.4          24.5159             1045.35
1141.45
         beta_1         0.172222         0.109699          -0.0427835
0.387228
         beta_2    -8.59546e-010     8.63049e-005        -0.000169155
0.000169154


           Asymptotic Correlation Matrix of Parameter Estimates

                   alpha          rho       beta_0       beta_1       beta_2
     alpha            -1            1       -0.025        0.016      -0.0026
       rho             1           -1        0.028       -0.022        0.009
    beta_0        -0.025        0.028            1         -0.7         0.54
    beta_1         0.016       -0.022         -0.7            1        -0.95
    beta_2       -0.0026        0.009         0.54        -0.95            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	10	1.08e+003	44.3	1.09e+003	79.9	-0.372
312	10	1.16e+003	108	1.15e+003	87.7	0.428
625	10	1.21e+003	123	1.2e+003	95.9	0.394
1250	10	1.29e+003	108	1.31e+003	113	-0.465

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 A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

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

Warning: Likelihood for fitted model larger than the Likelihood for model A3.

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-202.244681	5	414.489362
A2	-197.498037	8	410.996073
A3	-792.622946	6	1597.245893
fitted	-201.467856	5	412.935712
R	-212.606673	2	429.213345

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	30.2173	6	<.0001
Test 2	9.49329	3	0.0234
Test 3	1190.25	2	<.0001
Test 4	-1182.31	1	<.0001

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 less than .05. A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .05. You may want to consider a
different variance model

The p-value for Test 4 is less than .05. You may want to try a different

```
model


 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

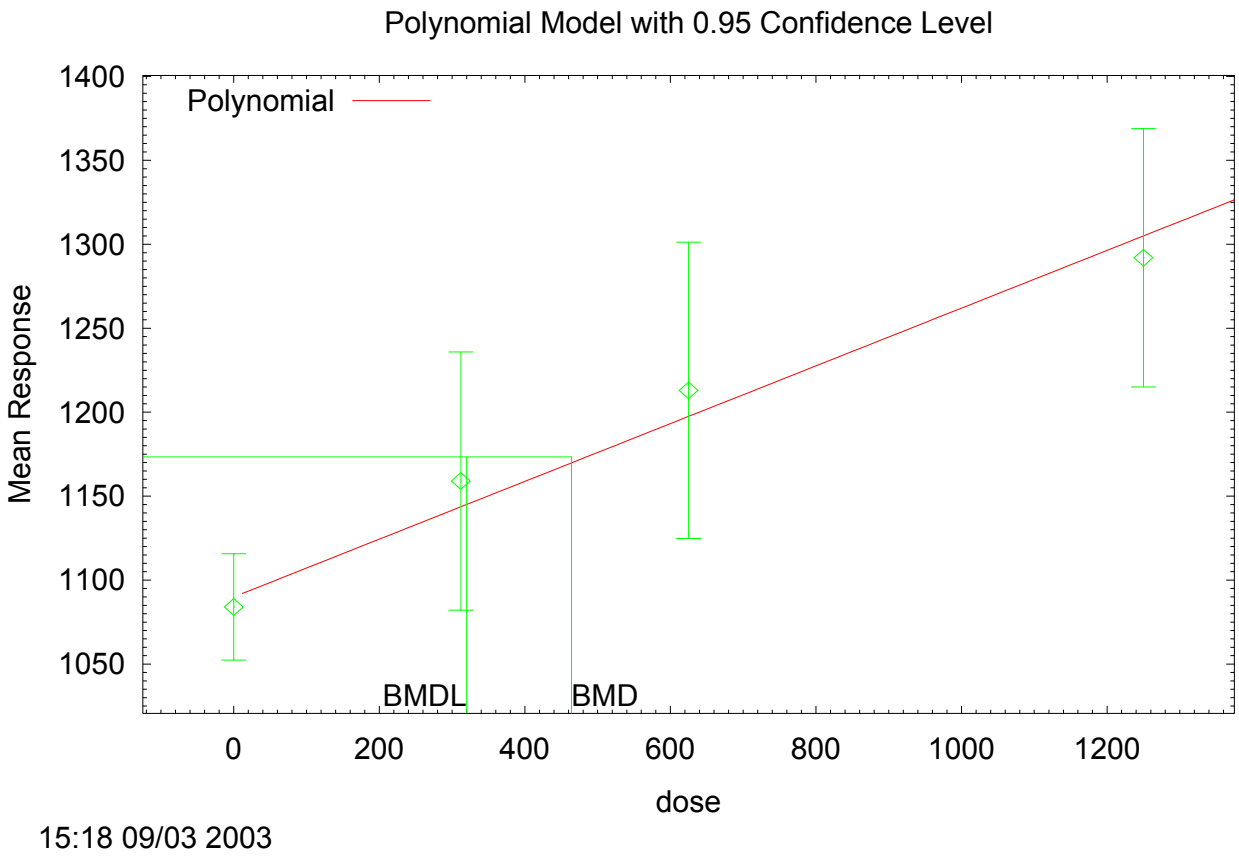

Confidence level =          0.95

             BMD =        464.11


            BMDL =       320.109

BMDL computation failed for one or more point on the BMDL curve.

                    The BMDL curve will not be plotted
```


