EPA

TOXICOLOGICAL REVIEW

OF

METHYL ETHYL KETONE

(CAS No. 78-93-3)

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

September 2003

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Note: This document may undergo revisions in the future. The most up-to-date version will be made available electronically via the IRIS Home Page at http://www.epa.gov/iris.

CONTENTS —TOXICOLOGICAL REVIEW OF
METHYL ETHYL KETONE (CAS No. 78-93-3)

LIST OF TABLES

LIST OF FIGURES

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 methyl ethyl ketone. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of methyl ethyl ketone.

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.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Susan Rieth
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

AUTHORS

Susan Rieth
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Karen Hogan
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Mark H. Follansbee, Ph.D.
Syracuse Research Corporation
Scarborough, ME

Peter McClure, Ph.D., DABT
Syracuse Research Corporation
North Syracuse, NY

Regina McCartney
Syracuse Research Corporation
Cincinnati, OH

Syracuse Research Corporation staff performed work under Contract No. 68-C-00-122, Work Assignment 2-06.

REVIEWERS

This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Director has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and

Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information; and the Regional Offices.

INTERNAL EPA REVIEWERS

Katherine Anitole, Ph.D.
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, DC

Daniel Axelrad, M.P.P.
Office of Policy, Economics, and Innovation
National Center for Environmental Economics
U.S. Environmental Protection Agency
Washington, DC

Philip Bushnell, Ph.D.
Office of Research and Development
National Health and Environmental Effect Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Audrey Cummings, Ph.D.
Office of Research and Development
National Health and Environmental Effect Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Robert Dewoskin, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Gary Foureman, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

David Herr, Ph.D.
Office of Research and Development
National Health and Environmental Effect Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Tracey Woodruff, Ph.D., M.P.H.
Office of Policy, Economics, and Innovation
National Center for Environmental Economics
U.S. Environmental Protection Agency
San Francisco, CA

EXTERNAL PEER REVIEWERS

Bryan Hardin, Ph.D.
Global Tox
Assistant Surgeon General, U.S. Public Health Service (retired)

Dale Hattis, Ph.D.
Clark University
Worcester, MA

Arthur R. Gregory, Ph.D.
Techto Enterprises
Luray, VA

Rochelle W. Tyl, Ph.D.
Research Triangle Institute
Research Triangle Park, NC

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

1. INTRODUCTION

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

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

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ 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 these hazard identification and dose-response assessments for methyl ethyl ketone 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, 1991a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *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 was based on the CASRN and at least one common name. At a minimum, the following data bases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. For this toxicological review, updated literature searches for 1987 to July 2003 were conducted for MEK. Literature searches were also conducted from 1991 to July 2003 for 2-butanol and from 1965 to July 2003 for 3-hydroxy-2-butanone and 2,3-butanediol. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Methyl ethyl ketone (MEK) is also known as 2-butanone, butanone, ethyl methyl ketone, and methyl acetone. Some relevant physical and chemical properties of MEK are listed below (ATSDR, 1992; CRC, 1994; HSDB, 1999; NTP, 2002):

CAS registry number: 78-93-3
Chemical formula: C_4H_8O
Molecular weight: 72.11
Density: 0.805 g/mL @ 20°C
Vapor pressure: 77.5 mm Hg @ 20°C
Water solubility: 275 mg/mL @ 20°C
Conversion factor: 1 ppm = 2.95 mg/m^3, 1 mg/m^3 = 0.340 ppm @ 25°C, 760 mm Hg

At room temperature, MEK is a clear liquid with a fragrant mint-like odor. It is flammable, with a flash point of -3°C. MEK is strongly reactive with a number of chemicals and chemical classes, including potassium tert-butoxide, chloroform, hydrogen peroxide, and strong oxidizers (e.g., chlorosulfonic, sulphuric, and nitric acids). It can also react with bases and strong reducing agents. Vigorous reactions occur with chloroform in the presence of bases, and explosive peroxides are formed when added to hydrogen peroxide and nitric acid. ACGIH (2001) recommends an 8-hour time-weighted average threshold limit value (TWA-TLV) of 200 ppm (590 mg/m^3) MEK. Similarly, the Occupational Safety and Health Administration (OSHA) has promulgated an 8-hour permissible exposure limit (PEL) of 200 ppm (590 mg/m^3) MEK (OSHA, 1993).

MEK is used as a solvent in the application of protective coatings (varnishes) and adhesives (glues and cements), in magnetic tape production, in smokeless powder manufacture, in the dewaxing of lubricating oil, in vinyl film manufacture, and in food processing. Its use as a component in adhesives used to join PVC pipes is a potential route for entry of the chemical into potable water (ATSDR, 1992). It is also commonly used in paint removers, cleaning fluids, acrylic coatings, pharmaceutical production, and colorless synthetic resins, and as a printing catalyst and carrier (Merck Index, 2001). MEK may be found in soil and water in the vicinity of some hazardous waste sites. MEK has been detected as a natural component of numerous foods, including: raw chicken breast, milk, nuts (roasted filberts), cheese (Beaufort, Gruyere, and cheddar), bread dough and nectarines at concentrations ranging from 0.3 to 19 ppm (ATSDR, 1992; HSDB, 1999; WHO, 1992). MEK is also found in tobacco smoke and volatile releases from building materials and consumer products (ATSDR, 1992). WHO (1992) estimated levels of daily MEK intake from different sources as follows: foodstuffs – 1,590 µg/day; drinking

water (2 liters) – 3.2 µg/day; rural outdoor air – 36 µg/day; urban outdoor air ≤760 µg/day; and tobacco smoke ≤1,620 µg/day.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

3.1.1. Oral Exposure

Case reports provide qualitative evidence that MEK is absorbed by the gastrointestinal tract following oral exposure in humans; however, they do not provide information regarding the extent of absorption following ingestion. For example, a woman accidentally ingested an unknown quantity of MEK and presented with symptoms of metabolic acidosis and a blood concentration of 95 mg/100 mL (13.2 mM) MEK (Kopelman and Kalfayan, 1983). A man who intentionally ingested 100 mL of liquid cement containing a mixture of acetone (18%), MEK (28% or about 37 mg/kg), and cyclohexanone (39%) was treated by gastric lavage 2 hours after ingestion. Three hours later, he had a plasma level of about 110 µg/mL MEK (Sakata et al., 1989).

Experimental data from rodents indicate that orally administered MEK is absorbed from the gastrointestinal tract and rapidly eliminated. Oral administration (gavage) of 1,690 mg/kg of MEK to four male Sprague-Dawley rats resulted in a mean peak plasma concentration of 94.1 mg/100 mL after 4 hours that decreased to 6.2 mg/100 mL 18 hours after exposure (Dietz and Traiger, 1979; Dietz et al., 1981). Thrall et al. (2002) reported mean peak concentrations in exhaled air 1 hour after an oral gavage dose of 50 mg/kg MEK to three male F344 rats, providing further support that MEK is absorbed from the digestive tract.

3.1.2. Inhalation Exposure

Data from humans and rats suggest that MEK is well absorbed during inhalation exposure due to its high blood/air solubility ratio (Perbellini et al., 1984; Sato and Nakajima, 1979; Thrall et al., 2002). Perbellini et al. (1984) investigated the uptake and kinetics of MEK in groups of industrial workers occupationally exposed to MEK. In one group, the concentration of MEK in environmental air was compared to MEK in the alveolar air of exposed workers (n = 82) by simultaneous collection of air samples into glass tubes via instantaneous sampling methods and gas chromatography (GC) analysis. Most of the measurements were made at environmental concentrations at or below 100 ppm. The alveolar air concentration of MEK in the exposed workers was highly correlated with the environmental air concentration and averaged 30% of the latter. From these survey results, the investigators estimated a pulmonary retention of 70% in

workers exposed to concentrations less than 300 ppm for 4 hours. Perbellini et al. (1984) presented a physiologically-based mathematical model for MEK that suggests that steady-state concentrations are reached within 8 hours when exposures are between 50 and 100 ppm, depending on the physical work load. In a controlled exposure experiment, pulmonary uptake in volunteers ranged from 51 to 55% of the inspired quantity at 200 ppm MEK for 4 hours in an exposure chamber (Liira et al., 1988). Liira et al. (1990a) found the pulmonary retention of MEK in five human volunteers similarly exposed to MEK to be $55.8 \pm 9.1\%$. Exercise increased the pulmonary uptake of MEK due to the greater ventilatory rate (Liira et al., 1988). Liira et al. (1990b) and Imbriani et al. (1989) reported that human inhalation exposure to MEK exhibited dose-dependent saturation. Dick et al. (1988) exposed 24 volunteers (12 men and 12 women) to MEK at 200 ppm for 4 hours and reported that alveolar breath samples (exhaled air) reached steady-state concentrations by 2 hours, stabilizing at 5–6% of the exposure concentration. There is no apparent explanation for the much lower pulmonary retention reported by Dick et al. (1988) as compared to Liira et al. (1988, 1990a).

Kessler et al. (1988) reported a pulmonary retention of 40% for rats exposed to concentrations less than or equal to 180 ppm for up to 14 hours.

3.1.3. Dermal Exposure

The percutaneous absorption of MEK appears to be rapid (Munies and Wurster, 1965; Wurster and Munies, 1965). These authors reported that MEK was present in the exhaled air of human subjects within 2.5–3.0 minutes after application to normal skin of the forearm, and the concentration of MEK in exhaled air reached a plateau in approximately 2 hours. The rate of absorption was slower when MEK was applied to dry skin, where a plateau for the concentration of MEK in expired air was attained in 4–5 hours. By contrast, absorption of MEK to moist skin was very rapid. MEK was detected in expired air in measurable concentrations within 30 seconds after application of MEK to the skin of the forearm, and a maximum concentration in expired air was achieved in 10–15 minutes, decreasing thereafter. Munies and Wurster (1965) concluded that the rapid percutaneous absorption of MEK is related to its olive oil-water partition coefficient of 0.93, as reported by GC analysis.

The percutaneous absorption data of Munies and Wurster (1965) have been used to calculate the following minimum rates of percutaneous penetration of MEK: 0.46 µg/cm^2/minute for dry or normal skin and 0.59 µg/cm^2/minute for moist skin (JRB Associates, 1980 as cited in WHO, 1992). Ursin et al. (1995) also studied the *in vitro* permeability of MEK through living

human skin. Ursin et al. (1995) measured the permeability of various solvents, including MEK, through a 0.64 cm² sample of living skin tissue separating a two-chamber diffusion cell. All skin samples were first calibrated for relative permeability using tritiated water. The authors concluded that MEK has a permeability rate of 53±29 g/m²/hour, which is equivalent to approximately 0.0066 cm/hour (Ursin et al., 1995) or approximately 88.3 µg/cm²/minute [53 g/m²-hour) x (1 hour/60 minute) x (100 µg/1 g) x (1 m²/10⁴ cm²) = 88.3 µg/cm²-minute]. The permeability absorption values from these studies differ by 2 orders of magnitude. The values reported by Munies and Wurster (1965) may be low because the analysis was based solely on the amount of MEK exhaled from the lungs, thereby not considering all routes of MEK elimination (WHO, 1992).

Brooke et al. (1998) studied the dermal uptake of MEK from the vapor phase. Groups of four volunteers were exposed for 4 hours to MEK in an inhalation chamber either 'whole body' or via the 'skin only' at 200 ppm MEK. For skin-only exposures, volunteers wore air masks that delivered room air. Uptake was assessed by monitoring levels of MEK in blood, single breath, and urine following exposure. Brooke et al. (1998) reported that dermal absorption of MEK contributed approximately 3–3.5% of the total body burden.

3.2. DISTRIBUTION

No studies were located regarding the distribution of MEK following oral or dermal exposure in humans or animals. In a study of MEK-exposed industrial workers (n = 23), Perbellini et al. (1984) compared the concentration of MEK in venous blood to alveolar air. Samples were collected simultaneously toward the end of the work shift and analyzed by gas chromatography-mass spectrometry (GC/MS). The level of MEK in the blood was significantly correlated with the environmental concentration, indicating rapid transfer from the lungs to the blood. Information on the distribution of MEK following inhalation exposure in humans also comes from an examination of postmortem tissues reported by Perbellini et al. (1984). The distribution of MEK in human tissues was examined in two solvent-exposed workers who died suddenly of heart attacks at the workplace (Perbellini et al., 1984). Postmortem determinations of the MEK tissue/air solubility ratio for human kidney, liver, muscle, lung, heart, fat, and brain revealed similar solubility in all these tissues, with the tissue/air ratio ranging from 147 (lung) to 254 (heart) (Perbellini et al., 1984). The available data suggest that MEK does not accumulate in fatty tissues in humans. Blood/tissue solubility ratios for several tissues approach unity (Perbellini et al., 1984). Since the results have also been repeated in rats (Thrall et al., 2002),

MEK is not expected to accumulate in any particular tissue (Perbellini et al., 1984).

3.3. METABOLISM

The available evidence indicates that the metabolism of MEK is similar in humans and experimental animals. As shown in Figure 1, the majority of MEK is metabolized to 3-hydroxy-2-butanone, which is subsequently metabolized to 2,3-butanediol. A small portion is reversibly converted to 2-butanol. Evidence supporting common metabolic pathways for MEK in humans and experimental animals is presented below.

In humans exposed to airborne MEK, 2-butanol and 2,3-butanediol have been identified as MEK metabolites in serum, while 3-hydroxy-2-butanone and 2,3-butanediol have been identified as urinary metabolites of MEK (Perbellini et al., 1984; Liira et al., 1988, 1990a). From a study of the kinetics of inhaled MEK in human volunteers (200 ppm for 4 hours), it was estimated that 3% of the absorbed dose was exhaled as unchanged MEK, 2% of the absorbed dose was excreted in urine as 2,3-butanediol, and the remainder of the absorbed dose entered into mainstream intermediary metabolism and was transformed to simple compounds such as carbon dioxide and water (Liira et al., 1988). Results from this study suggest that MEK is rapidly and nearly completely metabolized in humans exposed to 200 ppm MEK for 4 hours.

In humans, MEK has also been identified as a minor but normal constituent of urine, as a constituent in the serum and urine of diabetics, and in expired air. Its production in the body has been attributed to isoleucine catabolism (WHO, 1992). MEK was detected in the blood of more than 75% of the participants of the general population in the Third National Health and Nutrition Examination Survey (NHANES III) (Ashley et al., 1994; Churchill et al., 2001). Median blood levels were 5.4 ppb. Investigators looked for associations between MEK blood levels and self-reported chemical exposures as collected via NHANES questionnaire. Blood MEK levels were positively associated with mean daily alcohol intake, and were generally not associated with other environmental exposure variables (Churchill et al., 2001).
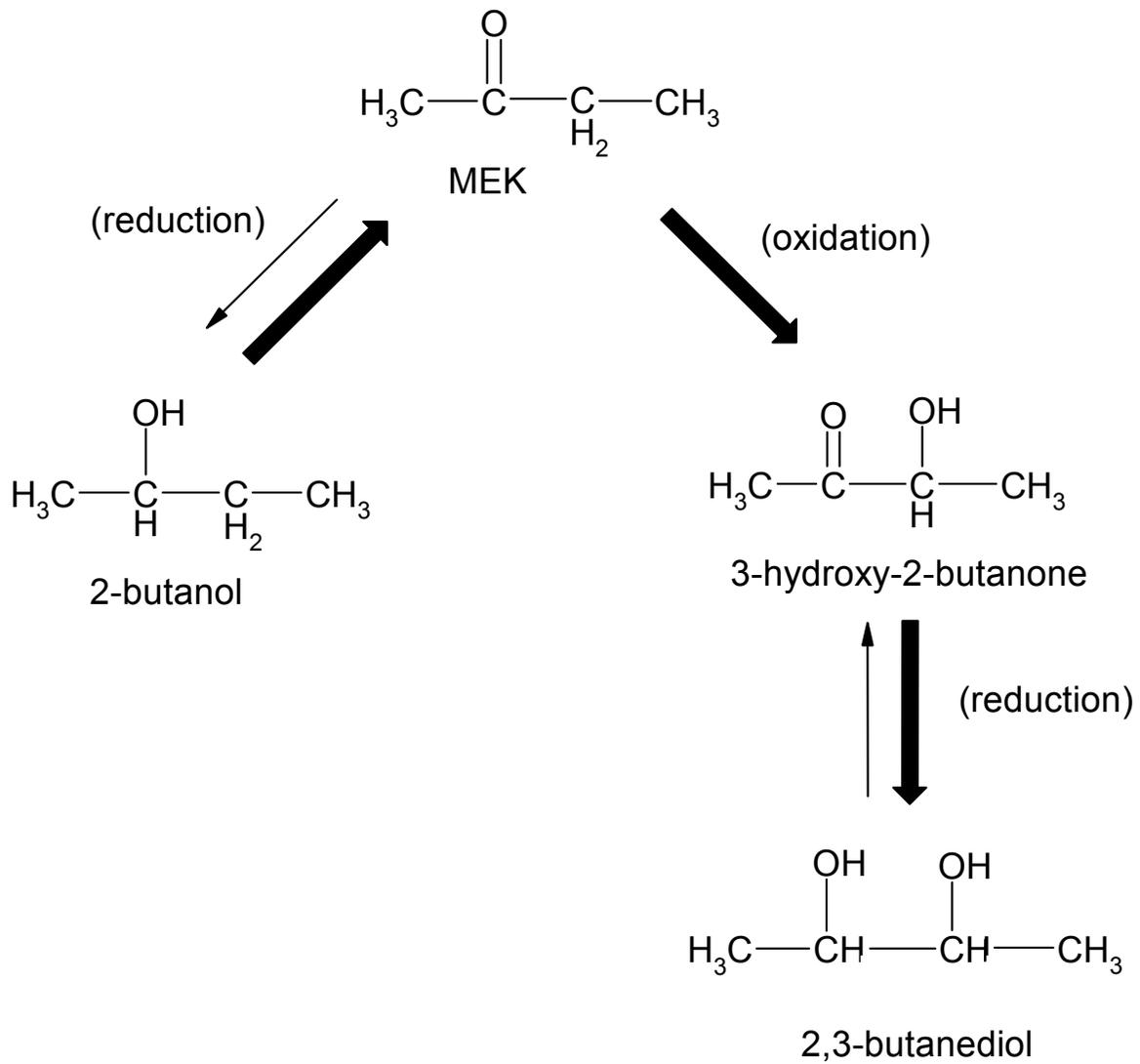
Figure 1. Proposed pathways for methyl ethyl ketone metabolism

Source: Adapted from DiVincenzo et al. (1976).

In rats and guinea pigs, the metabolism of MEK may follow one of two pathways (Dietz et al., 1981; DiVincenzo et al., 1976). The majority of MEK is oxidized by the cytochrome P450 monooxygenase system (P450IIE1 and IIB isozymes) to the primary metabolite, 3-hydroxy-2-butanone (3H-2B), which is subsequently reduced to 2,3-butanediol (2,3-BD) (Dietz and Traiger, 1979; Traiger et al., 1989; Brady et al., 1989; Raunio et al., 1990). A small portion of absorbed MEK is reduced to 2-butanol, which is rapidly oxidized back to MEK. Based on the data from Traiger and Bruckner (1976), Dietz et al. (1981) established that approximately 96% of an administered oral dose of 2-butanol is oxidized *in vivo* to MEK within 16 hours of oral administration. Dietz et al. (1981) reported that no significant difference in area under the curve (AUC) of MEK blood concentration was observed after oral dosing of rats with either 1,776 mg/kg 2-butanol or 1,690 mg/kg MEK (10,899±842 or 9,868±566 mg-hour/liter, respectively).[1] Peak concentrations of MEK and its downstream metabolites were similar whether MEK or 2-butanol were administered (Dietz et al., 1981), with a shift of approximately 4 hours to reach peak concentrations when MEK was administered:

	Peak Blood Concentration	
	Administration of 1,776 mg/kg 2-butanol	Administration of 1,690 mg/kg MEK
MEK	0.78 mg/ml at 8 hr	0.95 mg/ml at 4 hr
3H-2B	0.04 mg/ml at 12 hr	0.027 mg/ml at 8 hr
2,3-BD	0.21 mg/ml at 18 hr	0.26 mg/ml at 18 hr

Dietz et al. (1981) provides further support for the rapid conversion of orally administered 2-butanol to MEK. Ultimately, 2-butanol and MEK are metabolized through the same intermediates as shown in Figure 1.

DiVincenzo et al. (1976) identified the metabolites of aliphatic ketones in the serum of guinea pigs after administering a single dose of methyl n-butyl ketone, methyl isobutyl ketone, or MEK. The hepatic cytochrome P450-mediated metabolism of MEK (Figure 1) produced hydroxylated metabolites (3-hydroxy-2-butanone and 2,3-butanediol) that were eliminated in the urine (DiVincenzo et al., 1976). Male Sprague-Dawley rats given a single oral dose of MEK at 1,690 mg/kg exhibited blood concentrations of MEK and metabolites 4 hours after dosing as

---

[1] On a molar basis, the administered concentrations of 2-butanol and MEK are 0.024 and 0.023 mol/kg, respectively.

follows: MEK (94.1 mg/100 mL), 2-butanol (3.2 mg/100 mL), 3-hydroxy-2-butanone (2.4 mg/100 mL), and 2,3-butanediol (8.1 mg/100 mL) (Dietz and Traiger, 1979; Dietz et al., 1981). After 18 hours, blood concentrations of the parent compound and metabolites were: MEK (6.2 mg/100 mL), 2-butanol (0.6 mg/100 mL), 3-hydroxy-2-butanone (1.4 mg/100 mL), and 2,3-butanediol (25.6 mg/100 mL) (Dietz and Traiger, 1979).

Interestingly, the data of Dietz et al. (1981) demonstrated a peak blood concentration of MEK approximately 4 hours after oral administration of Sprague-Dawley rats to 1,690 mg/kg MEK, while Thrall et al. (2002) found peak concentrations in exhaled air at 1 hour after oral gavage of 50 mg/kg MEK to F344 rats. Thrall et al. (2002) concluded that the differences in MEK dose level (approximately 35-fold), rat strain used, and overnight fasting may explain the discrepancy between these findings.

Gadberry and Carlson (1994) showed that the *in vitro* hepatic oxidation of 2-butanol to MEK is inducible by pretreatment with ethanol (an inducer of P450IIE1) and phenobarbital (an inducer of P450IIB and IVB), but not beta-naphthaflavone (an inducer of P450IA1). By contrast, *in vitro* studies showed that 2-butanol oxidation in the lung was not inducible by any of the treatments. A daily dose of 1.4 mL/kg MEK for 3 days increased the amounts of ethanol- and phenobarbital-inducible cytochrome P450 isoforms (P450IIE1 and P450IIB) as demonstrated by *in vitro* assays (Raunio et al., 1990). Because MEK is an inducer of microsomal P450 activity, repeated MEK exposure may enhance the body's capacity for metabolism of subsequent exposures.

3.4. ELIMINATION AND EXCRETION

In human studies involving acute inhalation exposure, the urinary excretion of MEK and metabolites and the exhalation of unchanged MEK have been estimated to account for only a small percentage (0.1–3%) of the absorbed dose (Perbellini et al., 1984; Liira et al., 1988). The remainder of the absorbed dose is expected to have undergone rapid transformation to carbon dioxide and water through intermediary metabolic pathways (Liira et al., 1988). Nevertheless, the presence of unchanged MEK in urine has been proposed as a marker of exposure since strong positive correlations have been reported between MEK levels in urine and MEK levels in air (Perbellini et al., 1984; Liira et al., 1988; Imbriani et al., 1989; Sia et al., 1991; ACGIH, 2001).

MEK is rapidly cleared from the blood with a reported plasma half-life in humans of 49–96 minutes, exhibiting a biphasic elimination: $t_{1/2}$ alpha = 30 minutes and $t_{1/2}$ beta = 81 minutes (Liira et al., 1988). Dick et al. (1988) collected blood samples from 20 volunteers (sex not specified) who were exposed to 100 or 200 ppm MEK for 4 hours. Blood samples were obtained from each subject at 2 and 4 hours from the start of exposure and 15 and 20 hours post exposure. Assuming first-order kinetics, Dick et al. (1988) estimated an elimination half-life of 49 minutes for MEK. MEK was not detected in blood at 20 hours post exposure. Given the rapid clearance of MEK demonstrated by Liira et al. (1990b) and Dick et al. (1988), it is unlikely that MEK would accumulate with chronic exposure.

Based on the strong correlation between urinary MEK concentration and environmental exposure, a biological exposure index of 2 mg/L MEK in urine measured at the end of the work shift has been adopted to monitor occupational exposure to MEK (ACGIH, 2001).

3.5. PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELS

Physiologically-based pharmacokinetic (PBPK) models of MEK are available for humans (Liira et al., 1990b; Leung, 1992) and rats (Dietz et al., 1981; Thrall et al., 2002). PBPK models are unavailable for other species. The structural differences and limited data sets used to calibrate and test the rat and human models limits their application. The human PBPK model (Liira et al., 1990b; Leung, 1992) was developed to describe the dose-dependent elimination kinetics of MEK in humans following inhalation exposure to low concentrations of MEK. Liira et al. (1990b) exposed two men in an inhalation chamber for 4 hours in separate exposures to 25, 200, or 400 ppm MEK. Venous blood samples were taken during each exposure and for 8 hours thereafter. The metabolism of MEK was assumed to occur only in the liver and was described by Michaelis-Menten kinetics. The model, which is based on the spreadsheet model of Johanson and Naslund (1988), contained eight compartments describing the kinetics of MEK in lungs, GI tract, liver, richly perfused tissue, poorly perfused tissue, fat, muscle, and blood (see Table 1 for

model parameters). The elimination rate for MEK was calculated by the following equation:

$$\text{elimination rate} = V_{max} \times C_h / (K_m + C_h)$$

where:

$C_h =$ MEK concentration in hepatic venous blood;

$V_{max} =$ 30 µmol/minute (obtained by applying best fit of simulated curves to experimental MEK blood concentration); and

$K_m =$ 2 µM (obtained by applying best fit of simulated curves to experimental MEK blood concentration).

Liira et al. (1990b) reported that model predictions were similar to observed blood concentrations of MEK in 17 male volunteers exposed to 200 ppm. The authors also concluded that the kinetic constants were fairly representative of healthy male subjects.

Research utilizing rats (Dietz and Traiger, 1979; Dietz et al., 1981) identified the pathways of MEK metabolism and permitted a calculation of rate constants for the elimination of MEK and its metabolites from the blood as well as for the metabolic transformations. The data were used as the basis for a PBPK model for MEK (Dietz et al., 1981) to predict blood concentrations of 2-butanol and its metabolites. More specifically, the model was used to predict concentrations of MEK (i.e., 2-butanone), 3-hydroxy-2-butanone, and 2,3-butanediol in Sprague-Dawley rats after oral administration of 2-butanol or MEK, as well as after intravenous administration of 3-hydroxy-2-butanone or 2,3-butanediol.

The model contains two compartments (in the blood and the liver) where metabolism occurs. The differential equations are based upon a perfusion-limited model, and account for: (1) the elimination of 2-butanol and its metabolites from the blood at rates linearly proportional to blood concentrations, (2) transport between the blood and liver compartments, and (3) metabolic conversions in the liver. Metabolic conversions were described with Michaelis-Menten saturation kinetics and included rates for bidirectional conversions between 2-butanol and MEK, unidirectional conversion of MEK to 3-hydroxy-2-butanone, and bidirectional conversions between 3-hydroxy-2-butanone and 2,3-butanediol. Kinetic constants in the model were estimated by successive curve fitting of submodels to *in vivo* blood concentration data from groups of 5 rats following: (1) a single gavage administration of 1,690 mg/kg MEK, (2) a single gavage administration of 1,776 mg/kg 2-butanol, and (3) intravenous injections of 3-hydroxy-2-butanone and 2,3-butanediol at 400 or 800 mg/kg. Equations describing the metabolic conversion of MEK to 3-hydroxy-2-butanone included a competitive inhibition of its conversion

that was attributed to the presence of the competitive substrate, 2-butanol. In addition, a distribution coefficient was included to account for the unexpectedly low observed concentration of 3-hydroxy-2-butanone in the blood. The authors hypothesized that this was due to partitioning, binding, or altered transport rates from the liver. The "adjustments" resulted in an improved fit between model simulations and experimentally observed blood concentrations of MEK and 2-butanol following oral administration of 1,690 mg/kg MEK, but predicted blood concentrations of 3-hydroxy-2-butanone and 2,3-butanediol were about 20–30% lower than the observed values.[2] There were no comparisons reported for model predictions with data not used to derive the model parameters.

Thrall et al. (2002) developed a PBPK model for MEK in F344 rats, from experimentally determined partition coefficients using *in vitro* vial equilibration technique and *in vivo* measurements of MEK uptake in rats exposed to 100 to 2,000 ppm MEK in a closed, recirculating gas uptake system. The model included both a saturable metabolic pathway described by Michaelis-Menten kinetic constants and a nonsaturable first-order pathway. The model provided adequate predictions (based on visual inspection) of exhaled MEK concentrations following inhalation, intravenous, intraperitoneal, or oral administration of MEK to rats. One notable difference between the Thrall et al. (2002) and Dietz et al. (1981) models is the peak exhaled breath concentrations following oral gavage. Dietz et al. (1981) found peak MEK concentrations in blood 4 hours after oral gavage (1,690 mg/kg MEK), whereas the Thrall et al. (2002) study found peak MEK concentrations in exhaled air 1 hour after oral gavage (50 mg/kg MEK).

The Thrall et al. (2002) model could be extended to humans by substituting human parameter values for rat parameter values. Use of such a model for risk assessment purposes would still be dependent upon sufficient validation or comparisons of model predictions with relevant human data. This has not been carried out to date.

In summary, three PBPK models have been developed based on a limited number of data sets in rats and humans. The predictive capabilities of these models have not been adequately tested, and none of the models were parameterized for rats and humans to sufficiently support an

---

[2] The model parameters for the Dietz et al. (1981) model are not provided in Table 1 because relatively few of the values were provided by the authors. The rate constants that were provided were not readily interpretable in the framework shown in Table 1. The physiological constants appropriate for converting the available parameters to reflect the equivalent framework were also not available.

extrapolation of rat dose-response data to humans based upon an equivalent internal human dose metric. Data to support the use of the PBPK models for route-to-route extrapolation are also limited or not available.

Table 1. Kinetic parameters used for PBPK models for MEK kinetics in humans and rats

Parameter	Tissue/Kinetic Parameter	Human model, Liira et al. (1990b)		Rat model, Thrall et al. (2002)
Body weight (kg)		70		0.25
Blood flows to tissues at rest, L/min and (% of cardiac output)[a]	Lungs	5.05		-[d]
	GI tract	1.2	(21)	-
	Liver	1.6	(28)	(25)
	Richly perfused tissues	2.1	(37)	(51)
	Poorly perfused tissues	0.1	(2)	(20)
	Fat	0.25	(4)	(4)
	Muscle	0.5	(9)	-
Tissue volume, L and (% body weight)	Lungs	2.0		-
	GI tract	2.4		-
	Liver	1.5		(4)
	Richly perfused tissues	2.1		(5)
	Poorly perfused tissues	12.5		(74)
	Fat	14.5		(8)
	Muscle	16.5		-
Tissue/air partition coefficient[b]	Lungs	103		-
	GI tract	107		-
	Liver	107		152
	Richly perfused tissues	107		-
	Poorly perfused tissues	107		-
	Fat	162		101
	Muscle	103		185
	Blood	125		138.5
Ventilation at rest (L/hr)	Alveolar	403		5.4
	Pulmonary	672		-
Hepatic metabolism[c]	V_{max}, mg/h-kg	1.85		5.44
	K_m, mg/L	0.14		0.63
	First-order rate constant (h^{-1})	-		4.1

[a] Cardiac output for humans taken to be the total of the blood flows, or 5.75 L/min.
[b] Tissue air partition coefficient as reported by the autopsy study by Fiserova-Bergerova and Diaz (1986).
[c] Human metabolic parameters were reported by Liira et al. (1990b) as V_{max}=30 µmol/minute and K_m =2 µM.
[d] Parameter not used in model or not reported.

4. HAZARD IDENTIFICATION

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

All dose conversions made in this chapter are made assuming conditions of standard temperature and pressure.

4.1.1. Oral Exposure

Kopelman and Kalfayan (1983) described a case report of nonoccupational, acute toxicity from ingestion of MEK. A 47-year-old woman who inadvertently ingested an unknown amount of MEK was unconscious, hyperventilating, and suffering from severe metabolic acidosis upon hospital admission. Her plasma concentration of MEK was 950 mg/L. After a complete and uneventful recovery, she was discharged from the hospital.

4.1.2. Inhalation Exposure

4.1.2.1. *Acute Exposure*

As with other small molecular weight, aliphatic, or aromatic organic chemicals used as solvents (e.g., acetone or toluene), acute inhalation exposure to high concentrations of MEK vapors is expected to cause reversible central nervous system depression; however, evidence for such effects in humans is limited to a single case report (Welch et al., 1991). In an extensive series of studies involving 4-hour exposure of human subjects to 200 ppm (590 mg/m³) MEK, National Institute for Occupational Safety and Health (NIOSH) investigators found no statistically significant increase in reported symptoms of throat irritation, nor did they find marked performance changes in a series of tests of psychomotor abilities, postural sway, and moods (Dick et al., 1984, 1988, 1989, 1992).

Welch et al. (1991) reported that a 38-year-old male worker exposed to paint base containing MEK and toluene in an enclosed, unventilated garage exhibited neurological symptoms. Exposure occurred at an unknown concentration of MEK for an acute, but unspecified, period of time. Initial symptoms included nausea, headache, dizziness, and respiratory distress. Over the next several days, the subject experienced impaired concentration, memory loss, tremor, gait ataxia, and dysarthria. Subsequent MRI evaluation revealed fluid

accumulation in the left parietal area. The condition was diagnosed as toxic encephalopathy with dementia and cerebellar ataxia. Some neurological deficits persisted for more than 30 months following the acute exposure. It is not clear from this report whether the central nervous symptom effects were due to exposure to MEK, toluene, or a combination of solvents.

In a series of studies by NIOSH investigators (Dick et al., 1984, 1988, 1989), volunteers (male and female) underwent a single 4-hour exposure to 200 ppm (590 mg/m^3) MEK, after which the following neurobehavioral tests were conducted: psychomotor tests (choice reaction time, visual vigilance, dual task, and memory scanning), postural sway, and a profile of mood states. No statistically significant changes in neurobehavioral performance were observed (Dick et al., 1984, 1988, 1989). Dick et al. (1984, 1988) evaluated the performance of 16–20 volunteers on three performance tasks before, during, and after MEK exposure. Dick et al. (1989) evaluated 12 male and 13 female volunteers for neurobehavioral performance changes and biochemical indicators during and after MEK exposure. In a more recent study by Dick et al. (1992), exposure of 13 men and 11 women (ages ranged from 18 to 32 years) to 200 ppm MEK for 4 hours in an environmental chamber found no statistically significant increase in airway irritation reported by volunteers. Ingested ethanol (95%, 0.84 mL/kg) was used as a positive control for neurobehavioral effects. The volunteers were evaluated by the same battery of psychomotor tests noted for the earlier studies, a sensorimotor test, and a test of mood to measure neurobehavioral effects. Additionally, chemical measurements of MEK concentrations (venous blood and expired breath) and reports of sensory and irritant effects were recorded. MEK exposure produced statistically significant performance effects on 2 of 32 measures (choice reaction time in males only and percent incorrect responses for dual task in females only). Given the number of comparisons performed, the number of statistically significant associations was consistent with the number expected by chance alone. The authors concluded that the observed effects of MEK exposure could not be attributed directly to chemical exposure.

Muttray et al. (2002) exposed 19 healthy male volunteers to 200 ppm (590 mg/m^3) MEK or filtered air for 4 hours in a crossover study design. Mucociliary transport time was measured, as well as collection of nasal secretions for cytokines (tumor necrosis factor-alpha and interleukins 6, 8, and 1-beta). The study also assessed acute symptoms via a 17-part questionnaire that assessed irritation of mucous membranes, difficulties in breathing, and pre-narcotic symptoms. The volunteers did not report nasal irritation. The only statistically significant (p = 0.01) change was a 10% increase in mucociliary transport time (median values were 660 seconds for sham exposure as compared with 600 seconds after exposure to MEK), an indicator of subclinical rhinitis. The biological significance of this effect is not clear.

In an earlier study, ten volunteers were exposed to several concentrations of MEK for 3 to 5 minutes to determine a concentration that would be satisfactory for industrial exposure and a concentration that would be "unpleasant" or objectionable. Volunteers exposed to 100 ppm (295 mg/m^3) MEK reported only slight nose and throat irritation, while mild eye irritation was reported by some subjects at 200 ppm (590 mg/m^3), and exposure to 300 ppm (885 mg/m^3) was "conclusively rejected" as an 8-hour exposure (Nelson et al., 1943).

4.1.2.2. *Case Studies of Long-term Human Exposure to MEK*

Although MEK is a widely used industrial solvent, evidence that MEK may induce general solvent-like effects such as peripheral or central nerve fiber degeneration in humans is restricted to a small number of case reports and occupational studies. Three case studies demonstrated adverse effects following repeated exposure to MEK. First, Seaton et al. (1992) reported that a maintenance fitter was exposed to MEK for 2–3 hours/day for 12 years. Exposure was via both dermal and inhalation routes. The worker had developed slurred speech, cerebral ataxia, and sensory loss in his arms and on the left side of his face. Nuclear magnetic resonance imaging showed severe cerebellar and brainstem atrophy; however, nerve conduction studies were normal. A survey of his work area revealed peak MEK concentrations in excess of 1,695 ppm (5,000 mg/m^3) during some operations and 10-minute concentrations of approximately 305 ppm (900 mg/m^3).

