DRAFT - DO NOT CITE OR QUOTE

EPA/635/____
NCEA-S-1399

# TOXICOLOGICAL REVIEW

# OF

# METHYL ETHYL KETONE

(CAS No. 78-93-3)

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

*March 2003*

**NOTICE**

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

U.S. Environmental Protection Agency
Washington DC

## DISCLAIMER

This document is a preliminary draft for review purposes only and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

**CONTENTS —TOXICOLOGICAL REVIEW for 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 301-345-2870.

## AUTHORS, CONTRIBUTORS, AND REVIEWERS

### Chemical Manager/Author

Susan Rieth
National Center for Environmental Assessment
IRIS Program
U.S. EPA Office of Research and Development
Washington, D.C.

### Contributing Author

Karen Hogan
National Center for Environmental Assessment
IRIS Program
U.S. EPA Office of Research and Development
Washington, D.C.

### Authors

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

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

Regina McCartney, M.S.
Syracuse Research Corporation
Cincinnati, OH

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

Daniel Axelrad, M.P.P.
Office of Policy, Economics, and Innovation
National Center for Environmental Economics

Philip Bushnell, Ph.D.
Office of Research and Development
National Health and Environmental Effect Research Laboratory

Audrey Cummings, Ph.D.
Office of Research and Development
National Health and Environmental Effect Research Laboratory

Robert Dewoskin, Ph.D.
Office of Research and Development
National Center for Environmental Assessment

Gary Foureman, Ph.D.
Office of Research and Development
National Center for Environmental Assessment

David Herr, Ph.D.
Office of Research and Development
National Health and Environmental Effect Research Laboratory

Tracey Woodruff, Ph.D, MPH
Office of Policy, Economics, and Innovation
National Center for Environmental Economics

**External Peer Reviewers**
Name
Affiliation

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

# 1. INTRODUCTION

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

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

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ air breathed. Another form in which risk is presented is as 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 databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. For this toxicological review, update literature searches for 1987 to October 2002 were conducted for MEK. In addition, literature searches were conducted from 1991 to October 2002 for 2-butanol and from 1965 to October 2002 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 is also known as 2-butanone, butanone, ethyl methyl ketone, methyl acetone, and MEK. Some relevant physical and chemical properties of methyl ethyl ketone (MEK) are listed below (ATSDR, 1992; CRC, 1994; HSDB, 1999; NTP, 2002):

CAS Registry number: 78-93-3
Structural 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 chemical classes, including strong oxidizers (chlorosulfonic, sulphuric, and nitric acids), potassium tert-butoxide, chloroform, and hydrogen peroxide. 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. MEK is also incompatible with 2-propanol. ACGIH (2001) has recommended an 8-hour time-weighted average threshold limit value (TWA-TLV) of 200 ppm (590 mg/m$^3$, assuming 25° C, and 760 mm Hg [standard temperature and pressure]) for MEK, to protect against effects on the central nervous system. The Occupational Safety and Health Administration (OSHA) has also promulgated an 8-hour permissible exposure limit (PEL) of 200 ppm for 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. 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, 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 (ATSDR, 1992). MEK is also found in tobacco smoke and volatile releases from building materials and consumer products (ATSDR, 1992).

# 3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

## 3.1. ABSORPTION

### 3.1.1. Oral Exposure

Case reports provide qualitative evidence that MEK is absorbed 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 MEK of about 110 µg/mL (Sakata *et al.*, 1989).

Experimental data from rodents indicate that orally administered MEK is absorbed from the gastrointestinal (GI) tract and rapidly eliminated. Oral administration (gavage) of 1690 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 after 18 hours (Dietz and Traiger, 1979; Dietz *et al.*, 1981). Thrall *et al.* (2002) reported mean peak MEK 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 the high blood/air solubility ratio of MEK (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 alveolar air of exposed workers (n = 82) by simultaneous collection of air samples into glass tubes via instantaneous sampling methods and analysis by gas chromatography (GC). 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 is reached within 8 hours at an exposure concentration 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 of MEK administered at 200 ppm for 4 hours in an exposure chamber (Liira *et al.*, 1988). Liira *et al*. (1990a) found pulmonary retention of MEK in five human volunteers similarly exposed to MEK to be 55.8±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 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 minimum rates of percutaneous penetration of MEK: 0.46 $\mu g/cm^2$/minute for dry or normal skin and 0.59 $\mu g/cm^2$/minute for moist skin (JRB Associates, Inc., 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 $g/m^2$/hour, which is equivalent to approximately 0.0066 cm/hour (Ursin *et al*., 1995) or approximately 88.3 $\mu g/cm^2$/minute [53 $g/m^2$-hour) x (1 hour/50 minute) x (106 µg/1 g) x (1 $m^2/10^4$ $cm^2$) = 88.3 $\mu g/cm^2$-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 exhalation of MEK from the lungs, thereby not considering all routes of elimination for MEK (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 (the level of the U.K. Occupational Exposure Standard). For skin-only exposures, volunteers wore air masks that delivered room air. Uptake was assessed by monitoring of MEK in blood, single breath, or 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

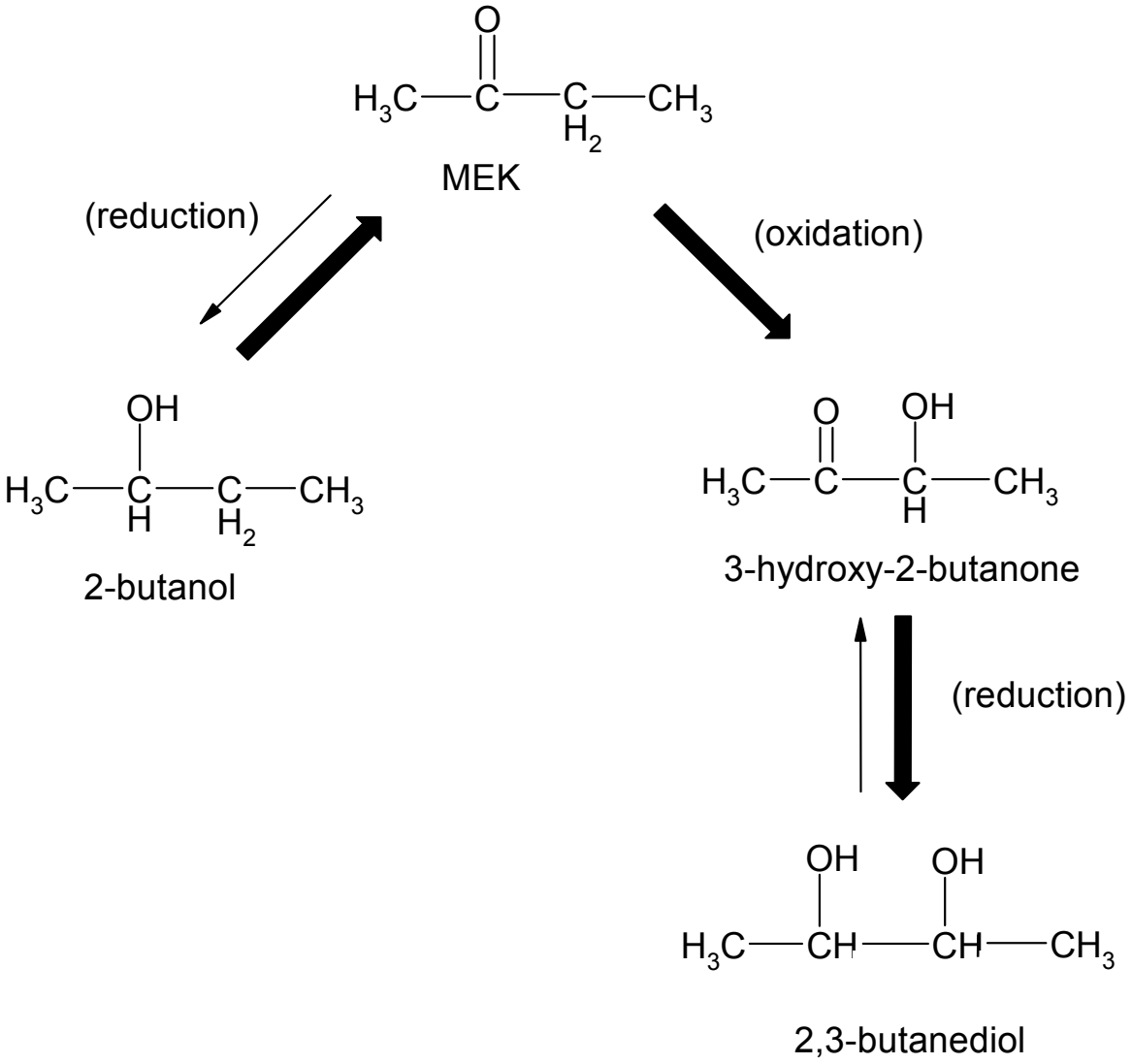
No studies were located regarding the distribution of MEK following oral or dermal exposure in humans or animals. In a study of industrial workers, Perbellini *et al*. (1984) compared the concentration of MEK in the venous blood to the alveolar air concentrations of the MEK-exposed workers (n = 23), which were collected simultaneously toward the end of the work shift and analyzed by GC and mass spectrometry (MS). The concentration 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 post-mortem 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). These post-mortem 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 will not accumulate in fatty tissues in humans. Blood/tissue solubility ratios for several tissues approach unity (Perbellini *et al.*, 1984). These results have also been repeated in rats (Thrall *et al.*, 2002). Therefore, 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, and a small portion is reversibly converted to 2-butanol. The 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, and 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, that 2% of the absorbed dose was excreted in urine as 2,3-butanediol, and that the remainder of the absorbed dose entered into mainstream intermediary metabolism and was transformed to simple compounds, like 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.

**Figure 1.** Proposed pathways for methyl ethyl ketone metabolism. Adapted from DiVincenzo *et al.*, 1976.



In humans, MEK has also been identified as a minor but normal constituent of urine, serum and urine of diabetics, and 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. The origins of MEK in blood at the levels documented in NHANES III remain uncertain. Blood MEK values did not reflect environmental exposures well, but were associated with mean daily daily alcohol intake (Churchill *et al*., 2001).

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, which is subsequently reduced to 2,3-butanediol (Dietz and Traiger, 1979; Traiger *et al.*, 1989). 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 (2-butanone) 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 with either 1776 mg/kg of 2-butanol or 1690 mg/kg MEK (10,899±842 vs. 9868±566 mg-hour/liter, respectively). In these rat studies, MEK blood concentrations peaked within 7–8 hours after 2-butanol administration and 4–5 hours after MEK administration (Dietz *et al*., 1981), providing 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 the test compound: methyl n-butyl ketone, methyl isobutyl ketone, or methyl ethyl ketone (MEK). The hepatic cytochrome P450-mediated metabolism of MEK produced hydroxylated metabolites, 3-hydroxy-2-butanone and 2,3-butanediol, as shown in Figure 1, which were eliminated in the urine (DiVincenzo *et al.*, 1976). Male Sprague-Dawley rats given a single oral dose of MEK at 1690 mg/kg exhibited blood concentrations of MEK and metabolites 4 hours after dosing as 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 as follows: 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 1690 mg/kg MEK, while in F344 rats a notable difference in peak exhaled breath concentration following oral gavage was reported by Thrall *et al.* (2002). Thrall *et al.* (2002) found peak MEK concentrations in exhaled air 1 hour after oral gavage of 50 mg/kg MEK. 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 their results and those of Dietz *et al.* (1981).

Gadberry and Carlson (1994) showed that the *in vitro* hepatic oxidation of 2-butanol to MEK in the liver 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 MEK at 1.4 mL/kg 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 both a substrate for cytochrome P450 metabolism and 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 of MEK 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 bi-phasic 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 MEK at 100 or 200 ppm 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 exposures, 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). No PBPK models are available 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 to MEK for 4 hours in separate exposures to 25, 200, or 400 ppm. 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),

$K_m$ = 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, and concluded that the kinetic constants were fairly representative of healthy male subjects in general.