Polynomial Model with 0.95 Confidence Level
Polynomial
Mean Response
1400
1350
1300
1250
1200
1150
1100
1050
BMDL
BMD
0
200
400
600
800
1000
1200
dose
15:18 09/03 2003

```
================================================================
         Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\AMARCUS\MY
DOCUMENTS\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\AMARCUS\MY
DOCUMENTS\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.plt
                                              Tue Sep 02 23:38:05 2003
 ================================================================

 Male kidney  power model  std dev =1 free parms
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = Dose
  The power is not restricted
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 5
   Total number of records with missing values = 1
   Maximum number of iterations = 1000
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                User Inputs Initial Parameter Values
                         alpha =        0.001
                           rho =            1
                       control =         1100
                         slope =          0.1
                         power =       0.6667


           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho      control        slope        power

     alpha            1           -1        -0.35        -0.46         0.52

       rho           -1            1         0.35         0.46        -0.52

   control        -0.35         0.35            1       -0.027       -0.045

     slope        -0.46         0.46       -0.027            1        -0.99

     power         0.52        -0.52       -0.045        -0.99            1


                          Parameter Estimates

       Variable           Estimate            Std. Err.
          alpha       1.23823e-039        5.77128e-038
            rho            13.9292             6.59632
        control            1080.89             14.8542
          slope            9.88816             15.5551
          power            0.40519             0.24176


     Table of Data and Estimated Values of Interest

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

```
   0    10  1.08e+003         44.3    1.08e+003         47.4         0.0657
 312    10  1.16e+003          108    1.18e+003         88.4         -0.263
 625    10  1.21e+003          123    1.22e+003          107        -0.0201
1250    10  1.29e+003          108    1.26e+003          137         0.243


 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 A3:        Yij = Mu(i) + e(ij)
          Var{e(ij)} = alpha*(Mu(i))^rho

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


 Warning: Likelihood for fitted model larger than the Likelihood for model A3.

                      Likelihoods of Interest

            Model      Log(likelihood)   DF        AIC
              A1         -202.244681      5     414.489362
              A2         -197.498037      8     410.996073
              A3         -216.945928      6     445.891856
           fitted        -199.320949      5     408.641898
              R          -212.606673      2     429.213345


                    Explanation of Tests

 Test 1:  Does response and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)    d.f        p-value

   Test 1              30.2173          6      3.574e-005
   Test 2              9.49329          3          0.0234
   Test 3              38.8958          2         <.00001
   Test 4               -35.25          1         <.00001

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 less than .05.  A non-homogeneous variance
model appears to be appropriate

The p-value for Test 3 is less than .05.  You may want to consider a
different variance model