Callender (1995) reported that a 31-year-old male engineer developed severe chronic headache, dizziness, loss of balance, memory loss, fatigue, tremors, muscle twitches, visual disturbances, throat irritation, and tachycardia after working for 7 months in a quality assurance laboratory where he was exposed daily to MEK and fumes from burning fiberglass material. Personal protection equipment and formal safety training were not provided. Based on a physical examination, neuropsychological tests (Poet Test Battery and WHO Neurobehavioral Core Test Battery), electroencephalographic tests, evoked brain potential tests, nerve conduction velocity tests, rotational and visual reflex testing, vestibular function testing, and SPECT and MRI scans of the brain, the patient was diagnosed with chronic toxic encephalopathy, peripheral neuropathy, vestibular dysfunction, and nasosinusitis. Information concerning the exposure levels and subsequent possible progression or regression of these conditions was not provided.

In a third case, a 27-year-old man developed multifocal myoclonus, ataxia, and postural tremor after occupational exposure (through dermal and inhalation pathways) over a 2-year period to solvents containing 100% MEK (Orti-Pareja et al., 1996). The actual exposure levels

are unknown. The patient reported symptoms of dizziness, anorexia, and involuntary muscle movement, beginning about 1 month prior to admission. Neurological examination confirmed multifocal myoclonus, ataxia, and tremor. Symptoms of solvent toxicity disappeared after 1 month of cessation of exposure and treatment with clonazepam and propranolol. Symptoms did not reappear after withdrawal of the drugs.

4.1.2.3. *Occupational Studies of MEK Exposure*

Several occupational studies examined the effects of chronic exposure to MEK. WHO (1992) reported the results of an occupational study by Freddi et al. (1982) of 51 Italian workers chronically exposed to MEK. The authors reported that MEK exposure was associated with slightly, but not statistically significant, reduced nerve conduction velocities (distal axonopathy) and other symptoms such as: headache, loss of appetite and weight, gastrointestinal upset, dizziness, dermatitis, and muscular hypotrophy, but no clinically recognizable neuropathy (Freddi et al., 1982). In addition, a brief report of dermatoses and numbness of fingers and arms in workers was reported following chronic exposure in a factory producing coated fabric (Smith and Mayers, 1944 as cited in WHO, 1992). MEK concentration in the factory was estimated to be 300–600 ppm (885–1,770 mg/m³) in the apparent absence of other solvents (Smith and Mayers, 1944 as cited in WHO, 1992). In both of these reports, the exposure concentration and duration are uncertain; thus, they are of limited utility in supporting an association between MEK exposure and persistent neurological impairment for dose-response assessment.

Oleru and Onyekwere (1992) examined the relative impacts of exposures to MEK, polyvinyl chloride, leather dust, benzene, and other chemicals for four operations (plastic, leather, rubber, and tailoring) at a Nigerian shoe factory that had been in existence for 30 years. MEK exposure occurred only in the leather unit where 43 workers were exposed to leather, dyes, MEK, and other unspecified solvents that were used to preserve leather. The concentration of MEK in the shoe factory was not measured. The workers were monitored for pulmonary function (forced ventilatory capacity and forced expiratory volume). The data were used to determine obstructive, restrictive, and mixed lung diseases among the study cohort (smoking status was assessed). The pulmonary function results were compared against prediction equations for nonindustrially exposed subjects. The subjects were given a questionnaire that assessed tiredness, headache, sleep disorder, dizziness, and drowsiness. The mean age of the MEK-exposed cohort was 32.8±4.03 years, and the mean duration of employment was 10.3±4.03 years. Incidences of self-reported symptoms of neurological impairment were elevated among the leather workers (MEK-exposed subgroup) compared with a referent group of tailors

(controls). Odds ratio (OR) analysis revealed that the following neurological indices were statistically significant: headache (27/43, OR = 6.2, p<0.005), sleep disorder (15/43, OR = 4.1, p<0.01), dizziness (15/43, OR = 16.6, p<0.005), and drowsiness (11/43, OR = 5.2, p<0.05). The authors did not report 95% confidence intervals for the odds ratios. Although the frequency of reported chest pain was statistically different from the reference population (p<0.05), the authors found that pulmonary toxicity (restrictive lung disease as determined by pulmonary function tests) was not statistically different from controls when age was considered. Association of the neurological effects reported by Oleru and Onyekwere (1992) with a specific chemical such as MEK is complicated by concurrent exposure to multiple solvents (including hexacarbon solvents whose neurotoxicity is reportedly exacerbated by MEK). In addition, the lack of a measured airborne concentration of MEK limits the utility of the data for use in dose-response assessment.

Mitran et al. (1997) and Mitran (2000) reported the results of a cross-sectional health study of workers in three Romanian factories exposed to MEK, acetone, or cyclohexane. The MEK group was composed of 41 exposed and 63 controls from a cable factory where a laquer containing MEK was applied as a coating. The mean age of the exposed subjects was 36±9.2 years and the mean length of exposure was 14±7.5 years. Workers were exposed to reported concentrations of 51–116 ppm (149–342 mg/m^3) MEK during an 8-hour shift. The control subjects were similar in age (36±12.3 years) and were reported to be matched for physical effort required for completion of work tasks, shift characteristics, and socioeconomic factors. Study participants completed a questionnaire about memory and subjective symptoms of neurological impairment, responded to questions about alcohol consumption, submitted to a clinical examination, submitted samples for identification of biological exposure markers, and underwent motor nerve function tests (conduction velocity, latency, amplitude, and duration of response following proximal and distal stimulation) and psychological tests. Psychological testing included tests for reaction times to auditory and visual stimuli, distributive attention, the Woodworth-Mathews personality questionnaire for psychoneurotic tendencies, and the labyrinth test to identify quality of attention. Nerve conduction testing was performed on the median and ulnar nerves of the arm of the dominant hand and the peroneal nerve of the ipsilateral leg.

Several neurotoxic symptoms were reported more frequently by MEK-exposed workers than control workers (Mitran et al., 1997). Percentages of MEK-exposed and control workers reporting neurotoxic symptoms were: 17% vs. 4.7% for mood disorders; 28% vs. 17% for irritability; 31% vs. 9.5% for memory difficulties; 19% vs. 6% for sleep disturbances; 41% vs. 7.8% for headache; and 24% vs. 7.8% for numbness of the hands or feet. Also reported more frequently by MEK-exposed workers than control workers were symptoms of ocular irritation

21

(41% vs. 7% in controls); upper respiratory tract irritation (28% vs. 11%); and various types of bone, muscle, or joint pain (e.g., 31% vs. 15% for muscular pains). In psychological tests, MEK-exposed workers were reported to have shown more "behavioral changes, such as emotional lability, low stress tolerance, and a tendency of hyperreactivity to conflict," but the data were not sufficiently reported by Mitran et al. (1997) to allow an independent assessment of the results. The only other information concerning these tests was a statement indicating that diffuse somatic neurotic changes were the dominant findings in exposed workers. Statistically significant decreases in mean nerve conduction velocities for the median, ulnar, and peroneal nerves in the MEK-exposed group were observed when compared with control means by 22, 28, and 26%, respectively (Mitran et al., 1997). Other statistically significant nerve conduction variables that were different in the MEK-exposed group included: increased proximal and distal latencies in the median nerve, increased proximal and distal latencies and decreased proximal amplitude in the ulnar nerve, and increased proximal latency and decreased distal amplitude in the peroneal nerve.

The Mitran et al. (1997) report has several weaknesses that limit its ability to support an association between long-term occupational exposure to MEK at concentrations below 200 ppm (590 mg/m^3) and persistent neurological impairment. The report does not provide information regarding important methodological details including: (1) criteria for selecting and matching the exposed and control workers (important confounding variables that can influence nerve conduction include the type of work [e.g., office vs. physical work], alcohol and tobacco consumption habits, and height and weight); (2) protocols for assessing exposure levels experienced by the workers; and (3) protocols used in the nerve conduction tests (e.g., it is not clear whether the exposed and control subjects were tested at the same location and time and under the same environmental conditions).

Two reviews (memorandum dated June 27, 2002, from William Boyes and David Herr, U.S. EPA to Susan Rieth, U.S. EPA; Graham, 2000) of the Mitran et al. (1997) report have noted that the differences in mean nerve conduction velocities between the two groups could be explained if the control subjects were tested under higher temperatures. Second, although there were statistically significant increases in self-reported neurological symptoms in the MEK-exposed group (e.g., numbness of hands and feet), the reviewers noted that the reliability of self-reported symptoms is widely recognized as suspect and subject to bias. Confidence in these findings would be increased if the study had demonstrated a correlation between subjects reporting symptoms and subjects with poor or subnormal nerve conduction velocity results, but this type of analysis was not presented. Third, the reviewers observed that the report provides no

indication of increasing response (either in prevalence of self-reported symptoms or nerve conduction results) with increasing indices of exposure. Confidence in the symptomatological and nerve-conduction findings would be increased if such dose-response relationships were demonstrated. Fourth, the pattern of changes in nerve conduction variables in the MEK-exposed group was not considered to be consistent with patterns demonstrated for compounds such as hexane and methyl n-butyl ketone (MnBK), which are well-known to cause peripheral neuropathy. A U.S. EPA memorandum dated June 27, 2002, from William Boyes and David Herr to Susan Rieth noted that, for this type of peripheral neuropathy, the distal latency of the peroneal nerve would be expected to be the most affected; however, the mean distal latency of the peroneal nerve in the MEK-exposed group was not different from that of the control group. Finally, the reviewers noted that the Mitran et al. (1997) results are only supported by inconclusive case reports of neuropathies in a few MEK-exposed individuals and are not consistent with results from well-conducted studies of animals. For example, a study of rats exposed to concentrations as high as 5,000 ppm (14,750 mg/m³) MEK, 6 hours/day, 5 days/week for up to 90 days looked for, but did not find, evidence for nerve fiber degeneration or gross neurobehavioral changes induced by MEK (Cavender et al., 1983, also reported in Toxigenics, 1981).

In summary, the human case reports and studies by Oleru and Onyekwere (1992) and Mitran et al. (1997) provide limited and equivocal evidence that repeated exposure to MEK in the workplace increases the hazard for persistent neurological impairment. The available occupational studies are limited by inadequate characterization of exposure, multiple solvent exposure, and study design problems.

Potential for Carcinogenic Effects in Humans

Several epidemiological studies evaluated the potential for carcinogenic effects in humans associated with MEK exposure. Two retrospective epidemiological mortality studies conducted by Alderson and Rattan (1980) and Wen et al. (1985) reported that deaths due to cancer were less than expected in industrial workers chronically exposed to MEK in dewaxing plants. Spirtas et al. (1991) and Blair et al. (1998) found no clear evidence of increased cancer risk from occupational exposure to MEK, but some evidence suggests an increased risk between multiple solvent exposure, which included MEK as a component, and certain cancers among workers in a degreasing plant. A case-control study of lymphoblastic leukemia in children and parental exposure to MEK (Lowengart et al., 1987) was considered exploratory and inconclusive.

In a historical prospective mortality study of 446 male workers in two MEK dewaxing plants, the number of observed deaths (46) was below the number expected (55.51), based on national mortality rates for the U.K. (Alderson and Rattan, 1980). The average follow-up was 13.9 years. Mortality due to cancer was less than expected (13 observed; 14.26 expected), although there was a significant increase in the number of deaths from tumors of the buccal cavity and pharynx (2 observed; 0.13 expected). Also, there were significantly fewer deaths from lung cancer (1 observed; 6.02 expected). Although statistically significant increases in the incidence of buccal or pharyngeal neoplasms was observed, the findings were regarded by the authors as due to chance since there were a small number of individuals affected, the researchers failed to include tobacco use in the study, and the number of separate comparisons between observed and expected rates. In view of the small number of individuals affected, the authors concluded that there was no clear evidence of cancer hazard in these workers.

A retrospective cohort study of 1,008 male oil refinery workers occupationally exposed to MEK in a lubricating-dewaxing solvent mixture (also containing benzene, toluene, hexane, xylene, and methyl isobutyl ketone) demonstrated a lower overall mortality for all causes, including cancer, than expected based on mortality data from the U.S. population (Wen et al., 1985). The increased incidence of buccal and pharyngeal neoplasms reported by Alderson and Rattan (1980) was not confirmed in this study. Although a statistically significant elevated risk of mortality from bone cancer was reported (SMR=10.34, 95% CI: 2.1-30.2, 3 observed), the investigators questioned the validity of this finding because two of the three observed bone cancers were not primary bone cancers and thus appeared to have been misclassified. The number of prostate cancer deaths was increased (SMR=1.82, 95% CI: 0.78-3.58, 8 observed, 4.4 expected), but the increase was not statistically significant. The risk of prostate cancer tended to increase with increasing duration of employment in the lube oil department, but not among workers in the solvent-dewaxing unit where the exposure to solvents (including MEK) primarily occurred. Thus, these epidemiological studies (Alderson and Rattan, 1980; Wen et al., 1985) showed no clear relationship between occupational exposure to MEK and the development of neoplasms in humans.

A retrospective cohort mortality study was conducted of aircraft maintenance workers employed for at least one year at Hill Air Force Base, Utah (Spirtas et al., 1991; with 10 years of follow-up reported by Blair et al., 1998). The MEK-exposed workers were from a total cohort of 14,457 subjects (222,426 person-years for male workers, and 45,359 person-years for female workers). The numbers of MEK-exposed workers were reported as 32,212 person-years for male workers and 10,042 person-years for female workers. Associations with cancer mortality

were also evaluated for 26 other specific chemical categories. Trends in mortality were assessed, although the data on MEK were limited due to a particular focus on potential carcinogenic risks posed by trichloroethylene. In general, the risks of mortality due to multiple myeloma, non-Hodgkin's lymphoma, and breast cancer were elevated for the entire cohort; the authors examined the relationship between the incidence of these cancers and several solvents (including MEK).

Spirtas et al. (1991) reported a significantly increased standard mortality ratio (SMR) for multiple myeloma among women exposed to MEK (SMR = 904, 95% CI: 109–3267, 2 observed), but not among men (SMR = 96, 95% CI: 2–536, 1 observed). The MEK-exposed subcohort was compared to age- and gender-matched incidences of multiple myeloma among the population of Utah. The authors applied an alternate analytical method by Thomas-Gart (TG), which adjusted for age at entry into follow up and competing causes of death to account for the small number of unexposed subjects in the subcohort. According to the TG analyses, the association was not statistically significant among women for multiple myeloma and exposures to MEK (n = 2, chi-square = 1.6, p = 0.204).

In the 10-year follow-up study, Blair et al. (1998) compared the mortality due to multiple myeloma, non-Hodgkin's lymphoma, and breast cancer among the MEK-exposed subcohort and internal referents (study subjects without occupational solvent exposure). During the 10-year follow-up period, one additional death due to multiple myeloma occurred in a female subject. The risk for multiple myeloma among females was elevated but was not statistically different from controls (relative risk = 4.6, 95% CI: 0.9–23.2, 3 observed). The finding is consistent with an earlier report by Spirtas et al. (1991) where the TG analysis was applied. As reported by the authors of the original (Spirtas et al., 1991) and follow-up (Blair et al., 1998) studies, the small number of cases and exposures to multiple solvents complicate attempts to relate the mortality excess for multiple myeloma to specific causes. In addition, given the multiple comparisons performed, some positive associations would be expected by chance alone. Thus, these studies (Spirtas et al., 1991; Blair et al., 1998) provide insufficient evidence that MEK is responsible for elevated risk of cancer.

In an exploratory case-control study, Lowengart et al. (1987) examined the relationship between acute lymphoblastic leukemia in children and parental exposure to MEK that occurred one year prior to conception until shortly before the diagnosis of leukemia. The mothers and fathers of children diagnosed with leukemia and individually matched controls (n = 123 matched pairs) were interviewed regarding occupational and home exposure to MEK, chlorinated

solvents, spray paints, dyes, pigments and cutting oils, personal and family medical history, and lifestyle habits associated with leukemia. The study investigators reported a statistically significant positive trend for risk of childhood leukemia based on father's frequency of use of all of the chemicals examined, including MEK. The authors reported an odds ratio for MEK that appeared elevated, but not statistically so, for the period of paternal exposure after birth of the child and acute lymphoblastic leukemia (OR = 3.0, 95% CI = 0.75–17.23; 9 exposed cases/3 exposed controls). There was no statistically significant association between a father's exposure one year before pregnancy or during pregnancy and leukemia in the child. No significant associations between leukemia and mothers' exposures to specific substances were found, although few mothers reported occupational exposure to the industrial solvents evaluated in the study. The investigation is considered an exploratory study, given that exposure levels were judged according to questionnaires only. Factors that could be confounding covariates such as other chemical exposures and personal lifestyle were not taken into account in the statistical analysis. The authors did not provide a biological rationale for why an elevated risk of childhood leukemia would be associated only with father's exposure after birth, but noted the possibility that recall bias could have influenced results (i.e., the possibility of better recall of more recent exposures). Thus, the findings of this study cannot be used to reliably examine the existence of an association between MEK and cancer.

In summary, the retrospective cohort studies of worker populations exposed to MEK (four studies of three different worker cohorts) provide no clear evidence of a cancer hazard in these populations. Because of various study limitations (including sample size, small numbers of cases, and multiple solvent exposures), these studies are not adequate to support conclusions about the carcinogenic potential of MEK in humans. A case-control study examining the association between paternal exposures to several solvents, including MEK, and childhood leukemia is exploratory in nature and cannot be used to reliably support the existence of any such association. Overall, the epidemiologic evidence from which to draw conclusions about carcinogenic risks in the human population is inconclusive. Although there is some suggestion of increased risk for some cancers (including bone and prostate) and multiple solvent exposure that includes MEK, there is no clear evidence for a relationship between these cancers and MEK exposure alone.

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

4.2.1. Oral Exposure

Information on the toxicity of MEK in experimental animals following oral exposure is limited to a few acute studies (see Section 4.4.1.1). No subchronic or chronic toxicity studies of MEK in experimental animals were located. Since 2-butanol is a metabolic precursor of MEK (Traiger and Bruckner, 1976), oral toxicity data on 2-butanol were evaluated to determine whether data gaps in the MEK oral exposure data base could be addressed by oral studies with 2-butanol. Similarly, the data base for the MEK metabolites, 3-hydroxy-2-butanone and 2,3-butanediol, were reviewed. No oral repeat-exposure animal studies or human exposure data were located for 2,3-butanediol. A 2-generation drinking water study of 2-butanol and a 13-week drinking water study with 3-hydroxy-2-butanone, however, provide information relevant to an assessment of the potential health effects of repeated exposure to MEK (see Section 4.3 for the 2-butanol study).

Gaunt et al. (1972) exposed CFE rats (15/sex/group) to 3-hydroxy-2-butanone in drinking water (0, 750, 3,000, or 12,000 ppm) for 13 weeks. According to the authors, the exposures are equivalent to mean intakes of 0, 80, 318, or 1,286 mg/kg-day for males and 0, 91, 348, or 1,404 mg/kg-day for females. Additional groups of 5 rats of each sex were exposed to 0, 3,000, or 12,000 ppm 3-hydroxy-2-butanone in their drinking water for 2 or 6 weeks. All rats were weighed weekly throughout the study and water and food consumption were measured once weekly over a 24-hour period. Urine was collected during the final week of treatment for appearance, microscopic constituents, glucose, bile salts, and blood. Also, a urine concentration test measured the specific gravity and volume of urine produced during a 6-hour period of water deprivation. At the end of the study, the animals were sacrificed and specimens of all major organs and tissues were examined histologically. Also, blood cell counts and blood chemistry were determined at the end of the exposure period. No animals died during the study, and all appeared normal. The 12,000-ppm rats showed a statistically significant (5-6%) reduction in body weight gain compared to controls at weeks 8 and 13 (study termination) for both sexes. In addition, a statistically significant increase in relative liver weight was observed among 12,000 ppm rats of both sexes exposed for 13 weeks (6.5% increase for males and 8.4% for females when compared to controls). The increased relative liver weight was not accompanied by changes in liver histology or in the activities of liver enzymes (LDH, SGPT, or SGOT), and was likely an adaptive response to the hepatic metabolism of 3-hydroxy-2-butanone. Slight, but

statistically significant, anemia was observed in both sexes of 12,000 ppm rats after 13 weeks of exposure (in males and females hemoglobin decreased by 4.9 and 4.2% as compared to controls and red blood cell count decreased by 5.4 and 8.3% with corresponding increases in reticulocytes, respectively). At study termination, the mean hemoglobin concentrations for all rats were 14.3, 13.8, 14.4, and 13.65 g/100 mL for 0, 750, 3,000, and 12,000 ppm, respectively. No other statistically significant effects were noted among rats exposed to 3-hydroxy-2-butanone compared with the controls. In this study, 3,000 ppm (318 mg/kg-day) was a NOAEL, and 12,000 ppm (1,286 mg/kg-day) was a LOAEL for slight anemia in CFE rats exposed to 3-hydroxy-2-butanone in drinking water for 13 weeks.

4.2.2. Inhalation Exposure

No chronic toxicity studies or cancer bioassays of inhalation exposure to MEK in experimental animals were located, although a number of less-than-lifetime inhalation toxicity studies have been reported. Since 2-butanol is a metabolic precursor of MEK (Traiger and Bruckner, 1976), inhalation toxicity data on 2-butanol were evaluated to determine whether the data gaps in the MEK inhalation exposure data base could be addressed by toxicity studies with 2-butanol. Similarly, the data bases for MEK metabolites (3-hydroxy-2-butanone and 2,3-butanediol) were reviewed. No repeat-exposure animal inhalation studies or human exposure data were located for 3-hydroxy-2-butanone or 2,3-butanediol. No chronic or subchronic inhalation toxicity studies with 2-butanol were found; however, a developmental inhalation toxicity study has been conducted (Nelson et al., 1989, 1990) (see Section 4.3.2.2).

Several repeat exposure inhalation studies of MEK in animals (all involving whole body chamber exposures) have been reported. Many of these studies have focused on the possible neurotoxicity of MEK, including the development of peripheral and central nerve fiber degeneration.

Cavender et al. (1983) exposed male and female Fischer 344 rats (15/sex/group) in a whole body dynamic air flow chamber to MEK 6 hours/day, 5 days/week for 90 days. The reported time-weighted average exposure concentrations (by gas-liquid chromatography) of MEK were 0, 1,254, 2,518, or 5,041 ppm (0, 3,700, 7,430, or 14,870 mg/m^3). The results of this study are also reported in a Toxic Substances Control Act (TSCA) Section 4 submission by Toxigenics (1981). All rats were observed twice daily for clinical signs and mortality. Food consumption and body weight were determined weekly. At the end of the 90-day exposure period, the eyes of each animal were examined by ophthalmoscopy, and neurological function

(posture, gait, tone and symmetry of facial muscles, and pupillary, palpebral, extensor-thrust and cross-extensor thrust reflexes) was evaluated. Clinical pathology evaluations, including urinalysis, hematology, and serum chemistry were performed at sacrifice for 10 animals/sex/group. At the study termination, 10 animals/sex/group were subject to routine gross pathology and histopathology. For routine histopathology, all tissues commonly listed on standard National Toxicology Program (NTP) protocols were examined microscopically. Organ weights were obtained for the brain, kidneys, spleen, liver, and testes. Special neuropathological studies were conducted on the remaining five male and five female rats from each group, including examination of Epon sections of the medulla and the sciatic nerve for pathologic changes, and evaluation of teased nerve fiber preparations of the tibial nerve (minimum of 50 individual nerve fibers/animal) by light microscopy for evidence of neuropathy.

Cavender et al. (1983) reported no signs of nasal irritation and no deaths during the 90-day study. Transient depressions in body weight gain compared to the control were seen in high dose (5,041 ppm) male and female rats early in the study. While statistically significant, the reductions did not exceed 8% of the control group weights for males or females. There were no treatment-related effects on food consumption or in the ophthalmological studies in any MEK-exposed rats. The evaluation of neurological function (i.e., assessments of posture, gait, facial muscular tone or symmetry, and four neuromuscular reflexes) revealed no abnormalities (Toxigenics, 1981). At all exposure concentrations, female rats exhibited statistically significant ($p<0.05$) dose-dependent increases in absolute liver weight when compared with controls. Relative liver weight was statistically increased in the 5,041 ppm females only when compared on a liver-to-brain weight basis (24% increase compared to controls) or liver-to-body weight basis (13% increase). In males, absolute and relative liver weights increased by 27% in the 5,041 ppm rats only. Other statistically significant differences in organ weights in 5,041 ppm female rats included decreased brain weights (absolute–5%, relative–9%), decreased spleen weights (absolute–5%), and increased kidney weights (relative–11%). Kidney weights were significantly increased (relative–6%) in 5,041 ppm male rats. Differences in the serum chemistry values for female rats in the 5,041 ppm exposure group included significant increases in serum potassium, alkaline phosphatase and glucose, and a significant decrease in SGPT activity compared to controls. No differences in serum chemistry between MEK-exposed males and control animals were observed. The only statistically significant difference in hematology parameters included higher mean corpuscular hemoglobin (average weight of hemoglobin per erythrocyte) in 5,041 ppm male and female rats, and higher mean corpuscular hemoglobin concentration (average hemoglobin concentration per erythrocyte) in 5,041 ppm females; the increase corresponded to a slight but not statistically significant decrease in number of red blood

cells. Hemoglobin concentrations were similar in the control and exposed groups. With the exception of larger urine quantity in 5,041 ppm males, no urinalysis parameters were significantly different in MEK-exposed rats.

Routine gross and histopathological examinations and the special neuropathology studies revealed no lesions that could be attributed to MEK exposure. Thus, while the increase in absolute liver weights in 5,041 ppm rats and altered serum enzyme activities in 5,041 ppm female rats indicated possible liver damage, no histopathological lesions in the liver were observed. The authors stated that the response may have been the result of a physiological adaptation mechanism. While decreased brain weights in the 5,041 ppm females suggest possible effects of MEK exposure on brain tissue, no histopathological lesions of the brain were observed and neurological function tests revealed no abnormalities.

Minimal to mild lesions of the upper or lower respiratory tract were noted in all control and MEK-exposed rats. The lesions were coded as chronic respiratory disease and consisted of "multifocal accumulation of lymphoid cells in the bronchial wall and peribronchial tissues with occasional polymorphonuclear cells (eosinophils) in the perivascular areas of small veins" (Toxigenics, 1981). Because the bronchial epithelium remained intact and exudates were not present in bronchial lumens, the lesions were considered pathologically insignificant. In addition, the authors reported an increased prevalence of nasal inflammation (including submucosal lymphocytic infiltration and luminal exudate) across the control and all exposure groups. There was no difference in the character or severity of lesions among the control and three treatment groups. The authors suggested that the pulmonary lesions were secondary to mycoplasma infection; unfortunately, no infectious agent was cultured to verify this etiology. While there is no indication that respiratory lesions are related to MEK exposure, the possibility exists that the outcome of the study may have been confounded by exposure to an unidentified infectious agent. The presence of lesions in the respiratory tract of all animals exposed via inhalation also prevents obtaining an unconfounded determination of any portal-of-entry effects.

In summary, review of the Cavender et al. (1983) findings reveals effects remote to the respiratory tract in the 5,041 ppm animals that are of uncertain biological significance, i.e., reduced body weight gain, statistically significant increases in relative liver weight (males and females) and altered serum liver enzymes (females), and decreased brain weight (females). As noted previously, reported liver effects are more likely indicative of a physiological adaptive response than toxicity. While the finding of decreased brain weight observed in female rats raises concerns, it is difficult to interpret. Generally, with a brain weight reduction of 5%, one

might expect evidence of corresponding pathology; however, no treatment-related brain pathology was observed in this study. The fact that the reduction in brain weight relative to controls was observed in only one sex also raises questions about the relevance of the finding. Thus, while the reduction in brain weight at 5,041 ppm is noteworthy, its biological significance is uncertain at this time.

LaBelle and Brieger (1955) exposed a group of 25 adult rats (strain and sex not specified) and 15 guinea pigs (strain and sex not specified) to 235±26 ppm (693±77 mg/m³) MEK 7 hours/day, 5 days/week for 12 weeks. A control group was included, but the number of control animals was not reported. At the end of the study, 15 rats were examined for histopathology (organs examined were not specified) and hematology (hemoglobin, erythrocyte, leukocyte, neutrophil, lymphocyte, and monocyte counts). The remaining 10 rats were reserved for growth studies. Growth study results demonstrated that 12 weeks of exposure to 235 ppm MEK reduced body weight gain (mean body weight was 95 g for exposed vs. 135 g for control); however, neither statistics nor standard deviation on the mean were provided. No adverse effects were reported for the exposed guinea pigs that could be attributed to MEK exposure. Information on the guinea pigs is only presented qualitatively in the study. In addition, the authors reported a 4-hour LC_{50} of 11,700±2,400 ppm (34,515±7,080 mg/m³) in rats exposed to MEK when narcosis preceded death. The study is inadequate for use in dose-response assessment since the study is poorly reported, only one exposure concentration was used in the chronic portion of the study, and relatively few toxicological parameters were measured.

Saida et al. (1976) found no evidence of peripheral neuropathy (as indicated by paralysis) following continuous exposure of 12 Sprague-Dawley rats (sex not specified) to 1,125 ppm (3,318 mg/m³) MEK for 16, 25, 35, or 55 days. Control animals were housed under similar environmental conditions without MEK exposure. At the end of the exposure period, rats were sacrificed and the sciatic nerve and foot muscle were excised. Spinal cord and dorsal root ganglion specimens were taken from the same rats. Additional studies were carried out with up to 5 months of exposure; no information regarding experimental procedures or endpoints evaluated was provided. No abnormal clinical findings were observed in animals exposed to MEK for any of the exposure periods (up to 55 days), although clinical observations were limited to the nervous system and the clinical data collected was only minimally described. Quantitative histology (neurofilaments/μm^2; frequency of inpouching of myelin sheath and denuded axons/mm²) showed no abnormality in rats exposed for up to 55 days. Although the authors reported that no abnormalities were observed in rats exposed as long as 5 months, no further details were provided.

Male Wistar rats (8 per group) were exposed to 0 or 200 ppm (0 or 590 mg/m^3) MEK 12 hours/day for 24 weeks (Takeuchi et al., 1983). Body weight and neurotoxicity endpoints (motor nerve conduction velocity, distal motor nerve latency, and tail nerve conduction velocity) were measured prior to exposure and every 4 weeks thereafter. After 24 weeks of exposure, the tail nerve from 1 rat per group was isolated for histopathology. The authors reported a slight increase in motor nerve conduction velocity and mixed nerve conduction velocity and a decrease in distal motor latency at 4 weeks of exposure, although no difference was observed after 8, 12, 16, 20, or 24 weeks. Microscopic examination of the tail nerves revealed no histopathological lesions after 24 weeks.

Garcia et al. (1978) examined behavioral effects of MEK exposure in rats. An increase in response rate (lever pressing to obtain a food reward) was reported in a group of six adult Sprague-Dawley rats (sex unspecified) exposed to MEK at concentrations between 25 and 800 ppm (74 and 2,360 mg/m^3) for 2 hours at approximately weekly intervals (the total number of exposures was not stated). Results at these exposure concentrations were not further reported. An increase in response rate (lever pressing to obtain a food reward) was also reported in a group of four rats exposed to 25 ppm for 6 hours compared to preexposure values for the same animals (Garcia et al., 1978). The effect persisted in some animals for several days. No statistics or standard deviation in the response rate was reported. The small number of measurements and variability in postexposure response rates complicate the interpretation of these findings.

Geller et al. (1979) studied behavioral effects in four male baboons (2-years-old) exposed continuously by inhalation to 100 ppm (295 mg/m^3) MEK for 7 days. Operant conditioning behavior conducted during exposure was compared to preexposure test scores. The operant behavior selected was a match-to-sample discrimination task. The experiment was designed to compare the performance of each baboon during exposure to its performance during a clean air exposure period in the same chamber immediately prior to each MEK exposure period. No effects on performance of the test in terms of the ability to discriminate visual stimuli were noted. Although reaction time increased, the extent varied considerably among the four animals. In two of the four baboons, response times returned to preexposure control values by day 7 of exposure. The exposure also increased the response time in a delayed "match to sample" task. This effect, however, was transient and disappeared during the course of repeated exposure. The authors suggested that this could be an early manifestation of the narcosis observed in rats in the acute toxicity (LC$_{50}$) study by LaBelle and Brieger (1955). Thus, Geller et al. (1979) found only transient neurological effects of MEK in primates at the concentrations studied. It should be

noted that each baboon was exposed to four different chemicals: acetone, MEK, methyl isobutyl ketone, and MEK plus methyl isobutyl ketone (in that order).

Couri et al. (1974) exposed 4 cats, 4 rats, 5 mice, and an unknown number of chickens to 1,500 ppm (4,425 mg/m^3) MEK 24 hours/day, 7 days/week for 7–9 weeks with no apparent adverse effects. No paralysis was seen in any of the animals, and MEK did not alter the histology of the nerves. In a dose range-finding study, an unknown concentration of MEK reportedly produced a statistically significant elevation in plasma cholinesterase levels in mice, rats, and chickens. The study was poorly reported and many experimental details required to evaluate study adequacy were not provided.

In addition to possible neurological effects, portal-of-entry and pulmonary effects of inhaled MEK have been studied. Five male Wistar rats were exposed to MEK (initially at 10,000 ppm, then reduced to 6,000 ppm) 8 hours/day, 7 days/week for 15 weeks (Altenkirch et al., 1978). The concentration of MEK was reduced from 10,000 ppm (29,493 mg/m^3) to 6,000 ppm (17,696 mg/m^3) due to severe irritation of the upper respiratory tract. The authors also reported that all animals in the MEK-exposed group were somnolent during exposure. The death of all of the rats at week 7 was attributed to bronchopneumonia rather than MEK exposure. The authors did not comment on possible connections between bronchopneumonia susceptibility and MEK exposure.

Toftgard et al. (1981) exposed 4 male Sprague-Dawley rats to 800 ppm (2,360 mg/m^3) MEK for 6 hours/day, 5 days/week for 4 weeks, and examined changes in enzymatic activity in rat liver. Increased absolute and relative liver weight when compared to controls (p<0.05) and slight reductions in the *in vitro* metabolic capacity of liver microsomes were reported in rats exposed to MEK.

In an earlier experiment intended to assess the effects of MEK on hepatic microsomal enzyme activity, Couri et al. (1977) continuously exposed an unreported number of young male Wistar rats to 750 ppm (2,210 mg/m^3) MEK for 7 or 28 days. After 7 days of exposure, there was a significant (p<0.005) reduction in hexobarbital sleep times (16.0±2.4 minutes for exposed vs. 26.0±2.4 minutes for control). In the group exposed for 28 days, the reduction in sleep times was less marked (the 28-day results were not reported quantitatively). The results are consistent with an earlier study (Raunio et al., 1990) reporting that pretreatment with MEK can induce hepatic detoxification capacity.

In summary, a number of less than lifetime inhalation studies of MEK have been conducted. In the 90-day inhalation study by Cavender et al. (1983), the only observed effects were decreased body weight gain, increased liver weight, altered enzyme levels, and decreased brain weight at 5,041 ppm (14,870 mg/m^3). Other studies of shorter duration have largely focused on neurological endpoints; many of these studies used either small numbers of animals or one exposure concentration. Data from these repeat inhalation exposure studies provide no evidence for MEK-induced nerve degeneration or other persistent neurological effects. Evidence is available suggesting that MEK can potentiate nerve degeneration produced by certain alkanes that can be metabolized to gamma-diketones, including n-hexane (Altenkirch et al., 1978) and MnBK (Saida et al., 1976). The evidence is summarized in Section 4.4.4.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Studies in Humans

No studies were located that examined the potential for MEK to induce developmental effects in humans after inhalation or oral exposure. Only one occupational study (Lemasters et al., 1999) is available that addresses the potential reproductive toxicity of MEK in humans. The investigators studied the male reproductive effects of solvent and fuel exposure at an aircraft maintenance facility. The study included 50 males who were exposed to a mixture of solvents and jet fuel on an Air Force installation and a control group of 8 unexposed men. In this prospective study, each subject was evaluated before the first exposure and at 15 and 30 weeks after exposures had begun. Industrial hygiene sampling and expired breath samples were collected to determine jet fuel exposure as measured by total naphthas, benzene, 1,1,1-trichloroethane, MEK, xylenes, toluene, and methylene chloride. Sperm parameters (concentration, motility, viability, morphology, morphometrics, and stability of sperm chromatin) were evaluated. Expired breath sampling demonstrated that exposures were generally low; all mean measures were below 6 ppm, which is less than 10% of the OSHA standard for all measured chemicals except benzene. Among the subjects, sheet metal workers had the highest mean breath levels for total solvents (24 ppb) and fuels (28.3 ppb). Mean values for most sperm measures remained in the normal range throughout the 30-week exposure period. When jobs were analyzed by exposure groups, some adverse changes were observed. The paint shop group, for example, had a significant decline in motility (19.5%) at 30 weeks. The authors noted a lack of a dose-response association for the observed spermatogenic changes. The study

is limited, since the exposure concentration and duration are unknown. Also, the results are confounded by exposure to other solvents and chemicals in the workplace.

4.3.2. Studies in Animals

4.3.2.1. *Oral Exposure*

No studies concerning reproductive or developmental toxicity of MEK exposure by the oral route are available. A study is available, however, of the reproductive and developmental toxicity of 2-butanol, a metabolic precursor of MEK (Cox et al., 1975). As described in Section 3.3, data from rats suggest that almost all (96%) of an administered dose of 2-butanol is converted to MEK (Traiger and Bruckner, 1976), and that both chemicals are metabolized through the same intermediates (DiVincenzo et al., 1976; Dietz et al., 1981) as shown in Figure 1. Thus, toxicity data from oral exposure to 2-butanol are considered relevant to MEK.