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. These 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, the blood and the liver (where metabolism occurs). The differential equations are based upon a perfusion -limited model, and account for the elimination of 2-butanol and its metabolites from the blood at rates linearly proportional to blood concentrations, transport between the blood and liver compartments, and 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 MEK at 1690 mg/kg, 2) a single gavage administration of 2-butanol at 1776 mg/kg, and 3) intravenous injections of 3-hydroxy-2-butanone and 2,3-butanediol, both at 400 and 800 mg/kg. The equations describing the metabolic conversion of MEK to 3-hydroxy-2-butanone included a competitive inhibition of the conversion of MEK to 3-hydroxy-2-butanone, 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. These "adjustments" resulted in an improved fit between model simulations and experimentally observed blood concentrations of MEK and 2-butanol following oral administration of 1690 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.[1] 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 exposure to 100 to 2000 MEK in a closed, recirculating gas uptake system. The PBPK model developed by Thrall *et al.* (2002) included both a saturable metabolic pathway described by Michaelis-Menten kinetic constants and a non-saturable first-order pathway. The Thrall *et al.* (2002) 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.* and Dietz *et al*. models is in peak exhaled breath concentration following oral gavage. Dietz *et al.* (1981) found peak MEK concentrations in blood 4 hours after oral gavage (1690 mg/kg), whereas the Thrall *et al*. (2002) study found peak MEK concentrations in exhaled air 1 hour after oral gavage (50 mg/kg).

The Thrall *et al.* (2002) model could be extended to humans by substituting human parameter values for the 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 upon a limited number of data sets in rats and humans. The predictive capabilities of these models have not been adequately tested,

---

[1] 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 few rate constants which were provided were not readily interpretable in the framework shown in Table 1, and physiological constants appropriate for converting the available parameters to reflect the equivalent framework were also not available.

and none of the models were parameterized for both rats and humans sufficient to support an 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) | | 0.70 | 0.25 |
| Blood flows to tissues at rest, L/min and (% of cardiac output)[a] | Lungs | 5.05 (50) | -[d] |
| | GI tract | 1.2 (11) | - |
| | Liver | 1.6 (15) | (25) |
| | Richly perfused tissues | 2.1 (19) | (51) |
| | Poorly perfused tissues | 0.1 (1) | (20) |
| | Fat | 0.25 (2) | (4) |
| | Muscle | 0.5 (5) | - |
| 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 10.8 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 non-occupational, 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 air concentrations of MEK vapors is expected to cause reversible central nervous system depression; however, evidence for such effects of MEK 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/m$^3$) 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).

In the only case report located, a 38-year-old male worker exposed to paint base containing MEK and toluene in an enclosed, unventilated garage exhibited neurological symptoms (Welch *et al.*, 1991). Exposure occurred at an unknown concentration of MEK for an acute, but unspecified, period of time. Initial symptoms were nausea, headache, dizziness, and respiratory distress. Over the next several days, he experienced impaired concentration, memory loss, tremor, gait ataxia, and dysathria. 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 CNS effects were due to exposure to MEK, toluene, or a combination of solvents.

In the 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 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 MEK at 200 ppm 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 comparison 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/m³) MEK or filtered air for 4 hours in a cross-over 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. The volunteers exposed to 100 ppm (295 mg/m$^3$) MEK reported only slight nose and throat irritation. Mild eye irritation was reported by some subjects at 200 ppm (590 mg/m$^3$). Exposure to 300 ppm (885 mg/m³) of MEK 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 few 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 daily for 12 years. Exposure was via both dermal and inhalation routes. He 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. The survey of his work area revealed MEK concentration in air in excess of 5000 mg/m³ (1695 ppm) during some operations and 10-minute concentrations of approximately 900 mg/m³ (305 ppm).

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 in this patient is not available.

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 in these workers was associated with slightly, but not significantly, reduced nerve conduction velocities (distal axonopathy) and various 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 (885–1770 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 polyvinyl chloride, MEK, leather dust, benzene, and other chemicals for four operations at a Nigerian shoe factory that had been in existence for 30 years. The four operations at the factory included plastic, leather, rubber, and tailoring. MEK exposure occurred only in the leather unit where 43 workers were exposed to leather, dyes, MEK, and other solvents (unspecified), which were used to preserve the 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). These 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 Nigerians. 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 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 these data for dose-response assessment.

Mitran *et al.* (1997, 2000) reported the results from a cross-sectional health study of workers in three Romanian factories exposed to acetone, MEK, 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. The mean length of exposure was 14±7.5 years. Workers were exposed to reported concentrations of 149–342 mg/m$^3$ (51–116 ppm) 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 tests 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 as follows: 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 (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 about these tests was a statement indicating that diffuse somatic neurotic changes were the dominant findings in exposed workers. Mean nerve conduction velocities for the median, ulnar, and peroneal nerves in the MEK-exposed group were statistically significantly decreased compared with control means by 22%, 28%, and 26%, respectively (Mitran *et al.*, 1997). Other nerve conduction variables that were statistically significantly different in the MEK-exposed group were: 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 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 [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 of the Mitran *et al.* (1997) report (Boyes and Herr, 2002; Graham, 2000) 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 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 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 symptomological 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 is not consistent with patterns demonstrated for the well-studied peripheral neuropathy from compounds that are metabolized to gamma-diketones (*e.g.*, hexane and methyl-n-butyl ketone). Boyes and Herr (2002) have 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 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 5000 ppm (14,750 mg/m$^3$) 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 equivocal evidence that repeated exposure to MEK in the workplace increases the hazard for persistent neurological impairment.

*Potential for Carcinogenic Effects in Humans*

Several epidemiological studies have 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 suggestive evidence of an increased risk between multiple solvent exposure, which included MEK, 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 operated by Shell Chemical Company, 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 reported as 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). The incidence of buccal or pharyngeal neoplasms was statistically significantly elevated, but this finding was regarded by the authors as due to chance given the small number of individuals affected, the failure to include tobacco use in the study, and the number of separate comparisons made between observed and expected rates. In view of the small numbers of individuals affected, the authors concluded that there was no clear evidence of cancer hazard in these workers.

A retrospective cohort study of 1008 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. A statistically significantly elevated risk of mortality from cancer of the bone was reported (SMR=10.34, 95% CI: 2.1-30.2, 3 observed); however, 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 exposure to solvents (including MEK) principally 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 only as person-years, with 32,212 for male workers and 10,042 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 rates of incidence 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 not statistically significantly elevated (relative risk = 4.6, 95% CI: 0.9–23.2, 3 observed); this finding is consistent with the earlier report by Spirtas *et al.* (1991) where the TG analysis was applied. As reported by the authors of the original and follow-up studies (Spirtas *et al.*, 1991; Blair *et al.*, 1998), the few cases and exposure 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 for 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 diagnosis of leukemia. The mothers and fathers of the children diagnosed with leukemia and individually matched controls (n = 123 matched pairs) were interviewed regarding occupational and home exposure to chlorinated solvents, MEK, spray paints, dyes and pigments, and cutting oils, personal and family medical history, and lifestyle habits associated with leukemia. The study 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). No significant association between leukemia and mothers' exposures to specific substances were found, although few mothers were occupationally

exposed to industrial solvents included in the categories of this experimental design. This study is considered as 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. 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), however, 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.

## 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 the data gaps in the MEK oral exposure database could be addressed by oral studies with 2-butanol. Similarly, the database 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 (a metabolic precursor of MEK) and a 13-week drinking water study with 3-hydroxy-2-butanone (a metabolite of MEK), 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, 3000, or 12,000 ppm) for 13 weeks. According to the authors, these exposures are equivalent to mean intakes of 0, 80, 318, or 1286 mg/kg-day for males and 0, 91, 348, or 1404 mg/kg-day for females. Additional groups of 5 rats of each sex were exposed to 0, 3000, 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, and glucose, bile salts, and blood. Also, a urine concentration test measured specific gravity and the 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 compared to controls). This increase in relative liver weight was not accompanied by changes in liver histology or in the activities of liver enzymes (LDH, SGPT, or SGOT). Thus, this is 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, 3000, 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, 3000 ppm (318 mg/kg-day) was a NOAEL, and 12,000 ppm (1286 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 database could be addressed by toxicity studies with 2-butanol. Similarly, the database for the 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, 1254, 2518, or 5041 ppm (0, 3700, 7430, 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 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 medulla and the sciatic and tibial nerves of the remaining five male and five female rats from each group.

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 (5041 ppm) male and female rats early in the study. While statistically significant, the reductions did not exceed 8% of the control group weights for either 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 as compared to controls. Relative liver weight was statistically increased in the 5041 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 were increased by 27% in the 5041 ppm rats only. Other statistically significant differences in organ weights in 5041 ppm female rats included decreased brain weights (absolute–5%, and relative–9%), decreased spleen weights (absolute–5%) and increased kidney weights (relative–11%); and in 5041 ppm male rats included increased kidney weights (relative–6%). Differences in the serum chemistry values for the female rats in the 5041 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 differences in hematology parameters were significantly higher mean corpuscular hemoglobin in 5041 ppm male and female rats and mean corpuscular hemoglobin concentration in 5041 ppm females; this 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 5041 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 5041 ppm rats and altered serum enzyme activities in 5041 ppm female rats were indicative of possible liver damage, no histopathological lesions in the liver were observed. The authors stated that response may have been the result of a physiological adaptation mechanism. While the decreased brain weights in the 5041 ppm females were an indication of possible direct effects of MEK on brain tissue, no histopathological lesions of the brain were observed.

Minimal to mild lesions of the upper or lower respiratory tract were noted in all control and MEK-exposed rats. These lesions of the respiratory tract 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 insignificant pathologically. In addition, the authors reported an increased prevalence of nasal inflammation (including submucosal lymphocytic infiltration and luminal exudate) across 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 tracts of all animals exposed via inhalation also prevents obtaining an unconfounded determination of any portal-of-entry effects.

Review of the Cavender *et al*. (1983) findings show the NOAEL to be 2518 ppm and the LOAEL to be 5041 ppm based on toxicity remote to the respiratory tract—*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 physiological adaptive response than toxicity. No histopathological lesions of the brain were observed in the exposed animals.

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 MEK at 235±26 ppm (693±77 mg/m$^3$) 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. The growth studies

suggest that 12 weeks of exposure to 235 ppm of MEK reduced body weight gain (mean body weight was 95 g for MEK vs. 135 g for control); however, neither statistics nor standard deviation on the mean are 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±2400 ppm (34,515±7080 mg/m$^3$) in rats exposed to MEK where narcosis preceded death. This study is inadequate for dose-response assessment; 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 1125 ppm (3318 mg/m$^3$) of MEK for periods of 16, 25, 35, or 55 days. Control animals were housed under similar environmental conditions without solvent 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 the 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/mm$^2$) showed no abnormality in rats exposed for up to 55 days. The authors reported that no abnormalities were observed in rats exposed as long as 5 months, but no further details were provided.

Male Wistar rats (8 per group) were exposed to 0 or 200 ppm (0 or 590 mg/m$^3$) of 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 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 various concentrations between 25 and 800 ppm (74 and 2360 mg/m$^3$) for 2 hours at approximately weekly intervals (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 pre-exposure values for the same animals (Garcia *et al.*, 1978). This 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 post-exposure response rates complicate interpretation of the findings.

Geller *et al.* (1979) studied behavioral effects in four male baboons (2 years old) exposed continuously by inhalation to MEK at a concentration of 100 ppm (295 mg/m$^3$) for 7 days. Operant conditioning behavior conducted during exposure was compared to pre-exposure test scores. The operant behavior selected was a match-to-sample discrimination task. The experimental protocol allowed the performance of each baboon during exposure to be compared to his performances during a clean air exposure in the same chamber immediately prior to each exposure. 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 pre-exposure control values by day 7 of exposure. Exposure to 100 ppm of MEK for 7 days 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, this report found only transient neurological effects of MEK in primates at the concentrations studied. It should be noted that each baboon in this study 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 1500 ppm (4425 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. This 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 6000 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 6000 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 and not MEK exposure. These authors did not comment on possible connections between bronchopneumonia susceptibility and exposure to MEK.