The p-value for Test 4 is less than .05.  You may want to try a different
model


 Benchmark Dose Computation
Specified effect =            1
```

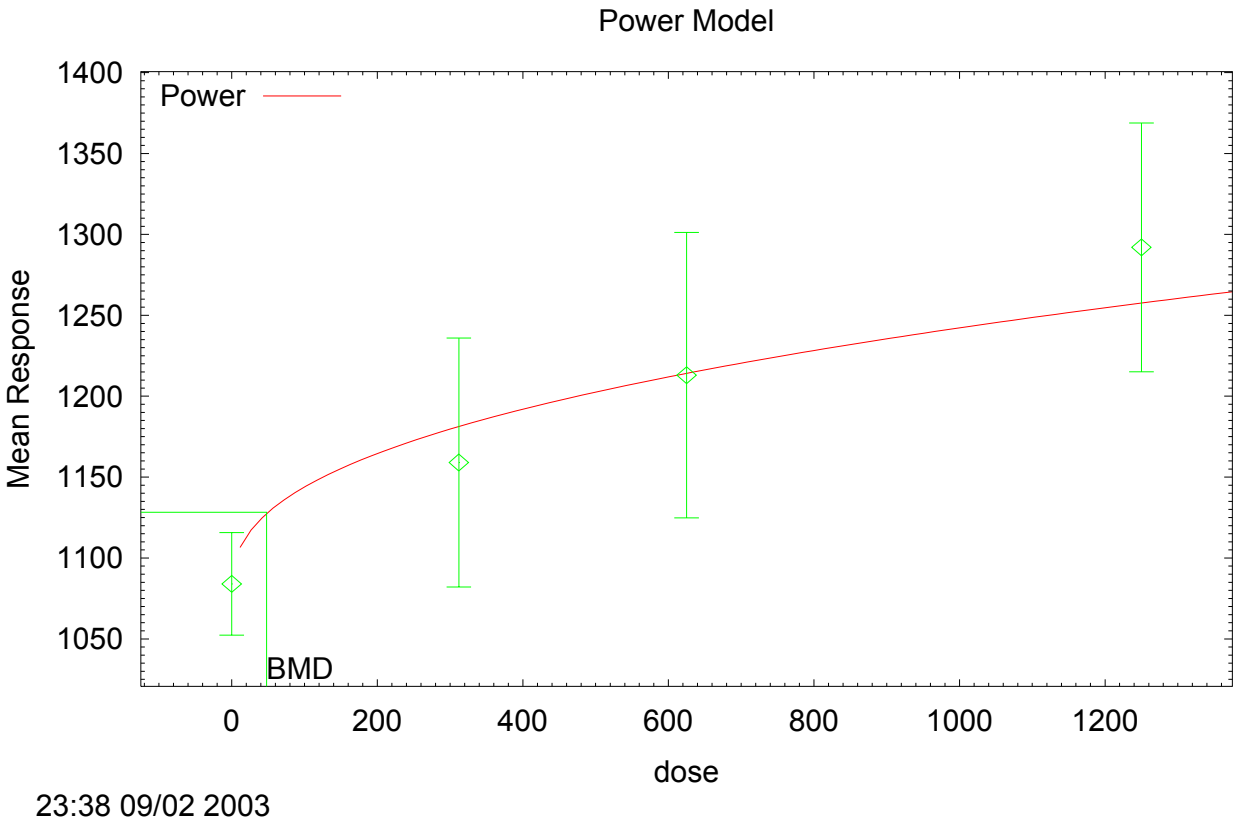
```
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       47.7706

Warning:  optimum may not have been found.  Bad completion code in Optimization
routine.
Warning:  optimum may not have been found.  Bad completion code in Optimization
routine.