Cox et al. (1975) conducted a multigeneration reproductive and developmental toxicity study of 2-butanol, which is quantitatively converted to MEK in the body. The study did not include statistical analyses of the results, although all collected data were fully reported. The results are also presented in abstract form by Gallo et al. (1977). Weanling FDRL-Wistar stock rats (30/sex/group) were given 2-butanol in drinking water at 0, 0.3, 1, or 3% solutions and a standard laboratory ration *ad libitum*. Weekly food consumption, fluid intakes, and body weights were examined to determine the efficiency of food utilization and to calculate the average daily intake of 2-butanol, which was reported by the authors for the initial 8 weeks of the study (intake was not reported for subsequent weeks) as: 0, 538, 1,644, and 5,089 mg/kg-day (males) and 0, 594, 1,771, and 4,571 mg/kg-day (females) for the 0, 0.3, 1, and 3% solutions, respectively. After 8 weeks of initial exposure, F0 males and females from each exposure group were mated to produce F1A litters which were delivered naturally and nursed through 21 days of lactation. F1A litters with more than 8 pups were randomly culled to 8 pups per litter on day 4 after birth. Pup and dam weights were recorded on days 4 and 21 after birth.   Various indices of reproductive performance were recorded (e.g., number of successful pregnancies, litter size, number of live pups at birth and end of lactation). Because increased mortality and decreased body weight occurred in the F1A litters at the 3% dose level (see below), all high-dose parents and F1A offspring were given drinking water without 2-butanol between days 10 and 21 of lactation and then 2% 2-butanol for the remainder of the experimental protocol. The average daily intake in mg/kg-day at the 2% (initially 3%) exposure level was not reported by the study investigators; therefore, average daily intakes of 3,384 mg/kg-day in males and 3,122 mg/kg-day

in females were estimated based on a linear regression analysis of the reported average intakes for males and females at drinking water concentrations of 0, 0.3, 1, and 3%.

After a 2-week post-lactation period, the F0 females were remated with males of their respective exposure groups to produce F1B litters. The F1B pregnancies of 20 pregnant rats per group were terminated on gestation day 20. Data recorded included numbers of corpora lutea, implant sites, and resorptions, number of live and dead fetuses, and the sex and weight of live fetuses. F1B fetuses were also examined for skeletal and visceral malformations and variations.

Selected male and female F1A rats (30 of each sex per exposure group) continued on their respective treatment protocols (0, 0.3, 1, or 2% 2-butanol) and mated at 12 weeks of age to produce F2 litters that were delivered and nursed through day 21 of lactation. Indices of second-generation reproductive performance were assessed, as were F2 pup weights at days 4 and 21. At day 21 of lactation, FIA adults were sacrificed. Major organs and tissues (35 in all) from 10 male and 10 female F1A rats per exposure group were examined histopathologically, and the liver and kidneys from all 30 F1A rats per sex/group were examined histopathologically.

At the highest exposure level (3%), net parental (F0) body weight gain was reduced compared with controls both in males (229 vs. 269 g in controls) and females (130 vs. 154 g in controls) during the 8 weeks of initial exposure. No differences were found in the efficiency of food utilization. Following birth of the first litter (F1A) of the parental generation, various reproduction and lactation responses were measured. The study authors reported no effects on reproductive parameters. Analysis of F0 male rate copulatory success (based on data in Appendix II) suggests a possible impact of 3% 2-butanol on male reproductive performance. The incidence of male F0 rats that did not successfully copulate with F0 females was: 0% (1/30), 0.3% (2/30), 1% (0/30), and 3% (6/30). Data from which to determine copulatory failure were not provided for other generations. In addition, reduced body weight gain in this high-dose group could have contributed to copulatory success. For these reasons, the biological significance of these data for the F0 generation males is uncertain.

When compared to the control group, the following effects were noted in the F1A litters from the high-dose (3%) group: reductions in the mean number of pups/litter born alive (8.46 vs. 10.3), the mean number of pups/litter alive before culling at 4 days (8.12 vs. 10.3), the mean number of pups/litter alive at 21 days (6.85 vs. 7.68), the mean body weight/pup after culling at 4 days (8.3 g vs. 10.7 g, from Appendix II of Cox et al., 1975), and the mean body weight/pup at 21 days (30 g vs. 49 g, from Appendix II of Cox et al., 1975). The high-dose mean F1A body

weights at 4 and 21 days represent 22 and 39% decreases, respectively, when compared to control values. The litter mean body weights were decreased relative to control at postnatal days 4 and 21 (5 and 4% for the 0.3% group, and 7 and 10% for the 1% group, respectively). Mean body weights and associated standard deviations were calculated from the individual litter means in Appendix II of the Cox et al. (1975) report and are summarized in Table 2.

Table 2. Mean F1A litter body weight on days 4 and 21 in rats exposed to 2-butanol in drinking water[a]

Dose[b] in mg/kg-day (%)	Number of litters, day 4	Mean litter body weight, day 4 (g)	Standard deviation, day 4	Number of litters, day 21	Mean litter body weight (g), day 21	Standard deviation, day 21
0	29	10.7	1.1	28	49	3.8
594 (0.3%)	27	10.2	1.3	27	47	3.9
1,771 (1%)	30	10.0	1.3	30	44	4.8
4,571 (3%)	26	8.3	1.8	26	30	11.9

[a] Mean litter body weights and standard deviations were calculated from individual F1A litter body weight means in Appendix II of Cox et al. (1975). Body weights were measured to the nearest 0.1 g. From the best available copy of the report, however, the value to the right of the decimal point could not be read clearly for day 21 body weight values. Therefore, day 21 body weight data in EPA tabulations may not, in all cases, be correctly reported to the nearest gram (e.g., 10.0 and 10.9 would be indistinguishable and both treated as 10).
[b] Doses are average daily intake for female rats for the initial 8 weeks of the study as reported by the authors.
Source: Adapted from Cox et al. (1975).

During the second pregnancy, the high-dose F0 dams receiving 2% 2-butanol exhibited reduced weight gain (94 g) compared to control, 0.3% and 1% dams (gains of 113, 111, and 120 g, respectively). The F1B fetuses of high-exposure dams showed a 10% reduction in average fetal weight compared with controls (3.74 ± 1.01 g vs. 4.14 ± 1.45 g, respectively). Standard deviations were calculated from the individual animal data in the appendix of the Cox et al. (1975) report. No differences in average fetal weight were observed at 0.3% (4.16 g) and 1% (4.38 g). The difference in the mean fetal weights of the adjusted high-dose (2%) and control groups was not statistically significant ($p > 0.05$) using a t-test, but when the F1B fetal weight data were fit by linear dose-response models, log-likelihood ratio tests indicated that mean body weights significantly decreased with increasing dose levels (see Appendix B, output B-3 for statistical test results).

The incidence of nidation, early fetal death, and late fetal death did not appear to be affected in the F1B litters of any exposure group compared with controls (Cox et al., 1975). The F1B fetuses in the 2% group showed increases in skeletal variations (missing sternebrae, wavy

ribs, and incomplete vertebrae ossification) when compared with the 1% dose group. When compared with control incidences, however, no differences were apparent (see Table 3). The investigators provided no explanation for the consistently lower responses observed in the 1% (mid-dose) group.

Table 3. Incidence of skeletal variations in F1B fetuses

Skeletal variation	Incidence (%), fetal basis [litter basis]			
	0	594 mg/kg-d (0.3%)	1,771 mg/kg-d (1%)	3,122 mg/kg-d (2%)
Missing sternebrae	51/235 (22%) [10/29 (34%)]	14/211 (7%) [9/27 (33%)]	11/254 (4%) [2/30 (7%)]	46/217 (21%) [13/29 (45%)]
Wavy ribs	41/235 (17%) [17/29 (59%)]	29/211 (14%) [14/27 (52%)]	20/254 (8%) [10/30 (33%)]	35/217 (16%) [17/29 (59%)]
Incomplete ossification	56/235 (24%) [17/29 (59%)]	56/211 (27%) [20/27 (74%)]	23/254 (9%) [10/30 (33%)]	69/217 (32%) [18/29 (62%)]

Source: Adapted from Cox et al. (1975).

F2 pups from the high-dose group (2%) showed a reduction in the mean pup body weight at postnatal day 4 (9.5 g vs. 10.0 g in the control) and in mean pup body weight at day 21 (35 vs. 40 g in the control). Mean body weights of F2 pups in the 0.3 and 1% groups were similar to controls at day 4 (9.7 and 9.6 g) and day 21 (39 and 39 g). Although body weight reductions in the high-dose F2 pups were not as great as those observed in the high-dose F1A pups, a continued decrease in body weight occurred in the high-dose pups at days 4 and 21 (reductions of 5% at day 4 and 13% at day 21 when compared with F2 controls).

No exposure-related changes in organ weights or increased incidence of lesions were found in the adult F1A rats sacrificed 21 days after the F2 birth, with the exception of specific histopathologic changes in the kidneys that were most prominent in males (Cox et al., 1975). Microcysts in the tip of the renal papilla were reported for rats receiving 2% 2-butanol, but not in control rats; however, the incidence was not reported. Slight to mild hydropelvis was also observed among control and 2-butanol-exposed rats, although no dose-related effect was observed. Other changes included tubular cast formation and foci of tubular degeneration and regeneration. Incidences of male F1A rats with these types of kidney changes were 0/30, 1/30, 1/30, and 8/30 for the control through high-dose groups, respectively. A similar increased incidence was not observed in females. The findings are consistent with the pattern for early

stages of α_{2u}-globulin-associated rat nephrotoxicity as described by the Risk Assessment Forum (U.S. EPA, 1991b). Testing was not conducted, however, to demonstrate the presence of the protein α_{2u}.

In summary, the results of the Cox et al. (1975) study demonstrate that the administration of 2-butanol in drinking water at concentrations as high as 3% did not affect reproductive performance in rats (with the possible exception of male rat copulatory success), but produced maternal toxicity accompanied by developmental effects at the highest exposure level. Decreased maternal weight gain, decreased F1A pup survival, and decreased F1A pup weights at days 4 and 21 were observed in the groups exposed to 3% 2-butanol in drinking water. At the next lower dose (1%) in this same generation, only reductions in F1A pup weights (7 to 10% at days 4 and 21) were observed; however, no similar reductions in body weight were observed in subsequent generations at the 1% dose level. The following effects were noted at the 2% level (the adjusted high-dose level administered following F1A postnatal day 21): decreased maternal body weight gain during the second pregnancy of the F0 dams (body weight gain was not measured during the first, F0, pregnancy nor during the F1A pregnancy), decreased F1B fetal weights when pregnancy was terminated at gestation day 20, and decreased F2 pup weights at days 4 and 21. Developmental endpoints were not affected at the 0.3% 2-butanol exposure levels in any of the generations. 2-Butanol treatment produced an increase in the incidence of kidney lesions in high-dose (3,384 mg/kg-day) male rats (F1A generation) that were exposed from gestation through 12 weeks after birth, mating, and gestation and lactation of the F2 generation; no other treatment-related histopathologic lesions were observed in adult rats. Thus, Cox et al. (1975) identified a LOAEL of 3,122 mg/kg-day (2% solution) and a NOAEL of 1,771 mg/kg-day (1% solution) based on decreased F1B fetal weights and decreased F1A and F2 pup body weights. The maternal LOAEL in this study was 3,122 mg/kg-day (2% solution) based on decreased weight gain, and the NOAEL was 1,771 mg/kg-day (1% solution).

It should be noted that the Cox et al. (1975) study protocol, although consistent with U.S. Food and Drug Administration (FDA) guidelines available at the time that the study was conducted, did not include the evaluation of certain parameters routinely measured in studies of more current design. Deficiencies included: lack of measurements of estrous cyclicity, sperm parameters, weights of uterus, epididymides, seminal vesicles, and brain; and less than complete clinical chemistry/hematology and histopathology. Water consumption was recorded in F0 and F1A rats prior to mating, but not during gestation and lactation. Consequently, more accurate measures of offspring exposure could not be developed. Statistical analyses were not performed by study investigators. In addition, changes in the drinking water concentration of high-dose

animals during the last 2 weeks of F0 lactation of F1A litters from 3% to 0% and then to 2% 2-butanol introduces some uncertainty in the exposure of high-dose animals.

4.3.2.2. *Inhalation Exposure*

No studies were located that specifically assessed the reproductive toxicity of inhaled MEK. Although no tests for reproductive function were performed, histological examination of the reproductive organs from rats of both sexes and mammary glands of female rats exposed subchronically to MEK at concentrations as high as 5,000 ppm (14,750 mg/m³) revealed no exposure-related lesions (Cavender et al., 1983). The data base on developmental toxicity of MEK by inhalation consists of several well-conducted studies.

Schwetz et al. (1974) exposed groups of 21-23 pregnant Sprague-Dawley rats (in whole body dynamic exposure chambers) to 1,000 or 3,000 ppm (2,950 or 8,850 mg/m³) MEK vapor, respectively, for 7 hours/day on gestation days 6-15. Sperm positive vaginal smear was designated as gestation day 0. Forty-three rats exposed to filtered room air served as controls. Another control group of 47 pregnant rats was sham exposed. The average measured concentrations in this study were 1,126 or 2,618 ppm (3,322 or 7,723 mg/m³). The following endpoints were used to assess exposure-related effects: maternal body weight, food intake, liver weight, SGPT activity levels, number of implantations, litter size, fetal anomalies, incidence of resorptions, and fetal body measurements. No evidence of maternal toxicity or change in the number of resorptions was reported at any concentration. Small, but statistically significant, decreases in fetal weight and crown-rump length were observed at 1,126 ppm, but not at 2,618 ppm. In the 1,126 ppm exposure group, mean litter weight decreased by 5% and crown rump length decreased by 3% when compared with air controls. Among 4 litters exposed to 2,618 ppm, 4 fetuses had rare gross malformations, two acaudate fetuses had an imperforate anus, and 2 fetuses had brachygnathia. No gross malformations were found in fetuses from the control or 1,126 ppm exposure groups. A statistically significant increase in the percentage of litters with fetuses exhibiting gross anomalies at 2,618 ppm was observed when compared with controls (19% vs. 0%; $p<0.05$). The malformations had not been observed previously in more than 400 historical control litters of this rat strain. The percentage of litters with specific skeletal variations (e.g., delayed ossification of skull or sternebrae) were not significantly different from control percentages in the 1,126 ppm group, but the 2,618 ppm group showed a statistically significant increase in the percentage of litters with sternebral skeletal variations (43% vs. 11% in concurrent controls; $p<0.05$). A statistically significant increase in the percent of litters exhibiting any skeletal anomaly was observed at 1,126 ppm (95%) but not at 2,618 ppm (81%),

when compared with the control (58%). Percentages of litters with specific soft tissue anomalies (e.g., subcutaneous edema or dilated ureters) were not significantly elevated in either exposure group. A statistically significant increase in the percent of litters with any soft tissue anomaly was observed at 2,618 ppm (76%) but not at 1,126 ppm (70%), when compared with the controls (51%).

The Schwetz et al. (1974) results indicate that 2,618 ppm was an adverse effect level for developmental effects in the absence of maternal toxicity, predominately on the strength of the findings for rarely occurring gross malformations that were not seen in the 1,126-ppm exposure groups or controls. The biological significance of the developmental findings for the 1,126-ppm exposure group is not clear. The decreased fetal weight and crown rump length reductions were very small (3–5% decrease), and statistical significance was not demonstrated for these variables at the higher exposure level. Likewise, the increased incidence of litters with any skeletal anomalies at 1,126 ppm (i.e., "total skeletal anomalies") was not statistically demonstrable at 2,618 ppm, and no incidences of specific skeletal anomalies were significantly elevated at 1,126 ppm. Thus, for this study, 1,126 ppm (7 hours/day on gestation days 6–15) is designated as a NOAEL, and 2,618 ppm is established as a LOAEL for developmental effects. Also, the highest exposure level, 2,618 ppm, is identified as a NOAEL for maternal toxicity for this study.

Deacon et al. (1981) attempted to repeat and improve upon the Schwetz et al. (1974) study. Deacon et al. (1981), also reported as Dow Chemical Corporation (1979), included an additional, lower exposure level (400 ppm). Groups of 26, 19, 19, and 18 Sprague-Dawley dams were exposed (in whole body dynamic exposure chambers) to nominal MEK concentrations of 0, 400, 1,000, and 3,000 ppm, respectively, for 7 hours/day on gestation days 6–15. The number of animals in the treatment groups are slightly smaller than the 20 animals/group recommended in current protocols. Average measured concentrations of MEK during the experiment were 412, 1,002, and 3,005 ppm (1,215, 2,955, and 8,865 mg/m^3). Dams exposed to 3,005 ppm MEK exhibited maternal toxicity consisting of a slight decrease in weight gain (326 g for 3,005 ppm group vs. 351 g for control; $p<0.05$ at gestation day 16), and increased water consumption on days 15–17 (82 mL/day for 3,005 ppm group vs. 69 mL/day for control; $p<0.05$ at gestation day 16) (Dow Chemical Corporation, 1979). None of the exposure levels produced statistically significant effects on: the incidences of pregnancy or resorption, the average number of implantations or live fetuses per dam, fetal weight, or length. No statistically significant differences in the incidence of external or soft-tissue alterations were observed between the exposed and control groups. Differences in the incidence of litters with two skeletal variations occurred in the 3,005 ppm exposure group when compared with the controls. The incidence of

41

extra ribs was 2/26 for control litters, compared with 0/19, 0/19, and 6/18 for 412, 1,002, and 3,005 ppm litters, respectively. This finding was statistically significant at the high dose under Fisher's Exact test. The respective incidences of delayed ossification of the cervical centra were 22/26, 15/19, 16/19, and 18/18, and were not statistically significant by Fisher's Exact test. Thus, this study found maternal toxicity (decreased weight gain) and fetal toxicity (increased incidence of extra ribs) at 3,005 ppm MEK 7 hours/day on gestation days 6–15 (LOAEL), but not at 412 or 1,002 ppm (NOAEL). The study thereby corroborates the developmental effect levels reported by Schwetz et al. (1974).

A subsequent inhalation developmental toxicity study in CD-1 mice (Schwetz et al., 1991; also reported as Mast et al., 1989 and NTP, 1990) verified the fetal effect levels established by the two developmental inhalation studies in Sprague-Dawley rats (Schwetz et al., 1974; Deacon et al., 1981). Groups of 10 virgin Swiss CD-1 mice and 33 sperm plug-positive (gestation day 0) females were exposed to mean concentrations of 0, 398±9, 1,010±28, and 3,020±79 ppm (0, 1,174±27, 2,980±83, and 8,909±233 mg/m^3) MEK by inhalation (in whole body dynamic exposure chambers) for 7 hours/day on gestation days 6–15 and were sacrificed on day 18 of gestation. At 0, 398, 1,010, or 3,020 ppm MEK, the number of gravid/mated mice were 26/33, 23/33, 26/33, and 28/33, respectively. In the dams, a slight, concentration-related increase in liver-to-body-weight ratio was observed. The increase achieved statistical significance at 3,020 ppm (increase of approximately 7% when compared with the control). Maternal body weight gain was similar across all groups. Two statistically significant developmental effects were observed, including: a 5% decrease in mean fetal weight (per litter) at 3,020 ppm in males when compared with controls and a 4% decrease for all fetuses combined when compared with controls; and a positive trend for an increased incidence of fetuses with misaligned sternebrae with increasing exposure level (incidences were 31/310, 27/260, 49/291, and 58/323 for the control through 3,020 ppm exposure groups, respectively). No statistically significant trend was found for the increased incidence of litters containing fetuses with misaligned sternebrae with increasing exposure level. For female fetuses at 3,020 ppm, the extent of the reduction in litter mean body weight (approximately 4%) was equivalent to the reduction noted in all fetuses and males, but it did not achieve statistical significance due to the relatively low fetal weight among female controls. No increase in the incidence of intrauterine death was observed in any of the exposed groups. No statistically significant increases in the incidence of malformations occurred, although 4 malformations (cleft palate, fused ribs, missing vertebrae, and syndactyly) were observed in exposed groups that were not seen in the control group or in contemporary control data. Based on the absence of both maternal and developmental toxic effects, a NOAEL of 1,010 ppm was established. Developmental and

maternal LOAELs were established at 3,020 ppm (7 hours/day on gestation days 6–15) for small, but statistically significant decreased fetal weight among males, increased incidence of misaligned sternebrae, and an increased maternal liver-to-body-weight ratio. The results are in accord with the developmental effect levels established by earlier studies in rats (Schwetz et al., 1974; Deacon et al., 1981).

While two other studies (Stoltenburg-Didinger et al., 1990; Stoltenburg-Didinger, 1991) involve inhalation exposure of rats to MEK during gestation (21 days) and lactation (21–30 days), their main focus was to compare the relative susceptibility of adult and juvenile rats to MEK potentiation of n-hexane peripheral neuropathy. The studies are not useful to assess the developmental toxicity of MEK alone since the available reports do not clearly describe details of the experimental conditions or the results from the groups exposed to MEK alone. Consequently, these particular studies are not further discussed in this document.

Three inhalation developmental studies in rodents demonstrated that exposure (7 hours/day on gestation days 6–15) to approximately 3,000 ppm (8,850 mg/m^3) MEK caused developmental toxicity in the presence of maternal toxicity in rats (Deacon et al., 1981) and mice (Schwetz et al., 1991), and developmental toxicity in the absence of maternal toxicity in one rat study (Schwetz et al., 1974).

Additional information relevant to the developmental toxicity of inhaled MEK is provided by the developmental inhalation toxicity study of Nelson et al. (1989, 1990), wherein the effects of exposure to industrial alcohols, including butanol isomers, were examined. Nelson et al. (1989, 1990) exposed gravid Sprague-Dawley rats by inhalation to 2-butanol at 0, 3,500, 5,000, or 7,000 ppm (0, 10,605, 15,150, or 21,210 mg/m^3) for 7 hours/day on gestation days 1–19. At these exposure concentrations, the number of gravid/mated rats were 15/16, 16/16, 14/15, and 11/15, respectively. Dams were sacrificed on gestation day 20 (sperm positive vaginal smear was gestation day zero), and fetuses were serially removed, weighed, sexed, and examined for external malformations. The frequency of visceral malformations and variations was determined in one-half of the fetuses, and the frequency of skeletal deviations was determined in the other half. Maternal toxicity was exhibited in the dams at all three exposure concentrations as statistically significant reductions in weight gain and food consumption (see Table 4). The authors reported narcosis (impairment of locomotor activity) at 5,000 ppm and above.

Table 4. Maternal and fetal effects in 2-butanol-exposed rats

Endpoint	Exposure Concentration (ppm)			
	0	3,500	5,000	7,000
Maternal Effects				
Day 20 weight gain (g) (estimated from a graph)	105	80*	76*	22*
Food consumption (week 3) (g)	126±15	113±13	112±17*	99±11*
Fetal Effects				
# resorptions/litter	1.5±1.3	1.6±1.4	1.5±0.9	3.8±2.2*
Fetal weight (g) Male Female	3.3±0.23 3.1±0.22	3.1±0.22 2.9±0.20	2.7±0.25* 2.6±0.23*	1.5±0.12* 1.4±0.18*

* Significantly different from control ($p < 0.05$).
Source: Adapted from Nelson et al. (1989).

Inhalation exposure to 2-butanol also produced statistically significant dose-related effects on certain fetal developmental indices. A statistically significant increase in the number of resorptions per litter was reported at 7,000 ppm (3.8±2.2) compared with the control (1.5±1.3). Fetal weights were reduced in all 2-butanol-exposed groups; differences were statistically significant when compared to the control at 5,000 and 7,000 ppm (see Table 4). External fetal malformations were not observed. A statistically significant increase in the incidence of pooled skeletal variations was observed at 7,000 ppm (100%) when compared to controls (32%). The authors did not report the nature of skeletal variations observed or the incidence of individual variations. Occasional visceral variations were seen; however, the authors did not attribute these to 2-butanol treatment. Although marked maternal toxicity was observed at 7,000 ppm (including weight gain that was less than 25% of the control), the increase in resorptions and skeletal variations at this concentration cannot necessarily be attributed to a direct effect of 2-butanol exposure. The types of developmental effects induced by inhalation exposure to 2-butanol at concentrations below 7,000 ppm during gestation are generally similar to those identified for inhalation exposure to MEK by Schwetz et al. (1974, 1991) and Deacon et al. (1981), and for oral exposure to 2-butanol by Cox et al. (1975). Body weight reductions were observed in 2-butanol-exposed rats and in MEK-exposed rats (Schwetz et al., 1974) and mice (Schwetz et al., 1991). No increase in the incidence of variations, however, was present in 2-butanol-exposed rats at concentrations that were associated with various skeletal variations in MEK-exposed rats and mice (Schwetz et al., 1974, 1991; Deacon et

al., 1981). Considering that the ability to detect a change in fetal weight (a continuous variable) is much greater than for other (dichotomous) fetal endpoints, changes in fetal weight are often observed at doses below those producing other signs of developmental toxicity (U.S. EPA, 1991a). Because the Nelson et al. (1989) study of 2-butanol included only 15–16 animals per group compared to the approximately 25 animals per group included in the MEK developmental toxicity studies (Schwetz et al., 1974, 1991; Deacon et al., 1981), it is possible that the 2-butanol study did not have sufficient power to detect anomalies.

To assess whether the magnitude of developmental effects associated with the inhalation of 2-butanol and MEK were similar, fetal weight changes observed in 2-butanol- and MEK-exposed animals were compared. Figure 2 shows the relationship between fetal weight (expressed as percent change from control) and exposure concentration for 2-butanol [based on data for male rat fetuses from Nelson et al. (1989)] and for MEK [based on data for rat fetuses from Schwetz et al. (1974) and mouse fetuses from Mast et al. (1989)/Schwetz et al. (1991)]. Although the range of exposure concentrations used in the 2-butanol study exceeded the range of exposure concentrations used in the MEK studies, visual inspection of the graph demonstrates that the dose-response curves for 2-butanol and MEK are consistent.

A summary of key repeat exposure reproductive and developmental toxicity studies in animals exposed to MEK and 2-butanol is available in Table 5.

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Data

4.4.1.1. *Oral Exposure*

Oral LD_{50} values for MEK include 5,522 and 2,737 mg/kg in rats (Smyth et al., 1962 and Kimura et al., 1971, respectively) and 4,044 mg/kg in mice (Tanii et al., 1986). A single gavage dose of 15 mmol/kg MEK (1,082 mg/kg) in corn oil produced no deaths or histological alterations in the livers of male Fischer 344 rats, but produced tubular necrosis in the kidneys (Brown and Hewitt, 1984).

Figure 2. Comparison of Fetal Body Weight Changes in Animals Exposed to MEK or 2-Butanol during Gestation
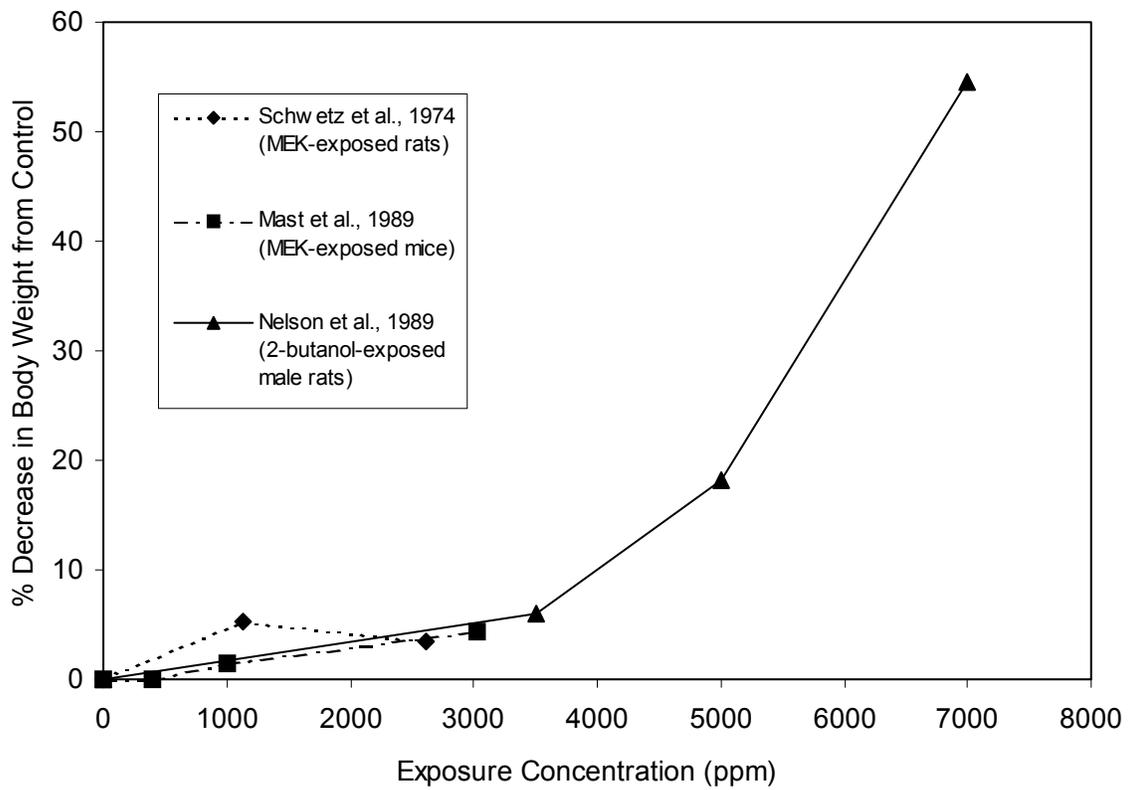
4.4.1.2. *Inhalation Exposure*

LaBelle and Brieger (1955) reported a 4-hour LC_{50} for MEK of $11,700\pm2,400$ ppm ($34,515\pm7,080$ mg/m^3) in rats. Several studies describe the behavioral effects of acute inhalation exposure of mice to MEK (Section 4.2.2.). Glowa and Dews (1987) exposed a group of 12 adult, male CD-1 mice to air concentrations of MEK that were increased at 30-minute intervals until the mice failed to respond to a visual stimulus (response to a visual stimulus and the response rate were used as indicators). The concentrations for each 30-minute period were 300, 1,000, 3,000, 5,600, and 10,000 ppm (885, 2,950, 8,850, 16,520, and 29,500 mg/m^3) MEK with a total exposure time of 2 hours. No effects were observed at 300 ppm, while a slight decrease in response rate was observed at 1,000 ppm and a 75% decrease in response rate was observed at 3,000 ppm. Most mice (incidence not reported) ceased to respond at 5,600 ppm, and all failed to respond at 10,000 ppm. The response rate returned to the control value 30 minutes after exposure ended. The EC_{50} (concentration expected to elicit a 50% decrease in response rate) was calculated to be 2,891 ppm (SD = 689 ppm). From these results, an EC_{10} (concentration estimated to elicit a 10% decrease in response rate) was calculated and dose-response estimates were derived. The concentrations of MEK producing a 10% decrease in response rate in 0.1, 1, and 10% of a population were calculated to be 17, 66, and 300 ppm, respectively (Glowa and Dews, 1987).

The EC_{50} established by Glowa and Dews (1987) for response to a visual stimulus in CD-1 mice (2,891 ppm) is similar to an EC_{50} for behavioral effects induced by MEK in Swiss mice. Groups of 10 adult male Swiss mice were exposed via whole-body inhalation chamber to MEK at 0, 1,602, 1,848, 2,050, or 2,438 ppm (0, 4,726, 5,452, 6,048, or 7,192 mg/m^3) for 4 hours (DeCeaurriz et al., 1983). Immediately after exposure, mice were subjected to the behavioral despair swimming test, where the decrease in total time of immobility during the first 3 minutes in a water bath was used as an indication of behavioral toxicity. MEK exposure produced a statistically significant ($p<0.05$) decrease in immobility in the behavioral despair swimming test at all exposure concentrations tested. Based on this data, the authors calculated a 50% decrease in immobility (ID_{50}) for MEK of 2,065 ppm. No other observations of the effects of inhalation exposure of mice to MEK were reported in this study.

4.4.2. Genotoxicity

MEK is not mutagenic as indicated by a number of conventional short-term assays for genotoxic potential. A battery of *in vitro* tests showed that MEK was not genotoxic in the

following assays: Salmonella (Ames) assay with or without metabolic activation, the L5178/TK$^{+/-}$ mouse lymphoma assay, and the BALB/3T3 cell transformation assay. MEK did not induce unscheduled DNA synthesis in rat primary hepatocytes (O'Donoghue et al., 1988). MEK also tested negative in a battery of *in vitro* tests (Salmonella, chromosome aberration, and sister chromatic exchange) conducted by the National Toxicology Program (NTP, undated). MEK was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 in the presence or absence of rat hepatic homogenates (Florin et al., 1980; Douglas et al., 1980; Zeiger et al., 1992). No induction of micronuclei was found in the erythrocytes of mice (O'Donoghue et al., 1988) or hamsters (WHO, 1992) after intraperitoneal injection with MEK. The only evidence of mutagenicity was mitotic chromosome loss at a high concentration in a study on aneuploidy in the diploid D61, M strain of the yeast *Saccharomyces cerevisiae* (Zimmermann et al., 1985); the relevance of this positive result to humans is unknown. Low levels of MEK combined with low levels of nocodazole (another inducer of aneuploidy) have also produced significantly elevated levels of aneuploidy in the *S. cerevisiae* test system (Mayer and Goin, 1987).

4.4.3. Carcinogenicity

As discussed in Section 4.2, no cancer bioassay is available from which to assess the carcinogenic potential of MEK in experimental animals by the oral or inhalation routes. In a skin carcinogenesis study designed to investigate the contribution of sulfur compounds to tumor induction by unrefined mineral oils, groups of 10 to 15 male C3H/He mice received dermal applications of various solvent mixtures, some containing MEK (Horton et al., 1965). Mice received 50 mg of a solution containing: (1) 25% MEK, 70% dodecylbenzene, and 5% benzyl disulfide; (2) 29% MEK, 70% dodecylbenzene, and 0.8% 2-phenylbenzothiophene; or (3) 17% MEK, 50% dodecylbenzene, and 33% decalin twice a week for 1 year. No skin tumors developed in the groups of mice treated with solvents containing 25% MEK with 5% benzyl disulfide (a weak accelerant for skin tumors in C3H mice). After 27 weeks, a single skin tumor developed in 1 of 10 mice treated with the solution containing 29% MEK, and after 51 weeks, a skin tumor developed in 1/15 mice treated with the solution containing 17% MEK and other solvents. This study is an inadequate test of MEK carcinogenicity due to concomitant exposure to sulfur-containing chemicals and dodecylbenzene (which are expected to accelerate the rate of skin tumor formation).

Using mechanism-based structure-activity relationship (SAR) analysis, it was determined that MEK is unlikely to be carcinogenic based on the lack of any structural features/alerts

indicative of carcinogenic potential (Woo et al., 2002).

4.4.4. MEK Potentiation of Peripheral Neuropathy from Chemicals Metabolized to Gamma-Diketones

A number of studies in experimental animals demonstrate that MEK potentiates the effects of known neurotoxicants (e.g., n-hexane, MnBK, and 2,5-hexanedione) (Saida et al., 1976; Altenkirch et al., 1978; Takeuchi et al., 1983). Saida et al. (1976) found peripheral neuropathy in rats after 25 days of continuous exposure to MEK and MnBK at concentrations of 1,125 ppm (3,319 mg/m³) MEK and 225 ppm MnBK. In contrast, rats exposed to 225 ppm MnBK alone developed peripheral neuropathy after 66 days. In a study with n-hexane and MEK, Altenkirch et al. (1978) reported that the onset of clinical and morphological effects was shortened and that the extent and severity of lesions in the peripheral and central nervous systems increased at an exposure of 9,000 ppm n-hexane and 1,000 ppm (2,950 mg/m³) MEK as compared to 10,000 ppm n-hexane alone. Altenkirch et al. (1982) also examined nervous system response to n-hexane and a mixture of n-hexane and MEK. Animals exposed continuously to 500 ppm n-hexane alone displayed hind limb paralysis after 9 weeks, as well as axonal lesions in peripheral nerves. In rats treated with a mixture of n-hexane (300 ppm) and MEK (200 ppm) similar clinical and pathological signs of neuropathy occurred one week earlier. Takeuchi et al. (1983) reported that distal motor nerve latency was significantly reduced at 4 weeks of exposure to 100 ppm n-hexane plus 200 ppm (590 mg/m³) MEK. While this effect did not persist, it was not seen with exposure to 100 ppm n-hexane alone or 200 ppm MEK alone. In addition, tail nerve conduction velocity in rats exposed to a mixture of 100 ppm n-hexane and 200 ppm MEK was statistically reduced as compared to control at 20 and 24 weeks of exposure, an effect that was not seen with exposure to n-hexane alone at 100 ppm. Microscopic examination of the tail nerves revealed no histopathological lesions after 24 weeks.

Evidence in humans that MEK has the capacity to interact with other solvents is less clear. In a series of studies in human volunteers by Dick et al. (1984, 1988, 1989, 1992), MEK-exposed groups (at 100 ppm) that were coexposed to relatively low levels (also around 100 ppm) of several other solvents including acetone, methyl isobutyl ketone and toluene, for 4 hours exhibited no evidence of neurotoxic interactions. Altenkirch et al. (1977) reported the occurrence of polyneuropathies in juveniles who sniffed glue thinner following the change in composition of the thinner from one containing n-hexane and other solvents to one that included MEK in the composition. A recent review (Noraberg and Arlien-Soborg, 2000) reported possible interactions following occupational exposure to mixtures of organic solvents containing

MEK, although because of the nature of the exposures, the studies cannot be used to establish a causal relationship between cases of neuropathy and specific chemical exposures. For example, Dyro et al. (1978) reported three cases of polyneuropathy in shoe factory workers exposed to MEK, acetone and toluene; the potential for dermal contact was noted but not further characterized. Allen et al. (1974) found evidence of neuropathies in 79 of 1,161 employees in a fabric plant where workers were regulatory exposed to methyl butyl ketone and MEK. Air concentrations of MEK reached levels as high as 5,000 mg/m^3 and employees washed their hands with these solvents. Upon removal of methyl butyl ketone from the plant and efforts to reduce solvent exposure, no new cases of neuropathy developed. Whether there were any interactive effects between MEK and methyl butyl ketone cannot be ascertained. Fagius and Gronquist (1978) performed a study of polyneuropathy in 42 steel plant workers exposed to 18 solvents, including MEK. Three possible cases of polyneuropathy were found (and none in a referent population). Measurement of 11 neurological tests revealed only weak and inconclusive evidence of decrements in peripheral nerve function in the solvent exposed population. Chia et al. (1993) investigated neurobehavioral effects in workers exposed to MEK, cyclohexanone, tetrahydrofuran and toluene in a video tape manufacturing facility in Singapore. Three of 7 neurobehavioral tests (indicative of visual motor control and recent memory impairment) revealed statistically significant differences between the exposed group and matched controls, although no dose-effect relation was observed. The possibility of extensive skin contact with the solvents was noted by the authors.