Toftgard *et al.* (1981) exposed 4 male Sprague-Dawley rats to 800 ppm (2360 mg/m$^3$) of MEK for 6 hours/day, 5 days/week, over 4 weeks and examined changes in enzymatic activity in rat

liver. Increased absolute and relative liver weight as 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 an air concentration of 750 ppm of MEK (2210 mg/m$^3$) for either 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 MEK 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). These results are consistent with an earlier report (Raunio *et al.*, 1990) that found 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 a 90-day inhalation study (Cavender *et al*., 1983), the only observed effects were decreased body weight gain, increased liver weight and altered enzyme levels, and decreased brain weight at a concentration of approximately 15,000 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 that suggests 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 methyl n-butyl ketone (Saida *et al.*, 1976). This 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 is available that addresses the potential reproductive toxicity of MEK in humans. Lemasters *et al.* (1999) studied the male reproductive effects of solvent and fuel exposure during aircraft maintenance. 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 revealed that exposures were generally low; all mean measures

were below 6 ppm, which is less than 10% of the OSHA standard for all chemicals except benzene. Sheet metal workers had the highest mean breath levels for both total solvents (24 ppb) and fuels (28.3 ppb). Mean values for most sperm measures remained in the normal range throughout the 30 weeks of exposure and measurement. When jobs were analyzed by exposure groups, some adverse changes were observed. The paint shop group had a significant decline in motility of 19.5% at 30 weeks. The authors noted a lack of a dose-response association for the observed spermatogenic changes. This 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 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 the majority 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, but all collected data were fully reported. The study 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. The average daily intake of 2-butanol as reported by the authors for the initial 8 weeks of the study (intake was not reported for subsequent weeks) was 0, 538, 1644, and 5089 mg/kg-day (males) and 0, 594, 1771, and 4571 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. 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 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. 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. The 2% 2-butanol exposure level is estimated to have produced average daily intakes of 3384 mg/kg-day in males and 3122 mg/kg-day in females based on a linear regression analysis of the reported average intakes for males and females in the 0, 0.3, 1, and 2% groups.

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) were 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. Following sacrifice and autopsy at day 21 of lactation, specimens of all major organs and tissues (35 in all) from 10 male and 10 female F1A rats per exposure group were processed histologically and examined microscopically. The liver and kidneys from all 30 F1A rats per sex in each group were examined histologically.

At the highest exposure level (3%), net parental (F0) body weight gain was reduced compared with controls both in males (229 g vs. 269 g in controls) and females (130 g 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. As compared to the control group, the following effects were noted in the litters (F1A) from the high-dose group (3%): reductions were seen 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, compared with control values. The litter mean body weight decreases relative to control at postnatal days 4 and 21 were 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 of F1A litter body weight means on days 4 and 21 in rats exposed to 2-butanol in drinking water. Source: Cox *et al*. (1975)[a].

| Dose[b] (mg/kg-day) | Number of litters day 4 | Mean body weight day 4 (g) | Standard deviation day 4 | Number of litters day 21 | Mean body weight day 21 (g) | Standard deviation day 21 |
|---|---|---|---|---|---|---|
| 0 | 29 | 10.7 | 1.1 | 28 | 49 | 3.8 |
| 594 | 27 | 10.2 | 1.3 | 27 | 47 | 3.9 |
| 1771 | 30 | 9.97 | 1.3 | 30 | 44 | 4.8 |
| 4571 | 26 | 8.3 | 1.8 | 26 | 30 | 11.9 |

[a] Means (and SD) were calculated from individual F1A litter body weight means in Appendix II of the Cox *et al.* (1975) report. Body weights were measured to the nearest 0.1 g, but from the best available copy of the report, data for day 21 could only be discerned to the nearest gram.
[b] Doses are average daily intake for female rats for the initial 8 weeks of the study as reported by the authors.

During the second pregnancy, the high-dose F0 dams receiving 2% 2-butanol exhibited reduced weight gain (gain of 94 g) compared to control, 0.3% or 1% dams (gains of 113, 111, or 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 body weight were observed at the two lower doses (0.3% – 4.16 g; 1% – 4.38 g). The difference in mean fetal body weights between the adjusted high-dose (2%) and control group was not statistically significant ($p>0.05$) using a t-test, but when the F1B fetal body 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-2 for statistical test results).

The incidences of nidation, early fetal deaths, and late fetal deaths 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. Source: Cox *et al.* (1975).

| Skeletal variation | Incidence (%), fetal basis (litter basis) | | | |
|---|---|---|---|---|
| | 0 | 594 mg/kg-d | 1771 mg/kg-d | 4571 mg/kg-d |
| Missing sternebrae | 51/235 (22%)<br>10/29 (34%) | 14/211 (7%)<br>9/27 (33%) | 11/254 (4%)<br>2/30 (7%) | 46/217 (21%)<br>13/29 (45%) |
| Wavy ribs | 41/235 (17%)<br>17/29 (59%) | 29/211 (14%)<br>14/27 (52%) | 20/254 (8%)<br>10/30 (33%) | 35/217 (16%)<br>17/29 (59%) |
| Incomplete ossification | 56/235 (24%)<br>17/29 (59%) | 56/211 (27%)<br>20/27 (74%) | 23/254 (9%)<br>10/30 (33%) | 69/217 (32%)<br>18/29 (62%) |

F2 pups from the high-dose group (2%) showed a reduction in the mean pup body weight at day 4 (9.5 g vs. 10.0 g in the control) and in mean pup body weight at 21 days (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 4 days (9.7 and 9.6 g) and 21 days (39 and 39 g). Although the 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 pups at days 4 and 21 (reductions of 5% at 4 days and 13% at 21 days compared with F2 controls).

No exposure-related changes in organ weights or increased incidences 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, which were most prominent in the 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 both 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. A similar increased incidence was not observed in females. These findings are consistent with the pattern of early stages of $\alpha_{2u}$-globulin-associated rat nephrotoxicity as described by the Risk Assessment Forum (U.S. EPA, 1991b). According to Agency guidance (U.S. EPA, 1991b), these species- and sex-specific renal effects are not appropriate to establish a critical effect for human health risk assessment.

In summary, the results of the Cox *et al.* (1975) study show that administration of 2-butanol in drinking water at concentrations as high as 3% did not affect reproductive performance variables in rats, 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 seen in the groups exposed to 3% 2-butanol in drinking water. At the next lower dose (1%) in this same generation, only reduced F1A pup weights were observed. At the 2% level (*i.e.*, the adjusted high-dose level administered following F1A postnatal day 21), the following effects were noted: 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 body 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. In addition, 2-butanol treatment did not increase the incidence of relevant neoplastic or non-neoplastic lesions in F1A generation rats that were exposed from gestation and continuing through 12 weeks after birth, mating, and gestation and lactation of the F2 generation. Thus, Cox *et al*. (1975) identified a LOAEL of 1771 mg/kg-day (1% solution) and a NOAEL of 594 mg/kg-day (0.3% solution) for decreased F1A pup body weights (particularly at postnatal day 21). In the F1B and F2 generations, the LOAEL was 3122 mg/kg-day (2% solution) and the NOAEL was 1771 mg/kg-day (1% solution) based on decreased F1B fetal body weights and decreased F2 pup body weights (particularly at postnatal day 21). The maternal LOAEL in this study was 3122 mg/kg-day (2% solution) based on decreased weight gain, and the NOAEL was 1771 mg/kg-day (1% solution). The highest dose level (3% solution or 4571 mg/kg-day) was a NOAEL for reproductive toxicity.

It should be noted that the Cox *et al.* study was conducted prior to the establishment of good laboratory practices (GLPs), and did not include as part of its protocol the evaluation of certain parameters routinely measured in studies of more current design. Among these deficiences are lack of measurements of estrous cyclicity, sperm parmeters, weights of uterus, epididymides and seminal vesicles, and brain, and less than complete histopathology.

#### 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 5000 ppm (14,750 mg/m$^3$) revealed no exposure-related lesions (Cavender *et al.*, 1983). The database on developmental toxicity of MEK by inhalation consists of several well-conducted studies.

Schwetz *et al.* (1974) exposed groups of 23 or 21 pregnant Sprague-Dawley rats (in whole body dynamic exposure chambers) to 1000 or 3000 ppm 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 1126 or 2618 ppm (3322 or 7723 mg/m$^3$). 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, and 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 tested. Small, but statistically significant, decreases in fetal weight and crown-rump length were observed at 1126 ppm, but not at 2618 ppm. In the 1126-ppm exposure group, the means of litter means were decreased by 5% for fetal body weight and 3% for crown rump length compared with air control values. Four fetuses from litters exposed to 2618 ppm showed rare gross malformations: two acaudate fetuses with an imperforate anus and 2 fetuses with brachygnathia. No gross malformations were found in fetuses from the control or 1126-ppm exposure groups. The percentage of litters with fetuses with gross anomalies was statistically significantly elevated at 2618 ppm compared with controls (19% vs. 0%; $p<0.05$). These 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 1126 ppm group, but the 2618 ppm group showed a statistically significant increase in the percentage of litters with sternebral skeletal variations (43% vs. 11% in concurrent controls; $p<0.05$). Percentage of litters with any skeletal anomaly was statistically significantly elevated at 1126 ppm (95%), but not at 2618 ppm (81%), compared with the control percentage (58%). Percentages of litters with specific soft tissue anomalies (*e.g.*, subcutaneous edema or dilated ureters) were not significantly elevated in either exposure group; the percentage of litters with any soft tissue anomaly was statistically significantly elevated at 2618 ppm (76%), but not at 1126 ppm (70%), compared with control values (51%).

The Schwetz *et al*. (1974) results indicate that 2618 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 1126-ppm exposure groups or controls. The biological significance of the developmental findings for the 1126-ppm exposure group is not clear. The decreased fetal body 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 1126 ppm (*i.e.*, "total skeletal anomalies") was not statistically demonstrable at 2618 ppm, and no incidences of specific skeletal anomalies were significantly elevated at 1126 ppm. Thus, for this study, 1126 ppm (7 hours/day on gestation days 6–15) is designated as a NOAEL, and 2618 ppm is established as a LOAEL for developmental effects. Also, the highest exposure level, 2618 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). While of high quality, this study predates the establishment of GLPs. 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, 1000, and 3000 ppm, respectively, for 7 hours/day on gestation days 6–15. The numbers of animals in the treatment groups are slightly smaller than the 20 animals/group recommended in current protocols. Average measured MEK concentrations during the experiment were 412, 1002, and 3005 ppm (1215, 2955, and 8865 mg/m$^3$). Dams exposed to 3005 ppm of MEK exhibited maternal toxicity: a slight decrease in weight gain (326 g for 3005 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 3005 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, or fetal weight and length. No statistically significant differences in the incidences of external or soft-tissue alterations were observed in the exposed versus the control groups. For example, in the 3005-ppm exposure group, two fetuses from two litters showed major malformations (one with multiple defects such as acaudia, imperforate anus, and multiple skeletal defects and another with the innominate artery missing) compared with one control fetus with similar multiple defects. Differences in the incidences of litters with two skeletal variations occurred in the 3005-ppm exposure group compared with the controls. The incidence of extra ribs was 2/26 for control litters, compared with 0/19, 0/19, and 6/18 for 412, 1002, and 3005 ppm litters, respectively (statistically significant at high dose by Fisher's Exact test). The respective incidences for delayed ossification of the cervical centra were 22/26, 15/19, 16/19, and 18/18 (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 3005 ppm (7 hours/day on gestation days 6–15) (LOAEL), but not at 412 or 1002 ppm (NOAEL), corroborating the developmental effect levels reported by Schwetz *et al*. (1974).

A subsequent inhalation developmental toxicity study in CD-1 mice verified the fetal effect levels established by the two developmental inhalation studies in Sprague-Dawley rats (Schwetz *et al.*, 1991; also reported as Mast *et al.*, 1989 and NTP, 1990). Groups of 10 virgin Swiss CD-1 mice and 33 sperm plug-positive (gestation day 0) females were exposed to mean MEK concentrations of 0, 398±9, 1010±28, and 3020±79 ppm (0, 1174±27, 2980±83, and 8909±233 mg/m$^3$) by inhalation (in whole body dynamic exposure chambers) for 7 hours/day on gestation days 6–15 and then sacrificed on day 18 of gestation. At these exposure concentrations (0, 398, 1010, or 3020 ppm), 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 ratios was observed. This increase achieved statistical significance at 3020 ppm (increase of approximately 7% compared with control). Maternal body weight gain was similar across all groups. Two statistically significant developmental effects were observed: a decrease in mean fetal body weight (per litter) at 3020 ppm in males (5% decrease

compared with controls) and for all fetuses combined (4% decrease compared with controls), and a positive trend for increasing 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 3020-ppm exposure groups). No statistically significant trend was found for increasing incidence of litters containing fetuses with misaligned sternebrae with increasing exposure level. For female fetuses at 3020 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 there were several malformations in one litter (cleft palate, fused ribs, missing vertebrae, syndactyly) in treated 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 1010 ppm was established. Developmental and maternal LOAELs were established at 3020 ppm (7 hours/day on gestation days 6–15) for small, but statistically significant, decreased fetal body weight among males, increased incidence of misaligned sternebrae, and an increased maternal liver-to-body-weight ratio. These results are in accord with the developmental effect levels established by earlier studies in rats (Schwetz *et al.*, 1974; Deacon *et al.*, 1981).