BMDL computation failed.
```


Power Model
Power
BMD
Mean Response
dose
1400
1350
1300
1250
1200
1150
1100
1050
0
200
400
600
800
1000
1200
23:38 09/02 2003

```
 ===================================================================
         Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
         Input Data File: C:\DOCUMENTS AND SETTINGS\AMARCUS\MY
DOCUMENTS\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.(d)
         Gnuplot Plotting File:  C:\DOCUMENTS AND SETTINGS\AMARCUS\MY
DOCUMENTS\IRIS\TOLUENE\TOLUENE_NTP_TABLE4.plt
                                                Tue Sep 02 23:47:38 2003
 ===================================================================

 Male kidney  power model  std dev =1 parms rho==0
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
  The power is not restricted
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 1
   Maximum number of iterations = 1000
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                User Inputs Initial Parameter Values
                         alpha =        0.001
                           rho =            1   Specified
                       control =         1100
                         slope =          0.1
                         power =       0.6667


           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho      control        slope        power

     alpha            1           -1         0.54        -0.47         0.44

       rho           -1            1        -0.54         0.47        -0.44

   control         0.54        -0.54            1        -0.69         0.64

     slope        -0.47         0.47        -0.69            1           -1

     power         0.44        -0.44         0.64           -1            1


                           Parameter Estimates

       Variable           Estimate             Std. Err.
          alpha            9067.79               369682
            rho                  0              5.74174
        control            1083.71              35.6717
          slope            1.24815              3.09369
          power           0.718076             0.338085


     Table of Data and Estimated Values of Interest

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

0	10	1.08e+003	44.3	1.08e+003	95.2	0.003
312	10	1.16e+003	108	1.16e+003	95.2	-0.0194
625	10	1.21e+003	123	1.21e+003	95.2	0.0236
1250	10	1.29e+003	108	1.29e+003	95.2	-0.00719

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	-202.244681	5	414.489362
A2	-197.498037	8	410.996073
fitted	-202.249668	4	412.499336
R	-212.606673	2	429.213345

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)	df	p-value
Test 1	30.2173	6	<.00001
Test 2	9.49329	3	0.0234
Test 3	0.00997428	1	0.9204

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 less than .05. Consider running a non-homogeneous variance model

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

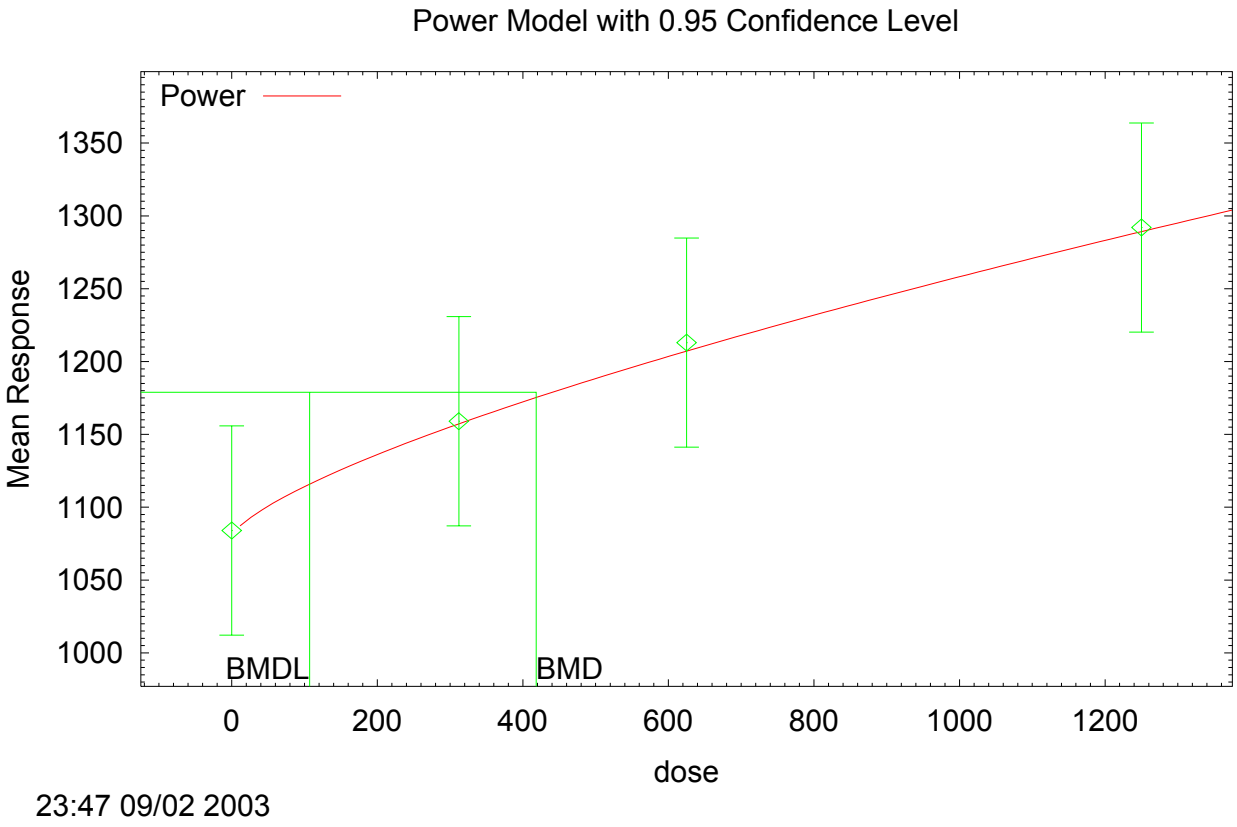
Benchmark Dose Computation
Specified effect = 1