None of the available occupational studies involving multiple chemical exposures as discussed above provide information adequate to establish whether MEK interacts with other neurotoxic solvents in humans. Further, the studies do not provide information to establish the lower limit of MEK exposure that may result in potentiation of effects by known neurotoxicants. From the review by Noraberg and Arlien-Soborg (2000), however, it appears that neurotoxicity was observed only in worker populations exposed to solvent mixtures where reported MEK air concentrations reached levels at or above the TLV (200 ppm; 590 mg/m^3).

The mechanism by which MEK potentiates the neurotoxicity of hexacarbon solvents is not entirely clear, although it appears to involve the biotransformation of these solvents to their toxic metabolites (such as 2,5-hexadione (2,5-HD), which is the putative moiety responsible for inducing neural damage associated with n-hexane exposure) (DiVincenzo et al., 1976; van Engelen et al., 1997; Ichihara et al., 1998). In the case of 2,5-HD, the potentiation effect appears to be due to the increased persistence of 2,5-HD in blood, probably due to inhibition by MEK of 2,5-HD phase II biotransformation that alters the metabolism and elimination of 2,5-HD (van

Engelen et al., 1997; Zhao et al., 1998; Yu et al., 2002). Consistent with this proposed mechanism of potentiation are the findings of Abdel-Rahman et al. (1976), wherein blood samples from rats exposed continuously to 400 ppm MnBK for 6 days revealed no detectable levels of MnBK, whereas MnBK was present at detectable levels (reported as 9.5 mg%) in blood samples from rats exposed continuously to a mixture of MnBK (225 ppm) and MEK (750 ppm). In contrast to n-hexane and MnBK, MEK is not metabolized to a gamma-diketone (a diketone in which the two carbonyl groups are separated by two carbons) (DiVincenzo et al., 1976). The difference is significant, since the gamma-diketones (in contrast to MEK's metabolites) have been associated with distal neurofilamentous axonopathy (Graham, 2000).

In general, potentiation of the neurotoxicity of other solvents by MEK has been demonstrated in experimental animals only at relatively high concentrations (>1,000 ppm or 2,950 mg/m³) where induction of hepatic enzymes (liver enzymes that are responsible for toxifying the gamma-diketones) is postulated as the mode of action. Studies of MEK potentiation of neurotoxicants at lower exposure concentrations have generally not been performed. One exception is Takeuchi et al. (1983) in which reversible potentiation of n-hexane neurotoxicity was observed at 200 ppm (590 mg/m³) MEK. The work of van Engelen et al. (1997) provides some insight into the lower limits of interactive effects of MEK and n-hexane in humans. Volunteers were exposed to n-hexane (approximately 60 minutes) with or without coexposure to MEK (200 or 300 ppm) for 15.5 minutes, while the concentration-time course of n-hexane (in exhaled alveolar air) and its metabolite 2,5-HD (in serum) were measured. Coexposure to 200 ppm MEK did not affect the concentration-time course of exhaled n-hexane or the rate of formation of serum 2,5-HD; however, MEK significantly decreased the rate of 2,5-HD formation (approximately 3-fold) at 300 ppm. Coexposure to 300 ppm MEK also significantly increased the time to reach peak concentration of 2,5-HD (T_{max}). At 200 ppm MEK, there was a trend to higher values of T_{max}, but the effect was not statistically significant. The investigators cautioned that their findings could not be used to predict interactive effects resulting from chronic exposure. The findings do suggest that, at least following short-term exposure, significant interactive effects may occur at levels somewhat above the TLV (200 ppm).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral Exposure

Data on the toxic effects associated with oral exposure of humans to MEK are limited to a single nonoccupational report of acute toxicity following accidental ingestion of MEK (Kopelman and Kalfayan, 1983). The report did not indicate any persistent adverse health effects. In laboratory animals, the data base on toxicity of MEK following oral exposure is limited to a small number of acute studies. LD_{50} values for adult mice and rats are 2–6 g/kg body weight, with death occurring within 1–14 days following a single oral dose (Tanii et al., 1986; Kimura et al., 1971; Smyth et al., 1962). The lowest, non-lethal acute oral dose producing an adverse effect is a report of renal tubule necrosis in F344 rats following a single oral dose of 1,082 mg/kg of MEK in corn oil (Brown and Hewitt, 1984).

Subchronic and chronic toxicity studies of oral MEK exposure are not available. Repeat-dose toxicity data are available, however, for 2-butanol (a metabolic precursor) and 3-hydroxy-2-butanone (a metabolite). In rats, the majority of an oral dose of 2-butanol is rapidly converted to MEK (Traiger and Bruckner, 1976; Dietz et al., 1981); both MEK and 2-butanol are transformed to common metabolites (3-hydroxy-2-butanone and 2,3-butanediol) in the rat (Dietz et al., 1981). In rats administered similar oral doses of MEK or 2-butanol, the elimination kinetics for the common metabolites are similar (Dietz et al., 1981).

The oral toxicity data base for 2-butanol consists of a two-generation reproductive and developmental toxicity study in rats (Cox et al., 1975). The administration of 2-butanol in drinking water before and during gestation and lactation at concentrations as high as 3% did not affect reproductive performance (with the possible exception of increased male copulatory failure), but did result in decreased pup survival and pup body weight gain in Wistar rats. A concentration of 2% in drinking water caused a reduction in fetal weights when pregnancies were terminated on gestation day 20 and decreased pup body weights when dams were allowed to deliver. Cox et al. (1975) reported a LOAEL of 3,122 mg/kg-day (2% solution) and a NOAEL of 1,771 mg/kg-day (1% solution) for decreased fetal weight and decreased pup body weight gain. The findings of developmental toxicity in rats exposed orally to 2-butanol is consistent with similar findings in inhalation developmental toxicity studies of MEK discussed in Section 4.3.2.2 (Schwetz et al., 1974, 1991; Deacon et al., 1981) and 2-butanol (Nelson et al., 1989, 1990). Given these observations, it is plausible that the developmental effects produced by 2-butanol and MEK are caused by MEK or a subsequent metabolite common to both.

In adult rats, exposure to 3% 2-butanol in drinking water for 8 weeks caused reduced weight gain in F0 males and females (Cox et al., 1975). F1 animals exposed to 2-butanol in drinking water at concentrations up to 2% for 12 weeks after birth and through mating, gestation, and lactation of F2 litters were subject to gross and histopathological examination. No exposure-related changes in organ weights or incidence of histopathologic lesions were observed with the exception of specific histopathologic changes of the kidney in male rats exposed to 2% 2-butanol. Changes were consistent with the pattern of early stages of α_{2u}-globulin-associated rat nephrotoxicity; however, testing needed to demonstrate the presence of α_{2u} was not conducted. Therefore, the relevance of this finding to humans is uncertain.

The oral toxicity data base for 3-hydroxy-2-butanone consists of a 13-week drinking water study in rats (Gaunt et al., 1972). Thirteen weeks of drinking water exposure to 3-hydroxy-2-butanone in CFE rats (15/sex/dose) did not produce a toxic effect aside from slight anemia (decreased hemoglobin concentration and red blood cell count) at the high dose (1,286 mg/kg-day) (Gaunt et al., 1972), an effect that has not been reported following exposure to 2-butanol (orally; Cox et al., 1975) or MEK (by inhalation; Cavender et al., 1983). In the Cavender et al. (1983) study, hemoglobin concentrations were unaffected by inhalation exposure to MEK; at 15,000 mg/m^3, there was a statistically significant increase in mean corpuscular hemoglobin that corresponded to a slight but insignificant decrease in red blood cells. Further, Gaunt et al. (1972) provides no information concerning the potential for developmental effects from exposure to 3-hydroxy-2-butanone. This observation further supports the use of 2-butanol, rather than a metabolite, as a surrogate for MEK.

In summary, information on the effects of MEK following repeat-dose oral exposure is limited to data for 2-butanol (a metabolic precursor) and 3-hydroxy-2-butanone (a metabolite). Because of the similarity in the effects of exposure to MEK and 2-butanol, as well as the finding that 2-butanol is rapidly converted to MEK in rats, 2-butanol is considered to be an appropriate surrogate for assessing MEK-associated toxicity. A multigeneration reproductive and developmental toxicity study of 2-butanol by Cox et al. (1975) identified developmental effects (reduced fetal and pup weight) as the most sensitive toxicologically relevant endpoint.

4.5.2. Inhalation Exposure

Evidence for neurotoxic effects following inhalation exposure to MEK is limited to a small number of case reports of neurological impairment in occupationally-exposed humans (Welch et al., 1991; Seaton et al., 1992; Callender, 1995; Orti-Pareja et al., 1996) and in one study of problematic design reporting increased incidence of subjectively reported neurological symptoms in MEK-exposed workers (Mitran et al., 1997; Graham, 2000). A few animal studies involving a single or limited number of inhalation exposures reported behavioral effects and narcosis (Nelson et al., 1989, 1990; Glowa and Dews, 1987). Several well-conducted studies in experimental animals, however, provide no convincing evidence that repeated exposure to MEK, by itself, is capable of producing persistent neurological effects. No persistent, treatment-related central or peripheral neural histopathology was observed in rats exposed for 90 days (6 hours/day, 5 days/week) to MEK at concentrations up to 5,041 ppm (14,870 mg/m^3) (Cavender et al., 1983). Repeated exposure of rats and mice to MEK at approximately 3,000 ppm (8,850 mg/m^3) (7 hours/day during days 6–15 of gestation) produced no overt neurological effects in the dams (Schwetz et al., 1974, 1991; Deacon et al., 1981).

Developmental effects following exposure to MEK have been described in experimental animals, but not humans. Three inhalation developmental studies in rodents demonstrated that MEK caused developmental toxicity in the presence of maternal toxicity in rats (Deacon et al., 1981) and mice (Schwetz et al., 1991), and in one rat study (Schwetz et al., 1974) in the absence of maternal toxicity. These inhalation studies provide evidence for developmental effects (decreased fetal weight and increased incidence of certain skeletal variants) in rats and mice exposed to 3,000 ppm MEK, 7 hours/day during gestation, but not at 1,000 ppm and lower. The observation of developmental delays following inhalation exposure to MEK is supported by the findings from studies of rats exposed orally (Cox et al., 1975) and by inhalation (Nelson et al., 1989, 1990) to 2-butanol, a metabolic precursor of MEK.

Available data provide no clear evidence for other systemic effects resulting from inhalation exposure to MEK. A subchronic inhalation study of MEK found no persistent body weight changes, gross behavioral changes, or histological changes in major tissues and organs in rats exposed 6 hours/day, 5 days/week for 90 days to concentrations as high as 5,000 ppm (14,750 mg/m^3) (Cavender et al., 1983). Some changes in organ weight (including increased liver weight and decreased brain weight) and clinical pathology parameters were observed; however, these were not supported by histological changes.

The available data provide no evidence for portal-of-entry effects following inhalation exposure to MEK. In a series of studies involving numerous volunteers, Dick et al. (1984, 1989, 1992) did not find any reported net effects related to irritation from MEK at exposures up to 200 ppm (590 mg/m^3) for up to 4 hours. In an earlier study involving few subjects and unclear exposure conditions, exposure to 300 ppm (885 mg/m^3) MEK was reported as intolerable (Nelson et al., 1943). Nasal irritation was noted in rats exposed to 6,000 ppm MEK for 15 weeks (Altenkirch et al., 1978), but not in other studies involving somewhat lower exposure concentrations. In the only available subchronic animal inhalation study of MEK (Cavender et al., 1983), no exposure-related upper respiratory irritation could be evaluated in rats exposed up to 5,000 ppm (14,750 mg/m^3) MEK for 90 days (confounding respiratory tract lesions were likely due to an infectious agent that occurred in all groups in this study including controls). In addition, respiratory irritation was not reported in dams exposed to 3,000 ppm (8,850 mg/m^3) MEK, 7 hours/day for days 6–15 of gestation (Schwetz et al., 1974, 1991; Deacon et al., 1981).

Results from studies of pregnant rodents exposed by inhalation to MEK indicate that developmental effects are the most sensitive, toxicologically relevant endpoint for inhalation exposure to MEK.

4.5.3. Mode of Action Information

The mode of action by which MEK induces toxicity has not been characterized.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under EPA's draft revised cancer guidelines (U.S. EPA, 1999), "*data are inadequate for an assessment of human carcinogenic potential*" for MEK, because studies of humans chronically-exposed to MEK are inconclusive, and MEK has not been tested for carcinogenicity in animals by the oral or inhalation routes. The majority of short-term genotoxicity testing of MEK has demonstrated no activity, and SAR analysis suggests that MEK is unlikely to be carcinogenic.

The few available epidemiological studies of MEK-exposed workers provide no clear evidence of a cancer hazard, but the studies are generally inadequate to discern an association between MEK exposure and an increased incidence of cancer (Alderson and Rattan, 1980; Wen et al., 1985; Spirtas et al., 1991; Blair et al., 1998). In these studies, the epidemiological

evidence is based on a small number of site-specific deaths, and studies that are confounded by exposure to multiple chemicals. A case-control study examining the association between paternal exposures to several solvents, including MEK, and childhood leukemia (Lowengart et al., 1987) is exploratory in scope and cannot be used to reliably support the existence of any such association. Overall, the epidemiologic evidence from which to draw conclusions about carcinogenic risks in the human population is inconclusive. Although there is some suggestion of increased risk for certain cancers (including bone and prostate) involving multiple solvent exposures that include MEK, there is no clear evidence for a relationship between these cancers and MEK exposure alone.

No cancer bioassay is available from which to assess the carcinogenic potential of MEK in experimental animals by the oral or inhalation routes. A skin carcinogenesis study by Horton et al. (1965) is an inadequate test of MEK's potential carcinogenicity due to concomitant exposure to chemicals that are expected to accelerate the rate of skin tumor formation.

MEK has not exhibited mutagenic activity in a number of conventional short-term test systems. *In vitro* tests showed that MEK was not genotoxic in the Salmonella (Ames) assay with or without metabolic activation, the L5178/TK$^{+/-}$ mouse lymphoma assay, and the BALB/3T3 cell transformation assay. The tests did not induce unscheduled DNA synthesis in rat primary hepatocytes, chromosome aberrations, or sister chromatic exchange (Florin et al., 1980; Douglas et al., 1980; O'Donoghue et al., 1988; NTP, undated; Zeiger et al., 1992). No induction of micronuclei was found in the erythrocytes of mice (O'Donoghue et al., 1988) or hamsters (WHO, 1992) after intraperitoneal injection with MEK. The only evidence of mutagenicity was mitotic chromosome loss at a high concentration in a study on aneuploidy in the diploid D61, M strain of the yeast *Saccharomyces cerevisiae* (Zimmerman et al., 1985); the relevance of this positive result to humans is unknown. In general, studies of MEK yielded little or no evidence of mutagenicity. SAR analysis suggests that MEK is unlikely to be carcinogenic based on the absence of any structural alerts indicative of carcinogenic potential (Woo et al., 2002).

4.7. SUSCEPTIBLE POPULATIONS AND LIFESTAGES

4.7.1. Possible Childhood Susceptibility

No specific data are available that assess the potential differences in susceptibility to adverse effects from MEK exposure between children and adults. At certain stages in their

development, children have differences in levels of cytochrome P450 enzymes and several phase II detoxification enzymes (e.g., N-acetyl transferases, UDP-glucuronyl transferases, and sulfotransferases) relative to adults (Leeder and Kearns, 1997; Vieira et al., 1996). Quantitative data on the possible contributions of these differences to potential age-related toxicity from MEK are lacking. Available results from animal inhalation developmental toxicity studies (Schwetz et al., 1974, 1991; Deacon et al., 1981) suggest that MEK or its metabolites may cross the placenta and may produce developmental effects. An exploratory case-control study showed an increased risk (not statistically significant) of childhood leukemia associated with paternal (but not maternal) exposure to MEK after birth of the child (Lowengart et al., 1987). Given the nature of the exposure information (self-reported via questionnaire), evidence for possible childhood susceptibility as provided by this study is considered very limited.

4.7.2. Possible Gender Differences

Available studies in humans and animals provide little evidence of any biologically relevant gender-related differences in the toxicity of MEK. Human occupational studies have failed to report sex-related differences in MEK toxicity by any route. The 90-day subchronic inhalation study by Cavender et al. (1983) suggests that female rats may be slightly more susceptible to the toxic effects of MEK (decreased absolute and relative brain and liver weight, as well as altered blood chemistry); however, the differences between the sexes were too small to specifically identify females as more susceptible to the effects of MEK than males. No gender-specific susceptibility was observed in offspring in any of the developmental studies (Schwetz et al., 1974, 1991; Deacon et al., 1981). While a possible effect on male copulatory success was noted in the Cox et al. (1975) multigeneration reproductive toxicity study, no effects on females were apparent.

4.7.3. Other

The potential exists for increased susceptibility to neurotoxicity, hepatotoxicity, and renal toxicity following exposure to MEK in combination with certain other solvents. MEK, for example, potentiates the neurotoxicity of hexacarbon solvents (n-hexane, MnBK, and 2,5-hexanedione) (Saida et al., 1976; Couri et al., 1977) and the liver and kidney toxicity of haloalkane solvents (carbon tetrachloride, trichloromethane, and chloroform) (Dietz and Traiger, 1979; WHO, 1992; Brown and Hewitt, 1984). Although the mode by which MEK potentiates the neurotoxicity of hexacarbon solvents is not entirely clear, it appears to involve alterations in their metabolism to toxic metabolites. Unlike other ketones, MEK is not metabolized to a

gamma-diketone and is not, therefore, associated with distal neurofilamentous axonopathy. The potentiating effects of MEK on the toxicity of other solvents have only been demonstrated at relatively high exposure concentrations (200–1,000 ppm or 590–2,950 mg/m^3).

No data are available concerning susceptibility of other specific lifestages (including elderly populations) to MEK toxicity. No toxicologic basis exists, metabolic or otherwise, to suspect that MEK is capable of exhibiting toxicity specific to these lifestages.

5. DOSE RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect

No studies examining the subchronic or chronic effects of oral exposure to MEK in humans or experimental animals were identified. The repeat-dose oral toxicity data base is limited to data for 2-butanol (a metabolic precursor) and 3-hydroxy-2-butanone (a metabolite).

The 2-butanol data consist of a 2-generation reproductive and developmental toxicity study of 2-butanol in the rat (Cox et al., 1975). For 3-hydroxy-2-butanone, a 13-week drinking water study in rats is available (Gaunt et al., 1972). No *in vivo* toxicity studies of repeat exposure (by any route) to 2,3-butanediol (the other main metabolite of MEK) are available. While the administration of 2-butanol in drinking water before and during gestation and lactation at concentrations as high as 3% did not affect reproductive performance (with the possible exception of effects on male copulatory success), it decreased pup survival and pup body weight gain. A concentration of 2% in drinking water reduced fetal weights when pregnancies were terminated on gestation day 20 and decreased pup body weight gains when dams were allowed to deliver. The finding of developmental toxicity in rats exposed orally to 2-butanol is consistent with similar findings in inhalation developmental toxicity studies of MEK (Schwetz et al., 1974, 1991; Deacon et al., 1981) and 2-butanol (Nelson et al., 1989, 1990) (see Table 5). Given these observations, it is plausible that the developmental effects produced by 2-butanol and MEK are caused by MEK or a subsequent metabolite common to both. The only other toxic effect associated with long-term oral exposure to 2-butanol is renal lesions in male rats at 2% in drinking water (3,384 mg/kg-day) (Cox et al., 1975).

While data from the 13-week drinking water study with 3-hydroxy-2-butanone in CFE rats (Gaunt et al., 1972) suggest adverse hematological effects (decreased hemoglobin concentration and red blood cell count), the effect was not observed in toxicity studies of 2-butanol (Cox et al., 1975) or MEK (Cavender et al., 1983). The study concerning exposure to 3-hydroxy-2-butanone in drinking water provides no information regarding the potential for developmental effects, which are the key effects seen with oral and inhalation exposure to 2-butanol and inhalation exposure to MEK. Thus, the slight anemia produced by oral exposure to 3-hydroxy-2-butanone is inconsistent with the effects seen following inhalation exposure to MEK, or oral or inhalation exposure 2-butanol. Hence, 3-hydroxy-2-butanone does not appear

to be an appropriate surrogate for assessing the toxicity of MEK.

Pharmacokinetic and toxicologic data support the use of 2-butanol as an appropriate surrogate for MEK. Pharmacokinetic findings in rats that support the use of 2-butanol as a surrogate for MEK include: (1) orally administered 2-butanol was almost completely converted to MEK and its metabolites within 16 hours; (2) peak MEK blood concentrations occurred at similar times after administration of 1,776 mg/kg 2-butanol (7–8 hours) or 1,690 mg/kg MEK (4–5 hours); and (3) common metabolites (3-hydroxy-2-butanone and 2,3-butanediol) were formed and eliminated with similar kinetics after the administration of 2-butanol or MEK (Traiger and Bruckner, 1976; Dietz et al., 1981). Comparable pharmacokinetic data for 2-butanol and MEK in humans are not available; however, evidence for metabolic conversion of MEK to 2-butanol in humans supports the assumption that rats and humans metabolize 2-butanol similarly. As discussed in Section 4.3, toxicologic findings that support the use of 2-butanol as a MEK surrogate include: (1) fetal weight deficits were critical effects in (a) studies of rats (Schwetz et al., 1974; Deacon et al., 1981) and mice (Schwetz et al., 1991) exposed to MEK by inhalation during gestation, (b) a two-generation reproductive and developmental toxicity study in rats exposed to 2-butanol in drinking water (Cox et al., 1975), and (c) a study of rats exposed to 2-butanol by inhalation during gestation (Nelson et al., 1989); and (2) the relationships between air concentrations and the degree of fetal weight changes were consistent for MEK and 2-butanol.

Thus, the reproductive and developmental drinking water toxicity study of 2-butanol in rats (Cox et al., 1975) was selected as the principal study for deriving an RfD for MEK. Cox et al. (1975) also served as the principal study for the RfD of 0.6 mg/kg-day that was previously entered in the IRIS data base in 1993. Developmental effects identified in this study included decreased pup survival and decreased neonatal body weight in F1A pups whose parents were exposed to 3% 2-butanol in drinking water before mating through day 10 of lactation. Decreased body weights, with no effect on survival, were observed in F1B fetuses and F1A and F2 pups that were exposed to 2% 2-butanol in drinking water (see Table 5).

5.1.2. Methods of Analysis

The RfD was derived using benchmark dose analysis of body weight data from offspring in the rat multigeneration drinking water toxicity study of 2-butanol (Cox et al., 1975). Details of the benchmark dose modeling results are presented in Appendices B-1 through B-5.

5.1.2.1. *Benchmark Dose Modeling*

The following data sets from the Cox et al. (1975) study were selected for benchmark dose modeling: fetal weight data from the F1B generation, and postnatal day 4 and day 21 pup weights from the F1A and F2 generations. Decreased F1A pup survival observed in the highest dose group (3% solution) is likely to have confounded the effects on surviving pup body weight (measured body weights represent survivors rather than all offspring born to 3% dams). Consequently, this exposure level does not help identify a level of exposure at which a less severe precursor of frank toxicity might occur. Because of this likely confounding, the modeling of the F1A pup body weight data did not include data from the high-dose group. Survival of fetuses or pups was not affected in any dose group in the F1B or F2 generations, so body weight data from all dose groups (0, 0.3, 1, and 2%) were included in the modeling for these generations.

Table 5. Summary of key repeat-exposure reproductive and developmental toxicity studies in animals exposed to MEK or 2-butanol

Study	Exposure protocol	Effects	LOAEL	NOAEL
Oral studies				
Cox et al. (1975) F0 generation F1A (first litter) F1B (second litter) F2 (F1A offspring)	FDRL-Wistar rats (F0), ~30/grp, exposed to 0, 0.3, 1, or 3% 2-butanol in drinking water for 8 weeks prior to mating, during F1A pregnancy and litter cast.[a] Starting at F1A postnatal day 21[a] through F2 gestation day 20: F0, F1B and F2 received 0, 0.3, 1, or 2% 2-butanol in drinking water.	F0: Decreased body weight (3%). F1A: Decreased pup survival (3%). Decreased pup weight, days 4 and 21 (3%). F1B: Decreased fetal weight (2%). F2: Decreased pup weight, days 4 and 21 (2%). [All pup and fetal weight comparisons were based on litter means and not individual pup/fetus data.]	3,122 mg/kg-day (2%) – fetal/pup body weight	1,771 mg/kg-day (1%)
Inhalation studies				
Schwetz et al. (1974)	Pregnant SD rats, 21-23/grp, exposed to 0, 1,126, or 2,618 ppm MEK, 7 hours/day on gestation days 6–15.	Developmental effects (percentage of litters with any soft tissue anomaly)	2,618 ppm (7,723 mg/m^3)	1,126 ppm (3,322 mg/m^3)
Deacon et al. (1981) also reported by Dow Chemical Corporation (1979)	Pregnant SD rats, 18-26/grp, exposed to 0, 412, 1,002, or 3,005 ppm MEK, 7 hours/day on gestation days 6–15.	Decreased maternal weight gain. Increased incidence fetal skeletal variations.	3,005 ppm (8,865 mg/m^3)	1,002 ppm (2,955 mg/m^3)
Nelson et al. (1989, 1990)	Pregnant SD rats, 15-16/grp, exposed to 0, 3,500, 5,000, or 7,000 ppm 2-butanol, 7 hours/day on gestation days 1–19.	Decreased maternal weight gain and food consumption. Decreased maternal locomotor activity. Decreased fetal weight.	5,000 ppm (15,150 mg/m^3)	3,500 ppm (10,605 mg/m^3)

Table 5. Summary of key repeat-exposure reproductive and developmental toxicity studies in animals exposed to MEK or 2-butanol

Study	Exposure protocol	Effects	LOAEL	NOAEL
Schwetz et al. (1991) also reported as Mast et al. (1989) and NTP (1990)	Pregnant CD-1 mice, 33/grp, exposed to 0, 398, 1,010, or 3,020 ppm <u>MEK</u>, 7 hours/day on gestation days 6–15.	Decreased body weight in male fetuses and both sexes combined [based on litter means]. Increased maternal liver-to-body weight ratio.	3,020 ppm (8,909 mg/m^3)	1,010 ppm (2,980 mg/m^3)

[a] In the 3% group, F0 dams and F1A pups received drinking water with no 2-butanol between days 10 and 21 post partum. Thereafter, the concentration was changed to 2%.

Continuous data models (linear, polynomial, or power), either with a constant variance or with variance as a power function of the mean value (using an additional model parameter), were fit to the data using U.S. EPA Benchmark Dose Software (version 1.3.1). The software was used to calculate potential points of departure for deriving the RfD by estimating the effective dose at a specified level of response (ED_x) and its 95% lower bound (LED_x). In the case of pup or fetal body weight, there is no specific decrement that is generally regarded as indicative of an adverse response. EPA's draft *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c) recommends, in the absence of some idea of the level of response to consider adverse, selecting as the benchmark response (BMR) level for continuous data a change in the mean equal to one control standard deviation from the control mean. Using data from Cox et al. (1975), one standard deviation from the control mean resulted in BMDs that corresponded to body weights 9 to 26% below the control mean (see Tables 6, 7 and 9) – values generally above the range of experimental data. Because an aim in BMD modeling is to select a BMD within the range of observation, other measures of the BMR were examined. A 5% reduction in fetal/pup body weight relative to the control was a response rate that fell within the range of experimental dose levels in the Cox et al. (1975) study, and consequently was selected as the benchmark response (BMR). In addition, an ED_{10} and LED_{10} for each endpoint were estimated as a consistent point of comparison across chemicals, as recommended in the Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c). These additional measures are provided in Appendix B.

Modeling of F1A Pup Body Weights

The overall means of the individual litter means for F1A pup body weights and their standard deviations in the control and two lowest exposure groups were calculated from litter data shown in Table 6.

Table 6. Mean litter pup body weight in F1A generation Wistar rats exposed to 2-butanol in drinking water in a two-generation reproductive and developmental toxicity study

Drinking water concentration (% 2-butanol by weight)	Maternal dose (mg/kg-day)[a]	Mean of litter means pup body weight postnatal day 4 (g ± standard deviation)[b] [number of litters]	Mean of litter means pup body weight postnatal day 21 (g ± standard deviation)[b] [number of litters]
0	0	10.7±1.1 [29]	49±3.8 [28]
0.3	594	10.2±1.3 [27]	47±3.9 [27]
1	1,771	10.0±1.3 [30]	44±4.8 [30]

[a] Average daily intake of 2-butanol as reported by Cox et al. (1975).
[b] The data reported herein differ from the summary data in Table 3 of Cox et al. (1975) because data for day 21 could only be discerned to the nearest gram from the best available copy of the study report.
Source: Adapted from Appendix II of Cox et al. (1975).

A linear continuous-variable model using constant variance (BMDS version 1.3.1) provided an adequate fit to the data (with a goodness-of-fit p value > 0.1) and was used to establish the ED_{05} (See Appendix B for benchmark dose software output). Other continuous variable models (polynomial and power) could not be fit to the data due to lack of degrees of freedom since the number of dose groups in the modeled data set were equal to or less than the number of parameters estimated in the models, and thus it was not possible to perform statistical tests typically used to determine adequacy of model fit. Visual inspection of a plot of the predicted and observed means also indicated a reasonable fit of the linear model to the data in the range nearest the point of departure (see Appendix B, outputs B-1 and B-2).

The model-predicted ED_{05} values associated with a 5% decrease in mean F1A pup body weight were 1,387 mg/kg-day for day 4 and 878 mg/kg-day for day 21. The corresponding LED_{05} values were 803 mg/kg-day for day 4 and 657 mg/kg-day for day 21.

Modeling of F1B Fetal Weights

The overall means of the individual litter means for F1B fetal weights and their standard deviations in the control and exposed groups were calculated from average fetus weight data for each litter presented in Appendix III of the Cox et al. (1975) report and are shown in Table 7.

Table 7. Litter mean fetal weight in F1B generation Wistar rats exposed to 2-butanol in drinking water in a two-generation reproductive and developmental toxicity study

Drinking water concentration (% 2-butanol by weight)	Maternal dose (mg/kg-day)	Number of litters	Mean of litter means fetal weight (g)	Standard deviation
0	0[a]	29	4.14	1.45
0.3	594[a]	27	4.16	0.69
1	1,771[a]	30	4.38	1.04
2	3,122[b]	29	3.74	1.01

[a] Average daily intake of 2-butanol as reported by the authors.
[b] Calculated based on a linear regression analysis of the reported average intakes and drinking water concentrations of 2-butanol.
Source: Adapted from Appendix III of Cox et al. (1975).

A constant variance polynomial continuous-variable model (BMDS version 1.3.1) provided the best fit to the data (as indicated by the lowest AIC with a goodness-of-fit p value > 0.1; see summary of goodness-of-fit statistics in Table 8) and was used to establish the ED_{05}. Fitting a model that described the variance as a power function of the mean value did not improve the fit as indicated by the AIC. Visual inspection of a plot of the predicted and observed means also indicated a reasonable fit of the polynomial model to the data in the range nearest the point of departure (see Appendix B, output B-3).

Table 8. Benchmark dose modeling results using litter mean body weight data for F1B fetuses

Model	GOFP	AIC	ED_{05} (mg/kg-day)	LED_{05} (mg/kg-day)
Linear	0.15	137.9	1,969	896
Polynomial	0.12	136.6	2,198	1,046
Power	0.17	137.0	2,980	1,578

GOFP = Goodness-of-fit p-value for chi-square.
AIC = Akaike's Information Criterion.
ED_{05} = Benchmark dose calculated by BMDS associated with a 5% decrease in mean fetal weight.
LED_{05} = 95% lower confidence limit on the ED_{05} as calculated by BMDS.
Source: Adapted from Cox et al. (1975).

The model-predicted ED_{05} associated with a 5% decrease in mean F1B fetal weight was 2,198 mg/kg-day. The corresponding LED_{05} was 1,046 mg/kg-day.

Modeling of F2 Pup Body Weights

The overall means of the individual litter means for F2 pup body weights at postnatal days 4 and 21 and their standard deviations in the control and exposed groups were calculated from litter averages presented in Appendix V of the Cox et al. (1975) report (see in Table 9).

Table 9. Litter mean pup body weight in F2 generation Wistar rats exposed to 2-butanol in drinking water in a two-generation reproductive and developmental toxicity study

Drinking water concentration (% 2-butanol by weight)	Maternal dose (mg/kg-day)	Mean of litter means pup body weight postnatal day 4 (g ± standard deviation)	Mean of litter means pup body weight postnatal day 21 (g ± standard deviation)
0	0[a]	10.0±1.4	40±6.1
0.3	594[a]	9.7±1.6	39±7.8
1	1,771[a]	9.6±2.3	39±9.4
2	3,122[b]	9.5±1.6	35±4.7

[a] Average daily intake of 2-butanol as reported by the authors.
[b] Calculated based on a linear regression analysis of the reported average intakes and drinking water concentrations of 2-butanol.
Source: Appendix V of Cox et al. (1975).

A linear continuous-variable model assuming constant variance (BMDS version 1.3.1) provided the best fit to the day 4 pup body weight (as indicated by the lowest AIC with a goodness-of-fit p value > 0.1), whereas a polynomial continuous-variable model assuming constant variance provided the best fit to the day 21 pup body weight. Both models were used to establish day 4 and day 21 ED_{05} values (see goodness-of-fit statistics in Table 10.) Fitting models that described the variance as a power function of the group means did not improve the fit as indicated by the AIC. Visual inspection of the plots of the predicted and observed means also indicated a reasonable fit of the selected models to the data in the range nearest the point of departure (see Appendix B, outputs B-4 and B-5).

Table 10. Benchmark dose modeling results using litter mean body weight data for F2 pups on postnatal days 4 and 21

Model	GOFP	AIC	ED$_{05}$ (mg/kg-day)	LED$_{05}$ (mg/kg-day)
Postnatal day 4				
Linear	0.88	227.9	3,471	1,347
Polynomial	0.61	227.9	3,471	1,347
Power	0.26	231.9	3,471	1,347
Postnatal day 21				
Linear	0.55	512.5	1,398	851
Polynomial	0.48	511.8	2,056	901
Power	0.26	515.5	2,508	919

GOFP = Goodness-of-fit p-value for chi-square.
AIC = Akaike's Information Criterion.
ED$_{05}$ = Benchmark dose calculated by BMDS associated with a 5% decrease in mean pup body weight.
LED$_{05}$ = 95% lower confidence limit on the ED$_{05}$ as calculated by BMDS.
Source: Adapted from Cox et al. (1975).

The model-predicted ED$_{05}$ values associated with a 5% decrease in mean pup body weight were 3,471 mg/kg-day for postnatal day 4 and 2,056 mg/kg-day for day 21. The corresponding LED$_{05}$ values were 1,347 and 901 mg/kg-day, respectively.

Comparison of Benchmark Dose Modeling Results

For oral exposure to 2-butanol, developmental effects on body weight from three generations of the Cox et al. (1975) study were modeled, including: fetal weight from the F1B generation, and pup body weight at postnatal days 4 and 21 from the F1A and F2 generations. The LED$_{05}$ values calculated from modeling these data sets are summarized in Table 11.

Table 11. Benchmark doses for developmental effects in rats from various generations and potential points of departure for the RfD

Endpoint	ED_{05} [a] (mg/kg-day)	LED_{05} [a] (mg/kg-day)
F1A pup body weight, postnatal day 4[b]	1,387	803
F1A pup body weight, postnatal day 21[b]	878	657
F1B fetal weight, gestation day 20	2,198	1,046
F2 pup body weight, postnatal day 4	3,471	1,347
F2 pup body weight, postnatal day 21	2,056	901

[a] ED_{05}: benchmark dose associated with a 5% decrement in litter mean pup or fetal weight compared with control mean.
  LED_{05}: 95% lower confidence limit on the ED.
[b] The data modeled excluded the high dose (3%).
  Source: Adapted from of Cox et al. (1975).

LED_{05} values from these data sets are within 2-fold of each other, suggesting that all the modeling results are equally plausible. The lowest point of departure, based on decreased pup body weight at postnatal day 21 in the F1A generation (LED_{05} = 657 mg/kg-day) was selected for derivation of the RfD as the most health protective value.

5.1.2.2. *Route-to-route Extrapolation*

As an alternative to using 2-butanol data as a surrogate for MEK, consideration was given to route-to-route extrapolation to derive oral doses from existing inhalation data for developing an RfD for MEK. Deficiencies in the absorption data, however, preclude the application of this method for MEK. In humans, the pulmonary retention value of 53% (±2%) reported by Liira et al. (1988) is based on acute (4-hour) exposure to 200 ppm (590 mg/m^3) MEK. In rats, the pulmonary retention data at similar exposure concentrations (200 ppm in humans compared to 180 ppm in rats) result from a longer period of exposure, 14 hours (Kessler et al., 1988). The pharmacokinetic data for MEK indicate that pulmonary retention is concentration-dependent (Liira et al., 1988), suggesting that absorption is limited by transport to the metabolizing enzymes in the liver, rather than metabolic capacity. Therefore, it cannot be assumed that the pulmonary retention value will be the same at exposures across a larger dose range. Developmental effects of MEK are produced by concentrations that are an order of magnitude greater than those used to calculate the rat pulmonary retention value. The toxicity of MEK may be a result of exposure to concentrations that exceed of the capacity for detoxification by a saturable enzyme mechanism. For this reason, it would be inappropriate to estimate the

pulmonary retention value at the effect levels identified by the inhalation developmental toxicity studies of MEK in rodents (Schwetz et al., 1974, 1991; Deacon et al., 1981), precluding the derivation of an oral RfD for humans based on extrapolation from inhalation effects in animals. Moreover, the route-to-route extrapolation would also require data on oral absorption of MEK in humans, and such data are not available. Consequently, these deficiencies in the data preclude route-to-route extrapolation as a basis for development of an oral RfD for MEK.