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), but 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 because the available reports do not clearly describe details of the experimental conditions or the results for the groups exposed to MEK alone. Consequently, these 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 3000 ppm 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. 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.

Gravid Sprague-Dawley rats were exposed by inhalation to 2-butanol at 0, 3500, 5000, or 7000 ppm (0, 10,605, 15,150, or 21,210 mg/m$^3$) for 7 hours/day on gestation days 1–19 (Nelson *et al.*, 1989, 1990). At these exposure concentrations, the number of gravid/mated rats were 15/16, 16/16, 14/15, and 11/15, respectively. For evaluation of developmental toxicology, dams were sacrificed on gestation day 20 (sperm positive vaginal smear was gestation day zero), and fetuses were

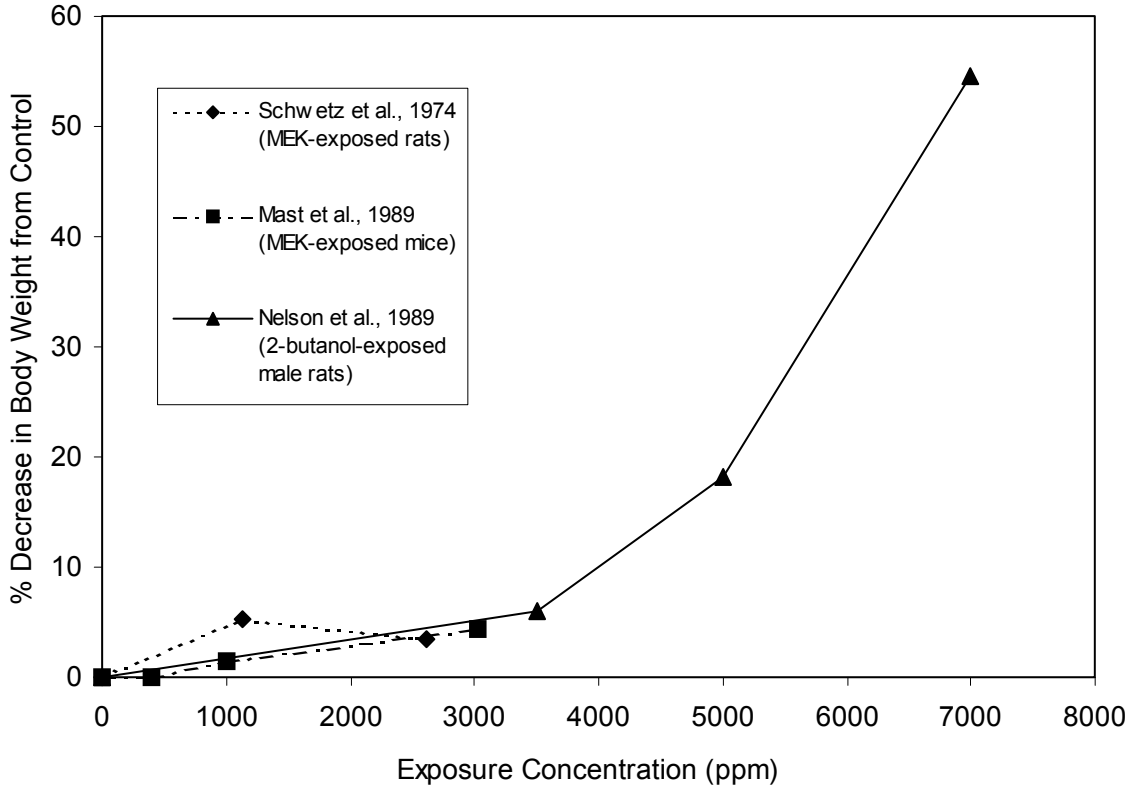
serially removed, weighed, sexed, and examined for external malformations. The frequency of visceral malformations and variations was determined in one-half of the fetuses, and the frequency of skeletal deviations was determined in the other half. Maternal toxicity was exhibited in the dams at all three exposure concentrations as statistically significant reductions in weight gain and food consumption. Maternal weight gain at day 20 was approximately 110 g for control, compared to 85 g, 84 g, and 25 g for 3500, 5000, and 7000 ppm 2-butanol, respectively. Likewise, maternal food consumption was reduced at all concentrations of 2-butanol, although at week 3 of gestation statistical significance was only seen at 5000 and 7000 ppm (126±15 g for control vs. 113±13 g, 112±17 g, and 99±11 g for 3500, 5000, and 7000 ppm 2-butanol). The authors reported narcosis (impairment of locomotor activity) at 5000 and above, but not at 3500 ppm. 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 7000 ppm (3.8±2.2) compared with the control (1.5±1.3). Fetal body weights were reduced in all MEK-exposed groups. The mean of litter means (±SD) for male and female fetal weights for the control group were 3.3±0.23 g and 3.1±0.22 g vs. 3.1±0.22 g and 2.9±0.20 g (3500 ppm), 2.7±0.25 g and 2.6±0.23 g (5000 ppm), and 1.5±0.12 g and 1.4±0.18 g (7000 ppm). The authors reported that decreased fetal body weights were significantly different from the control at 5000 and 7000 ppm. External fetal malformations were not observed. The incidence of pooled skeletal variations was statistically significantly increased at 7000 ppm (100%) compared to control (32%). The authors did not report the nature of skeletal variations observed or the incidence of the individual variations. Occasional visceral variations were seen; however, the authors did not attribute these to 2-butanol treatment. Given the marked maternal toxicity observed at 7000 ppm (including weight gain that was less than 25% of the control), the increase in resorptions and skeletal variations at this exposure concentration cannot necessarily be attributed to a direct effect of 2-butanol. The types of developmental effects induced by inhalation exposure to 2-butanol at concentrations below 7000 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). It has been shown that the ability to detect a change in fetal body weight (a continuous variable) is much greater than for other (dichotomous) fetal endpoints, and thus changes in fetal body 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, 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 inhalation exposure

to 2-butanol and MEK were similar, fetal body weight changes observed in both 2-butanol and MEK-exposed animals were compared. Figure 2 shows the relationship between fetal body 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 plots shows that the dose-response curves for 2-butanol and MEK are not inconsistent.

A summary table of key repeat exposure reproductive and developmental toxicity studies in animals exposed to MEK and 2-butanol is available in Section 5.1.1 (Table 3).

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



## 4.4. OTHER STUDIES

### 4.4.1. Acute Toxicity Data

#### 4.4.1.1. Oral Exposure

Oral $LD_{50}$ values for MEK include 5522 and 2737 mg/kg in rats (Smyth *et al.*, 1962 and Kimura *et al.*, 1971, respectively) and 4044 mg/kg in mice (Tanii *et al.*, 1986). Single gavage doses of 15 mmol/kg MEK in corn oil (1082 mg/kg) 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).

#### 4.4.1.2. Inhalation Exposure

LaBelle and Brieger (1955) reported a 4-hour $LC_{50}$ for MEK of 11,700±2400 ppm (34,515±7080 mg/m$^3$) in rats. Several studies have described the behavioral effects of acute inhalation exposure of mice to MEK (Section 4.2.2.1). 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 (both response to a visual stimulus and the response rate were used as indicators). The concentrations for each 30-minute period were 300, 1000, 3000, 5600, or 10,000 ppm (885, 2950, 8850, 16,520, or 29,500 mg/m$^3$) with a total exposure time of 2 hours. No effects were observed at 300 ppm, a slight decrease in response rate at 1000 ppm, and a 75% decrease in response rate at 3000 ppm. Most mice (incidence not reported) ceased to respond at 5600 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 2891 ppm (SD = 689 ppm). From these results, an $EC_{10}$ (*i.e.*, 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 (2891 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, 1602, 1848, 2050, or 2438 ppm (0, 4726, 5452, 6048, or 7192 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 these data in mice, the authors calculated a 50% decrease in immobility ($ID_{50}$) for MEK of 2065 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 Salmonella (Ames) assay with or without metabolic activation, the L5178/$TK^{+/-}$ mouse lymphoma assay, and the BALB/3T3 cell transformation assay, and 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* (Zimmerman *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, groups of 10 male C3H/He mice received dermal applications of 50 mg of a solution containing 17, 25, or 29% MEK in dodecylbenzene (50, 70, or 70%, respectively) twice a week for 1 year (Horton *et al.*, 1965). No skin tumors developed in the groups of mice treated with 17% MEK or 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 29% MEK and 0.8% 2-phenylbenzothiophene. 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 have demonstrated that MEK potentiates the effects of known neurotoxicants (e.g., n-hexane, methyl-n-butylketone, 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/methyl n-butyl ketone at concentrations of 1125 ppm (3319 mg/m³) MEK and 225 ppm methyl n-butyl ketone. In contrast, rats exposed to 225 ppm methyl n-butyl ketone 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 were increased at an exposure of 9000 ppm n-hexane and 1000 ppm (2950 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 to a mixture of n-hexane and MEK. Animals exposed continuously to 500 ppm n-hexane alone displayed hindlimb paralysis after 9 weeks, as well as axonal lesions in peripheral nerves. In rats treated with n-hexane/MEK (300 ppm/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 to 200 ppm MEK alone. In addition, tail nerve conduction velocity in rats exposed to 100 ppm n-hexane plus 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 and coworkers, MEK exposure groups (at 100 ppm) that were co-exposed 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 (Dick *et al.*, 1984, 1988, 1989, 1992). 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, these studies are not adequate 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 1161 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 5000 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 referant 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) conducted an investigation of neurobehavioral effects in workers in a video tape manufacturing facility in Singapore where there was exposure to MEK, cyclohexanone, tetrahydrofuran and toluene. 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. None of the available occupational studies involving multiple chemical exposure discussed above provide information adequate to establish whether MEK interacts with other neurotoxic solvents in humans. Further, these studies do not provide information from which one can 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 air concentrations 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 the metabolic conversion of n-hexane to 2,5-HD or to an intermediate metabolite to 2,5-HD (van Engelen *et al*., 1997; Zhao *et al*., 1998; Yu *et al*., 2002). In contrast to n-hexane and methyl n-butyl ketone, MEK is not metabolized to a gamma-diketone (*i.e.*, a diketone in which the two carbonyl groups are separated by two carbons) (DiVincenzo *et al.*, 1976). This difference is paramount, 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 (>1000 ppm or 2950 mg/m$^3$) where induction of hepatic enzymes (that are responsible for toxifying the gamma-diketones) is postulated as the mode of action. One exception is Takeuchi *et al.* (1983) in which reversible potentiation of n-hexane neurotoxicity was observed at MEK concentrations of 200 ppm (590 mg/m$^3$). The work of van Engelen *et al.* (1997) provides some insights into lower limits of interactive effects of MEK and n-hexane in humans. Volunteers were exposed to n-hexane (approximately 60 minutes) with or without co-exposure to MEK (200 or 300 ppm) for 15.5 minutes, and the concentration-time

course of n-hexane (in exhaled alveolar air) and its metabolite 2,5-HD (in serum) were measured. Co-exposure to 200 ppm MEK did not affect the concentration-time course of exhaled n-hexane. Co-exposure to 200 ppm MEK did not significantly affect the rate of formation of serum 2,5-HD, but 300 ppm MEK significantly decreased the rate of 2,5-HD formation (approximately 3-fold). Co-exposure 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 toxic effects of oral exposure of humans to MEK are limited to a single non-occupational report of acute toxicity following accidental ingestion of MEK. This report did not indicate any persistent adverse health effects. In laboratory animals, the database 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 1082 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 database for 2-butanol consists of a 2-generation reproductive and developmental toxicity study in rats (Cox *et al.*, 1975). Administration of 2-butanol in the drinking water before and during gestation and lactation at concentrations as high as 3% did not affect reproductive performance, but did result in decreased pup survival and pup body weight gain in Wistar rats. A concentration of 2% in drinking water caused a slight reduction in fetal weights when pregnancies were terminated on gestation day 20 and concentrations in the range of 1 to 2% in drinking water caused a slight decrease in pup body weights when dams were allowed to deliver. Thus, based on data from Cox *et al*. (1975), the LOAEL for the F1A generation was 1771 mg/kg-day (1%

solution) and the NOAEL was 594 mg/kg-day (0.3% solution) based on decreased F1A body weights. In the F1B and F2 generations, the LOAEL was 3122 mg/kg-day (2% solution) and the NOAEL was 1771 mg/kg-day (1% solution) for decreased F1B fetal body weights and decreased F2 body weights. The finding 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 at drinking water 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. These changes were consistent with the pattern of early stages of $\alpha_{2u}$-globulin-associated rat nephrotoxicity. According to Agency guidance (U.S. EPA, 1991b), these species- and sex-specific renal effects are not appropriate to use in establishing a critical effect for human health risk assessment.