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 418.374

BMDL = 107.032


Power Model with 0.95 Confidence Level
Power
Mean Response
1350
1300
1250
1200
1150
1100
1050
1000
BMDL
BMD
0
200
400
600
800
1000
1200
dose
23:47 09/02 2003

Appendix C: Benchmark Dose Modeling Results for the Derivation of the RfC (Zavalic et al., 1998a)

The age and alcohol-adjusted color confusion index scores reported by Zavalic et al. (1998a) were considered for dose-response modeling using the BMDS package (U.S. EPA, 2003). First, since the data had been expressed as adjusted scores, the adjusted control response was a negative number. BMDS requires that all model coefficients be non-negative or non-positive, so a negative control value would be incompatible with the positive slope that would describe these data. Accordingly, the adjusted control response was normalized to zero by adding 0.029, and the same amount was added to the other 2 adjusted means in order to maintain the same relationship among the responses. This type of transformation has no impact on the dose-response modeling. These data are summarized in Table C-1.

Given the number of dose groups, the only model available to fit the data was the continuous linear model. That is, since the other available models involve more model parameters, there are not enough degrees of freedom to evaluate Goodness-of-Fit statistics. The variances across the exposure groups were reasonably homogeneous, leading to an acceptable fit using the linear model Goodness-of-Fit P=0.24; see the outputs following this section for additional model fit information).

Since there is no level at which the color confusion index indicates a clearly adverse level, several benchmark responses (BMRs) in terms of increments of standard deviations were considered. These BMRs and their corresponding BMCs and BMCLs are summarized in Table C-2, including an equivalent estimate of the extra population risk anticipated to correspond to each BMR. Here the upper 98th percentile of the control distribution is taken to describe an upper limit of "normal" values.

Table C-1: Color confusion index scores, adjusted for alcohol consumption and age, in workers occupationally exposed to toluene - Zavalic et al., 1998a					
Exposure Group	Group Size	AACCI ± SD		Normalized[a] AACCI ± SD	
		Wednesday	Monday	Wednesday	Monday
0 ppm	90	-0.029 ± .06	-0.027 ± .06	0.0 ± .06	0.0 ± .06
32 ppm	46	0.001 ± .07	0.001 ± .07	0.030 ± .07	0.028 ± .07
132 ppm	37	0.039 ± .08	0.037 ± .08	0.068 ± .08	0.064 ± .08

[a] The value of the adjusted control mean response for each day was added to each of the means for that day.