Rat PBPK models that include oral, inhalation, and parenteral portals of entry have been developed recently (Thrall et al., 2002), but human PBPK models with both oral and inhalation portals of entry have not yet been developed. When appropriate human PBPK models are developed, the rat and human models could be used to estimate human oral exposure levels associated with an appropriate internal dose surrogate from the inhalation exposure levels in the rat developmental toxicity studies for MEK (Deacon et al., 1981; Schwetz et al., 1974).

5.1.3. RfD Derivation – Including Application of Uncertainty Factors

The LED_{05} of 657 mg/kg-day was used as the point of departure for calculating the RfD. This point of departure is associated with a 5% decrease in mean postnatal day 21 body weight of F1A Wistar rat pups exposed to 2-butanol in drinking water (Cox et al., 1975). Because 2-butanol was used as a surrogate for MEK, a molar adjustment was performed to account for the different molecular weights of the two chemicals. A total uncertainty factor of 1,000 was applied to this adjusted point of departure: 10 for extrapolation from animals to humans, 10 for extrapolation to the most susceptible humans, and 10 for data base deficiencies.

The following molar adjustment of the LED_{05} value was calculated to account for differences in the molecular weights of 2-butanol and MEK:

657 mg/kg-day 2-butanol × $\dfrac{72.1066 \text{ g/mol MEK}}{74.1224 \text{ g/mol 2-butanol}}$

= 639 mg/kg-day MEK

A 10-fold uncertainty factor was used to account for laboratory-animal to human interspecies differences. No information is available on the toxicity of MEK in humans exposed by the oral route. No other information is available to assess possible differences between animals and humans in pharmacodynamic responses to MEK. Rat and human PBPK models for

oral exposure to MEK could potentially be used to decrease pharmacokinetic uncertainty in extrapolating from rats to humans, but such models are not currently available.

A 10-fold uncertainty factor for intraspecies differences was used to account for potentially susceptible human subpopulations. In the absence of information on the variability in response of humans to MEK exposure, the default value of 10 was used.

A 10-fold uncertainty factor was used to account for deficiencies in the available MEK data base. While no oral data are available for MEK, the available pharmacokinetic and inhalation toxicity data support 2-butanol as an appropriate surrogate for MEK. Nonetheless, the use of 2-butanol data to estimate the toxicity associated with MEK exposure introduces some uncertainty in the assessment. Although no chronic studies are available, the data base includes a two-generation reproductive and developmental toxicity assay wherein rats were exposed to 2-butanol for 14–18 weeks with observed effects limited to reductions in body weight and histopathologic changes in the kidney of male rats only. The absence of any other organ-specific toxicity following a 14-18 week exposure to 2-butanol reduces the uncertainty associated with the lack of chronic toxicity data for MEK or 2-butanol.

An uncertainty factor to extrapolate from a LOAEL to a NOAEL was not necessary because BMD modeling was used to determine the point of departure. The dose corresponding to a 5% decrease in pup weight, relative to control, was selected as the point of departure. There is no specific decrement in fetal/pup weight that is generally recognized as indicative of an adverse effect. Further, there were no other effects in the range of the LED_{05} of 657 mg/kg-day 2-butanol. Therefore, no further adjustments were considered for identifying a level of oral exposure to MEK associated with a minimal level of risk.

Consistent with EPA practice (U.S. EPA, 1991a), an uncertainty factor was not used to account for extrapolation from less than chronic results because developmental toxicity (decreased pup body weight following in utero and neonatal exposure) was used as the critical effect. The developmental period is recognized as a susceptible lifestage where exposure during certain time windows are more relevant to the induction of developmental effects than lifetime exposure.

The RfD for MEK was calculated as follows:

$$RfD = LED_{05} \div UF$$
$$= 639 \text{ mg/kg-day} \div 1000$$
$$= 0.6 \text{ mg/kg-day}$$

5.1.4. Previous Oral Assessment

In the 1993 IRIS assessment of MEK, an RfD of 0.6 mg/kg-day was derived based on the NOAEL of 1,771 mg/kg-day for decreased fetal birth weight in the F1B generation of Wistar rats in the multigeneration drinking water study with 2-butanol by Cox et al. (1975). The 1993 assessment stated that "a combined uncertainty factor of 3000 was applied to account for four uncertainty factors assigned 10 for each factor, including: 10 for inter- and intraspecies extrapolations, 10 to adjust for subchronic-to-chronic extrapolation since long-term effects in the dams were not reported in the principal study; 10 for an incomplete data base that included a lack of both subchronic and chronic oral exposure studies for MEK; and 10 for lack of data for a second rodent species for either MEK or 2-butanol."

The 1993 and current RfDs differ in the approach used to derive the reference values and in the application of UFs. The current RfD was derived using BMD methods rather than the NOAEL as a point of departure. The UFs applied in the 1993 assessment did not conform with current practices. In particular, it is not current Agency practice to apply a subchronic to chronic uncertainty factor where the point of departure is based on developmental toxicity. Thus, the total UF used in the 1993 assessment was 3,000, whereas the total UF applied in the current assessment is 1,000.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

Several studies examining the health effects of inhalation exposure to MEK exist in experimentally- and occupationally-exposed humans as well as in experimental animals; however, many of these are inappropriate for use in dose-response assessment. For example, many occupational studies are complicated by insufficient data on exposure levels (duration and concentration) and potential simultaneous exposure to other solvents (often to hexacarbon

solvents for which MEK may potentiate toxicity). Therefore, it is not possible to identify effect levels from the available occupational reports for dose-response assessment. As with other small molecular weight, aliphatic or aromatic chemicals, acute exposure to high concentrations of MEK results in reversible central nervous system depression. Evidence for this effect in humans is limited to a few case reports involving combined exposure to MEK and toluene (Welch et al., 1991; Seaton et al., 1992; Callender, 1995; Orti-Pareja et al., 1996). The only other human data are from a series of studies involving acute, 4-hour exposures of volunteers (Dick et al., 1984, 1988, 1989, 1992) wherein no exposure-related changes were reported for performance on psychomotor and mood tests or incidences of irritation.

As discussed in Section 4.5.2, the range of toxic effects in animals resulting from inhalation exposure to MEK indicates that developmental effects are the most sensitive toxicologically-relevant endpoints. Inhalation exposure of experimental animals to 3,000 ppm MEK (7 hours/day on days 6–15 of gestation) resulted in developmental effects in rats and mice (Schwetz et al., 1974, 1991; Deacon et al., 1981). The most appropriate data for the derivation of an inhalation RfC for MEK are from inhalation developmental toxicity studies in rats (Deacon et al., 1981) and mice (Schwetz et al., 1991). The original laboratory reports are available for both, and the effect levels for the reported developmental effects are consistent (although the specific endpoints differ). In Sprague-Dawley rats, Deacon et al. (1981) reported fetal toxicity (increased incidence of skeletal variations) at 3,005 ppm (8,865 mg/m³) MEK 7 hours/day on gestation days 6–15. In CD-1 mice, Schwetz et al. (1991) found reduced fetal weight at 3,020 ppm (8,909 mg/m³) MEK 7 hours/day on gestation days 6–15, and a positive trend for increasing the incidence of fetuses with misaligned sternebrae. In each case, fetal effects were accompanied by slight maternal toxicity.

5.2.2. Methods of Analysis

The RfC was derived using benchmark analysis of developmental effects for rats and mice exposed to MEK during gestation (Deacon et al., 1981; Schwetz et al., 1991). NOAELs of 1,002 and 1,010 ppm (2,955 and 2,980 mg/m³) and LOAELs of 3,005 and 3,020 ppm (8,865 and 8,909 mg/m³) were established for rats and mice, respectively. Details of the benchmark dose modeling results are presented in Appendix B.

5.2.2.1. *Benchmark Dose Modeling*

In Sprague-Dawley rats, Deacon et al. (1981) reported a statistically significant increase in the incidence of litters with fetuses with extra ribs. In CD-1 mice, Schwetz et al. (1991) identified two statistically significant developmental effects in fetuses exposed to MEK: decreased fetal weight per litter (continuous data) and a trend for increasing the incidence of fetuses with misaligned sternebrae with increasing exposure level (dichotomous data). Data from each of these three endpoints have been analyzed by benchmark dose methods and examined for toxicological relevance.

Modeling of Incidence of Rat Litters with Fetuses with Extra Ribs

The incidence of extra ribs (litters with an affected fetus) as reported by Deacon et al. (1981) is shown in Table 12. The incidence of extra ribs in fetal Sprague-Dawley rats exposed to 3,005 ppm MEK 7 hours/day on gestation days 6–15 was statistically different from control.

Table 12. Incidence of extra ribs (litters with an affected fetus) in Sprague-Dawley rats exposed to MEK 7 hours/day on gestation days 6–15

Concentration (ppm)	Number of fetuses (litters)	Number of litters with fetuses with extra ribs	Mean percent of fetuses with extra ribs per litter
0	329 (26)	2 (2)	0.6 ± 0.3
412	237 (19)	0	0 ± 0
1,002	226 (19)	0	0 ± 0
3,005	229 (18)	7 (6)	3.1 ± 1.8

Source: Adapted from Deacon et al. (1981).

All nested models for dichotomous variables available in EPA's Benchmark Dose Software (BMDS version 1.3.1) were fit to the data in Table 12. The models – the nested logistic (NLogistic), NCTR, and Rai and vanRyzin models – allow for the possibility that the variance among the proportions of pups affected in individual litters is greater than would be expected if the pups were responding completely independently of each other (U.S. EPA, 2000c). A 5% increase in the incidence of extra ribs was selected as the benchmark response because it was a response rate that fell within the range of experimental dose levels used in the Deacon et al. (1981) study. All of the models provided similar fits to the data, based on the summary results reported in the BMDS output and the detailed examination of graphs and

goodness-of-fit statistics (summarized in Table 13). Model fits were not improved by the incorporation of litter size (as a litter-specific covariate) or intra-litter correlations, as determined by comparisons of AIC values. Since the fits were quite similar, only one set of model output (the NCTR model, fitting only slightly better than the others) is provided in Appendix B, output B-6.

Table 13. Benchmark concentration modeling results using litter incidence data for Sprague-Dawley rat fetuses with extra ribs exposed to MEK during gestation days 6-15

Nested Model	GOFP	AIC	EC_{05} (ppm)	LEC_{05} (ppm)
Log-Logistic	0.09	96.5	3,124	2,993
NCTR	0.51	96.5	3,317	2,993
Rai and vanRyzin	0.51	96.6	3,353	2,992

GOFP = Goodness-of-fit p-value for chi-square.
AIC = Akaike's Information Criterion.
EC_{05} = Benchmark concentration calculated by BMDS associated with a 5% extra risk of affected fetuses per litter.
LEC_{05} = 95% lower confidence limit on the EC_{05} as calculated by BMDS.
Source: Adapted from Deacon et al. (1981).

The model-predicted EC_{05} value associated with a 5% increased incidence of extra ribs was 3,317 ppm. The corresponding LEC_{05} was 2,993 ppm.

Modeling of Decreased Fetal Weight Data in Mice

The full laboratory report from Schwetz et al. (1991) is available in Mast et al. (1989). The overall means of the individual litter means for fetal weight and their standard deviations in the control and MEK-exposed groups are shown in Table 14 below.

Table 14. Litter mean fetal weight (both sexes combined) in CD-1 mice exposed to MEK 7 hours/day on gestation days 6–15

Concentration (ppm)	Number of litters	Fetal weight (mean of litter means) (g)	Standard deviation
0	26	1.35	0.07
398	23	1.35	0.06
1,010	26	1.33	0.07
3,020	28	1.29	0.08

Sources: Adapted from Mast et al. (1989) and Schwetz et al. (1991).

Models for continuous data (linear, polynomial, and power), either with constant variance or with variance as a power function of the mean value (using an additional model parameter), were fit to the data in Table 14 using EPA's Benchmark Dose Software (BMDS version 1.3.1). Since there is no specific decrement in fetal weight that is generally regarded as indicative of an adverse effect, a decrease in the mean fetal weight of 1 standard deviation of the control mean was selected as the benchmark response for this endpoint consistent with the recommendations of EPA's Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c). This benchmark response corresponds to a 5% decrease in the mean control group weight for this data set. A linear continuous-variable model assuming constant variance (BMDS version 1.3.1) provided the best fit to the data (as indicated by the lowest AIC with a goodness-of-fit p value > 0.1; see summary of goodness-of-fit statistics in Table 15.) Visual inspection of the graph of the predicted and observed means also indicated a reasonable fit of the selected model to the data in the range nearest the point of departure (see Appendix B, output B-7).

Table 15. Benchmark concentration modeling results using litter mean body weight data

Model	GOFP	AIC	EC (ppm MEK)	LEC (ppm MEK)
Linear	0.90	-442.2	3,339	2,273
Polynomial	0.66	-440.2	3,330	2,273
Power	0.28	-438.2	3,343	2,275

GOFP = Goodness-of-fit p-value for chi-square.
AIC = Akaike's Information Criterion.
EC = Benchmark concentration calculated by BMDS associated with a mean fetal weight 1 SD below the control mean.
LEC = 95% lower confidence limit on the EC as calculated by BMDS.
Sources: Adapted from Mast et al. (1989) and Schwetz et al. (1991).

The model-predicted EC associated with a mean fetal weight of 1 standard deviation below the control mean was 3,339 ppm MEK. The corresponding LEC was 2,273 ppm MEK.

Modeling of Misaligned Sternebrae Data in Mice

The other statistically significant effect identified by Schwetz et al. (1991) was an increased incidence of misaligned sternebrae in CD-1 mouse fetuses exposed to MEK. The complete laboratory report from Schwetz et al. (1991) is available in Mast et al. (1989). A summary of the incidence of misaligned sternebrae for individual fetuses (Mast et al., 1989) is shown in Table 16.

Table 16. Total number of fetuses (combined for both sexes) with misaligned sternebrae per exposure group in CD-1 mice exposed to MEK 7 hours/day on gestation days 6–15

Concentration (ppm)	Number of fetuses (litters)	Number of fetuses (litters) with misaligned sternebrae	Mean percent of fetuses with misaligned sternebrae per litter (mean ± SD)
0	310 (26)	31 (18)	9.7 ± 10.4
398	260 (23)	27 (14)	9.8 ± 11.2
1,010	291 (26)	49 (18)	17.4 ± 16.7
3,020	323 (28)	58 (21)	17.5 ± 14.9

Sources: Adapted from Mast et al. (1989) and Schwetz et al. (1991).

The nested, dichotomous-variable models available in BMDS version 1.3.1 were fit to the individual litter data for fetuses with misaligned sternebrae as reported in Appendix F of Mast et al. (1989). Each model was fit with and without litter size as a covariate. Including litter size as a covariate made very little difference in the goodness-of-fit statistics, indicating that litter size was not a significant explanatory variable for changes in the incidence of misaligned sternebrae (results not shown). Then each model was fit with and without intra-litter correlations. In each case, the model fit was linear and was better with the intralitter correlations included. All three nested models provided adequate fits to the data, based on the summary results reported in the BMDS output (see Appendix B, output 8). A more detailed examination of the graphs and residuals suggested that a nonlinear model should be considered, since the low- and mid-dose responses were not fitted by the models as closely as the high-dose response. Allowing the power parameter in each model to take a value less than one increased the AIC value for each

model. Therefore, the linear versions already fitted were used. A 10% extra risk for misaligned sternebrae was selected as the benchmark response since the model and data are most consistent in this range of the data set. Also, EPA's Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c) recommends estimation of a 10% BMR for a point of consistent comparison across chemicals. Further, the nested model did not provide a useful estimate of the lower bound for a BMR of 5% (the lower bound on the BMC was estimated as essentially zero). Effective concentrations associated with this BMR and their 95% lower confidence limits (LEC_{10}) are summarized in Table 17.

Table 17. Benchmark concentration modeling results using individual litter data for mouse fetuses with misaligned sternebrae exposed to MEK during gestation days 6-15 (without litter size as covariates)

Nested Model	GOFP	AIC	EC_{10} (ppm)	LEC_{10} (ppm)
NLOGISTIC	0.6349	937.1	3,197	1,714
Rai and Van Ryzin	0.5433	937.2	3,222	1,789
NCTR	0.4877	937.2	3,222	1,789

GOFP = Goodness-of-fit p-value for chi-square.
AIC = Akaike's Information Criterion.
EC_{10} = Concentration associated with a 10% extra risk for misaligned sternebrae in fetuses.
LEC_{10} = 95% lower confidence limit on the EC_{10}.
Source: Adapted from Mast et al. (1989).

Because the three model fits were very similar (Table17), an average of the three LEC_{10} values was calculated as the point of departure. The respective EC_{10} and LEC_{10} values calculated as an average of the three models are 3,214 and 1,764 ppm, respectively.

Comparison of Benchmark Dose Modeling Results

For inhalation exposure to MEK, the following three developmental endpoints from two species were evaluated: increased incidence of extra ribs in Sprague-Dawley rats (Deacon et al., 1981), decreased fetal weight, and increased incidence of misaligned sternebrae in CD-1 mice (Schwetz et al., 1991). The EC and LEC values for these developmental endpoints are summarized in Table 18. Benchmark modeling of the data produced similar points of departure for the three developmental endpoints observed in the two species (within 2-fold). The lowest point of departure of 1,764 ppm (5,202 mg/m^3) was based on the incidence of misaligned

sternebrae in mice exposed to MEK by inhalation for 7 hours/day on gestation days 6–15 and was therefore selected as the most health protective value for derivation of the RfC.

Table 18. Benchmark concentrations for developmental effects in mice and rats and potential points of departure for the RfC

Endpoint	Benchmark Response Level	EC, mg/m³ (ppm)[a]	LEC, mg/m³ (ppm)[a]
Increased incidence of extra ribs (rats) (Deacon et al., 1981)	5%	9,781 (3,317)	8,826 (2,993)
Decreased fetal weight (mice) (Schwetz et al., 1991)	1 s.d. = 5%	9,847 (3,339)	6,703 (2,273)
Increased incidence of misaligned sternebrae (mice) (Schwetz et al., 1991)	10%	9,478 (3,214)	5,202 (1,764)

[a] Sample calculation: (3,317 ppm x 72.1 mg/mmol)/24.45 = 9,781 mg/m³, assuming 25˚C and 760 mm Hg.

5.2.2.2. *Adjustment to a Human Equivalent Exposure Concentration*

By definition, the RfC is intended to apply to continuous lifetime exposures to humans (U.S. EPA, 1994b). Because the RfC values are often derived from studies using intermittent and less-than-lifetime exposures, EPA has established guidance (U.S. EPA, 1994b) for adjusting the exposures to an appropriate human equivalent via a simple concentration (C) × time (t) relationship (e.g., 8 hours @ 300 ppm = 24 hours @ 100 ppm). For developmental studies, the *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a) and the *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996) note that peak exposure may be a more relevant exposure metric for short half-life compounds, because the toxic effects may be due to absolute concentration at a specific critical period during fetal development. Some more recent studies suggest that area under the curve (AUC), the assumption underlying the C × t relationship, may be a more appropriate metric for some developmental toxicants than peak exposure. The latter has been demonstrated for certain agents with a short half-life in the body (U.S. EPA, 2002). In consideration of this information, EPA recommends that adjusted continuous exposures be used for inhalation developmental toxicity studies as for other health effects from inhalation exposure (U.S. EPA, 2002).

Duration adjustment is appropriate as the more health-protective procedure, unless there are pharmacokinetic data suggesting that the adjustment to a continuous exposure equivalent is inappropriate, or mode of action information suggests that a susceptible period of development is

specifically targeted (which would suggest that the peak dose may represent the effective dose). In applying these considerations to MEK, the critical effect is nonspecific developmental toxicity (developmental delays and variants), which suggests that duration adjustment may be appropriate. On the other hand, the available pharmacokinetic data indicate that MEK is rapidly absorbed, distributed, and metabolized, suggesting that duration adjustment may be less appropriate than peak exposure. Overall, the available pharmacokinetic, pharmacodynamic, and mechanism of action data for MEK do not provide sufficient evidence to support the use of either peak exposure level or AUC as the most appropriate metric for internal effective dose. Thus, it is appropriate to apply a health-protective duration adjustment to time-weight the intermittent exposures used in the principal study. The LEC of 1,764 ppm (5,202 mg/m³) for increased incidence of misaligned sternebrae in mice exposed to MEK (7 hours/day on days 6–15 of gestation) as reported by Schwetz et al. (1991) is adjusted from an intermittent exposure to continuous exposure (7 hours/day to 24 hours/day) as follows:

$$LEC(ADJ) = LEC \times \frac{7 \ hours/day}{24 \ hours/day}$$
$$= 5{,}202 \ mg\big/m^3 \times 7/24$$
$$= 1{,}517 \ mg\big/m^3$$

The RfC methodology provides a procedure for estimating the human concentration that corresponds to a given animal exposure concentration, i.e., the human equivalent concentration or HEC. Because the critical effect of MEK is extrarespiratory, it is appropriate to apply a factor to account for species differences in blood:air partition coefficients, assuming periodicity was attained (i.e., the ratio of the coefficients). According to EPA's RfC guidelines (U.S. EPA, 1994b), MEK is a category 3 gas because it is not active in the respiratory tract, is rapidly transferred between the lungs and blood, and the effects of inhalation exposure are extra-pulmonary. In humans, reported mean blood:air partition coefficients for MEK from three studies range from 125 to 202. The value of 125 was reported by Fiserova-Bergerova and Diaz (1986) using blood collected directly from human volunteers (n=5) and processed immediately. Perbellini et al. (1984) reported a blood:air partition coefficient of 183 based on blood collected from two cadavers (delay in blood sample collection and preservation procedures were not reported), and Sato and Nakajima (1979) reported a blood:air partition coefficient of 202 based on preserved blood (n=5) collected from a blood bank. Because the blood:air partition coefficient reported by Fiserova-Bergerova and Diaz (1986) was derived from samples that were

subject to immediate and minimal processing, most closely resembling the sample processing in test species, the human blood:air partition coefficient was estimated as 125. In the rat, the blood:air partition coefficients for MEK have been reported as 138 to 139 (Thrall et al., 2002). The RfC methodology stipulates that where the animal blood:air partition coefficient is greater than the human coefficient, a value of one is used for the ratio (U.S. EPA, 1994b). Therefore, the rat $LEC_{(ADJ)}$ is adjusted to a $LEC_{(HEC)}$ following the default procedure in the guidelines (U.S. EPA, 1994b) as follows:

$$LEC_{(HEC)} = LEC_{(ADJ)} \times \frac{Blood: Air\ Partition\ Coefficient\ rat}{Blood: Air Partition\ Coefficient\ human}$$
$$= 1{,}517\ mg/m^3 \times 1$$
$$= 1{,}517\ mg/m^3$$

The $LEC_{(HEC)}$ value of 1,517 mg/m^3 for a 10% extra risk of misaligned sternebrae is used to derive the RfC for MEK.

5.2.2.3. *PBPK Modeling*

Alternatively, PBPK modeling may be used to reduce uncertainty in the RfC resulting from extrapolating from mice or rats to humans. PBPK models for rats (Dietz et al., 1981; Thrall et al., 2002) and humans (Liira et al., 1990b) have been developed to describe the kinetics of MEK in blood. The existing human model (Liira et al., 1990b) is limited in that it includes only inhalation as a portal of entry, it was developed based on data from two healthy males, and comparisons of model predictions with data from other human subjects are not available. With sufficient model validation, the rat model from Thrall et al. (2002), for which the code is available, could be used to estimate human equivalent concentrations corresponding to the benchmark doses developed from the rat inhalation developmental toxicity study by Deacon et al. (1981). No mouse PBPK model has been developed, however, precluding calculation of chemical-specific human equivalent concentrations from the mouse inhalation developmental toxicity study by Mast et al. (1989).

5.2.3. RfC Derivation — Including Application of Uncertainty Factors

The $LEC_{(HEC)}$ of 1,517 mg/m³ (associated with a 10% extra risk of misaligned sternebrae in CD-1 mice exposed to MEK by inhalation 7 hours/day on days 6–15 of gestation; Schwetz et al., 1991) was used as the point of departure for calculating the RfC. A total uncertainty factor (UF) of 300 was applied to this point of departure: 3 for interspecies extrapolation, 10 for susceptible individuals, and 10 for an incomplete data base.

A 3-fold uncertainty factor was used for interspecies extrapolation, since this factor embodies two areas of uncertainty: pharmacokinetics and pharmacodynamics. In this assessment, the pharmacokinetic component is addressed by the calculation of the human equivalent concentration (HEC) according to the procedures in the RfC methodology (U.S. EPA, 1994b). Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty ($10^{0.5}$ or approximately 3).

A 10-fold uncertainty factor for intraspecies differences was used to account for potentially susceptible individuals within the human population. In the absence of information on the variability in response of humans to MEK exposure, the default value of 10 was used.

Consistent with EPA practice (U.S. EPA, 1991a), an uncertainty factor was not used to account for the extrapolation from less than chronic results because developmental toxicity resulting from a narrow period of exposure (gestation days 6-15) was used as the critical effect. The developmental period is recognized as a susceptible lifestage when exposure during certain time windows of development are more relevant to the induction of developmental effects than lifetime exposure.

A 10-fold uncertainty factor was used to account for data base deficiencies. As noted earlier, the minimum data base requirements for deriving an RfC are satisfied by the Cavender et al. (1983) study. Inhalation developmental toxicity studies are available in rats and mice (Deacon et al., 1981; Schwetz et al., 1991). The data base lacks a chronic inhalation toxicity study and multigeneration reproductive toxicity study. Neurotoxicity is adequately addressed by the subchronic inhalation study of Cavender et al. (1983), in which animals were examined for both neurological function and for central nervous system lesions with special neuropathological procedures. The results from this study indicate that MEK has little, if any, neurotoxic potential by itself when tested in adult laboratory animals under conditions of high-level repeated inhalation exposure. Consistent with this finding is a lack of mechanistic evidence for

neurotoxicity. The MEK data base does not, however, specifically include a developmental neurotoxicity study.

A UF for extrapolation from a LOAEL to a NOAEL was not necessary because BMD modeling was used to determine the point of departure. The exposure concentration corresponding to a 10% extra risk of misaligned sternebrae in CD-1 mice (Schwetz et al., 1991) was selected as the point of departure. There is no specific level of extra risk for a skeletal variant that is generally regarded as indicative of an adverse effect. Further, there were no effects in the range of the LEC_{HEC} of 1,517 mg/m^3 other than those that appeared to be related to a general delay in growth. Therefore, no further adjustments were considered for identifying a level of inhalation exposure to MEK associated with a minimal level of risk.

An RfC for MEK is calculated as follows:

$$RfC = LEC_{(HEC)} \div UF$$
$$= 1,517 \ mg/m^3 \div 300$$
$$= 5 \ mg/m^3$$

As noted in Section 5.2.4., the previous MEK RfC of 1 mg/m^3 incorporated a modifying factor of 3 to account for the lack of unequivocal data for respiratory tract (portal-of-entry) effects suggested by earlier studies with MEK (Altenkirch et al., 1978). More recent data concerning the portal-of-entry effects from MEK address the applicability of a modifying factor to this assessment. Dick et al. (1984, 1988, 1989, 1992) found no evidence of a statistically significant increase in respiratory tract irritation among humans who were exposed to MEK at 590 mg/m^3 for 4 hours. In addition, Oleru and Onyekwere (1992) found no statistically significant pulmonary effects among MEK-exposed leather workers (mean duration of employment was approximately 10 years). While these studies do not directly address the potential for portal-of-entry effects for MEK in continuous lifetime exposure scenarios as animal studies evaluating histology would, they do address the concerns raised in the 1992 IRIS assessment. Accordingly, a separate modifying factor to account for possible portal-of-entry effects is not included in this assessment.

5.2.4. Previous Inhalation Assessment

The previous RfC for MEK of 1 mg/m^3 was entered into IRIS in 1992, prior to the publication of EPA's RfC methodology (U.S. EPA, 1994b). The RfC was based on the Schwetz et al. (1991) developmental toxicity study in the mouse. In the previous assessment, a combined uncertainty factor of 3,000 was applied to a human equivalent concentration NOAEL (2,978 mg/m^3), which was not adjusted to a continuous exposure basis. The combined uncertainty factor accounted for interspecies extrapolation, intrahuman variability, and data base deficiencies (including a lack of chronic and reproductive toxicity studies). A modifying factor of 3 accounted for the lack of unequivocal data for respiratory tract (portal-of-entry) effects suggested by earlier studies with MEK (Altenkirch et al., 1978).

The current RfC is based on a BMD approach, rather than the NOAEL/LOAEL approach used previously, and a combined UF of 300 rather than 3,000. Difference in the UF values are accounted for by the calculation of an HEC according to the procedures in the RfC methodology, which supports an interspecies UF of 3 rather than 10, and by more recent data that addressed earlier portal-of-entry concerns, so that a modifying factor of 3 is no longer considered necessary.

5.3. CANCER ASSESSMENT

5.3.1. Oral Slope Factor

Not applicable.

5.3.2. Inhalation Unit Risk

Not applicable.

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

6.1. HUMAN HAZARD POTENTIAL

Methyl ethyl ketone (MEK, CASRN 78-93-3) has the chemical formula C_4H_8O (structural formula $CH_3COCH_2CH_3$) and a molecular weight of 72.11 g/mole. At room temperature, MEK is a clear liquid with a sharp, mint-like odor. MEK is flammable, with a flash point of $-3^\circ C$. MEK is strongly reactive with a number of chemical classes, particularly strong oxidizers. MEK is used as a solvent in the application of protective coatings and adhesives, as a paint remover, and in cleaning fluids. MEK is a natural component of many foods, and may also be found in soil and water in the vicinity of some hazardous waste sites. Other sources of potential exposure include drinking water, tobacco smoke, and volatile releases from building materials and consumer products (ATSDR, 1992).

Studies of the toxicokinetics of MEK reveal that the chemical is well absorbed by oral and inhalation routes, does not appear to accumulate in tissues, and undergoes relatively rapid clearance (on the order of hours) from the body, largely as a result of metabolism. MEK has been shown to induce microsomal P450 activity.

In general, the available human data do not produce a definitive picture of the possible adverse effects of long-term human exposure to MEK. Short-term inhalation exposure (4 hours) to MEK under experimental conditions at or near 200 ppm (590 mg/m^3) does not appear to pose an increased risk of neurologic or irritation symptoms (Dick et al., 1984, 1988, 1989, 1992). Although some evidence of persistent neurotoxicity is available from case reports of repeated exposure (especially when MEK exposure occurs in combination with other solvents), the case for a persistent neurotoxic effect of MEK exposure is not well supported in animal studies that have focused on the possible neurotoxicity of MEK, including the development of peripheral and central nerve fiber degeneration. Saida et al. (1976) found no evidence of peripheral neuropathy (as indicated by paralysis) following continuous exposure of 12 Sprague-Dawley rats to 1,125 ppm (3,318 mg/m^3) MEK for periods of 16 to 55 days. Cavender et al. (1983) found no neurological effects in special neuropathological studies of the medulla (a portion of the brain) and sciatic and tibial nerves of rats exposed to MEK at concentrations up to 5,041 ppm (14,870 mg/m^3) for 90 days. Takeuchi et al. (1983) exposed male Wistar rats (8 per group) to 200 ppm (590 mg/m^3) MEK 12 hours/day for 24 weeks and found no evidence of a persistent effect on

motor or mixed nerve conduction velocity, distal motor nerve latency, or histopathological lesions of tail nerves. Couri et al. (1974) exposed 4 cats, 4 rats, 5 mice, and an unknown number of chickens to 1,500 ppm (4,425 mg/m³) MEK 24 hours/day, 7 days/week for 7–9 weeks with no apparent adverse neurologic effects.

In experimental animals, the longest exposure study available for characterizing the health effects of repeated exposure to MEK is the 90-day inhalation study by Cavender et al. (1983), wherein no toxicity could be attributed to MEK at concentrations as high as 2,518 ppm (7,430 mg/m³). A two-generation reproductive and developmental toxicity study of Wistar rats exposed to 2-butanol, a metabolic precursor of MEK, in drinking water, reported no clear reproductive effects, but found body weight deficits in offspring and kidney histopathologic lesions in adult male rats at estimated dose levels of approximately 3,000 mg/kg-day (Cox et al., 1975). In addition, several developmental toxicity studies of rodents (exposed by inhalation 6–7 hours/day during gestation) reported reduced fetal weight and increased skeletal variations at exposure levels of approximately 1,000 ppm (3,000 mg/m³) MEK (Schwetz et al., 1974, 1991; Deacon et al., 1981). In the absence of chronic toxicity information for MEK by any route of exposure, the effects of lifetime exposure to MEK must necessarily remain somewhat uncertain. Available animal data consistently identify developmental effects in animals exposed to relatively high levels of MEK. It is therefore reasonable and prudent to state that MEK is a possible health hazard to humans who are repeatedly exposed to relatively high levels of MEK.

According to EPA's draft revised cancer guidelines (U.S. EPA, 1999), the hazard descriptor "*data are inadequate for an assessment of human carcinogenic potential*" is appropriate for MEK because cancer studies of humans chronically exposed to MEK are inconclusive, MEK has not been tested for carcinogenicity in animals by the oral or inhalation routes, and the majority of short-term genotoxicity testing of MEK has demonstrated no activity.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

There are no chronic or subchronic oral dose-response data for MEK in humans or animals. The only relevant data for the oral RfD assessment are derived from a study with 2-butanol, a metabolic precursor of MEK. The multigeneration reproductive and developmental toxicity drinking water study by Cox et al. (1975) reported decreased F1A and F2 pup body weights and decreased F1B fetal weights associated with 2-butanol exposure. Benchmark dose modeling of F1A pup body weight data (mean of litter means) at postnatal day 21 yields a point of departure (LED$_{05}$) of 657 mg/kg-day for 2-butanol (i.e., the lower 95% confidence limit on a dose producing a mean 5% decrease in body weight compared with control). Molar adjustment to account for differences in the molecular weights of 2-butanol and MEK yields a point of departure of 639 mg/kg-day. A combined uncertainty factor of 1,000 was applied to the point of departure and a chronic RfD of 0.6 mg/kg-day was derived. This RfD is the same as the RfD from the previous 1993 IRIS assessment. Confidence in the principal study is medium to low. Although the study was adequately-conducted and the critical effect demonstrated therein was supported by inhalation studies with MEK, a metabolic surrogate was used in place of MEK and the highest drinking water concentration was reduced during the study resulting in a need to estimate the actual exposure dose. Furthermore, certain parameters routinely evaluated in studies of more current design (e.g., estrous cyclicity, sperm parameters, and uterine weight) were not measured in Cox et al. (1975). Confidence in the data base is low, due to a lack of chronic exposure information from any route of exposure for MEK. Consequently, the RfD is based on developmental toxicity data for 2-butanol, a compound that is rapidly metabolized to MEK in rats and shows a time-course profile of metabolites following oral administration that is similar to the profile for MEK. Although similar developmental effects were reported following oral and inhalation exposure to 2-butanol and by inhalation exposure to MEK, the lack of oral data for MEK itself and the absence of data in a second species precludes any higher level of data base confidence. Reflecting the medium to low confidence in the principal study and low confidence in the data base, confidence in the RfD is low.

6.2.2. Noncancer/Inhalation

In humans, a number of studies examining the toxicity of MEK following inhalation exposure exist. The available data include case reports, occupational studies, and controlled short-term tests with volunteers. Uncertainty in exposure levels and multiple chemical exposure

precludes dose-response assessment using case reports or occupational studies. The majority of short-term human studies reported no effects after 4 hours of exposure to 200 ppm (590 mg/m^3) (Dick et al., 1984, 1988, 1989, 1992).

In experimental animals, sufficient evidence is available to conclude that developmental effects may result from inhalation exposure to MEK. The developmental effects occur at concentrations between approximately 1,000 and 3,000 ppm (3,000 and 9,000 mg/m^3) MEK 7 hours/day on days 6–15 of gestation (Schwetz et al., 1974, 1991; Deacon et al., 1981). By comparison, this concentration range is not far from the range of exposure levels that have been reported in human case reports of toxicity: 300–600 ppm (885–1,770 mg/m^3) (Smith and Mayers, 1944) and 305–1,695 ppm (900–5,000 mg/m^3) (Seaton et al., 1992) (see Section 4.1.2.).

In the previous IRIS assessment from 1992, an RfC of 1 mg/m^3 was derived based on a NOAEL of 2,978 mg/m^3 for decreased fetal weight in MEK-exposed mice (Schwetz et al., 1991). In the current assessment, benchmark dose models were employed to derive the point of departure for the RfC. From the rat and mouse data on developmental effects produced by inhalation exposure to MEK (exposed 7 hours/day on days 6–15 of gestation), potential points of departure were derived from data sets from the developmental toxicity studies of Deacon et al. (1981) and Schwetz et al. (1991). The lowest of the LECs was selected as the point of departure (1,764 ppm or 5,202 mg/m^3; the lower 95% confidence limit on the concentration associated with a 10% extra risk of misaligned sternebrae in mice) was adjusted from an intermittent exposure (7 hours/day) to continuous exposure (24 hours/day) and to a human equivalent concentration (HEC) by accounting for differences in the blood:air partition coefficients that have been reported for rats and humans (HEC = 1,517 mg/m^3). To this HEC, a combined uncertainty factor of 300 was applied to account for the pharmacodynamic portion of interspecies uncertainty, susceptible individuals within the human population, and data base deficiencies, yielding an RfC of 5 mg/m^3.