The oral toxicity database 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 (1286 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.* 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 concentration that corresponded to a slight but not significant 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 few 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). Several well-conducted studies in experimental animals, however, provide no convincing evidence that 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 5041 ppm (Cavender *et al.*, 1983). Repeated exposure of rats and mice to MEK at approximately 3000 ppm (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 body weight and increased incidence of delayed skeletal development) in rats and mice exposed to MEK at 3000 ppm, 7 hours/day during gestation, but not at 1000 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.

The available data provide no evidence for other systemic effects resulting from inhalation exposure to MEK. A subchronic inhalation study of MEK found no persistent body weight, 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 5000 ppm (Cavender *et al*., 1983). Although some changes in organ weight and clinical pathology parameters were observed, 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 and co-workers 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 (Dick *et al.*, 1984, 1989, 1992). In an earlier study involving few subjects and unclear exposure conditions, exposure to 300 ppm (885 mg/m$^3$) was reported as intolerable (Nelson *et al.*, 1943). Nasal irritation was noted in rats exposed to 6000 ppm 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 5000 ppm MEK for 90 days (confounding respiratory tract lesions likely due to an infectious agent occurred in all groups in this study including controls). In addition, respiratory irritation was not reported in dams exposed to 3000 ppm, 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 the draft revised cancer guidelines (U.S. EPA, 1999), *data are inadequate for an assessment of human carcinogenic potential* of 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 each is confounded by exposure to multiple chemicals. A case-control study examining the association between paternal exposures to several solvents, including MEK, and childhood leukemia 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 some cancers (including bone and prostate) and multiple solvent exposures that include MEK.

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 (Horton *et al.*, 1965) is an inadequate test of MEK 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, and 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 MEK 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.

### 4.7.2. Possible Gender Differences

Available studies in humans and animals have not demonstrated 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 were 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 sensitive to the effects of MEK than males. In addition, no gender-specific susceptibility was observed in offspring in any of the developmental studies (Schwetz *et al.*, 1974, 1991; Deacon *et al.*, 1981).

### 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 potentiates the neurotoxicity of hexacarbon solvents (n-hexane, methyl-n-butylketone and 2,5-hexanedione) (Saida *et al.*, 1976; Couri *et al.*, 1977, respectively) and the liver and kidney toxicity of haloalkane solvents (carbon tetrachloride, trichloromethane, and chloroform) (Dietz and Traiger, 1979; WHO, 1992; Brown and Hewitt, 1984, respectively). The mode by which MEK potentiates the neurotoxicity of hexacarbon solvents is not entirely clear, but 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. These potentiating effects of MEK on the toxicity of other solvents, however, have only been demonstrated at relatively high exposure concentrations (200–1000 ppm).

No data are available concerning susceptibility of other specific lifestages (including elderly populations) to MEK toxicity, although there exists no toxicologic basis, metabolic or otherwise, to suspect MEK 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 database 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. Administration of 2-butanol in the drinking water before and during gestation and lactation at concentrations as high as 3% did not affect reproductive performance, but did result in decreased pup survival and pup body weight gain. A concentration of 2% in drinking water caused reduction in fetal weights when pregnancies were terminated on gestation day 20 and concentrations in the range of 1 to 2% in drinking water caused a decrease in pup body weights 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 4). 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 (Cox *et al*., 1975), a response that has the hallmark characteristics of $\alpha_{2u}$-globulin nephrotoxicity and is therefore problematic as the basis for any dose-response assessment (U.S. EPA, 1991b).

Data from the 13-week drinking water study with 3-hydroxy-2-butanone in CFE rats (Gaunt *et al.*, 1972) are suggestive of an adverse hematological effect (decreased hemoglobin concentration and red blood cell count). This effect was not observed, however, in toxicity studies of 2-butanol (Cox *et al.*, 1975) or MEK (Cavender *et al.*, 1983). This study of drinking water exposure to 3-hydroxy-2-butanone provides no information concerning 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 toxicity of MEK.

Pharmacokinetic and toxicologic data support the use of 2-butanol as an appropriate surrogate for MEK. Pharmacokinetic findings in rats supporting 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 1776 mg/kg 2-butanol (7–8 hours) or 1690 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 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 supporting the use of 2-butanol as a MEK surrogate include: 1) fetal body weight deficits were critical effects in studies of rats (Schwetz *et al*., 1974; Deacon *et al*., 1981) and mice (Schwetz *et al*., 1991) exposed to MEK by inhalation during gestation, in a two-generation reproductive and developmental toxicity study in rats exposed to 2-butanol in drinking water (Cox *et al*., 1975), and in a study of rats exposed by inhalation during gestation to 2-butanol (Nelson *et al*., 1989); and 2) the relationships between air concentrations and degree of fetal body weight changes were consistent for MEK and 2-butanol.

Thus, for derivation of an MEK RfD, the reproductive and developmental drinking water toxicity study of 2-butanol in rats (Cox *et al.*, 1975) was selected as the principal study. 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 database 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 and through day 10 of lactation, and slight decrease in neonatal body weight at 1% 2-butanol. Decreased body weights were also observed in F1B fetuses and F2 pups that were exposed to 2% 2-butanol in drinking water. Based on these developmental endpoints, a LOAEL of 1771 mg/kg-day (1% solution) and a NOAEL of 594 mg/kg-day (0.3% solution) were identified for decreased F1A pup body weights (at days 4 and 21), and a LOAEL of 3122 mg/kg-day (2% solution) and a NOAEL of 1771 mg/kg-day (1% solution) were identified for decreased F1B fetal body weights and decreased F2 pup body weights (see Table 4).

Table 4. Summary table 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)<br><br>F0 generation<br>F1A (first litter)<br>F1B (second litter)<br>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]<br><br>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%).<br>F1A: Decreased pup survival (3%). Decreased pup weight, days 4 and 21 (1% & 3%).<br>F1B: Decreased fetal weight (2%).<br>F2: Decreased pup weight, days 4 and 21 (2%).<br><br>[All pup and fetal body weight comparisons were based on litter means and not individual pup/fetus data.] | F1A: 1771 mg/kg-day (1%) – body weight (10% lower than control)<br><br>F1B & F2: 3122 mg/kg-day (2%) – fetal/pup body weight | F1A: 594 mg/kg-day (0.3%)<br><br>F1B & F2: 1771 mg/kg-day (1%) |
| ***Inhalation studies*** | | | | |
| Schwetz *et al*. (1974) | Pregnant SD rats, 21-23/grp, exposed to 0, 1126, or 2618 ppm **MEK**, 7 hours/day on gestation days 6–15. | Developmental effects (percentage of litters with any soft tissue anomaly). | 2618 ppm (7723 mg/m$^3$) | 1126 ppm (3322 mg/m$^3$) |

Table 4. Summary table of key repeat-exposure reproductive and developmental toxicity studies in animals exposed to MEK or 2-butanol.

| Study | Exposure protocol | Effects | LOAEL | NOAEL |
|---|---|---|---|---|
| Deacon *et al*. (1981) also reported by Dow Chemical Corporation (1979) | Pregnant SD rats, 18-26/grp, exposed to 0, 412, 1002, or 3005 ppm **MEK**, 7 hours/day on gestation days 6–15. | Decreased maternal weight gain.<br>Increased incidence fetal skeletal variations. | 3005 ppm (8865 mg/m$^3$) | 1002 ppm (2955 mg/m$^3$) |
| Nelson *et al.* (1989, 1990) | Pregnant SD rats, 15-16/grp, exposed to 0, 3500, 5000, or 7000 ppm **2-butanol**, 7 hours/day on gestation days 1–19. | Decreased maternal weight gain and food consumption.<br>Decreased maternal locomotor activity.<br>Decreased fetal weight. | 5000 ppm (15,150 mg/m$^3$) | 3500 ppm (10,605 mg/m$^3$) |
| Schwetz *et al*. (1991) also reported as Mast *et al*. (1989) and NTP (1990) | Pregnant CD-1 mice, 33/grp, exposed to 0, 398, 1010, or 3020 ppm **MEK**, 7 hours/day on gestation days 6–15. | Decreased body weight in male fetuses and both sexes combined [based on litter means].<br>Increased maternal liver-to-body weight ratio. | 3020 ppm (8909 mg/m$^3$) | 1010 ppm (2980 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%.

### 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, B-2 and B-3.

#### 5.1.2.1. Benchmark Dose Modeling

Several data sets from the Cox *et al*. (1975) study were selected for benchmark dose modeling: fetal body 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 (*i.e.*, 3% solution) is likely to have confounded the effects on surviving pup body weight (i.e., measured body weights are representative of survivors only and not 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.

Models for continuous data (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). This 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. Consequently, for each generation, a 5% decrease in the mean pup or fetus body weight per litter (compared with the control mean) was selected as the benchmark response because it was a response rate that fell within the range of experimental dose levels used in the Cox *et al.* study. 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.

##### 5.1.2.1.1. Modeling of F1A Pup Body Weights

The means of the 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 5).

Table 5. Means of litter means for postnatal day 4 and 21 pup body weight in the F1A generation of Wistar rats exposed to 2-butanol in drinking water in a two-generation reproductive and developmental toxicity study. Source: Appendix II of Cox *et al.* (1975).

| 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 | 1771 | 9.97±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.

A constant variance linear continuous-variable model (BMDS version 1.3.1) provided an adequate fit to the data (with a goodness-of-fit p value > 0.1). See Appendix B for benchmark dose software output. The other continuous variable models (polynomial and power) could not be fit to the data due to lack of degrees of freedom (i.e., 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).

The model-predicted $ED_{05}$ values associated with a 5% decrease in mean F1A fetal body weight were 1387 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.

#### 5.1.2.1.2. Modeling of F1B Fetal Body Weights

The means of the litter means for F1B fetal body 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 6.

Table 6. Means of litter means for fetal body weight in the F1B generation of Wistar rats exposed to 2-butanol in drinking water in a two-generation reproductive and developmental toxicity study. Source: Appendix III of Cox *et al.* (1975).

| Drinking water concentration (% 2-butanol by weight) | Maternal dose (mg/kg-day) | Number of litters | Mean of litter means fetal body weight (g) | Standard deviation |
|---|---|---|---|---|
| 0 | 0[a] | 29 | 4.14 | 1.45 |
| 0.3 | 594[a] | 27 | 4.16 | 0.69 |
| 1 | 1771[a] | 30 | 4.38 | 1.04 |
| 2 | 3122[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.

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 7). 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).

Table 7. Benchmark dose modeling results using litter mean body weight data for F1B fetuses from Cox *et al.* (1975).

| Model | GOFP | AIC | $ED_{05}$ (mg/kg-day) | $LED_{05}$ (mg/kg-day) |
|---|---|---|---|---|
| Linear | 0.15 | 137.9 | 1969 | 896 |
| Polynomial | 0.12 | 136.6 | 2198 | 1046 |
| Power | 0.17 | 137.0 | 2980 | 1578 |

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 body weight.
$LED_{05}$ = 95% lower confidence limit on the $ED_{05}$ as calculated by BMDS.

The model-predicted $ED_{05}$ associated with a 5% decrease in mean F1B fetal body weight was 2198 mg/kg-day. The corresponding $LED_{05}$ was 1046 mg/kg-day.

#### 5.1.2.1.3. Modeling of F2 Pup Body Weights

The mean of the 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 as shown in Table 8.