Table C-2: BMC Summary for Selected BMRs			
BMR - standard deviation increment	BMC, ppm	BMCL, ppm	Extra Risk[a] (%)
1	132	99	13
0.5	66	49	4
0.25	33	25	2

[a] Percent of population exposed at the BMC who would be expected to exceed the 98th percentile of the control distribution of adjusted scores, assuming the data are normally distributed and that the fitted model is plausible.

```
 ======================================================================
        Polynomial Model. Revision: 2.2   Date: 9/12/2002
        Input Data File: F:\USER\KHOGAN02\_BMDS\TOLUENE_AACCI.(d)
        Gnuplot Plotting File:   F:\USER\KHOGAN02\_BMDS\TOLUENE_AACCI.plt
                                              Wed Nov 19 09:20:49 2003
 ======================================================================

 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 = ppm
  rho is set to 0
  Signs of the polynomial coefficients are not restricted
  A constant variance model is fit

  Total number of dose groups = 3
  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 =                0.00453706
              rho =                         0   Specified
            beta_0 =                0.00627109
            beta_1 =               0.000482846



                Parameter Estimates

                          95.0% Wald Confidence Interval
    Variable            Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
     alpha            0.00449393       0.00048319          0.00354689          0.00544096
     beta_0           0.00382987       0.00626203         -0.00844349           0.0161032
     beta_1          0.000508721     9.90263e-005         0.000314633         0.000702809


      Asymptotic Correlation Matrix of Parameter Estimates

           alpha                beta_0       beta_1
   alpha               1    -6.4e-009     6.1e-009
   beta_0      -6.4e-009            1        -0.58
   beta_1       6.1e-009        -0.58            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       90          0         0.06      0.00383        0.067         -0.542
 32       46       0.03         0.07       0.0201        0.067              1
132       37      0.068         0.08        0.071        0.067          -0.27


 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                381.721859       4    -755.443718
      A2                384.000080       6    -756.000161
     fitted             381.034967       2    -758.069935
      R                 368.752511       2    -733.505021

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               30.4951          4          <.0001
 Test 2               4.55644          2          0.1025
 Test 3               1.37378          1          0.2412

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
```

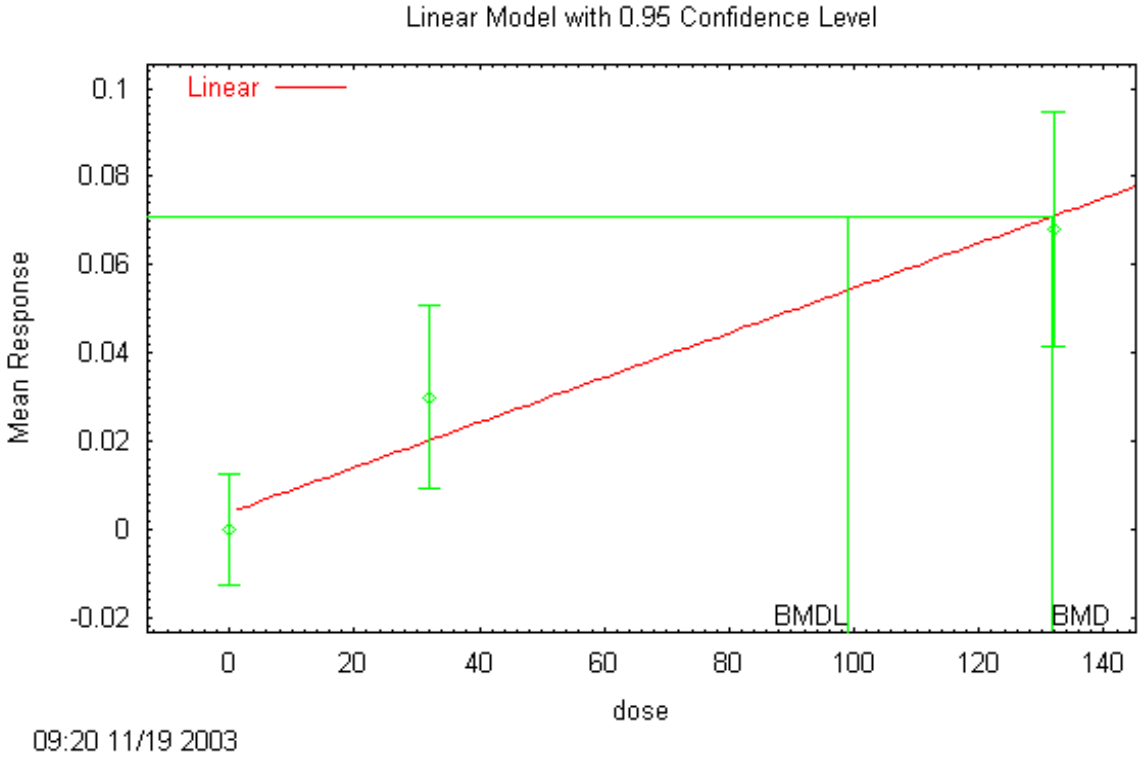
```
 Benchmark Dose Computation
Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

      BMD =              131.775


      BMDL =             98.9107
```