Confidence in the principal study (Schwetz et al., 1991) is high; it is well-designed and it tested several exposure concentrations over a reasonable range that included maximum tolerated doses for dams and fetuses. Also, animal studies in a second species (rats) corroborate the effect level for developmental toxicity. Confidence in the data base is medium. The data base lacks chronic exposure toxicity information from any route of exposure, and no multigenerational reproductive toxicity studies are available for MEK itself. The subchronic inhalation study by Cavender et al. (1983) satisfies the minimum inhalation data base requirements for derivation of an RfC. Well-conducted studies in experimental animals provide no convincing evidence that

repeated inhalation exposure to MEK itself (at much higher exposure levels than those in the workplace) is capable of producing persistent neurological effects. Portal-of-entry concerns are addressed by studies in human volunteers showing no net irritation following a 4-hour exposure to 200 ppm (590 mg/m^3). Reflecting high confidence in the principal study and medium confidence in the data base, confidence in the RfC is medium.

6.2.3. Cancer/Oral and Inhalation

Data in both humans and animals are inadequate to evaluate potential associations between cancer and MEK exposure by any route. Available studies in humans are insufficient to evaluate the potential carcinogenicity of MEK. In animals, no chronic study exists for MEK by any route of exposure; short term tests for genotoxicity have generally been negative. Under the draft revised cancer guidelines (U.S. EPA, 1999), the *data are inadequate for an assessment of human carcinogenic potential* of MEK. Accordingly, the data do not support the derivation of an oral slope factor or inhalation unit risk for MEK.

7. REFERENCES

Abdel-Rahman, MS; Hetland, LB; Couri, D. (1976) Toxicity and metabolism of methyl n-butyl ketone. Am Ind Hyg Assoc 37:95-102.

ACGIH (American Conference of Governmental Industrial Hygienists). (2001) Threshold limit values for chemical substances and physical agents and biological exposure indices 2001. ACGIH, Cincinnati, OH.

Alderson, M; Rattan, N. (1980) Mortality of workers on the isopropyl plant and two MEK dewaxing plants. Br J Ind Med 37:85-9.

Allen, N; Mendell, JR; Billmaier, D. (1974) An outbreak of a previously undescribed toxic polyneuropathy due to industrial solvent. Trans Am Neurol Assoc 99:74-79.

Altenkirch, H; Mager, J; Stoltenburg, G; et al. (1977) Toxic polyneuropathies after sniffing a glue thinner. J Neurol 214:137-52.

Altenkirch, H; Stoltenburg, G; Wagner, HM. (1978) Experimental studies on hydrocarbon neuropathies induced by methyl-ethyl-ketone (MEK). J Neurol 219:159-170.

Altenkirch, H; Wagner, HM; Stoltenburg, G; et al. (1982) Nervous system responses of rats to subchronic inhalation of n-hexane and n-hexane + methyl-ethyl-ketone mixtures. J Neurol Sci 57(2-3):209-19.

ATSDR (Agency for Toxic Substances and Disease Registry). (1992) Toxicological profile for 2-butanone. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Ashley, DL; Bonin, MA; Cardinali, FL; et al. (1994) Blood concentrations of volatile organic compounds in a nonoccupationally exposed US population and in groups with suspected exposure. Clin Chem 40:1401-4.

Blair, A; Hartge, P; Stewart, PA; et al. (1998) Mortality and cancer incidence of aircraft maintenance workers exposed to trichloroethylene and other organic solvents and chemicals: Extended follow up. Occup Environ Med 55(3):161-71.

Brady, JF; Li, D; Ishizaki, H; et al. (1989) Induction of cytochromes P450IIE1 and P450IIB1 by secondary ketones and the role of P450IIE1 in chloroform metabolism. Toxicol Appl Pharmacol 100:342-349.

Brooke, I; Cocker, J; Delic, JI; et al. (1998) Dermal uptake of solvents from the vapour phase: an experimental study in humans. Ann Occup Hyg 42(8):531-40.

Brown, EM; Hewitt,WR. (1984) Dose-response relationships in ketone-induced potentiation of chloroform hepato- and nephrotoxicity. Toxicol Appl Pharmacol 76:437-53.

Callender, TJ. (1995) Neurotoxic impairment in a case of methyl ethyl ketone exposure. Arch Environ Health 50(5):392.

Cavender, FL; Casey, HW; H Salem; et al. (1983) A 90-day vapor inhalation toxicity study of methyl ethyl ketone. Fundam Appl Toxicol 3(4):264-70. (The data are the same as Toxigenics, 1981.)

Chia, SE; Ong, CN; Phoon, WH; et al. (1993) Neurobehavioural effects on workers in a video tape manufacturing factory in Singapore. NeuroTox 14:51-6.

Churchill, JE; Ashley, DL; Kaye, WE. (2001) Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Arch Environ Health 56:157-166.

Couri, D; Hetland, LB; O'Neill, JJ; et al. (1974) Comments on a plastics industry neurotoxicity in relationship to methylbutyl ketone. *In: Proceedings of the 5th Annual Conference on Environmental Toxicology*, Fairborn, Ohio. Wright Patterson Air Force Base, Ohio, Aerospace Medical Research Laboratory. AMRL Technical Report No. 74-125. pp. 109-120.

Couri, D; Hetland, LB; Abdel-Rahman, MS; et al. (1977) The influence of inhaled ketone solvent vapors on hepatic microsomal biotransformation activities. Toxicol Appl Pharmacol 41:285-289.

Cox, GE; Bailey, DE; Morgareidge, K. (1975) Toxicity studies in rats with 2-butanol including growth, reproduction and teratologic observations. Food and Drug Research Laboratories, Inc. Waverly, NY. Report No. 91MR R 1673.

CRC. (1994) CRC Handbook of chemistry and physics. 75th Edition. Lide, DR, ed. CRC Press, Boca Raton, FL.

Deacon, MM; Pilny, MD; John, JA; et al. (1981) Embryo- and fetotoxicity of inhaled methyl ethyl ketone in rats. Toxicol Appl Pharmacol 59:620-2.

DeCeaurriz, J; Desiles, JP; Bonnet, P; et al. (1983) Concentration-dependent behavioral changes in mice following short-term inhalation exposure to various industrial solvents. Toxicol Appl Pharmacol 67:383-9.

Dick, R; Setzer, J; Wait, R; et al. (1984) Effects of acute exposure of toluene and methyl ethyl ketone on psychomotor performance. Int Arch Occup Environ Health 54:91-109.

Dick, RB; Brown, WD; Setzer, JV; et al. (1988) Effects of short duration exposures to acetone and methyl ethyl ketone. Toxicol Lett 43:31-49.

Dick, R; Setzer, J; Taylor, B; et al. (1989) Neurobehavioral effects of short duration exposures to acetone and methyl ethyl ketone. Br J Ind Med 46:111-21.

Dick, RB; Krieg Jr., EF; Setzer, J; et al. (1992) Neurobehavioral effects from acute exposures to methyl isobutyl ketone and methyl ethyl ketone. Fundam Appl Toxicol 19(3):453-73.

Dietz, FK; Traiger, GJ. (1979) Potentiation of CCl4 of hepatotoxicity in rats by a metabolite of 2-butanone: 2,3-butanediol. Toxicology 14:209-15.

Dietz, FK; Rodriguez-Giaxola, M; Traiger, GJ; et al. (1981) Pharmacokinetics of 2-butanol and its metabolites in the rat. J Pharmacokin Biopharmacol 9(5):553-76.

DiVincenzo, GD; Kaplan, CJ; Dedinas, J. (1976) Characterization of the metabolites of methyl n-butyl ketone, methyl iso-butyl ketone and methyl ethyl ketone in guinea pig serum and their clearance. Toxicol Appl Pharmacol 36:511-22.

Douglas, GR; Nestmann, ER; Betts, JL; et al. (1980) Mutagenic activity in pulp mill effluents. Water chlorination. *In: Water chlorination environmental impact and health effects*, Volume 3. Jolley, R.L., W.A. Brungs, R.B. Cumming, et al., Eds. p. 865-80.

Dow Chemical Corporation. (1979) Teratologic evaluation of inhaled methyl ethyl ketone in rats. OTS Fiche #0205871. Document No. 878211793. (These data are the same as Deacon et al., 1981.)

Dyro, FM (1978) Methyl ethyl ketone polyneuropathy in shoe factory workers. Clin Toxicol 13:371-6.

Fagius, J; Gronqvist, B. (1978) Function of peripheral nerves and signs of polyneuropathy in solvent-exposed workers at a Swedish steelworks. Acta Neurol Scandinav 57:305-316.

Fiserova-Bergerova, V; Diaz, ML. (1986) Determination and prediction of tissue-gas partition coefficients. Int Arch Occup Environ Health 58:75-87.

Florin, I; Rutberg, L; Curvall, M; et al. (1980) Screening of tobacco smoke constituents for mutagenicity using the Ames test. Toxicology 18:219-32.

Freddi, A; Paci, A; Vittori, O; et al. (1982) [Clinical and electromyographic study of workers exposed to methyl ethyl ketone vapor.] Ann Med Perugia 73:111-36. (in Italian). As cited in WHO, 1992.

Gadberry, MG; Carlson, GP. (1994) 2-Butanol metabolism by rat hepatic and pulmonary cytochromes P450. Toxicol Lett 74(3):203-9.

Gallo, MA; Oser, BL; Cox, GE; et al. (1977) Studies on the long-term toxicity of 2-butanol. Toxicol Appl Pharmacol 41:135. (Abstract).

Garcia, CR; Geller, I; Kaplan, HL. (1978) Effects of ketones on lever-pressing behavior of rats. Proc West Pharmacol Soc 21:433-38.

Gaunt, IF; Brantom, PG; Kiss, IS; et al. (1972) Short-term toxicity of acetoin (acetylmethylcarbinol) in rats. Food Cosmet Toxicol 10(2):131-41.

Geller, I; Gause, E; Kaplan, H; et al. (1979) Effects of acetone, methyl ethyl ketone and methyl isobutyl ketone on a match-to-sample task in the baboon. Pharmacol Biochem Behav 11:401-6.

Glowa, JR; Dews, PB. (1987) Behavioral toxicology of volatile organic solvents. IV. Comparisons of the rate-decreasing effects of acetone, ethyl acetate, methyl ethyl ketone, toluene, and carbon disulfide on schedule-controlled behavior of mice. J Am Coll Toxicol 6(4):461-9.

Graham, DG. (2000) Critical analysis of Mitran et al. (1997). Neurotoxicity associated with occupational exposure to acetone, methyl ethyl ketone, and cyclohexanone. Environ Res 73:181-3.

Horton, A; Bingham, E; Burton, M; et al. (1965) Carcinogenesis of the skin. III. The contribution of elemental sulfur and of organic sulfur compounds. Cancer Res 25:1759-63.

HSDB (Hazardous Substance Data Bank). (1999) National Library of Medicine, Bethesda, MD. Online at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Ichihara, G; Saito, I; Kamijima, M; et al. (1998) Urinary 2,5-hexanedione increases with potentiation of neurotoxicity in chronic coexposure to n-hexane and methyl ethyl ketone. Int Arch Occup Environ Health 71(2):100-4.

Imbriani, M; Ghittori, S; Pezzagno, G; et al. (1989) Methyl ethyl ketone (MEK) in urine as biological index of exposure. G Ital Med Lav 11(6):255-61.

Johanson, G; Naslund, PH. (1988) Spreadsheet programming--A new approach in physiologically-based modeling of solvent toxicokinetics. Toxicol Lett 41:115-27.

JRB Associates, Inc. (1980) Support document, Chapter IV. Health effects of methyl ethyl ketone. Washington, DC, U.S. Environmental Protection Agency, 58 pp. Contract No. 68-01-4839, Task No. 11, Project No. 2-800-08-107-04. As cited in WHO, 1992.

Kessler, W; Denk, B; Filser, JG. (1988) Species-specific inhalation pharmacokinetics of 2-nitropropane. *In*: *Biologically Based Methods for Cancer Risk Assessment*, Series 159, C.C. Travis, Ed. NATO Advanced Science Institute. p. 123-40.

Kimura, ET; Ebert, DM; Dodge, PW. (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704.

Kopelman, P; Kalfayan, P. (1983) Severe metabolic acidosis after ingestion of butanone. Br Med J 286:21-2.

LaBelle, CW; Brieger, H. (1955) The vapor toxicity of a composite solvent and its principal components. Am Med Assoc Arch Ind Health 12:623-7.

Leeder, JS; Kearns, GL. (1997) Pharmacogenetics in pediatrics: implications for practice. Pediatr Clin North Am 44(1):55-77.

Lemasters, GK; Olsen, DM; Yiin, JH; et al. (1999) Male reproductive effects of solvent and fuel exposure during aircraft maintenance. Reprod Toxicol 13(3):155-66.

Leung, H-W. (1992) Use of physiologically based pharmacokinetic models to establish biological exposure indices. Am Indust Hyg Assoc J 53(6):369-74.

Liira, J; Riihimaki, V; Pfaffli, P. (1988) Kinetics of methyl ethyl ketone in man: absorption, distribution and elimination in inhalation exposure. Int Arch Occup Environ Health 60(3):195-200.

Liira, J; Riihimaki, V; Engstrom, K. (1990a) Effects of ethanol on the kinetics of methyl ethyl ketone in man. Br J Indust Med 47:325-330.

Liira, J; Johanson, G; Riihimaki, V. (1990b) Dose-dependent kinetics of inhaled methyl ethyl ketone in man. Toxicol Lett 50(2-3):195-201.

Lowengart, RA; Peters, JM; Cicioni, C; et al. (1987) Childhood leukemia and parents' occupational and home exposures. J Natl Canc Inst 79(1):39-46.

Mast, TJ; Dill, JA; Evanoff, JJ; et al. (1989) Inhalation developmental toxicology studies: Teratology study of methyl ethyl ketone in mice. Final Report. Prepared by Pacific Northwest Laboratory, Battelle Memorial Institute, for the National Toxicology Program, Washington, DC. PNL-6833 UC-408. (Data are the same as Schwetz et al., 1991)

Mayer, VW; Goin, CJ. (1987) Effects of chemical combinations on the induction of aneuploidy in Saccharomyces cerevisiae. Mutat Res 187(1):21-30.

Merck Index. (2001) The Merck Index: an encyclopedia of chemicals, drugs, and biologicals. 13th edition. Budavari, S. (ed). Merck & Co., Inc., Whitehouse Station, New Jersey. p. 1084.

Mitran, E; Callender, T; Orha, B; et al. (1997) Neurotoxicity associated with occupational exposure to acetone, methyl ethyl ketone, and cyclohexanone. Environ Res 73:181-8.

Mitran, E. (2000) Reply. Environ Res 82(2):184-5.

Munies, R; Wurster, DE. (1965) Investigation of some factors influencing percutaneous absorption--III. Absorption of methyl ethyl ketone. J Pharm Sci 54:1281-4.

Muttray, A; Jung, D; Klimmek, L; et al. (2002) Effects of an external exposure to 200 ppm methyl ethyl ketone on nasal mucosa in healthy volunteers. Int Arch Occupat Environ Health 75(3):197-20.

Nelson, KW; Ege, JF; Ross, M; et al. (1943) Sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 25:282-85.

Nelson, BK; Brightwell, WS; Khan, A; et al. (1989) Lack of selective developmental toxicity of three butanol isomers administered by inhalation to rats. Fundam Appl Toxicol 12:469-79.

Nelson, BK; Brightwell, WS; Krieg Jr, EF. (1990) Developmental toxicology of industrial alcohols: a summary of 13 alcohols administered by inhalation to rats. Toxicol Ind Health 6(3-4):373-87.

Newton, PE; Wooding, WL; Bolte, HF; et al. (2001) A chronic inhalation toxicity/oncogenicity study of methylethylketoxime in rats and mice. Inhal Toxicol 13(12):1093-116.

Noraberg, J; Arlien-Soborg, P. (2000) Neurotoxic interactions of industrially used ketones. NeuroToxicology 21(3):409-418.

National Research Council (NRC). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

NTP (National Toxicology Program). (Undated). Methyl ethyl ketone: chemical health and safety information. http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/Res_Stat/iH_Res_Stat_Frames.html.

NTP. (1990) Inhalation developmental toxicology studies: teratology study of methyl ethyl ketone (CAS No. 78-93-3) in Mice NTP Study: TER88046. Research Triangle Park, NC. (Data are the same as Mast et al., 1989)

NTP. (1999) NTP technical report on the toxicity studies of methyl ethyl ketoxime (CAS No. 96-29-7) administered in drinking water to F344/N rats and B6C3F1 mice. Toxicology Report Series No. 51. NIH Publication 99-3947. July.

NTP. (2002) Chemical health and safety data. Online at <http://ntp-server.niehs.nih.gov/cgi/iH_Indexes/ALL_SRCH/iH_ALL_SRCH_Frames.html>.

O'Donoghue, JL; Haworth, SR; Curren, RD; et al. (1988) Mutagenicity studies on ketone solvents: methyl ethyl ketone, methyl isobutyl ketone, and isophorone. Mutat Res 206(2):149-61.

Oleru, UG; Onyekwere, C. (1992) Exposures to polyvinyl chloride, methyl ketone and other chemicals. The pulmonary and non-pulmonary effect. Int Arch Occ Environ Health 63(7):503-7.

Orti-Pareja, M; Jimenez-Jimenez, FJ; Miquel, J; et al. (1996) Reversible myoclonus, tremor, and ataxia in a patient exposed to methyl ethyl ketone. Neurol 46(1):272.

OSHA (Occupational Safety and Health Administration). (1993) 29 CFR Part 1910. Air contaminants rule. Federal Register 58(124):35338-51.

Perbellini, L; Brugnone, F; Mozzo, P; et al. (1984) Methyl ethyl ketone exposure in industrial workers. Uptake and kinetics. Int Arch Occup Environ Health 54:73-81.

Raunio, H; Liira, J; Elovaara, E; et al. (1990) Cytochrome P450 isozyme induction by methyl ethyl ketone and m-xylene in rat liver. Toxicol Appl Pharmacol 103(1):175-9.

Saida, K; Mendell, JR; Weiss, HS. (1976) Peripheral nerve changes induced by methyl n-butyl ketone and potentiation by methyl ethyl ketone. J Neuropath Exp Neurol 35(3):205-25.

Sakata, M; Kikuchi, J; Masanobu, H; et al. (1989) Disposition of acetone, methyl ethyl ketone and cyclohexanone in acute poisoning. Clin Toxicol 27:67-77.

Sato, A; Nakajima, T. (1979) Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. Br J Ind Med 36:231-34.

Schulze, GE; Derelanko, MJ. (1993) Assessing the neurotoxic potential of methyl ethyl ketoxime in rats. Fundam Appl Toxicol 21(4):476-85.

Schwetz, BA; Leong, BKJ; Gehring, PJ. (1974) Embryo- and fetotoxicity of inhaled carbon tetrachloride, 1,1-dichloroethane and methyl ethyl ketone in rats. Toxicol Appl Pharmacol 28:452-64.

Schwetz, BA; Mast, TJ; Weigel, RJ; et al. (1991) Developmental toxicity of inhaled methyl ethyl ketone in mice. Fundam Appl Toxicol 16:742-8.

Seaton, A; Jellinek, EH; Kennedy, P. (1992) Major neurological disease and occupational exposure to organic solvents. Quart J Med 84(305):707-12.

Sia, GL; Ong, CN; Chia, SE; et al. (1991) Environmental and biological monitoring of methyl ethyl ketone (MEK). Environ Monit Assess 19(1-3):401-11.

Smith, AR; Mayers, MR. (1944) Study of poisoning and fire hazards of butanone and acetone. NY State Dept Labor Ind Bull April:174-176. As cited in WHO, 1992.

Smyth, HF, Jr.; Carpenter, CP; Weil, CS; et al. (1962) Range-finding toxicity data: list VI. Am Ind Hyg J 1962:95-107.

Spirtas, R; Stewart, PA; Lee, JS; et al. (1991) Retrospective cohort mortality study of workers at an aircraft maintenance facility: I. Epidemiological results. II. Exposures and their assessment. Br J Ind Med 48(8):515-37.

Stoltenburg-Didinger, G. (1991) The effect of pre- and postnatal exposure to organic solvents on the development of the cerebellar cortex in the rat. Prog Histochem Cytochem 23(1-4):227-34.

Stoltenburg-Didinger, G; Altenkirch, H; Wagner, M. (1990) Neurotoxicity of organic solvent mixtures: embryotoxicity and fetotoxicity. Neurotoxicol Teratol 12(6):585-9.

Takeuchi, Y; Ono, Y; Hisanaga, N; et al. (1983) An experimental study of the combined effects of n-hexane and methyl ethyl ketone. Br J Ind Med 40:199-203.

Tanii, H; Tsuji, H; Hashimoto, K. (1986) Structure–toxicity relationship of monoketones. Toxicol Lett 30:3-7.

Thrall, KD; Soelberg, JJ; Weitz, KK; et al. (2002) Development of a physiologically based pharmacokinetic model for methyl ethyl ketone in F344 rats. J Toxicol Environ Health 65(13):881-96.

Toftgard, R; Nilsen, O; Gustafsson, J-A. (1981) Changes in rat liver microsomal cytochrome P-450 and enzymatic activities after the inhalation of n-hexane, xylene, methyl ethyl ketone weeks. Scand J Work Environ Health 7:31-7.

Toxigenics. (1981) 90-Day vapor inhalation toxicity study of methyl ethyl ketone in albino rats. Submitted to Chemical Industry Institute of Toxicology, Research Triangle Park, NC. Doc. ID 878212064. Microfiche No. 205953. (Data are the same as Cavender et al., 1983)

Traiger, GJ; Bruckner, JV. (1976) The participation of 2-butanone in 2-butanol-induced potentiation of carbon tetrachloride hepatotoxicity. J Pharmacol Exp Ther 196:493-500.

Traiger, GJ; Bruckner, JV; Jiang, W-D; et al. (1989) Effect of 2-butanol and 2-butanone on rat hepatic ultrastructure and drug activity. J Toxicol Environ Health 28(2):235-48.

Tyl, RW; Gerhart, JM; Myers, CB; et al. (1996) Reproductive toxicity evaluation of methylethyl ketoxime by gavage in CD rats. Fundam App Toxicol 31(2):149-61.

Ursin, C; Hansen, CM; Van Dyk, JW; et al. (1995) Permeability of commercial solvents through living human skin. Am Indust Hygiene Assoc J 56 (7):651-60.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014-34025.

U.S. EPA. (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006-34012.

U.S. EPA. (1988) Recommendations for and documentation of biological values for use in risk assessment. EPA 600/6-87/800.

U.S. EPA. (1991a) Guidelines for developmental toxicity risk assessment. Federal Register 56:63798-63826.

U.S. EPA. (1991b) Alpha 2u-globulin: association with chemically induced renal toxicity and neoplasia in the male rat. Risk Assessment Forum, Washington, DC. EPA/625/3-91/019F.

U.S. EPA. (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity: notice of availability. Federal Register 59:53799.

U.S. EPA. (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. EPA/600/8-90/066F.

U.S. EPA. (1995) Use of the benchmark dose approach in health risk assessment. EPA/630/R-94/007.

U.S. EPA. (1996) Reproductive toxicity risk assessment guidelines. Federal Register 61(212):56274-56322.

U.S. EPA. (1998a) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926-26954.

U.S. EPA. (1998b) Science policy council handbook: peer review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-98/001.

U.S. EPA. (1999) Guidelines for carcinogen risk assessment [review draft]. Risk Assessment Forum, Washington, DC; NCEA-F-0644. <http://www.epa.gov/ncea/raf/cancer.htm>

U.S. EPA. (2000a) Science policy council handbook: peer review. Second edition. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA/100/B-00/001.

U.S. EPA. (2000b) Science policy council handbook: risk characterization. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-00-002.

U.S. EPA. (2000c) Benchmark dose technical guidance document [external review draft]. EPA/630/R-00/001.

U.S. EPA. (2000d) Supplementary guidance for conducting health risk assessment of chemical mixtures. Office of Research and Development, Risk Assessment Forum, Washington, DC. EPA /630/R-00/002.

U.S. EPA. (2002) A review of the reference dose and reference concentration processes. December 2002. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC. EPA/630/P-02/002F.

van Engelen, JG; Rebel-de Haan, W; Opdam, JJ; et al. (1997) Effect of coexposure to methyl ethyl ketone (MEK) on n-hexane toxicokinetics in human volunteers. Toxicol Appl Pharmacol 144:385-95.

Vieira, I; Sonnier, M; Cresteil, T. (1996) Developmental expression of CYP2E1 in the human liver: hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238:476-83.

Volkel, W; Wolf, N; Derelanko, M; et al. (1999) Slow oxidation of acetoxime and methylethyl ketoxime to the corresponding nitronates and hydroxy nitronates by liver microsomes from rats, mice, and humans. Toxicol Sci 47(2):144-50.

Welch, L; Kirshner, H; Heath, A; et al. (1991) Chronic neuropsychological and neurological impairment following acute exposure to a solvent mixture of toluene and methyl ethyl ketone (MEK). J Toxicol Clin Toxicol 29(4):435-45.

Wen, CP; Tsai, SP; Weiss, NS; et al. (1985) Long-term mortality study of oil refinery workers. IV. Exposure to the lubricating-dewaxing process. J Natl Cancer Inst 74:11-8.

Woo, Y-T; Lai, D; McLain, JL; et al. (2002) Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products. Environ Health Persp 110(Suppl. 1):75-87.

WHO (World Health Organization). (1992) Methyl ethyl ketone. Environmental Health Criteria. Volume 143.

Wurster, DE; Munies, R. (1965) Factors influencing percutaneous absorption II: absorption of methyl ethyl ketone. J Pharm Sci 54:554-6.

Yu, RC; Hattis, D; Landaw, EM; et al. (2002) Toxicokinetic interaction of 2,5-hexanedione and methyl ethyl ketone. Arch Toxicol 75:643-652.

Zeiger, E; Anderson, B; Haworth, S; et al. (1992) Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19(Suppl 21):2-141.

Zhao, W; Misumi, J; Yasui, T; et al. (1998) Effects of methyl ethyl ketone, acetone, or toluene coadministration on 2,5-hexanedione concentration in the sciatic nerve, serum and urine of rats. Int Arch Occup Environ Health 71:236-244.

Zimmermann, FK; Mayer, VM; Scheel, I; et al. (1985) Acetone, methyl ethyl ketone, ethyl acetate, acetonitrile and other polar aprotic solvents are strong inducers of aneuploidy in *Saccharomyces cerevisiae*. Mutat Res 149(3):339-51.

APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

The support document and IRIS Summary for MEK have undergone both internal peer review by scientists within EPA and a more formal external peer review by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer 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 questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's response to these comments follows. EPA also received scientific comments from the public. These comments and EPA's response are included in a separate section of this appendix.

Comments from External Peer Review

A. General Questions

1. Charge Question: Is the document logical, clear and concise? Are the arguments presented in an understandable manner?

Comments: The external peer reviewers generally considered the document to be clearly written, with arguments presented in an understandable manner.

2. Charge Question: Are you aware of any other data/studies that are relevant to the assessment of adverse effects, both cancer and noncancer, from exposure to MEK?

Comments: One reviewer identified a study that demonstrates the potential for MEK to potentiate the neurotoxic potential of 2-hexanone in rats (Abdel-Rahman et al., 1976). Another reviewer suggested that the toxicity literature for methyl ethyl ketoxime (MEKO) might be relevant to the evaluation of the toxicity of MEK. The reviewer indicated that MEKO is metabolized to hydroxylamine and MEK, and pointed to a two-generation reproductive toxicity study by Tyl et al. (1996), in which the hydroxylamine was considered responsible for observed hematopoietic effects and MEK was considered responsible for observed central nervous system depression. The reviewer noted that there was no evidence of structural or functional reproductive toxicity and no developmental/postnatal toxicity associated with MEKO exposure.

Response: The results from Abdel-Rahman et al. (1976) were incorporated into Section 4.4.4. of the Toxicological Review.

EPA reviewed the literature for MEKO, and considered the pattern of toxicity associated with this chemical to be substantially different from that associated with MEK. In the rat, the major target of MEKO toxicity is the erythrocyte; MEKO induces a methemoglobinemia and a responsive Heinz body anemia. Liver lesions, secondary to the destruction of erythrocytes, have also been observed in rats and mice exposed to MEKO. NTP (1999) notes that although there is strong circumstantial evidence that the erythrotoxicity is due to the hydrolysis product, hydroxylamine, the evidence is not unequivocal. Other effects associated with MEKO include degeneration of olfactory epithelium and hyperplasia of the urinary bladder transitional epithelium, neither of which could be attributed to the ketone metabolite (MEK). In a study of neurotoxic potential, there was no evidence of cumulative neurotoxicity in a 13-week study at exposure levels that produced effects on the hematopoietic system. MEKO induced liver tumors in rats and mice. The mechanism of tumor induction has not been well defined, although one proposed mechanism includes the oxidation of the oxime to nitronates of secondary-nitroalkanes, which are mutagenic and tumorigenic in rodents. MEKO is weakly genotoxic (Schulze and Derelanko, 1993; Tyl et al., 1996; NTP, 1999; Volkel et al., 1999; Newton et al., 2001). Given the differences in the pattern of toxicity between MEK and MEKO and the difficulties associated with attributing effects to the parent compound or its metabolites (hydroxylamine and MEK), the literature for MEKO was not considered useful in the qualitative characterization of MEK toxicity. Further, the available pharmacokinetic data for MEKO does not establish the percent of MEKO that is metabolized to MEK, and thus does not support a more quantitative analysis of the exposure level of MEK present as a metabolite of MEKO associated with reported health effects. Accordingly, the MEKO toxicity literature was not included in the health assessment for MEK.

B. Reference Dose (RfD)

1. Charge Question: The RfD is based on data for 2-butanol, a metabolic precursor of MEK, from Cox et al. (1975). Is the use of 2-butanol as a surrogate for MEK adequately supported?

Comments: All of the reviewers agreed that the use of 2-butanol to establish the RfD for MEK was appropriate given the available data set. One reviewer recommended a more detailed examination of pharmacokinetics and peak internal concentrations to determine whether a dosimetric adjustment should be made to equate MEK generated internally from 2-butanol with

MEK absorbed directly from the environment. Another reviewer suggested using data from MEKO, which generates MEK *in vivo*.

Response: EPA believes that the available data from Dietz et al. (1981) and Traiger and Bruckner (1976) adequately demonstrate that the elimination profiles of MEK and metabolites in blood following oral administration of MEK and 2-butanol are comparable, in terms of both area under the curve (AUC) and peak internal dose. Additional information on peak blood concentrations from Dietz et al. (1981) was added to Section 3.3. of the Toxicological Review to better describe these elimination profiles.

For the reasons discussed above, MEKO was not considered by EPA to be a suitable surrogate for assessing MEK toxicity.

2. Charge Question: Reduced pup weight in the F1A generation, particularly at postnatal day 21, served as the critical effect. Do you consider this effect to be a biologically relevant response?

Comments: Reviewers had different opinions as to whether reduced F1A pup weight on postnatal day 21 was the most appropriate, biologically relevant response. One reviewer agreed that the effect was biologically relevant, while another agreed that reduced pup weight on postnatal day 21 was a biologically relevant response. The reviewer added that the significance and magnitude of the response made it of questionable use in establishing an RfD. A third reviewer commented that body weight data from F2 pups on postnatal day 21 is more objectively justifiable as the critical effect, and has the advantage of being derived from three dose levels rather than from the truncated 2-dose data set for F1A neonates. Another reviewer considered that pup weight at postnatal day 4 is more closely analogous to fetal growth inhibition and reduced birth weight in humans, although the postnatal day 21 body weight reduction provided supportive information.

Response: All of the peer reviewers were of the opinion that reduced pup or fetal weight was a biologically relevant response, but did not agree on which generation or postnatal day from the multigeneration reproductive toxicity study was most appropriate for evaluating human health. The LED_{05} values (95% lower confidence limit on the effective dose, ED) from the five data sets were within twofold of each other, suggesting that responses in different generations and at different times in development were reasonably comparable. In the absence of common recommendations from the external peer reviewers, EPA retained as the critical data set for the

RfD the F1A pup body weight data on postnatal day 21, since this data set produced the lowest, and thus most health protective point of departure.

3. Charge Question: Do you agree with the application of a benchmark dose (BMD) approach to identify a point of departure using data from the Cox et al. (1975) study? Would use of a NOAEL/LOAEL approach be preferable?

Comments: Three of the reviewers considered BMD methodology the most appropriate way to analyze the Cox et al. (1975) data. One of the three reviewers suggested that EPA present the results of a NOAEL/LOAEL approach in addition to the BMD methodology. The fourth reviewer strongly disagreed with the use of BMD modeling for this particular data set, but also considered the NOAEL/LOAEL approach to be inappropriate. The reviewer objected to using the BMD methodology because the choice of a weight reduction as large as 5% implies a tolerance for potential adverse effects in humans far larger than should be considered acceptable as a functional equivalent of a NOAEL or LOAEL. The reviewer further observed that a one standard deviation change in human birth weight corresponds to a 500 g or so shift, or about 15% of approximate average birth weights of 3,400 g. This reduction in birth weight would be associated with an excess mortality of about 10/1000 and elevated risks for other conditions and a 5% shift would be expected to cause one-third this level of impact. Overall, the reviewer objected to the use of fetal growth inhibition as an effect for deriving an RfD since it has been this reviewer's experience that dose-response relationships for fetal growth inhibition do not support nonlinearity.

Response: Consistent with the input of 3 out of the 4 reviewers, EPA agrees that a BMD analysis is the most appropriate method of analysis for deriving the RfD for MEK, and is preferable to the application of a NOAEL/LOAEL approach. For this reason, a NOAEL/LOAEL approach is not presented.

EPA does not agree with the reviewer who stated that the selected benchmark response (BMR) of 5% is too large. In the absence of some idea of a specific level of response to consider adverse, EPA's "Benchmark Dose Technical Guidance" (U.S. EPA, 2000c) recommends a change in the mean equal to one standard deviation from the control mean. This level of response "gives an excess risk of approximately 10% for the proportion of individuals below the 2nd percentile or above the 98th percentile of controls for normally distributed effects." EPA is not aware of precedents for using BMR values as small as 0.1 or 1% as suggested by the

reviewer. Furthermore, a BMR of 5%, in this case, is within the range of experimental dose levels used in the Cox et al. (1975) study, and for that reason is supportable.

4. Charge Question: Are the appropriate uncertainty factors applied? Is the explanation for each transparent?

Comments: Two reviewers considered that the uncertainty factors have been appropriately applied. One reviewer indicated that a data base uncertainty factor of 10 is overly cautious, and suggested that a 3-fold uncertainty factor might be more appropriate. The fourth reviewer offered an alternative approach to the BMC (or NOAEL) divided by uncertainty factors. The reviewer also suggested a target RfD be derived that would produce no more than an expected one-in-one million extra burden of infant mortality, and that this target be tied to day 4 body weight reduction (in the F1A and F2 litters). This approach would reduce the RfD by about ninefold from the value of 0.7 mg/kg-day presented in the external review draft.

Response: EPA considers a data base uncertainty factor of 10 to be appropriately cautious for the MEK RfD, particularly given the lack of oral toxicity data specific to MEK.

At this time, it is EPA's position that the extrapolation from an exposure level associated with a one-in-one million extra burden of infant mortality is not supportable, since the uncertainty associated with projecting a dose corresponding to a one-in-one million extra burden level from the available animal data, i.e., Cox et al. (1975), would be substantial. Thus, EPA is retaining the current BMD approach and uncertainty factors presented in the external review draft.

C. Reference Concentration (RfC)

1. Charge Question: The RfC for MEK derived in 1993 is based on reduced fetal weight as reported in the mouse developmental toxicity study of Schwetz et al. (1991). The RfC derived in the reassessment is based on a different developmental endpoint – increased incidence of misaligned sternebrae – from the same Schwetz et al. (1991) study. Do you consider an increased incidence of this skeletal variant to be a biologically relevant endpoint? Would an alternative endpoint (e.g., reduced fetal weight in the mouse) be more appropriate?

Comments: None of the reviewers offered an opinion that misaligned sternebrae was not a biologically significant endpoint of toxicity. One reviewer who considered misaligned sternebrae to be biologically relevant and preferable to the use of reduced fetal weight noted the

considerable difference of opinion among experts in evaluating increased incidence of misaligned sternebrae in mouse developmental toxicology. A second reviewer considered reduced fetal weight in the mouse to be the most appropriate endpoint in this data set because body weight is a continuous rather than dichotomous variable, and because misaligned sternebrae were elevated on a per fetus basis rather than a per litter basis (the latter considered the more appropriate statistical unit). A third reviewer stated that, based on the reviewer's 40 years of experience performing developmental toxicity studies in rodents, fetal weight per litter is usually the most sensitive indicator of fetal toxicity. The reviewer also stated that the presentation of skeletal effects data as the number of affected fetuses/group was not appropriate, since the unit for statistical analysis is the dam or the litter, rather than the fetus. The reviewer recommended the use of more sophisticated statistical software programs (e.g., SUDAAN) that are designed to analyze correlated data. The fourth reviewer noted the highly nonlinear dose-response relationship for misaligned sternebrae, and suggested that fetal weight reduction might be preferable for addressing human health risk at low doses since it has a more linear dose-response relationship.