Table 8. Means of litter means for postnatal day 4 and 21 pup body weight in the F2 generation of Wistar rats exposed to 2-butanol in drinking water in a two-generation reproductive and developmental toxicity study. Source: Appendix V of Cox *et al.* (1975).

| 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)[c] |
|---|---|---|---|
| 0 | 0[a] | 10.0±1.4 | 40±6.1 |
| 0.3 | 594[a] | 9.7±1.6 | 39±7.8 |
| 1 | 1771[a] | 9.6±2.3 | 39±9.4 |
| 2 | 3122[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.
[c] The data reported herein differ from the data in Table 10 of Cox *et al*. (1975) because data for day 21 could only be discerned to the nearest gram from the best available copy of Appendix V.

A constant variance linear continuous-variable model (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). A constant variance polynomial continuous-variable model provided the best fit to the day 21 pup body weight. (See goodness-of-fit statistics in Table 9.) 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).

Table 9. Benchmark dose modeling results using litter mean body weight data for F2 pups on postnatal days 4 and 21 from Cox *et al.* (1975).

| Model | GOFP | AIC | $ED_{05}$ (mg/kg-day) | $LED_{05}$ (mg/kg-day) |
|---|---|---|---|---|
| *Postnatal day 4* | | | | |
| Linear | 0.88 | 227.9 | 3471 | 1347 |
| Polynomial | 0.61 | 227.9 | 3471 | 1347 |
| Power | 0.26 | 231.9 | 3471 | 1347 |
| *Postnatal day 21* | | | | |
| Linear | 0.55 | 512.5 | 1398 | 851 |
| Polynomial | 0.48 | 511.8 | 2056 | 901 |
| Power | 0.26 | 515.5 | 2508 | 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 fetal body weight.
$LED_{05}$ = 95% lower confidence limit on the $ED_{05}$ as calculated by BMDS.

The model-predicted $ED_{05}$ values associated with a 5% decrease in mean pup body weight were 3471 mg/kg-day for day 4 and 2056 mg/kg-day for day 21. The corresponding $LED_{05}$ values were 1347 and 901 mg/kg-day, respectively.

#### 5.1.2.1.4. 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: fetal body weight from the F1B generation and pup body weight at post natal days 4 and 21 from the F1A and F2 generations. The $LED_{05}$ values calculated from modeling these data sets are shown in Table 10.

Table 10. Benchmark doses for developmental effects in rats from various generations of Cox *et al*. (1975) and potential points of departure for the MEK RfD.

| Endpoint | $ED_{05}$ [a] (mg/kg-day) | $LED_{05}$ [a] (mg/kg-day) |
|---|---|---|
| F1A pup body weight, day 4[b] | 1387 | 803 |
| F1A pup body weight, day 21[b] | 878 | 657 |
| F1B fetal body weight, gestation day 20 | 2198 | 1046 |
| F2 pup body weight, day 4 | 3471 | 1347 |
| F2 pup body weight, day 21 | 2056 | 901 |

[a] $ED_{05}$: benchmark dose associated with a 5% decrement in litter mean pup or fetus body weight compared with control mean.
$LED_{05}$: 95% lower confidence limit on the ED.
[b] The data modeled excluded high dose (3%).

The $LED_{05}$ values from these data sets are within 2-fold of each other; therefore, all the modeling results are equally plausible. The lowest point of departure, based on the 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 development of an RfD for MEK. Unfortunately, deficiencies in the absorption data preclude the application of these methods for MEK. For a route-to-route extrapolation, a number of assumptions would need to be made with regard to the inhalation and oral absorption efficiencies 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 concentrations of 200 ppm (590 mg/m$^3$). 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 derivation of an oral RfD for humans based upon extrapolation from inhalation effects in animals. Moreover, the route-to-route extrapolation would require data on oral absorption of MEK in humans; 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 (*e.g.*, 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). A total uncertainty factor of 1000 was applied to this point of departure: 10 for extrapolation from animals to humans, 10 for extrapolation to the most sensitive humans, and 10 for database deficiencies.

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 sensitive human subpopulations. Although the RfD is based on a potentially sensitive population (developing fetus and neonates), this uncertainty factor was not reduced because of the lack of human oral exposure information in the range of responses to MEK in human subpopulations.

A 10-fold uncertainty factor was used to account for deficiencies in the available MEK database. No oral data are available for MEK; however, the available pharmacokinetic and inhalation toxicity data support 2-butanol as an appropriate surrogate for MEK. Although no chronic studies are available, the database 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. The absence of 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.

A 5% decrease in pup weight, relative to control, was selected to help identify the point of departure. Although dose-response data suggested a trend of decreased body weight with increasing dose, the 2-generation Cox *et al.* study provides no evidence that this effect was associated with permanent functional alterations. In this 2-generation study, reduction in offspring body weight (as high as 43% in F1A rats on postnatal day 21) was not associated with impairment of reproductive performance or other toxicologically relevant endpoints evaluated in the study. Further, the pup body weight reductions in the first set of offspring (F1A) in the low- and mid-dose groups were less apparent in the next generation (F2). In the F2 generation on postnatal day 21, the only group of pups with body weights not similar to the control was the high-dose group; these offspring were born to dams with body weights 13% lower than the control. Since there were no other effects in the range of the $LED_{05}$ of 657 mg/kg-day, 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 sensitive lifestage where exposure during certain time windows of development are more relevant to induction of developmental effects than lifetime exposure.

The RfD for MEK was calculated as follows:

$$\begin{aligned} RfD &= LED_{05} \div UF \\ &= 657 \text{ mg/kg-day} \div 1000 \\ &= 0.7 \text{ mg/kg-day} \end{aligned}$$

### 5.1.4. Previous Oral Assessment

In the previous IRIS assessment entered on IRIS in 1993, an RfD of 0.6 mg/kg-day was derived for MEK based on the NOAEL of 1771 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: a (10) for inter- and intraspecies extrapolations, a (10) to adjust for subchronic-to-chronic extrapolation since long-term effects in the dams were not reported in the principal study; a (10) for an incomplete database that included a lack of

both subchronic and chronic oral exposure studies for MEK; and a (10) for lack of data for a second rodent species for either MEK or 2-butanol."

## 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 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). Due to uncertainty in the exposure levels or concomitant exposure to other chemicals, it is not possible to identify effect levels from the 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 CNS 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 endpoint. Inhalation exposure of experimental animals to 3000 ppm MEK (7 hours/day on days 6–15 of gestation) resulted in developmental effects, but no persistent neurological effects (Schwetz *et al.*, 1974, 1991; Deacon *et al.*, 1981). The most appropriate data for derivation of an inhalation RfC for MEK are from inhalation developmental toxicity studies in two species: 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 developmental effects reported are consistent (although the specific endpoints differ). In Sprague-Dawley rats, Deacon *et al.* (1981) reported fetal toxicity (increased incidence of skeletal variations) at 3005 ppm (8865 mg/m$^3$) (7 hours/day on gestation days 6–15). In CD-1 mice, Schwetz *et al.* (1991) found reduced fetal body weight at 3020 ppm (8909 mg/m$^3$) (7 hours/day on gestation days 6–15) and a positive trend for increasing 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 2955 and 2980 mg/m$^3$ and LOAELs of 8865 and 8909 mg/m$^3$ 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 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.

##### 5.2.2.1.1. Modeling of Incidence of Rat Litters with Fetuses with Extra Ribs

The incidences of extra ribs (litters with an affected fetus) as reported by Deacon *et al.* (1981) are shown in Table 11. The incidence of extra ribs among fetal Sprague-Dawley rats exposed to 3005 ppm MEK 7 hours/day on gestation days 6–15 was statistically different from the incidence among controls.

Table 11. Incidence of extra ribs (litters with an affected fetus) in Sprague-Dawley rats exposed to MEK 7 hours/day on gestation days 6–15. Source: Deacon *et al.* (1981).

| Concentration (ppm) | Number of fetuses (litters) | Number of fetuses (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 |
| 1002 | 226 (19) | 0 | 0 ± 0 |
| 3005 | 229 (18) | 7 (6) | 3.1 ± 1.8 |

All nested models for dichotomous variables available in the EPA Benchmark Dose Software (BMDS version 1.3.1) were fit to the data in Table 11. These models – the nested logistic (NLogistic), the NCTR, and the 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, 2002c). 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.* study. All of these models provided similar fits to the data, based on the summary results reported in the BMDS output, and detailed examination of the graphs and goodness-of-fit statistics (summarized in Table 12). Model fits were not improved by incorporation of litter size (as a litter-specific covariate) or by incorporation of 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.

Table 12. Benchmark concentration modeling results using litter incidence data for rat fetuses with extra ribs exposed to MEK during gestation from Deacon *et al.* (1981).

| Nested Model | GOFP | AIC | $EC_{05}$ (ppm) | $LEC_{05}$ (ppm) |
|---|---|---|---|---|
| Log-Logistic | 0.09 | 96.5 | 3124 | 2993 |
| NCTR | 0.51 | 96.5 | 3317 | 2993 |
| Rai and vanRyzin | 0.51 | 96.6 | 3353 | 2992 |

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.

The model-predicted $EC_{05}$ associated with a 5% increased incidence of extra ribs was 3317 ppm. The corresponding $LEC_{05}$ was 2993 ppm.

**5.2.2.1.2. Modeling of Decreased Fetal Body Weight Data in Mice**

The full laboratory report from Schwetz *et al.* (1991) is available in Mast *et al.* (1989). The mean of the litter means for fetal body weights (and their standard deviations) in the control and MEK-exposed groups presented in Table 7 of Mast *et al.* (1989) are shown in Table 13 below.

Table 13. Means of litter means for fetal body weight (both sexes combined) in CD-1 mice exposed to MEK 7 hours/day on gestation days 6–15. Source: Mast *et al.* (1989); Schwetz *et al*. (1991).

| Concentration (ppm) | Number of litters | Fetal body weight (mean of litter means in g) | Standard deviation |
|---|---|---|---|
| 0 | 26 | 1.35 | 0.07 |
| 398 | 23 | 1.35 | 0.06 |
| 1010 | 26 | 1.33 | 0.07 |
| 3020 | 28 | 1.29 | 0.08 |

Models for continuous data (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 in Table 13 using U.S. EPA Benchmark Dose Software (version 1.3.1). A decrease in the mean fetal body weight of 1 standard deviation of the control mean was selected as the benchmark response for this endpoint consistent with the recommendations of the Benchmark Dose Technical Guidance Document (U.S. EPA, 2000c). This corresponds to a 5% decrease in the mean control group weight for this data set. A constant variance linear 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 14.) Visual inspection of the plot 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).

Table 14. Benchmark concentration modeling results using litter mean body weight data from Mast *et al.* (1989), Schwetz *et al.* (1991).

| Model | GOFP | AIC | EC (ppm) | LEC (ppm) |
|---|---|---|---|---|
| Linear | 0.90 | -442.2 | 3339 | 2273 |
| Polynomial | 0.66 | -440.2 | 3330 | 1561 |
| Power | 0.28 | -438.2 | 3343 | 2275 |

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 body weight 1 SD below the control mean.
LEC = 95% lower confidence limit on the EC as calculated by BMDS.

The model-predicted EC associated with a mean fetal body weight of 1 standard deviation below the control mean was 3339 ppm. The corresponding LEC was 2273 ppm.

#### 5.2.2.1.3. Modeling of Misaligned Sternebrae Data in Mice

The other statistically significant developmental effect identified by Schwetz *et al.* (1991) was 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 15.

Table 15. 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. Source: Mast *et al.* (1989); Schwetz *et al*. (1991).

| Concentration (ppm) | Number of fetuses (litters) | Number of fetuses (litters) with misaligned sternebrae | Mean percent of fetuses with misaligned sternebrae per litter |
|---|---|---|---|
| 0 | 310 (26) | 31 (18) | 9.7 ± 10.4 |
| 398 | 260 (23) | 27 (14) | 9.8 ± 11.2 |
| 1010 | 291 (26) | 49 (18) | 17.4 ± 16.7 |
| 3020 | 323 (28) | 58 (21) | 17.5 ± 14.9 |

The nested, dichotomous-variable models available in the EPA BMD software were fit to the individual litter data for fetuses with misaligned sternebrae as reported in Appendix F of Mast *et al.* (1989). First, each model was fit without litter size as a covariate, and then the models were fit with 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 misaligned sternebrae incidence (results not shown). Then each model was fit with and without intra-litter correlations. In each case, the model fit was better with the intra-litter correlations included. These model fits were linear. All three nested models provided adequate fits to the data, based on the summary results reported in the BMDS output (see Appendix B for benchmark dose software output). A more detailed examination of the graphs and residuals suggested that a non-linear 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 the data are most consistent in this range of the data set. Also, the Benchmark Dose Technical Guidance Document recommends estimation of a 10% BMR for a point of consistent comparison across

chemicals (U.S. EPA, 2000c). Effective concentrations associated with this BMR and their 95% lower confidence limits ($LEC_{10}$s) are summarized in Table 16.