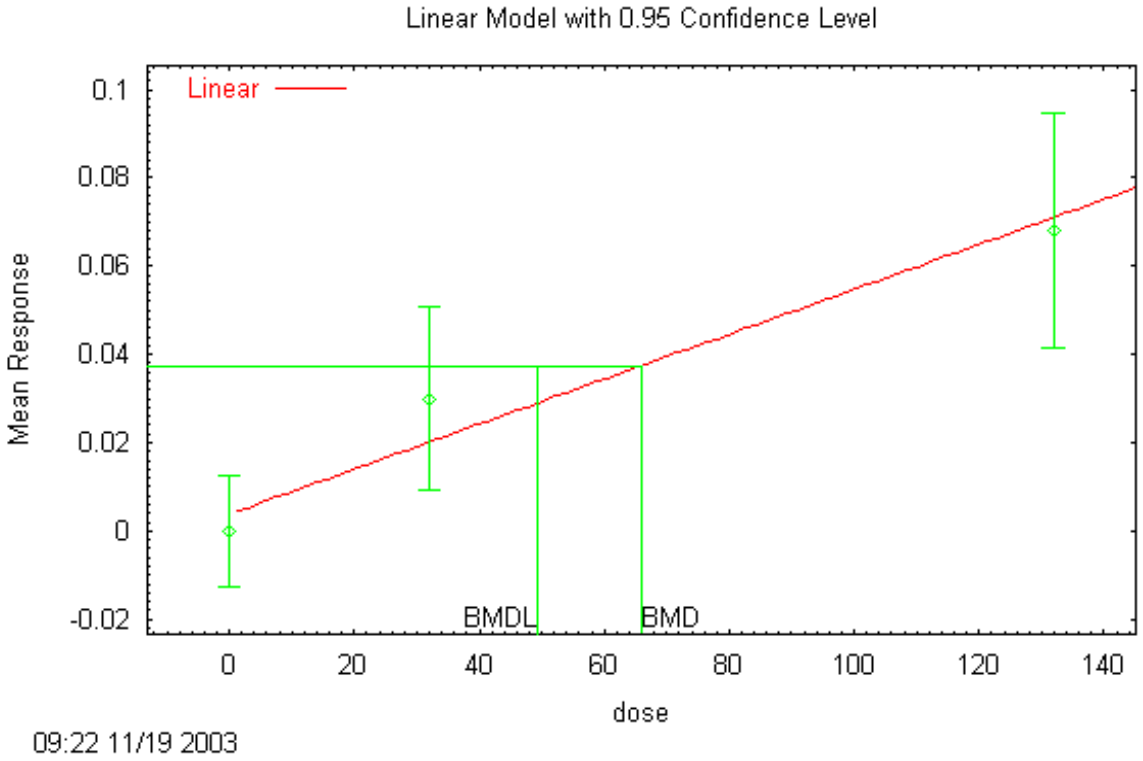
```
 Benchmark Dose Computation
Specified effect =           0.5

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

      BMD =              65.8875


      BMDL =             49.4554
```



```
 Benchmark Dose Computation
Specified effect =          0.25

Risk Type        =     Estimated standard deviations from the control mean


Confidence level =          0.95

      BMD =              32.9437


      BMDL =             24.7277
```