Response: Based on external peer reviewers' comments and further analysis of the data from Schwetz et al. (1991), EPA gave further consideration to the use of fetal weight data as the basis for the point of departure for the RfD rather than incidence of misaligned sternebrae. Points that support the use of fetal weight rather than misaligned sternebrae included the fact that the incidence of misaligned sternebrae was highly nonlinear with dose, and the incidence showed a high degree of variability. Mast et al. (1989) also reported a positive trend (not statistically significant) for reduced ossification of the sternebrae, raising the possibility that misaligned sternebrae may reflect a more general growth delay. Selection of the critical effect (i.e., reduced fetal weight or incidence of misaligned sternebrae) was further deliberated during consensus review. In general, consensus reviewers did not find the arguments for using fetal weight as the more supportable critical effect to be compelling. Thus, the decision was made to use the more health protective endpoint, misaligned sternebrae, as the critical effect.

2. Charge Question: Do you agree with the application of a benchmark dose (BMD) approach to identify a point of departure using data from Schwetz et al. (1991)? Would use of a NOAEL/LOAEL approach be preferable?

Comments: All four reviewers considered BMD methodology the most appropriate way to analyze the data for deriving the point of departure for the RfC. One reviewer added that BMD methodology was appropriate as long as it was applied to the data for the quantal endpoint of

misaligned sternebrae. Another reviewer suggested presenting the results of the NOAEL/LOAEL approach in addition to the BMD methodology.

Response: A BMD methodology was retained for the analysis of the RfC, consistent with the external peer reviewer feedback.

3. Charge Question: The State of California has developed a draft Reference Exposure Level (REL), which is comparable to EPA's RfC, of 3 mg/m^3 based on the Mitran et al. (1997) occupational study that reported various neurological effects in MEK-exposed workers. Because of certain critical limitations in this study, it was not selected as the basis for the RfC. Is this decision adequately supported? More generally, is the weight of evidence for the neurotoxic potential of MEK adequately described?

Comments: Two of the reviewers agreed that Mitran et al. (1997) suffered from too many deficiencies to serve as a reliable basis for the RfC. A third reviewer commented that if the worker study used by the State of California was adequately and appropriately performed and reported, then it is a valid basis for the RfC, especially since it provided data in humans.

The fourth reviewer appears to have misinterpreted the question as asking if the REL of 3 mg/m^3 was comparable to the external review draft RfC of 15 mg/m^3. The reviewer also stated that consistency between the proposed RfC and proposed RfD could be accomplished by developing and applying PBPK models in rats, mice and humans, such that toxicologically equivalent doses (based on either Cmax in the plasma or AUC during key periods in development) could be projected. The reviewer suggested using another calculation based on a test of acute behavioral toxicity. Specifically, the reviewer recommended using Glowa and Dews (1987) in mice to define a point of departure based on sophisticated neurobehavioral modeling of interindividual variability for a sensitive neurobehavioral change.

Two of the four reviewers commented on the adequacy of the description of the weight-of-evidence for the neurotoxic potential of MEK in humans. One of the two considered the evidence to be adequately described, while the second did not think the IRIS assessment adequately addressed or discussed the neurotoxic potential of MEK, but did not further identify areas where discussion could be improved.

Response: As supported by two of the external peer reviewers, EPA continues to consider the Mitran et al. (1997) study to be too limited to use for RfC determination. In the absence of

specific feedback on how to improve the discussion of the neurotoxic potential of MEK, the relevant sections of the Toxicological Review were not substantially revised.

EPA agrees that the development of a PBPK model for MEK that would support extrapolation between species and between routes of exposure would inform this assessment. Given the need for timely completion of the MEK IRIS assessment, it was decided to move forward with information contained in the published peer reviewed literature available at the time of the reassessment. A PBPK model that supports interspecies and interroute extrapolation was not available at the time of the assessment.

An analysis of interindividual variability among mice, as proposed by one of the reviewers, was not undertaken. The Glowa and Dews (1987) study examined schedule-controlled behavior in mice exposed to high concentrations of MEK for up to 2 hours. Because developmental toxicity served as the critical effect for the RfC, and because repeat-dose toxicity studies with MEK do not provide evidence of neurotoxicity, the proposed analysis of interindividual variability was not considered appropriate.

4. Charge Question: Has the matter of MEK's capacity to produce interactions with other toxicants (e.g., n-hexane) been sufficiently acknowledged and accommodated in the assessment?

Comments: In general, the external peer reviewers considered the discussion of MEK's capacity to interact with other toxicants to be sufficiently addressed. One reviewer directed EPA to a study by Couri et al. (1977) that reported the potential for MEK to increase potentiation of the end toxicant, 2,5-hexanedione (2,5-HD) from 2-hexanone. Another reviewer noted that the mechanism of potentiation as described in the assessment was not completely correct, and noted that the positive interaction between MEK and 2,5-HD in producing neurotoxicity was best understood by competitive inhibition by MEK of the enzymes responsible for metabolic detoxification of 2,5-HD.

Response: EPA reviewed the Couri et al. (1977) study, in which the exposure of rats to MEK in combination with methyl n-butyl ketone reduced hexobarbital sleep time. Blood levels of 2,5-HD and 2-hexanone were not measured, nor were other measures of neurotoxicity evaluated. It was determined that the study did not add appreciably to the current discussion of evidence for MEK's potentiation of neurotoxicants. The discussion of the mechanism of potentiation was revised, however, to state that potentiation of n-hexane neurotoxicity appears to be due to the

increased persistence of 2,5-HD in blood, probably due to the inhibition of 2,5-HD phase II biotransformation by MEK.

5. Charge Question: Are the appropriate uncertainty factors applied? Is the explanation for each transparent? Considering the nature of the critical effect, is an additional factor needed to reduce the point of departure to one that poses minimal health risk.

Comments: Three of the four reviewers considered the assigned uncertainty factors (UFs) to be appropriate and sufficiently justified. The three reviewers stated that no additional UF was needed. One of the three reviewers observed that mice typically exhibit spontaneous incidences of many skeletal effects, such as misaligned sternebrae, so the specific findings may be mouse specific. One reviewer commented that an additional factor was needed to reduce the point of departure to one that poses minimal health risk.

Response: Consistent with the comments of the majority of external peer reviewers, the UFs applied in deriving the RfC were retained.

D. Cancer Weight-of-Evidence Evaluation

1. Charge Question: The weight-of-evidence characterization is discussed in Section 4.6. Have appropriate criteria been applied from EPA's draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999)?

Comments: All four reviewers were of the same opinion that the 1999 draft revised cancer guidelines have been appropriately applied in characterizing the cancer weight-of-evidence. One reviewer noted that the Cox et al. (1975) multigeneration study in rats indicates that the F2 offspring were transferred to a chronic drinking water study of MEK, and questioned whether the report from that chronic study might be available. A second reviewer noted that the summary of carcinogenicity incorrectly suggested that MEK can produce bone and prostate cancer.

Response: The laboratory that performed the multigeneration study (Food and Drug Research Laboratories, Inc.) is no longer in operation. EPA's literature search did not identify a chronic drinking water study of MEK in the published literature, and does not have knowledge of an unpublished version of a chronic study. The summary statement that mentioned bone and prostate cancers was revised to state: "Although there is some suggestion of increased risk for

some cancers (including bone and prostate) and multiple solvent exposure that includes MEK, there is no clear evidence for a relationship between these cancers and MEK exposure alone."

E. Questions Regarding the Study by Cox et al. (1975)

The multigeneration reproductive and developmental toxicity study by Food and Drug Research Laboratories, Inc. (Cox et al., 1975) serves as the principal study for the RfD. EPA sought independent peer review of this laboratory report from two external peer reviewers because the study's findings were not published in the peer-reviewed literature and because the study was conducted prior to the introduction of Good Laboratory Practices.

1. Charge Question: Was the study design adequate?

Comments: One of the two reviewers considered the study to be adequately designed. A second reviewer commented that the study was based on early FDA testing guidelines, but did not consider the design adequate.

Response: EPA recognizes that the Cox et al. (1975) study design, which was based on early FDA testing guidelines, does not evaluate all endpoints that are covered under current testing protocols. Major deficiencies are discussed in Section 4.3.2.1. Discussion of other study design limitations have been added to the Toxicological Review.

2. Charge Question: Were the study findings adequately reported?

Comments: One of the two reviewers indicated that findings were, in general, adequately reported. The reviewer noted the absence of discussion of male reproductive effects in terms of mating success and failure, and in particular pointed to data in Appendix II of Cox et al. (1975) that suggested a potential adverse impact on reproductive performance, but not on the fertility of high-dose males. The reviewer summarized the high dose male data as follows:

Male Rat Copulatory Success When Breeding F1A Litters*				
Copulation	**Control**	**0.3%**	**1%**	**3%**
Failed	1	2	0	6
Succeeded	29	28	30	24
Total	30	30	30	30

* Successful mating was judged by the presence of a vaginal plug or sperm in a
vaginal smear. Males that failed, by the end of the second cycle, to mate successfully
according to that measure were replaced by a previously successful male. In Appendix
II of Cox et al. (1975), the second entry of a male rat number was interpreted as
indicative of a mating failure.
Source: Adapted from Cox et al., 1975.

In light of the data on F0 male copulatory success, the reviewer also questioned whether the high dose is a NOAEL for reproductive toxicity for both males and females. The reviewer concluded with the observation that even if an effect were determined to be statistically or toxicologically significant, it is unlikely to have altered selection of the critical effect. The second reviewer noted the following reporting inadequacies: presentation of means/group, with no variance term (e.g., standard deviation or standard error) and no statistical analyses of any parameter.

Response: A discussion of the incidence of F0 male rats that did not successfully copulate with F0 females was added to the Toxicological Review. The identification of the high-dose group as a NOAEL for reproductive toxicity was qualified with the observation of a possible increase in male copulatory failure at that dose level.

EPA recognizes that the study presents means/group without a variance term. Because appendices to the report provided individual litter data, statistical analyses were conducted for certain data sets and standard deviations were calculated. Standard deviations were included in the Toxicological Review.

3. Charge Question: Were the authors' conclusions supported by the results?

Comments: One reviewer maintained that the conclusions are supported by the study results. A second reviewer did not, in all instances, find the conclusions adequately supported. In the absence of statistical analyses, the reviewer considered the authors' conclusions to be "'cautious' and very conservative (e.g., effects may have been observed by the authors did not designate them as 'significant' or necessarily treatment- or concentration-related)."

Response: EPA conducted independent statistical analysis of the data from Cox et al. (1975) and based its conclusions on the findings from those analyses. Therefore, the assessment for MEK was not necessarily limited by instances where the study authors' conclusions were necessarily "cautious."

4. Charge Question: Are there any notable limitations or deficiencies in this study?

Comments: One reviewer did not identify any notable limitations or deficiencies in the study. The second reviewer, however, identified the following deficiencies: (1) no water consumption during gestation and lactation, so maternal intake cannot be correlated with maternal and offspring effects; (2) no dosed water during the last two weeks of F0 lactation of F1A litters; (3) the high concentration was changed from 3.0 to 0% during the last two weeks of lactation, to 2% for the postwean F1A offspring; (4) no information was presented on the concentration provided to the F0 parents after the weaning of F1A litters; (5) only minimal types of assessment at minimal times were assessed for critical parameters (e.g., body weights, feed and water consumption, clinical signs, etc.); (6) limited numbers of F1A adult animals/group (and none of the F0 and F2) were assessed for clinical chemistry/hematology and histopathology; (7) data were presented as means/group with no variance terms and no statistical analyses; and (8) the conclusions were "tepid," probably due to the lack of statistical analyses and the very limited assessments performed, with no summarized information or conclusions for effects at 3%.

Response: Additional discussion of the specific limitations identified by one reviewer was included in Section 4.3.2.1. of the Toxicological Review.

5. Charge Question: Is EPA's summary of the study in the Toxicological Review and analysis of the study findings appropriate?

Comments: One reviewer concurred with the use of the Cox et al. (1975) study and the selection of reduced neonatal body weight as the critical effect, but questioned the designation of 1% 2-butanol as the LOAEL for neonatal body weight, which the study authors regarded as a no-effect level. The reviewer stated that day 4 and day 21 body weight reductions in the F1A generation did not appear to be statistically significant in the mid-dose (1%) group, and the designation of the 1% solution as the LOAEL was not adequately justified. A second reviewer noted that EPA focused on the few strengths of the study and ignored its weaknesses. In particular, the reviewer noted that effects on body weight in male F1A pups on postnatal day 21 occurred in the absence

of dosing (since treatment was discontinued for the last two weeks of F1A lactation), and questioned how EPA could assign a dose to this effect.

Response: EPA reconsidered the designation of NOAELs and LOAELs from the Cox et al. (1975) study. Rather than designate these values for each generation, a NOAEL and LOAEL for the study as a whole were established. Although the F1A pup body weights for the low- and mid-dose groups were 4 to 10% lower than the control, a similar reduction was not observed in subsequent generations (i.e., F1B and F2). Therefore, the mid-dose group (1%) was considered to be the NOAEL and the 2% group the LOAEL, consistent with the interpretation of the study investigators.

EPA was similarly concerned with the interpretation of the F1A data set at the high dose resulting from changes in the 2-butanol drinking water concentration during lactation. Accordingly, EPA did not use pup body weight data from the high-dose group in the dose-response analysis of F1A pup body weight. Body weight data from the control, low- and mid-dose groups only were used.

6. Charge Question: Overall, was the study as designed, performed, and reported of sufficient quality to use as the basis for the RfD?

Comments: One reviewer considered the study to be suitable as the basis for the RfD, while the second did not. The second reviewer noted that for its time (1973 to 1975) the study was conducted as well or better than many, and more or less followed FDA multigeneration study design. In the opinion of this reviewer, however, the study is not supportable when compared against current guidelines as the basis for the RfD.

Response: EPA acknowledges the limitations of the Cox et al. (1975) study design when compared against current protocols for multigeneration studies. The study shows dose-related effects, however, that are consistent across generations and appear to be attributable to 2-butanol exposure. If this study was deemed to be inadequate, there would be no alternative basis for derivation of an RfD for MEK. Because the study deficiencies identified by an external peer reviewer were a function of the age of the study and not inappropriate study conduct, and because compound-related effects were reported, EPA decided to retain the Cox et al. (1975) study as the principal study for the RfD.

F. Additional Comments from External Peer Reviewers

Comment: Several of the reviewers offered editorial comments and suggestions to improve the clarity of the text.

Response: In most instances, the suggested editorial changes and revisions to the text were incorporated.

Comment: One reviewer suggested that the EPA create a PBPK model for dosimetic comparisons across species. The reviewer considered the data on percentage retention as a function of the duration of exposure to be an excellent basis for calibrating human PBPK models. Because absorption and therefore metabolism appear to be dose dependent, the reviewer suggested that PBPK modeling was needed to sort out the likely changes in the relationship between external and internal exposure levels between the high doses where testing was done and the lower environmental doses to which humans might be exposed.

Response: PBPK models have been developed for MEK in humans and in the rat, but a mouse model has not been developed. Development of a mouse model would require parameterization for the mouse, validation, and calibration against an additional data set. In addition, such a model would need to be subject to peer review. Given the need for timely reassessment of the health effects of MEK and associated reference values, EPA decided to move forward with the published peer reviewed literature at hand at the time of the reassessment. EPA is open to considering other PBPK models that are developed in the future.

Comment: One reviewer pointed out a conceptual or typographic error in Table 1 (Kinetic parameters used for PBPK models for MEK kinetics in humans and rats). This reviewer noted that because the entire cardiac output must pass through the lungs before returning to the heart, the blood flow to the lungs must be listed as 100%, and the flows to the remaining tissues should also total 100%.

Response: The table was corrected as suggested by the reviewer.

Comment: Two reviewers offered additional comments on the summary of the Cox et al. (1975) study. One reviewer recommended that the observations in the F1A and F2 litters be discussed in terms of statistical significance (or lack thereof), and that the results of the F0 male rat's copulatory success be noted. The second reviewer disagreed with the LOAEL and NOAEL designations for fetal growth inhibition, and recommended that the decrease in pup weight would

better be described as a continuous function of dose with a defined slope than as something that becomes statistically significant only at defined high doses.

Response: EPA believes that an analysis of the biological significance of the findings of the Cox et al. (1975) study is more relevant than a statistical analysis of the results. To that end, the discussion of the biological significance of the study findings was expanded. Further, statistical analysis was conducted as part of the BMD analysis of the data; BMD software outputs are provided in Appendix B of the Toxicological Review. While the analysis does not include pair wise statistical tests, it does include a log-likelihood ratio test that provides an indication of whether or not pup/fetal body weights changed significantly with increasing dose levels. This type of analysis appears to be consistent with the recommendations of the second reviewer.

Comments from the Public

Comment: One public commenter concurred with the use of the Cox et al. (1975) study as an appropriate basis for the RfD, but found inadequate justification for modifying the NOAEL and LOAEL from that presented previously on IRIS. The commenter noted that the IRIS Summary (posted in 1993) for MEK considered 1,771 mg/kg-day to be a NOAEL, whereas this dose level was considered to be a LOAEL in the external review draft based on reduced pup weight observed in F1A litters, but not F1B or F2 litters. The commenter also noted that the study was recently reviewed by EPA scientists as part of an OECD SIDS Dossier and SIAR for 2-butanol, and in that context 1,771 mg/kg-day was regarded as a NOAEL.

Response: A similar comment was received from one of the external peer reviewers. As discussed above, the summary of the Cox et al. (1975) study was revised such that a NOAEL and LOAEL for the study as a whole were identified. Because the body weight reductions compared to control in the low- and mid-dose groups in F1A pups were not observed in subsequent generations (i.e., F1B and F2), a NOAEL and LOAEL for the study as a whole of 1% (1,771 mg/kg-day) and 2% (3,122 mg/kg-day), respectively, were presented.

Comment: A commenter did not consider the 5% decrease in mean pup or fetus body weight to be sufficiently justified, particularly when 5% is less than one standard deviation and the purported effect was not observed at 1,771 mg/kg-day in the F1B and F2 litters. The commenter indicated that it is more scientifically reasonable to use a 10% reduction in pup weight as the benchmark response. The commenter also considered the inconsistent selection of a benchmark response rate (either 5% or 10%) to be arbitrary.

Response: One standard deviation as a benchmark response (BMR) serves as a recommended point of comparison across assessments, but is generally considered the last choice as the basis for the BMR if there is no other basis for a biologically relevant degree of change in response. In the case of pup body weight data from the Cox et al. (1975) study upon which the RfD was based, there is no specific decrement that is generally regarded as indicative of a biologically relevant response. EPA considered the use of one standard deviation as the BMR. Using data from Cox et al. (1975), one standard deviation from the control mean resulted in BMDs that corresponded to body weights 9 to 26% below the control mean (see Tables 6, 7 and 9) – values generally above the range of experimental data. Because an aim in BMD modeling is to select a BMD within the range of observation, other measures of the BMR were examined. A 5% reduction in fetal/pup body weight relative to the control was a response rate that fell within the range of experimental dose levels in the Cox et al. (1975) study, and consequently was selected as the benchmark response (BMR). In addition, an ED_{10} and LED_{10} for each endpoint were estimated as a consistent point of comparison across chemicals, as recommended in the Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c). These additional measures are provided in Appendix B. The basis for the selection of a BMR of 5% is discussed in Section 5.1.2.1. of the Toxicological Review.

In the case of the RfC, two data sets from Schwetz et al. (1991) were analyzed by BMD methods. A decrease in mean fetal weight of one standard deviation of the control mean was selected as the BMR for this endpoint. The BMR corresponds to an approximately 5% decrease in mean body weight for the data set, and a BMD generally within the range of experimental dose levels used in the Schwetz et al. (1991) study. Ten percent extra risk was used as a BMR for the dichotomous response, misaligned sternebrae, also from Schwetz et al. (1991) because the BMR corresponds to a BMD within the range of the experimental dose levels. EPA also tried using 5% extra risk as a BMR in the analysis of misaligned sternebrae, but the nested model could not provide a useful estimate of the lower bound on the BMD for this BMR (i.e., the lower bound on the BMD was estimated as essentially zero). The basis for selecting these BMRs is discussed in Sections 5.2.2.1.2. and 5.2.2.1.3. of the Toxicological Review.

Comment: A commenter stated that decreased fetal birth weight, rather than an increased incidence of misaligned sternebrae, is the more scientifically sound basis for the RfC. The commenter noted that misaligned sternebrae represents an anomalous skeletal variation that was seen in only one of four developmental toxicity studies of MEK. The commenter also observed that since the control standard deviation is 10%, approximately 15% of the control litters have an "adverse finding," suggesting that the benchmark response is too restrictive.

Response: As discussed in the response to external peer reviewer comments, EPA reevaluated study findings for misaligned sternebrae as reported by Schwetz et al. (1991) and Mast et al. (1989) and the dose-response analyses for both misaligned sternebrae and fetal weight from this study. The endpoints were given further consideration as potential critical effects. Based on deliberations during consensus review, it was decided to use misaligned sternebrae as the endpoint yielding the more health protective point of departure.

Comment: One commenter stated that EPA did not present an adequate scientific justification for applying a duration adjustment to the inhalation developmental toxicity study and, at the very least, the additional conservatism added by the application of this factor should be explicitly recognized. The commenter pointed to the statement in the Toxicological Review that "MEK is rapidly absorbed, distributed, and metabolized, suggesting that duration adjustment may be inappropriate," and to EPA's conclusions that ultimately there was not "sufficient evidence to argue convincingly for either peak exposure level or area under the curve."

Response: Duration adjustment of the exposure concentrations in the developmental study of MEK (Schwetz et al., 1991) was performed consistent with recent EPA guidance, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002). The document recommends that duration adjustment procedures to continuous exposure based on C × t be used as a default procedure for inhalation developmental toxicity studies as it is for other health effects from inhalation exposure. The recommendation is based on evidence that shows that some agents cause developmental toxicity more as a function of peak concentration, whereas the effects of other agents are related to area-under-the-curve (AUC). The latter is true even of some developmental toxicants with a short half-life. In the absence of data that supports peak concentration or AUC as more closely correlated with developmental toxicity, EPA's 2002 review document recommends duration adjustment as the more health protective default procedure. As noted in the Toxicological Review of MEK, because the data are insufficient to argue convincingly for either peak exposure level or AUC as the most appropriate metric, the more health protective procedure (duration adjustment) was applied as a policy matter. The text of the Toxicological Review was revised to better support this decision.

Comment: One commenter disagreed with EPA's interpretation of the high-exposure level (5,000 ppm) in the Cavender et al. (1983) study as a LOAEL based on reduced body weight, increased liver weight, and decreased brain weight. The commenter believed that these organ weight changes, in the absence of histopathology, should not be considered adverse effects and that the 5,000 ppm exposure level should be considered a NOAEL. The commenter also noted

that an OECD SIDS Dossier and SIAR for 2-butanol identified a NOAEL of 5,000 ppm on the basis that the changes in organ weight and clinical pathology parameters were not supported by histological changes.

Response: EPA gave further consideration to the biological significance of the findings in the 5,000 ppm animals in the Cavender et al. (1983) study, and in particular the organ weight findings. Although the decrease in brain weight in female high-dose animals is of some concern, EPA agrees that this effect, in the absence of corresponding histopathology and functional abnormalities, cannot be clearly characterized as being of toxicological relevance. The text was revised to further emphasize the difficulty in interpreting the significance of these findings. In light of these uncertainties, characterization of the 5,000 ppm exposure level as a LOAEL and the mid-dose group (2,518 ppm) as a NOAEL were dropped.

Comment: A commenter agreed with EPA's characterization of the Mitran et al. (1997) study, but noted that the California Office of Environmental Health Hazard Assessment (OEHHA) has withdrawn its draft Reference Exposure Level (REL) based on the study.

Response: According to Dr. Andrew Salmon of Cal EPA's OEHHA (May 27, 2003), the REL for MEK has not been withdrawn. A draft of the REL is still available; however, OEHHA is not working to finalize the MEK assessment at this time. One of the primary issues encountered in reviewing the REL concerned the Mitran et al. (1997) study that serves as the basis for the draft REL. Cal EPA's science review panel expressed concerns with the findings of the study. Their concerns are consistent with those raised by EPA in review of the MEK literature.

APPENDIX B: BENCHMARK DOSE MODELING RESULTS AND OUTPUT

Output B-1:   Reduced Pup Body Weight in Wistar Rats, F1A Generation at Postnatal Day 4 (Cox et al., 1975)
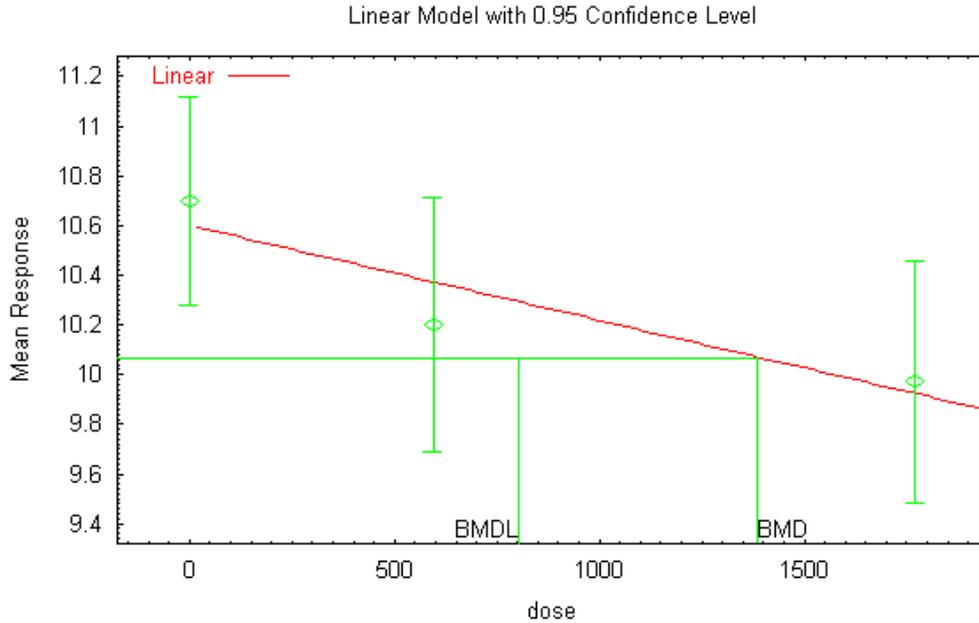
Linear Model with 0.95 Confidence Level

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

   The form of the response function is:

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

   Dependent variable = MEAN
   Independent variable = Dose
   rho is set to 0
   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 =        1.52807
                          rho =              0    Specified
                       beta_0 =        10.5909
                       beta_1 =    -0.00038168

                      Parameter Estimates
        Variable           Estimate              Std. Err.
          alpha            1.48828               0.226961
         beta_0            10.5956               0.193304
         beta_1         -0.00038201              0.000176104

          Asymptotic Correlation Matrix of Parameter Estimates

                    alpha         beta_0         beta_1
      alpha             1      -9.3e-010       1.9e-009
     beta_0     -9.3e-010              1          -0.73
     beta_1      1.9e-009          -0.73              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       29     10.7         1.1         10.6         1.22          2.48
  594       27     10.2         1.3         10.4         1.22         -3.73
 1771       30     9.97         1.3         9.92         1.22          1.25

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         -59.705513         4     127.411027
            A2         -59.191916         6     130.383833
          fitted       -60.097751         2     124.195502
            R          -62.891337         2     129.782675


 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           7.39884               4         0.02474
   Test 2           1.02719               2         0.5983
   Test 3          0.784476               1         0.3758

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 =          0.05
Risk Type        =      Relative risk
Confidence level =          0.95

          BMD =      1386.83
          BMDL =      803.086


Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Relative risk
Confidence level =          0.95
          BMD =      2773.65
          BMDL =      1606.17
```

Output B-2:   Reduced Pup Body Weight in Wistar Rats, F1A Generation at Postnatal Day 21 (Cox et al., 1975)

Linear Model with 0.95 Confidence Level

17:10 03/03 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 = 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 = 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 =        17.7256
                        rho =              0    Specified
                     beta_0 =        48.8619
                     beta_1 =     -0.00278464

                     Parameter Estimates
      Variable          Estimate              Std. Err.
        alpha            17.1217               2.62635
        beta_0           48.8645               0.66404
        beta_1        -0.00278278           0.000601426

        Asymptotic Correlation Matrix of Parameter Estimates
               alpha         beta_0         beta_1
    alpha          1         -2e-007        2.5e-007
    beta_0    -2e-007              1           -0.74
    beta_1   2.5e-007          -0.74               1
```

B-3

```
     Table of Data and Estimated Values of Interest
Dose       N     Obs Mean    Obs Std Dev   Est Mean    Est Std Dev   Chi^2 Res.
------     ---    --------    -----------   --------    -----------   ----------
    0      28        49          3.8          48.9         4.14         0.917
  594      27        47          3.9          47.2         4.14        -1.38
 1771      30        44          4.8          43.9         4.14         0.463

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        -163.160835        4    334.321669
            A2        -162.157710        6    336.315419
           fitted     -163.214728        2    330.429455
             R        -172.764782        2    349.529563

 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           21.2141             4         <.0001
   Test 2            2.00625            2         0.3667
   Test 3            0.107786           1         0.7427
```

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 =          0.05
Risk Type        =      Relative
Confidence level =          0.95
         BMD =       877.979
        BMDL =       656.797


Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Relative
Confidence level =          0.95
         BMD =       1755.96
        BMDL =       1313.59
```
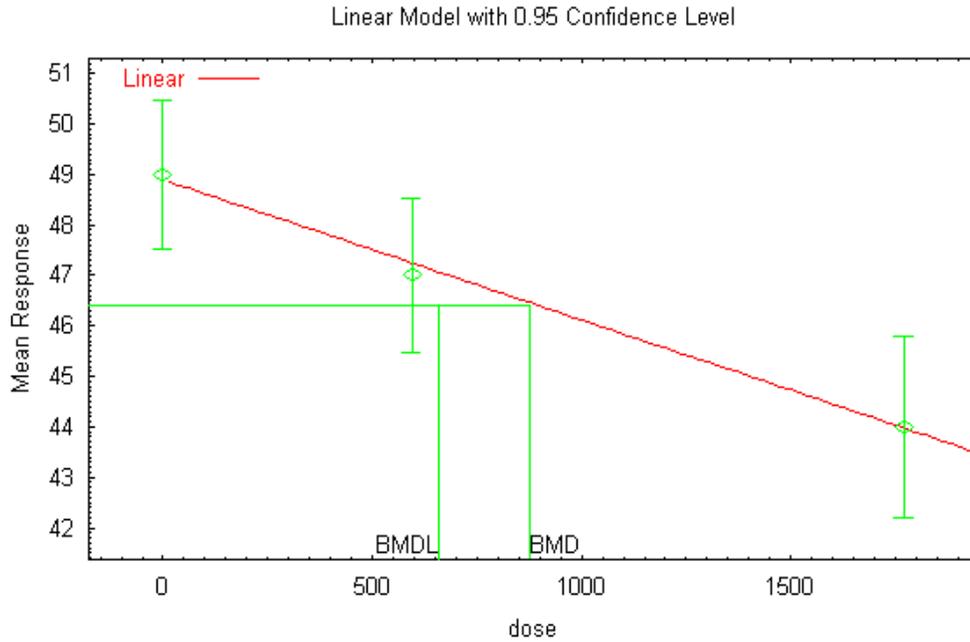
Output B-3:   Reduced Fetal Weight in Wistar Rats, F1B Generation (Cox et al., 1975)

Polynomial Model with 0.95 Confidence Level

17:16 03/03 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 = dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                      alpha =       1.18178
                        rho =             0    Specified
                     beta_0 =       4.07806
                     beta_1 =             0
                     beta_2 = -1.71632e-007

                       Parameter Estimates
       Variable          Estimate              Std. Err.
         alpha            1.16541               0.15369
         beta_0           4.25434               0.132747
         beta_1                 0                    NA
         beta_2      -4.4034e-008          2.57616e-008

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
     has no standard error.

         Asymptotic Correlation Matrix of Parameter Estimates
                 alpha         beta_0        beta_2
     alpha           1      -9.1e-008      1.2e-007
     beta_0  -9.1e-008              1         -0.65
     beta_2   1.2e-007          -0.65             1
```

The following parameter(s) have been estimated at a boundary point or have been specified.
Correlations are not computed:
beta_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   29   4.14   1.45   4.25   1.08   -3.07
   594   27   4.16   0.69   4.24   1.08   -1.97
  1771   30   4.38   1.04   4.12   1.08   7.33
  3122   29   3.74   1.01   3.83   1.08   -2.29

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   -65.068238   5   140.136476
           A2   -57.686139   8   131.372278
         fitted   -66.301668   2   136.603336
            R   -67.746445   2   139.492890

 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   20.1206   6   0.0001602
   Test 2   14.7642   3   0.00203
   Test 3   2.46686   1   0.1163

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
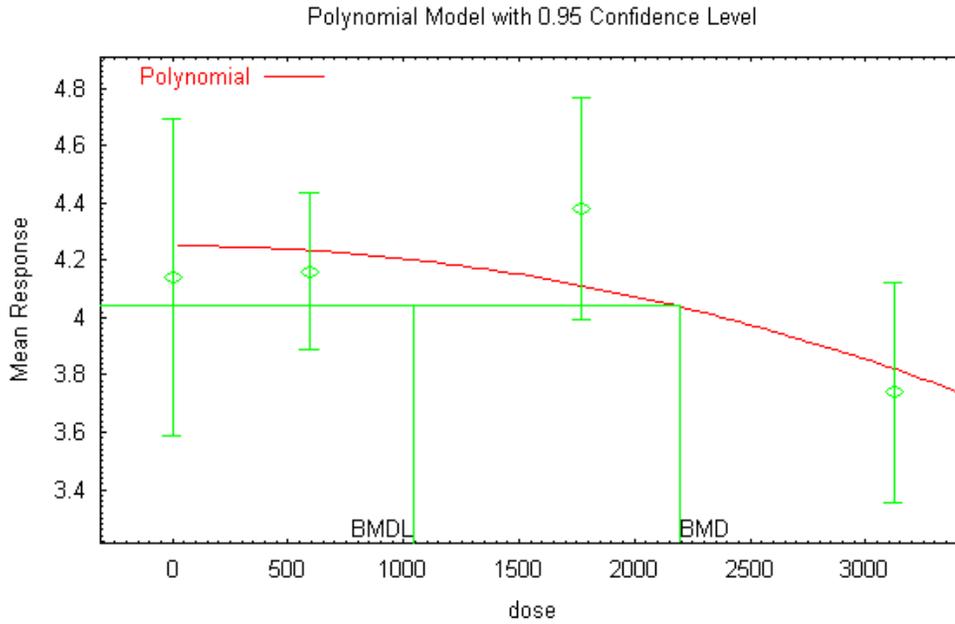
The p-value for Test 2 is less than .05. Consider running a non-homogeneous variance model

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

Benchmark Dose Computation
Specified effect =   0.05
Risk Type   =   Relative risk
Confidence level =   0.95
           BMD =   2197.9
          BMDL =   1046.23

Benchmark Dose Computation
Specified effect =   0.1
Risk Type   =   Relative risk
Confidence level =   0.95
           BMD =   3108.29
          BMDL =   2085.07

Output B-4: **Reduced Pup Body Weight in Wistar Rats, F2 Generation at Postnatal Day 4 (Cox et al., 1975)**

Linear Model with 0.95 Confidence Level
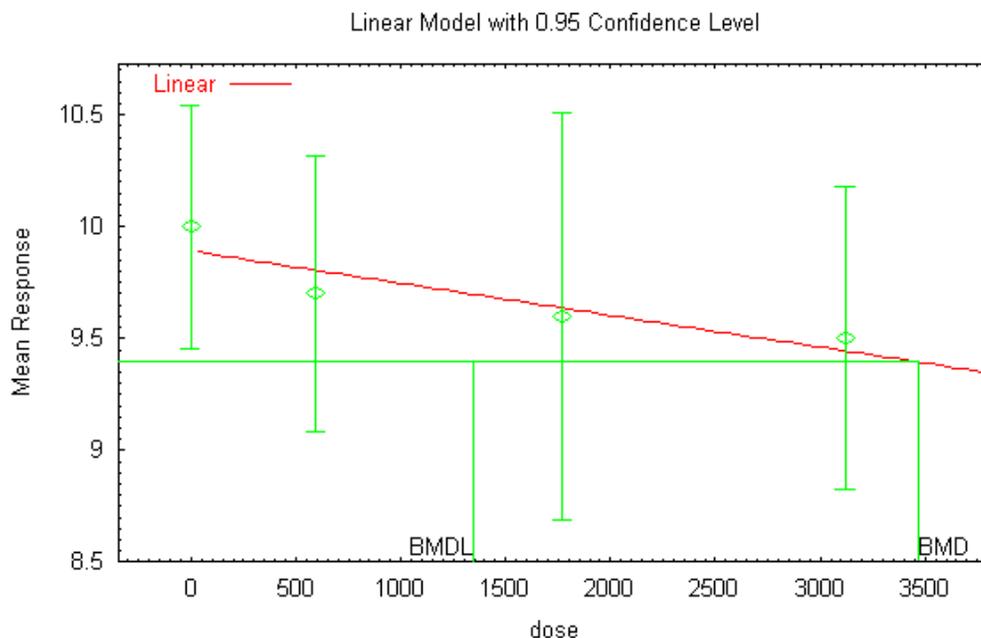
08:22 03/04 2003

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

                  Default Initial Parameter Values
                        alpha =      3.09184
                          rho =            0    Specified
                       beta_0 =      9.89257
                       beta_1 = -0.000140383

                       Parameter Estimates
      Variable           Estimate              Std. Err.
        alpha            2.98335               0.407875
        beta_0           9.89404               0.249663
        beta_1        -0.000142507             0.00014249

        Asymptotic Correlation Matrix of Parameter Estimates
                  alpha         beta_0         beta_1
   alpha              1       -4.5e-008       5.4e-008
   beta_0     -4.5e-008              1          -0.74
   beta_1      5.4e-008          -0.74              1
```

B-7

```
     Table of Data and Estimated Values of Interest
 Dose       N     Obs Mean    Obs Std Dev    Est Mean    Est Std Dev    Chi^2 Res
 ------     ---   --------    -----------    --------    -----------    ----------
     0      28       10          1.4          9.89         1.73          1.72
   594      28       9.7         1.6          9.81         1.73         -1.77
  1771      27       9.6         2.3          9.64         1.73         -0.651
  3122      24       9.5         1.6          9.45         1.73          0.707

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        -111.850740         5     233.701481
           A2        -107.811454         8     231.622908
         fitted      -111.977995         2     227.955990
            R        -112.478139         2     228.956278


 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           9.33337              6        0.02517
   Test 2           8.07857              3        0.04442
   Test 3           0.254509             2        0.8805
```

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 =          0.05
Risk Type        =      Relative risk
Confidence level =          0.95
          BMD =        3471.42
          BMDL =       1347.21

Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Relative risk
Confidence level =          0.95
          BMD =        6942.85
          BMDL =       2694.43
```
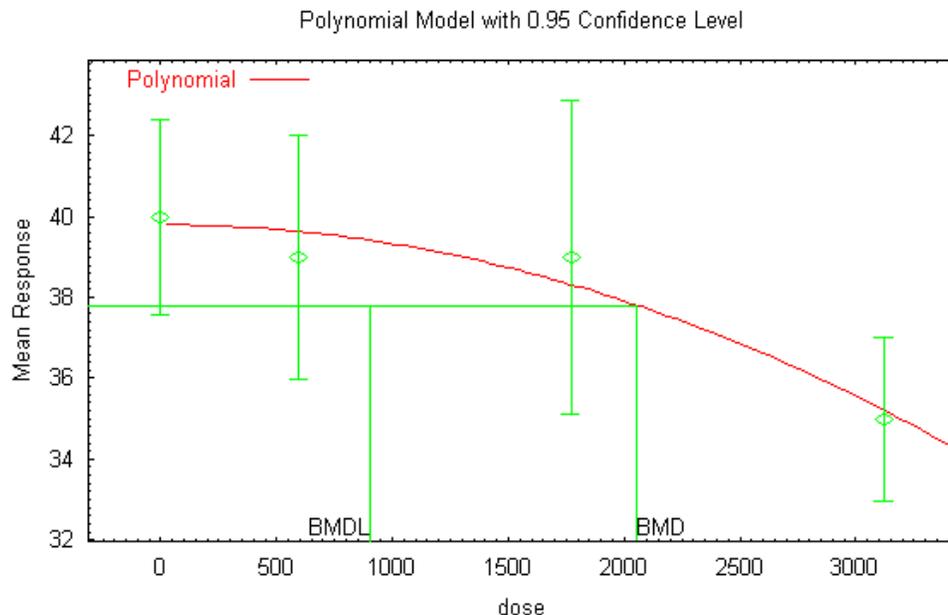
Output B-5: **Reduced Pup Body Weight in Wistar Rats, F2 Generation at Postnatal Day 21 (Cox et al., 1975)**

Polynomial Model with 0.95 Confidence Level

17:26 03/03 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 = dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit
   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

                 Default Initial Parameter Values
                        alpha =      52.6945
                          rho =            0    Specified
                       beta_0 =      39.6222
                       beta_1 =            0
                       beta_2 = -6.24081e-007

                       Parameter Estimates
        Variable           Estimate              Std. Err.
         alpha             50.8889                7.0912
         beta_0            39.7965              0.899513
         beta_1                  0                  NA
         beta_2       -4.70576e-007          1.85023e-007

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus
     has no standard error.