Table 16. Benchmark concentration modeling results using individual litter data for mouse fetuses with misaligned sternebrae exposed to MEK during gestation (without litter size as covariates). Source: Mast *et al.* (1989).

| Nested Model | GOFP | AIC | $EC_{10}$ (ppm) | $LEC_{10}$ (ppm) |
|---|---|---|---|---|
| NLOGISTIC | 0.6349 | 937.1 | 3197 | 1714 |
| Rai and Van Ryzin | 0.5433 | 937.2 | 3222 | 1789 |
| NCTR | 0.4877 | 937.2 | 3222 | 1789 |

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 $EC_{10}$.

Because the three model fits are very similar (Table 16), an average of the three $LEC_{10}$s was calculated as the point of departure. The respective $EC_{10}$ and $LEC_{10}$ values calculated as an average of the three models are 3214 and 1764 ppm, respectively.

#### 5.2.2.1.4. Comparison of Benchmark Dose Modeling Results

For inhalation exposure to MEK, three developmental endpoints from two species were modeled: increased incidence of extra ribs in Sprague-Dawley rats (Deacon *et al.*, 1981) and decreased fetal body 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 shown in Table 17. 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 1764 ppm (5202 $mg/m^3$) based on the incidence of misaligned sternebrae in mice exposed to MEK by inhalation for 7 hours/day on gestation days 6–15 was selected as the most health protective value for derivation of the RfC.

Table 17. Benchmark concentrations for developmental effects in mice and rats and potential points of departure for the MEK 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% | 9781 (3317) | 8826 (2993) |
| Decreased fetal body weight (mice) (Schwetz *et al.*, 1991) | 1 s.d. ≈ 5% | 9847 (3339) | 6705 (2273) |
| Increased incidence of misaligned sternebrae (mice) (Schwetz *et al.*, 1991) | 10% | 9478 (3214) | 5202 (1764) |

[a] Sample calculation: (3317 ppm x 72.1 mg/mmol)/24.45 = 9781 mg/m$^3$, 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) x time (t) relationship (*e.g.*, 8 hours @ 300 ppm = 24 hours @ 100 ppm). For developmental studies, the 1991 *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a) and the 1996 *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 x t relationship, may be a more appropriate metric for some developmental toxicants than peak exposure (U.S. EPA, 2002). In consideration of this information, EPA recommends that adjustment to continuous exposures be used for inhalation developmental toxicity studies as for other health effects from inhalation exposure (U.S. EPA, 2002).

Therefore, unless there are pharmacokinetic data suggesting that adjustment to a continuous exposure equivalent is inappropriate, or mode of action information suggests that a susceptible period of development that is specifically targeted (which would suggest that the peak dose may be representative of the effective dose), duration adjustment is appropriate as the more health-protective procedure. In applying these considerations to MEK, the critical effect is non-specific developmental toxicity (developmental delays), 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 inappropriate. Overall, the available pharmacokinetic, pharmacodynamic, and mechanism of action data for MEK do not provide sufficient evidence to argue convincingly for either peak exposure level or area under the curve 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 5202 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 (*i.e.*, 7 hours of exposure/day to 24 hours/day) as follows:

$$LEC(ADJ) = LEC \times \frac{7\ hours}{24\ hours}$$
$$= 5202\ mg/m^3 \times 7/24$$
$$= 1517\ mg/m^3$$

The RfC methodology provides for deriving an estimate of the human concentration that would correspond 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:

$$LEC_{(HEC)} = LEC_{(ADJ)} \times \frac{B{:}\,A\,rat}{B{:}\,A\,human}$$
$$= 1517\ mg/m^3 \times 1$$
$$= 1517\ mg/m^3$$

The $LEC_{(HEC)}$ value of 1517 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 1517 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 100 was applied to this point of departure: 3 for interspecies extrapolation, 10 for sensitive individuals, and 3 for incomplete database.

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 sensitive individuals within the human population. This uncertainty factor was not reduced because of the lack of human inhalation exposure information addressing potentially sensitive populations. Although the RfC is based on a potentially sensitive population (developing fetus), this uncertainty factor was not reduced because of the lack of information on the range of responses associated with inhalation exposure to MEK in human subpopulations.

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 resulting from a narrow period of exposure (gestation days 6-15) was used as the critical effect. The developmental period is recognized as a sensitive lifestage where exposure during certain time windows of development are more relevant to induction of developmental effects than lifetime exposure.

A 3-fold uncertainty factor was used to account for database deficiencies. In this case, a partial factor is applied ($10^{0.5}$ or approximately 3). As noted earlier, the minimum database requirements for derivation of an RfC are satisfied by the Cavender *et al.* (1983) study. Data from an oral multigeneration reproductive and developmental toxicity study (Cox *et al.*, 1975) with a metabolic precursor, 2-butanol, demonstrated no systemic toxicity or reproductive effects in rats dosed for 14–18 weeks and confirmed developmental toxicity as the critical endpoint. Histological information available on the reproductive organs from the subchronic inhalation study by Cavender *et al.* (1983) gives additional indication that MEK is not likely to be a reproductive toxicant. 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 CNS 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 developmental toxicity studies revealed no evidence of neurotoxicity potential in developing populations, although specific tests for neurological toxicity were not performed.

An RfC for MEK is calculated as follows:

$$\begin{aligned} RfC &= LEC_{(HEC)} \div UF \\ &= 1517\ mg/m^3 \div 100 \\ &= 15\ mg/m^3 \end{aligned}$$

As noted in Section 5.2.4. below, 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 publication of the RfC methodology (U.S. EPA, 1994b). This RfC was based on the Schwetz *et al.* (1991) developmental toxicity study in the mouse. In the previous assessment, a combined uncertainty factor of 3000 was applied to a human equivalent concentration NOAEL (2978 mg/m$^3$), which was not adjusted to a continuous exposure basis. The combined uncertainty factor accounted for interspecies extrapolation, intrahuman variability, and database deficiencies (including a lack of chronic and reproductive toxicity studies), and 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).

## 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° 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).

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 1125 ppm (3318 mg/m$^3$) of 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 5041 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$^3$) of 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 1500 ppm (4425 mg/m$^3$) 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 2518 ppm (7430 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 relevant systemic toxic effects or reproductive effects, but found body weight deficits in offspring at estimated dose levels of approximately 2000 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 body weight and increased skeletal variations at exposure levels of approximately 1000 ppm (or 3000 mg/m³) MEK (Schwetz *et al*., 1974, 1991; Deacon *et al*., 1981). Despite the considerable information available on the suspected target tissue, neurological, for this solvent that show no effects from exposure durations up to and including subchronic (approximately 10% of the lifespan), the effects of lifetime exposure to MEK must necessarily remain somewhat uncertain due the lack of chronic toxicity information for MEK by any route of exposure. Available animal data consistently identify developmental effects in animals exposed to relatively high levels of MEK, i.e., greater than 500 mg/m$^3$. It is therefore reasonable and prudent to state that MEK is a possible health hazard to humans repeatedly exposed to MEK relatively high levels of MEK.

According to the U.S. EPA (1999) draft revised cancer guidelines, 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 come 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) study reported decreased F1A and F2 pup body weights and decreased F1B fetal body weights associated with 2-butanol exposure. Benchmark dose modeling of F1A pup body weight data (mean of litter means) at post natal day 21 yields a point of departure ($LED_{05}$) of 657 mg/kg-day (*i.e.*, the lower 95% confidence limit on a dose producing a mean 5% decrease in body weight compared with control). To this point of departure, a combined uncertainty factor of 1000 was applied, and a chronic RfD of 0.7 mg/kg-day was derived. This RfD is similar to 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, the study was conducted prior to the implementation of GLPs, and 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*. Confidence in the database 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 database confidence. Reflecting the medium to low confidence in the principal study and low confidence in the database, 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 level 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 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 MEK concentrations between 3000 and 9000 mg/m$^3$ (exposed 7 hours/day on days 6–15 of gestation) (Schwetz *et al.*, 1974, 1991; Deacon *et al.*, 1981). For comparison, this concentration range is not far from the range of exposure concentrations that have been reported in human case reports of toxicity: 885–1770 mg/m$^3$ (Smith and Mayers, 1944) and 900–5000 mg/m$^3$ (Seaton *et al.*, 1992) (see Section 4.1.2.2). In the previous IRIS assessment from 1993, an RfC of 1 mg/m$^3$ was derived based on a NOAEL of 2978 mg/m$^3$ for decreased fetal body 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 three data sets from the developmental toxicity studies of Deacon *et al.* (1981) and Schwetz *et al.* (1991). The lowest of the three LECs was selected as the point of departure (5202 mg/m$^3$; the lower 95% confidence limit on the concentration associated with a 10% extra risk for 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 = 1517 mg/m$^3$). To this HEC, a combined uncertainty factor of 100 was applied to account for the pharmacodynamic portion of interspecies uncertainty, sensitive individuals within the human population, and database deficiencies, yielding an RfC of 15 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 both 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 is lacking 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 database 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 590 $mg/m^3$. Reflecting high confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

### 6.2.3. Cancer/Oral and Inhalation

Data in both humans an 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, data are inadequate for the derivation of an oral slope factor or inhalation unit risk for MEK.

## 7. REFERENCES

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

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

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

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

Altenkirch, H., H.M. Wagner, G. Stoltenburg,, and P.S. Spencer. (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, D.L., M.A. Bonin, F.L., Cardinali, J.M. McCraw, and J.V. Wooten (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., P. Hartge, P.A. Stewart, *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.

Boyes, W.K, and D.W. Herr. (2002) Memo to Susan Rieth, U.S. EPA, NCEA, entitled: Comments on Reported Methyl Ethyl Ketone Neurotoxicity in Mitran *et al.*, 1997. June 27, 2002.

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

Brown, E.M. and W.R. Hewitt. (1984) Dose-response relationships in ketone-induced potentiation of chloroform hepato- and nephrotoxicity. Toxicol Appl Pharmacol 76: 437-53.

Browner, C. (1995) Memorandum from EPA Administrator, Carol Browner, dated March 21, 1995. Subject: Guidance on Risk Characterization.

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

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

Chia, S.E., C.N. Ong, W.H. Phoon, K.T. Tan, and J. Jeyaratnam. (1993) Neurobehavioural effects on workers in a video tape manufacturing factory in Singapore. NeuroTox 14:51-6.

Churchill, J.E., D.L. Ashley, and W.E. Kaye. (2001) Recent chemical exposures and blood volatile organic compound levels in a large population-based sample. Arch Environ Health 56:157-166.

Couri, D., L.B. Hetland, J.J. O'Neill, *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., L.B. Hetland, M.S. Abdel-Rahman, and H. Weiss. (1977) The influence of inhaled ketone solvent vapors on hepatic microsomal biotransformation activities. Toxicol Appl Pharmacol 41:285-9.

Cox, G.E., D.E. Bailey, and K. Morgareidge. (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, D.R., Ed. CRC Press, Boca Raton, FL.

Deacon, M.M., M.D. Pilny, J.A. John, *et al.* (1981) Embryo- and fetotoxicity of inhaled methyl ethyl ketone in rats. Toxicol Appl Pharmacol 59:620-2.

DeCeaurriz, J., J.P. Desiles, P. Bonnet, *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., J. Setzer, R. Wait, *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, R.B., W.D. Brown, J.V. Setzer, *et al.* (1988) Effects of short duration exposures to acetone and methyl ethyl ketone. Toxicol Lett 43:31-49.

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

Dick, R.B., E.F. Krieg Jr., J. Setzer, B. Taylor. (1992) Neurobehavioral effects from acute exposures to methyl isobutyl ketone and methyl ethyl ketone. Fundam Appl Toxicol 19(3):453-73.