          Asymptotic Correlation Matrix of Parameter Estimates
                  alpha        beta_0        beta_2
       alpha          1       2.4e-008      4.8e-008
      beta_0   2.4e-008             1          -0.62
      beta_2   4.8e-008         -0.62             1
```

The following parameter(s) have been estimated at a boundary point or have been specified.
Correlations are not computed:
beta_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     27       40          6.1          39.8         7.13          0.77
   594     28       39          7.8          39.6         7.13         -2.47
  1771     25       39          9.4          38.3         7.13          2.38
  3122     23       35          4.7          35.2         7.13         -0.677
```

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        -253.632495        5    517.264989
             A2        -247.410830        8    510.821659
           fitted      -253.876754        2    511.753508
              R        -257.015989        2    518.031978
```

 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           19.2103             6        0.0002473
   Test 2           12.4433             3         0.006009
   Test 3          0.488518             1          0.4846
```

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 =        0.05
Risk Type         =     Relative risk
Confidence level =        0.95
          BMD =        2056.33
          BMDL =        900.888

Benchmark Dose Computation
Specified effect =        0.1
Risk Type         =     Relative risk
Confidence level =        0.95
          BMD =        2908.09
          BMDL =        1801.78
```

Output B-6: Increased Incidence of Extra Ribs in Sprague-Dawley Rats (Deacon et al., 1981)

NCTR Model with 0.95 Confidence Level
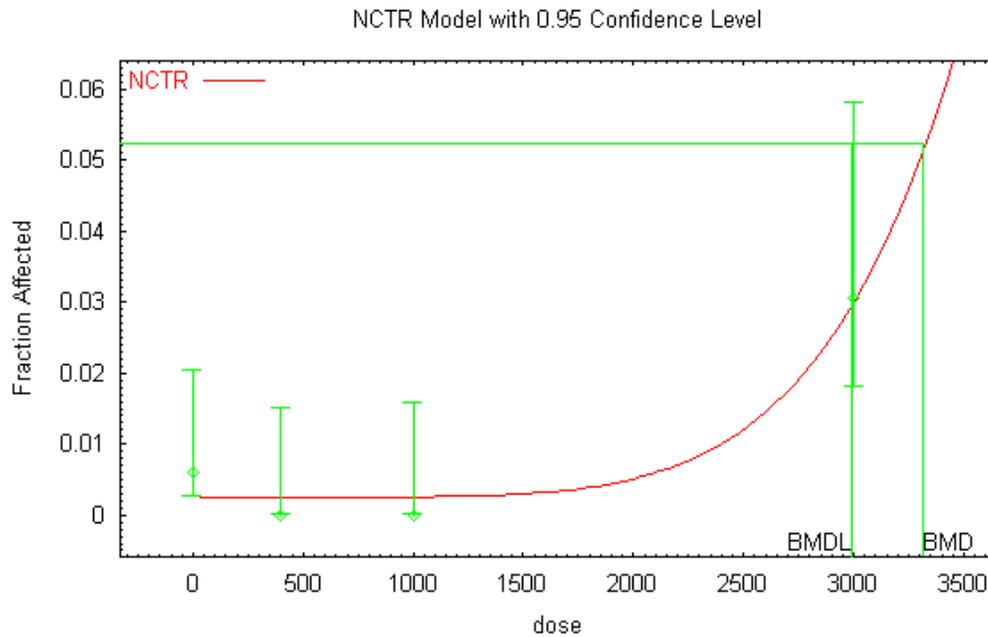
16:36 03/05 2003

```
BMDS MODEL RUN: NCTR Model
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The probability function is:

 Prob. = 1 - exp[-(alpha + th1*Rij) - (beta + th2*Rij)*Dose^rho],

         where Rij is the centralized litter specific covariate.

 Restrict Power rho >= 1.

 Total number of observations = 82
 Total number of records with missing values = 0
 Total number of parameters in model = 9
 Total number of specified parameters = 6

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

****  We are sorry but Relative Function and Parameter Convergence   ****
****  are currently unavailable in this model.  Please keep checking ****
****  the web sight for model updates which will eventually          ****
****  incorporate these convergence criterion.  Default values used. ****

 User specifies the following parameters:
          theta1 =              0
          theta2 =              0
            phi1 =              0
            phi2 =              0
            phi3 =              0
            phi4 =              0

                Default Initial Parameter Values
                      alpha =    0.00255273
                       beta = 2.32553e-013
                        rho =       3.18392

Warning: Maximum iteration may be not large enough. Iterations reach the maximum.

                    Parameter Estimates
      Variable          Estimate              Std. Err.
```

B-11

```
        alpha           0.00252937           0.00183262
         beta         1.0926e-022          3.33415e-019
          rho             5.87127              381.143

                    Analysis of Deviance Table
          Model      Log(likelihood)     Deviance  Test DF     P-value
     Full model          -29.869
    Fitted model        -45.2723         30.8066      80           1
   Reduced model        -51.542          43.3461      81        0.9998

        AIC:              94.5446  AIC=-2*Log(likelihood)-2*p=96.5446

                    Goodness  of  Fit

     Dose   Litter_Size   Est._Prob.   Expected   Observed
    ------------------------------------------------------------
     0.0000        4         0.003        0.010        0
     0.0000        8         0.003        0.020        0
     0.0000       10         0.003        0.025        0
     0.0000       11         0.003        0.056        0
     0.0000       12         0.003        0.121        0
     0.0000       13         0.003        0.263        1
     0.0000       14         0.003        0.177        0
     0.0000       15         0.003        0.076        0
     0.0000       16         0.003        0.040        1
     0.0000       17         0.003        0.043        0

   400.0000        8         0.003        0.040        0
   400.0000        9         0.003        0.023        0
   400.0000       11         0.003        0.028        0
   400.0000       12         0.003        0.061        0
   400.0000       13         0.003        0.197        0
   400.0000       14         0.003        0.212        0
   400.0000       15         0.003        0.038        0

  1000.0000        5         0.003        0.013        0
  1000.0000        6         0.003        0.015        0
  1000.0000        9         0.003        0.023        0
  1000.0000       10         0.003        0.051        0
  1000.0000       11         0.003        0.113        0
  1000.0000       12         0.003        0.031        0
  1000.0000       13         0.003        0.067        0
  1000.0000       14         0.003        0.072        0
  1000.0000       15         0.003        0.154        0
  1000.0000       16         0.003        0.041        0

  3000.0000        4         0.030        0.122        0
  3000.0000        8         0.030        0.244        1
  3000.0000        9         0.030        0.274        0
  3000.0000       11         0.030        1.006        2
  3000.0000       12         0.030        0.366        1
  3000.0000       13         0.030        1.188        1
  3000.0000       14         0.030        1.280        1
  3000.0000       15         0.030        0.914        0
  3000.0000       16         0.030        0.488        0
  3000.0000       17         0.030        0.518        1
  3000.0000       19         0.030        0.579        0

   Chi-square =      34.20    DF = 35    P-value = 0.5064
```

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of control group: 12.653846

```
Benchmark Dose Computation
Specified effect =          0.05
Risk Type        =      Extra risk
Confidence level =      0.950000
          BMD =          3317.45
          BMDL =         2992.69
```

B-12

Output B-7: Reduced Fetal Weight in CD-1 Mice (Schwetz et al., 1991/Mast et al., 1989)

Linear Model with 0.95 Confidence Level
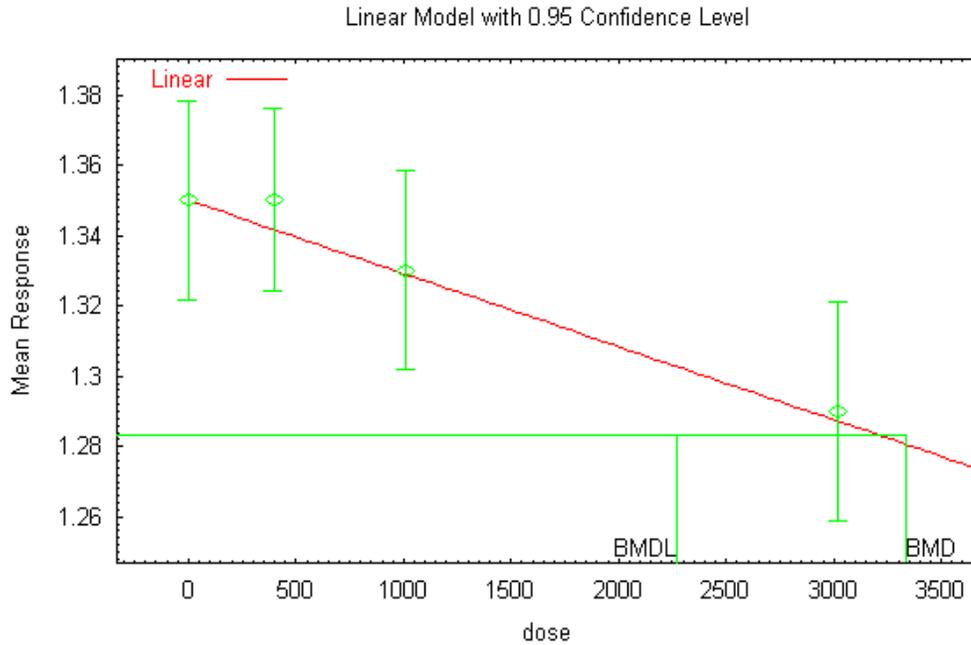
16:43 03/13 2003

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

                Default Initial Parameter Values
                      alpha =    0.0050202
                        rho =            0   Specified
                     beta_0 =      1.35314
                     beta_1 = -2.09075e-005

                     Parameter Estimates
        Variable          Estimate              Std. Err.
          alpha          0.00483473          0.000673703
          beta_0             1.3529           0.00958809
          beta_1       -2.08259e-005          5.75863e-006

        Asymptotic Correlation Matrix of Parameter Estimates
                  alpha          beta_0          beta_1
     alpha            1       -8.6e-009         6e-009
    beta_0    -8.6e-009              1           -0.7
    beta_1       6e-009           -0.7              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     26      1.35        0.07         1.35       0.0695        -1.08
  398     23      1.35        0.06         1.34       0.0695         1.78
 1010     26      1.33        0.07         1.33       0.0695        -0.697
 3020     28      1.29        0.08         1.29       0.0695        -0.0018


  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         223.195553         5     -436.391107
             A2         224.250451         8     -432.500903
           fitted       223.094409         2     -442.188818
              R         216.935622         2     -429.871245

 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           14.6297             6        0.002162
   Test 2            2.1098             3         0.5499
   Test 3          0.202289             2         0.9038


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 =       3338.74
        BMDL =       2272.53
```

Output B-8:   Incidence of Misaligned Sternebrae in CD-1 Mice (Schwetz et al., 1991/Mast et al., 1989)

Nested Logistic Model with 0.95 Confidence Level

15:40 03/25 2003
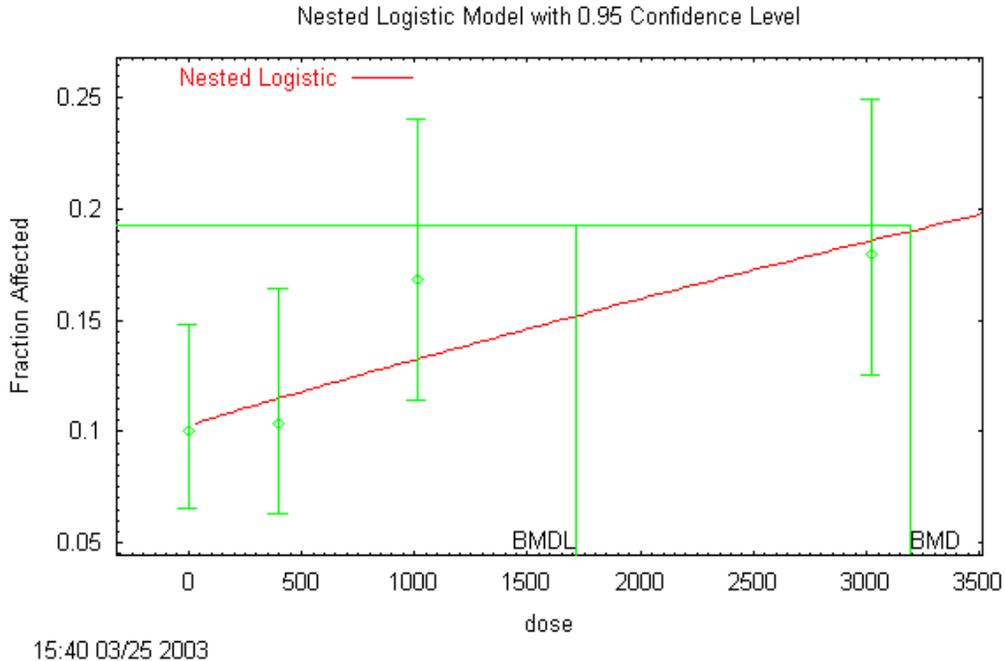
```
BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

 The probability function is:
 Prob. = alpha + theta1*Rij + [1 - alpha - theta1*Rij]/

                   [1+exp(-beta-theta2*Rij-rho*log(Dose))],

         where Rij is the litter specific covariate.

 Restrict Power rho >= 1.

 Total number of observations = 103
 Total number of records with missing values = 0
 Total number of parameters in model = 9
 Total number of specified parameters = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

 User specifies the following parameters:
          theta1 =            0
          theta2 =            0

                Default Initial Parameter Values
                    alpha =       0.102954
                     beta =       -10.2672
                   theta1 =              0    Specified
                   theta2 =              0    Specified
                      rho =              1
                    phi1 =      0.0232129
                    phi2 =      0.0522594
                    phi3 =       0.107766
                    phi4 =      0.0855581
```

B-15

```
                        Parameter Estimates
          Variable         Estimate            Std. Err.
            alpha          0.102937            0.0156965
            beta           -10.2671            0.457475
             rho              1                 Bounded
            phi1           0.023208            0.0571841
            phi2           0.052033            0.0519001
            phi3           0.107815                0
            phi4           0.0855465               0

                     Analysis of Deviance Table
        Model      Log(likelihood)  Deviance   Test DF    P-value
     Full model      -377.311
    Fitted model     -462.552       170.481      97     5.9568151e-006
   Reduced model     -478.095       201.568     102        <.0001

          AIC:           937.104

                           Litter Data
            Lit.-Spec.              Litter                    chi-squared
     Dose      Cov.      Est._Prob.  Size   Expected  Observed  Residual
    -------------------------------------------------------------------
      0.0000    5.0000     0.103       5     0.515        0      -0.7246
      0.0000    7.0000     0.103       7     0.721        0      -0.8397
      0.0000   10.0000     0.103      10     1.029        1      -0.0278
      0.0000   11.0000     0.103      11     1.132        4       2.5634
      0.0000   11.0000     0.103      11     1.132        1      -0.1183
      0.0000   11.0000     0.103      11     1.132        4       2.5634
      0.0000   11.0000     0.103      11     1.132        0      -1.0122
      0.0000   11.0000     0.103      11     1.132        1      -0.1183
      0.0000   11.0000     0.103      11     1.132        1      -0.1183
      0.0000   11.0000     0.103      11     1.132        1      -0.1183
      0.0000   11.0000     0.103      11     1.132        0      -1.0122
      0.0000   12.0000     0.103      12     1.235        2       0.6484
      0.0000   12.0000     0.103      12     1.235        1      -0.1995
      0.0000   12.0000     0.103      12     1.235        0      -1.0474
      0.0000   12.0000     0.103      12     1.235        2       0.6484
      0.0000   12.0000     0.103      12     1.235        0      -1.0474
      0.0000   12.0000     0.103      12     1.235        0      -1.0474
      0.0000   13.0000     0.103      13     1.338        0      -1.0802
      0.0000   13.0000     0.103      13     1.338        1      -0.2730
      0.0000   14.0000     0.103      14     1.441        2       0.4308
      0.0000   14.0000     0.103      14     1.441        2       0.4308
      0.0000   14.0000     0.103      14     1.441        4       1.9726
      0.0000   14.0000     0.103      14     1.441        1      -0.3400
      0.0000   15.0000     0.103      15     1.544        1      -0.4016
      0.0000   15.0000     0.103      15     1.544        1      -0.4016
      0.0000   16.0000     0.103      16     1.647        1      -0.4584

    398.0000    6.0000     0.115       6     0.691        0      -0.7873
    398.0000    8.0000     0.115       8     0.921        0      -0.8737
    398.0000    8.0000     0.115       8     0.921        0      -0.8737
    398.0000    8.0000     0.115       8     0.921        2       1.0227
    398.0000    8.0000     0.115       8     0.921        0      -0.8737
    398.0000   10.0000     0.115      10     1.152        0      -0.9416
    398.0000   10.0000     0.115      10     1.152        2       0.6934
    398.0000   11.0000     0.115      11     1.267        1      -0.2045
    398.0000   11.0000     0.115      11     1.267        1      -0.2045
    398.0000   11.0000     0.115      11     1.267        1      -0.2045
    398.0000   11.0000     0.115      11     1.267        1      -0.2045
    398.0000   11.0000     0.115      11     1.267        1      -0.2045
    398.0000   12.0000     0.115      12     1.382        0      -0.9967
    398.0000   12.0000     0.115      12     1.382        4       1.8879
    398.0000   13.0000     0.115      13     1.497        0      -1.0207
    398.0000   13.0000     0.115      13     1.497        0      -1.0207
    398.0000   13.0000     0.115      13     1.497        1      -0.3390
    398.0000   13.0000     0.115      13     1.497        5       2.3877
    398.0000   13.0000     0.115      13     1.497        0      -1.0207
    398.0000   14.0000     0.115      14     1.612        2       0.2506
    398.0000   14.0000     0.115      14     1.612        3       0.8972
    398.0000   15.0000     0.115      15     1.728        1      -0.4476
    398.0000   15.0000     0.115      15     1.728        2       0.1675

   1010.0000    7.0000     0.133       7     0.934        4       2.6566
   1010.0000    9.0000     0.133       9     1.200        3       1.2930
   1010.0000    9.0000     0.133       9     1.200        1      -0.1439
```

B-16

```
1010.0000    9.0000    0.133     9    1.200    0    -0.8623
1010.0000   10.0000    0.133    10    1.334    4     1.7669
1010.0000   10.0000    0.133    10    1.334    0    -0.8837
1010.0000   10.0000    0.133    10    1.334    0    -0.8837
1010.0000   11.0000    0.133    11    1.467    0    -0.9025
1010.0000   11.0000    0.133    11    1.467    5     2.1736
1010.0000   11.0000    0.133    11    1.467    2     0.3279
1010.0000   11.0000    0.133    11    1.467    1    -0.2873
1010.0000   11.0000    0.133    11    1.467    1    -0.2873
1010.0000   11.0000    0.133    11    1.467    3     0.9432
1010.0000   11.0000    0.133    11    1.467    3     0.9432
1010.0000   12.0000    0.133    12    1.600    0    -0.9191
1010.0000   12.0000    0.133    12    1.600    0    -0.9191
1010.0000   12.0000    0.133    12    1.600    4     1.3782
1010.0000   12.0000    0.133    12    1.600    3     0.8039
1010.0000   12.0000    0.133    12    1.600    2     0.2295
1010.0000   12.0000    0.133    12    1.600    1    -0.3448
1010.0000   12.0000    0.133    12    1.600    0    -0.9191
1010.0000   12.0000    0.133    12    1.600    1    -0.3448
1010.0000   13.0000    0.133    13    1.734    5     1.7594
1010.0000   13.0000    0.133    13    1.734    2     0.1435
1010.0000   14.0000    0.133    14    1.867    4     1.0820
1010.0000   14.0000    0.133    14    1.867    0    -0.9471

3020.0000    7.0000    0.188     7    1.317    1    -0.2493
3020.0000    9.0000    0.188     9    1.693    0    -1.1128
3020.0000    9.0000    0.188     9    1.693    0    -1.1128
3020.0000    9.0000    0.188     9    1.693    3     0.8586
3020.0000   10.0000    0.188    10    1.882    1    -0.5361
3020.0000   10.0000    0.188    10    1.882    2     0.0720
3020.0000   10.0000    0.188    10    1.882    0    -1.1443
3020.0000   10.0000    0.188    10    1.882    3     0.6802
3020.0000   11.0000    0.188    11    2.070    4     1.0932
3020.0000   11.0000    0.188    11    2.070    0    -1.1722
3020.0000   11.0000    0.188    11    2.070    2    -0.0395
3020.0000   11.0000    0.188    11    2.070    2    -0.0395
3020.0000   11.0000    0.188    11    2.070    3     0.5269
3020.0000   11.0000    0.188    11    2.070    3     0.5269
3020.0000   12.0000    0.188    12    2.258    2    -0.1367
3020.0000   12.0000    0.188    12    2.258    2    -0.1367
3020.0000   12.0000    0.188    12    2.258    4     0.9236
3020.0000   12.0000    0.188    12    2.258    1    -0.6669
3020.0000   12.0000    0.188    12    2.258    1    -0.6669
3020.0000   12.0000    0.188    12    2.258    1    -0.6669
3020.0000   13.0000    0.188    13    2.446    2    -0.2223
3020.0000   13.0000    0.188    13    2.446    0    -1.2193
3020.0000   13.0000    0.188    13    2.446    0    -1.2193
3020.0000   14.0000    0.188    14    2.634    0    -1.2394
3020.0000   14.0000    0.188    14    2.634    8     2.5248
3020.0000   14.0000    0.188    14    2.634    6     1.5837
3020.0000   15.0000    0.188    15    2.822    3     0.0792
3020.0000   15.0000    0.188    15    2.822    4     0.5248
```

Combine litters with adjacent levels of the litter-specific covariate within dose groups until
the expected count exceeds 3.0, to help improve the fit of the X^2 statistic to chi-squared.

```
                        Grouped Data
                 Mean                          chi-squared
     Dose   Lit.-Spec. Cov.   Expected  Observed   Residual
     ---------------------------------------------------------
     0.0000       8.2500        3.397        5       0.8439
     0.0000      11.0000        7.926        8       0.0250
     0.0000      12.0000        7.411        5      -0.8347
     0.0000      13.3333        4.117        3      -0.5126
     0.0000      14.0000        4.323        7       1.1913
     0.0000      15.0000        3.088        2      -0.5680
     0.0000      16.0000        1.647        1      -0.4584

   398.0000      7.5000        3.455        2      -0.7181
   398.0000      9.3333        3.225        2      -0.6046
   398.0000     11.0000        6.335        5      -0.4572
   398.0000     12.3333        4.262        4      -0.1068
   398.0000     13.0000        5.989        6       0.0037
   398.0000     14.0000        3.225        5       0.8116
   398.0000     15.0000        3.455        3      -0.1981
```

B-17

```
1010.0000          8.3333             3.334         8      2.0448
1010.0000          9.6667             3.867         4      0.0520
1010.0000         10.6667             4.268         5      0.2664
1010.0000         11.0000             7.335        10      0.7333
1010.0000         12.0000            12.803        11     -0.3660
1010.0000         13.0000             3.467         7      1.3456
1010.0000         14.0000             3.734         4      0.0954

3020.0000          8.0000             3.010         1     -1.0137
3020.0000          9.0000             3.387         3     -0.1797
3020.0000         10.0000             7.526         6     -0.4641
3020.0000         11.0000            12.418        14      0.3657
3020.0000         12.0000            13.547        11     -0.5513
3020.0000         13.0000             7.338         2     -1.5363
3020.0000         14.0000             7.903        14      1.6564
3020.0000         15.0000             5.645         7      0.4271
```
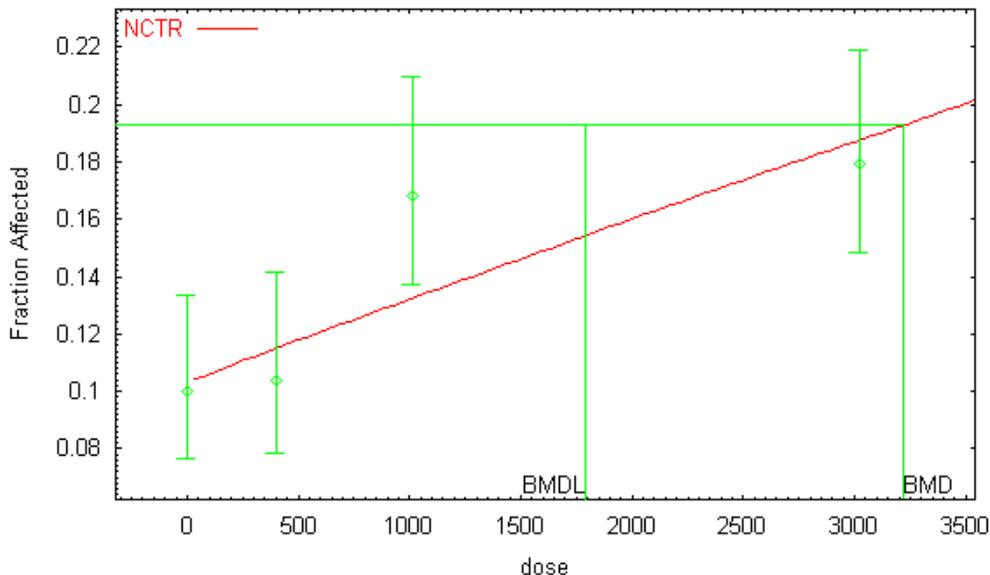
 Chi-square =   19.17   DF = 22   P-value = 0.6349

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific
covariate of control group: 11.923077

Benchmark Dose Computation

```
Specified effect =           0.1
Risk Type         =      Extra risk
Confidence level =           0.95
            BMD =        3196.69
           BMDL =        1714.11
```

NCTR Model with 0.95 Confidence Level

10:36 03/26 2003

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

 The probability function is:

 Prob. = 1 - exp[-(alpha + th1*Rij) - (beta + th2*Rij)*Dose^rho],

          where Rij is the centralized litter specific covariate.

 Restrict Power rho >= 1.

 Total number of observations = 103
 Total number of records with missing values = 0
 Total number of parameters in model = 9
 Total number of specified parameters = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

****  We are sorry but Relative Function and Parameter Convergence   ****
****  are currently unavailable in this model.  Please keep checking  ****
****  the web sight for model updates which will eventually           ****
****  incorporate these convergence criterion.  Default values used.  ****

 User specifies the following paramters:
          theta1 =            0
          theta2 =            0

                  Default Initial Parameter Values
                      alpha =     0.109279
                       beta = 3.27008e-005
                        rho =            1
                       phi1 =    0.0233469
                       phi2 =    0.0519936
                       phi3 =      0.10788
                       phi4 =    0.0854085

                       Parameter Estimates
         Variable         Estimate              Std. Err.
           alpha          0.109279              0.0217683
            beta       3.27008e-005           0.000239898
             rho                 1               0.915599
```

B-19

```
              phi1            0.0233469               0.0343278
              phi2            0.0519936               0.0511353
              phi3             0.10788                0.0674803
              phi4            0.0854085               0.0488455

                          Analysis of Deviance Table
            Model      Log(likelihood)    Deviance  Test DF    P-value
        Full model        -377.311
       Fitted model       -462.583        170.542     98       <.0001
      Reduced model       -478.095        201.568    102       <.0001

              AIC:          935.165 =-2L+2p=-2(-462.583)+2(6)=937.166

                          Goodness   of   Fit
        Dose   Litter_Size  Est._Prob.    Expected    Observed
        -----------------------------------------------------------
        0.0000          5        0.104       0.518          0
        0.0000          7        0.104       0.725          0
        0.0000         10        0.104       1.035          1
        0.0000         11        0.104       9.110         12
        0.0000         12        0.104       7.453          5
        0.0000         13        0.104       2.692          1
        0.0000         14        0.104       5.797          9
        0.0000         15        0.104       3.106          2
        0.0000         16        0.104       1.656          1

      398.0000          6        0.115       0.691          0
      398.0000          8        0.115       3.684          2
      398.0000         10        0.115       2.302          2
      398.0000         11        0.115       6.331          5
      398.0000         12        0.115       2.763          4
      398.0000         13        0.115       7.482          6
      398.0000         14        0.115       3.223          5
      398.0000         15        0.115       3.453          3

     1010.0000          7        0.133       0.929          4
     1010.0000          9        0.133       3.581          4
     1010.0000         10        0.133       3.979          4
     1010.0000         11        0.133      10.214         15
     1010.0000         12        0.133      12.734         11
     1010.0000         13        0.133       3.449          7
     1010.0000         14        0.133       3.714          4

     3020.0000          7        0.188       1.315          1
     3020.0000          9        0.188       5.071          3
     3020.0000         10        0.188       7.513          6
     3020.0000         11        0.188      12.396         14
     3020.0000         12        0.188      13.523         11
     3020.0000         13        0.188       7.325          2
     3020.0000         14        0.188       7.889         14
     3020.0000         15        0.188       5.635          7

     Chi-square =     25.56   DF = 27    P-value = 0.5433
```

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of control group: 11.923077

```
   Benchmark Dose Computation
Specified effect =          0.1
Risk Type        =      Extra risk
Confidence level =      0.950000
          BMD =        3221.96
          BMDL =       1788.93
```

B-20

RaiVR Model with 0.95 Confidence Level
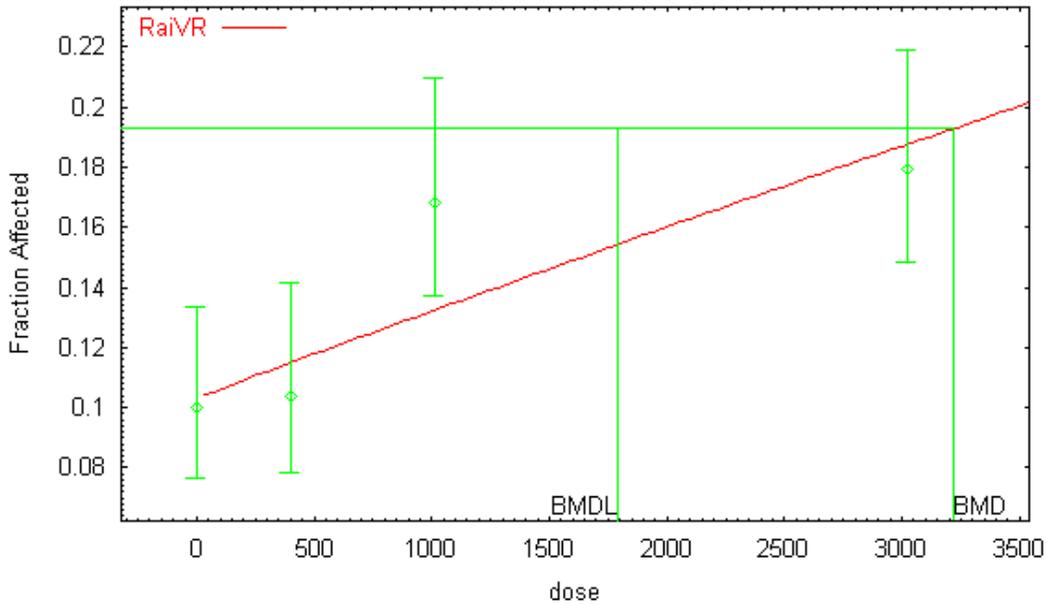
10:37 03/26 2003

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

 The probability function is:

 Prob. = [1-exp(-Alpha-Beta*Dose^Rho)]*exp(-(Th1+Th2*Dose)*Rij),

          where Rij is the litter specific covariate.

 Restrict Power rho >= 1.

 Total number of observations = 103
 Total number of records with missing values = 0
 Total number of parameters in model = 9
 Total number of specified parameters = 2

 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008

Relative Function and Parameter Convergence are currently unavailable in this model.

 User specifies the following parameters:
         theta1 =            0
         theta2 =            0

                 Default Initial Parameter Values
                         alpha =     0.109279
                          beta = 3.27008e-005
                           rho =            1
                          phi1 =    0.0233469
                          phi2 =    0.0519936
                          phi3 =      0.10788
                          phi4 =    0.0854085

                         Parameter Estimates
         Variable        Estimate              Std. Err.
           alpha         0.109279             0.0217683
            beta      3.27008e-005          0.000239898
             rho                1             0.915599
            phi1        0.0233469             0.0343278
```

B-21

```
        phi2            0.0519936              0.0511353
        phi3            0.10788                0.0674803
        phi4            0.0854085              0.0488455

                     Analysis of Deviance Table
        Model      Log(likelihood)    Deviance  Test DF    P-value
    Full model       -377.311
   Fitted model      -462.583          170.542     98      <.0001
  Reduced model      -478.095          201.568    102      <.0001

           AIC = -2L+2p=-2(-462.583)+2(6)=937.166

                      Goodness  of  Fit
     Dose   Litter_Size   Est._Prob.   Expected    Observed
   -----------------------------------------------------------
     0.0000         5        0.104        0.518         0
     0.0000         7        0.104        0.725         0
     0.0000        10        0.104        1.035         1
     0.0000        11        0.104        9.110        12
     0.0000        12        0.104        7.453         5
     0.0000        13        0.104        2.692         1
     0.0000        14        0.104        5.797         9
     0.0000        15        0.104        3.106         2
     0.0000        16        0.104        1.656         1

   398.0000         6        0.115        0.691         0
   398.0000         8        0.115        3.684         2
   398.0000        10        0.115        2.302         2
   398.0000        11        0.115        6.331         5
   398.0000        12        0.115        2.763         4
   398.0000        13        0.115        7.482         6
   398.0000        14        0.115        3.223         5
   398.0000        15        0.115        3.453         3

  1010.0000         7        0.133        0.929         4
  1010.0000         9        0.133        3.581         4
  1010.0000        10        0.133        3.979         4
  1010.0000        11        0.133       10.214        15
  1010.0000        12        0.133       12.734        11
  1010.0000        13        0.133        3.449         7
  1010.0000        14        0.133        3.714         4

  3020.0000         7        0.188        1.315         1
  3020.0000         9        0.188        5.071         3
  3020.0000        10        0.188        7.513         6
  3020.0000        11        0.188       12.396        14
  3020.0000        12        0.188       13.523        11
  3020.0000        13        0.188        7.325         2
  3020.0000        14        0.188        7.889        14
  3020.0000        15        0.188        5.635         7

  Chi-square =      25.56    DF = 26     P-value = 0.4877
```

To calculate the BMD and BMDL, the litter specific covariate is fixed at the mean litter specific covariate of control group: 11.923077

```
   Benchmark Dose Computation
Specified effect =            0.1
Risk Type         =       Extra risk
Confidence level  =       0.950000
         BMD =         3221.96
         BMDL =        1788.93
```