Dietz, F.K. and G.J. Traiger. (1979) Potentiation of CCl4 of hepatotoxicity in rats by a metabolite of 2-butanone: 2,3-butanediol. Toxicology 14:209-15.

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

DiVincenzo, G.D., C.J. Kaplan, and J. Dedinas. (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, G.R., E.R. Nestmann, J.L. Betts, *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, F.M. (1978) Methyl ethyl ketone polyneuropathy in shoe factory workers. Clin Toxicol 13:371-6.

Fagius, J., and B. Gronqvist. (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., and M.L. Diaz. (1986) Determination and prediction of tissue-gas partition coefficients. Int Arch Occup Environ Health 58:75-87.

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

Freddi, A., A. Paci, O. Vittori, *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, M.G. and G.P. Carlson. (1994) 2-Butanol metabolism by rat hepatic and pulmonary cytochromes P450. Toxicol Lett 74(3):203-9.

Gallo, M.A., B.L. Oser, G.E. Cox, and D.E. Bailey. (1977) Studies on the long-term toxicity of 2-butanol. Toxicol Appl Pharmacol 41:135. (Abstract).

Garcia, C.R., I. Geller, and H.L. Kaplan. (1978) Effects of ketones on lever-pressing behavior of rats. Proc West Pharmacol Soc 21:433-38.

Gaunt, I.F., P.G. Brantom, I.S. Kiss, P. Grasso, and S.D. Gangolli. (1972) Short-term toxicity of acetoin (acetylmethylcarbinol) in rats. Food Cosmet. Toxicol 10(2):131-41.

Geller, I., E. Gause, H. Kaplan, and R.J. Hartmann. (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, J.R. and P.B. Dews. (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, D.G. (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., E. Bingham, M. Burton, and R. Tye. (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., I. Saito, M. Kamijima, X. Yu, E. Shibata, M. Toida, and Y. Takeuchi. (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., S. Ghittori, G. Pezzagno, and E. Capodaglio. (1989) Methyl ethyl ketone (MEK) in urine as biological index of exposure. G Ital Med Lav 11(6):255-61.

Johanson, G. and P.H. Naslund. (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., B. Denk, and J.G. Filser. (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, E.T., D.M. Ebert, and P.W. Dodge. (1971) Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19:699-704.

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

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

Leeder, J.S. and G.L. Kearns. (1997) Pharmacogenetics in pediatrics: Implications for practice. Pediatr Clin North Am 44(1):55-77.

Lemasters, G.K., D.M. Olsen, J.H. Yiin, *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., V. Riihimaki, and P. Pfaffli. (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., V. Riihimaki, and K. Engstrom. (1990a) Effects of ethanol on the kinetics of methyl ethyl ketone in man. Br J Indust Med 47:325-330.

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

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

Mast, T.J., J.A. Dill, J.J. Evanoff, *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. (These data are the same as Schwetz *et al.*, 1991.)

Mayer, V.W. and C.J. Goin. (1987) Effects of chemical combinations on the induction of aneuploidy in Saccharomyces cerevisiae. Mutat Res 187(1):21-30.

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

Mitran, E., T. Callender, B. Orha, *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. and D.E. Wurster. (1965) Investigation of some factors influencing percutaneous absorption--III. Absorption of methyl ethyl ketone. J Pharm Sci 54:1281-4.

Muttray, A., D. Jung, L. Klimmek, and C. Kreiner. (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, K.W., J.F. Ege, M. Ross, *et al.* (1943) Sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 25:282-85.

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

Nelson, B.K., W.S. Brightwell, and E.F. Krieg Jr. (1990) Developmental toxicology of industrial alcohols: a summary of 13 alcohols administered by inhalation to rats. Toxicol Ind Health 6(3-4):373-87.

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

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. (These data are the same as Mast *et al.*, 1989.)

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

O'Donoghue, J.L., S.R. Haworth, R.D. Curren, *et al.* (1988) Mutagenicity studies on ketone solvents: methyl ethyl ketone, methyl isobutyl ketone, and isophorone. Mutat Res 206(2):149-61.

Oleru, U.G. and C. Onyekwere. (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., F.J. Jimenez-Jimenez, J. Miquel, *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., F. Brugnone, P. Mozzo, *et al.* (1984) Methyl ethyl ketone exposure in industrial workers. Uptake and kinetics. Int Arch Occup Environ Health 54:73-81.

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

Saida, K., J.R. Mendell, and H.S. Weiss. (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., J. Kikuchi, H. Masanobu, *et al.* (1989) Disposition of acetone, methyl ethyl ketone and cyclohexanone in acute poisoning. Clin Toxicol 27:67-77.

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

Schwetz, B.A., B.K.J. Leong, and P.J. Gehring. (1974) Embryo- and fetotoxicity of inhaled carbon tetrachloride, 1,1-dichloroethane and methyl ethyl ketone in rats. Toxicol Appl Pharmacol 28:452-64.

Schwetz, B.A., T.J. Mast, R.J. Weigel, *et al.* (1991) Developmental toxicity of inhaled methyl ethyl ketone in mice. Fundam Appl Toxicol 16:742-8.

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

Sia, G.L., C.N. Ong, S.E. Chia, *et al.* (1991) Environmental and biological monitoring of methyl ethyl ketone (MEK). Environ Monit Assess 19(1-3):401-11.

Smith, A.R. and M.R. Mayers. (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, H.F., Jr., C.P. Carpenter, C.S. Weil, *et al.* (1962) Range-finding toxicity data: list VI. Am Ind Hyg J 1962:95-107.

Spirtas, R., P.A. Stewart, J.S. Lee, *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., H. Altenkirch, and M. Wagner. (1990) Neurotoxicity of organic solvent mixtures: embryotoxicity and fetotoxicity. Neurotoxicol Teratol 12(6):585-9.

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.

Takeuchi, Y., Y. Ono, N. Hisanaga, *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., H. Tsuji, and K. Hashimoto. (1986) Structure–toxicity relationship of monoketones. Toxicol Lett 30:3-7.

Thrall, KD., J.J. Soelberg, K.K. Weitz, and A.D. Woodstock. (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.,O. Nilsen, and J-A. Gustafsson. (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. (These data are the same as Cavender *et al.*, 1983)

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

Traiger, G.J., J.V. Bruckner, W-D. Jiang, F.K. Dietz, and P.H. Cooke. (1989) Effect of 2-butanol and 2-butanone on rat hepatic ultrastructure and drug activity. J Toxicol Environ Health 28(2):235-48.

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

U.S. EPA. (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, NCEA-F-0644, July. Risk Assessment Forum, Washington, DC. 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. (2000) 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, J.G., W. Rebel-de Haan, J.J. Opdam, and G.J. Mulder. (1997) Effect of coexposure to methyl ethyl ketone (MEK) on n-hexane toxicokinetics in human volunteers. Toxicol Appl Pharmacol 144:385-95.

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

Welch, L., H. Kirshner, A. Heath, *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, C.P., S.P. Tsai,, N.S. Weiss, *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., D. Lai, J.L. McLain, 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, D.E. and R. Munies. (1965) Factors influencing percutaneous absorption II: absorption of methyl ethyl ketone. J Pharm Sci 54:554-6.

Yu, R.C., D. Hattis, E.M. Landaw, and J.R. Froines. (2002) Toxicokinetic interaction of 2,5-hexanedione and methyl ethyl ketone. Arch Toxicol 75:643-652.

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

Zhao, W., J. Misumi, T. Yasui, K. Aoki, and T. Kimura. (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, F.K., V.M. Mayer, I. Scheel, and M.A. Resnick. (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.

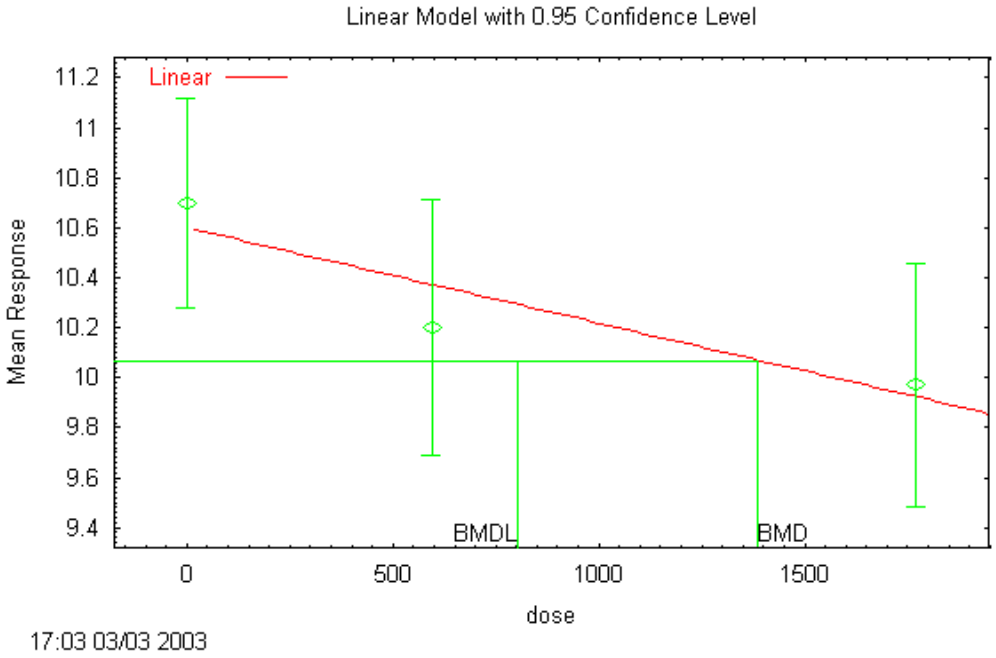
## APPENDIX A: Summary of External Peer Review and Public Comments and Disposition

*To be added following the completion of the external peer review.*

# APPENDIX B: Benchmark Dose Modeling Results and Output

## APPENDIX B CONTENTS

**Output B-1: Cox *et al.* (1975), Reduced Pup Body Weight in Wistar Rats, F1A Generation at Postnatal Day 4**

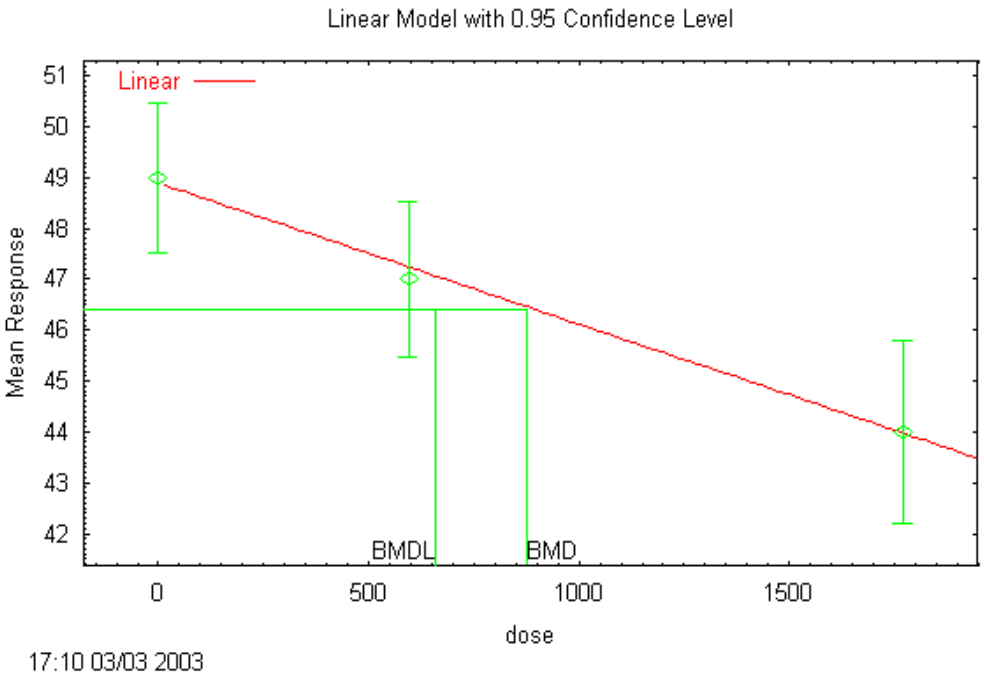



```
BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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




```
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 |

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

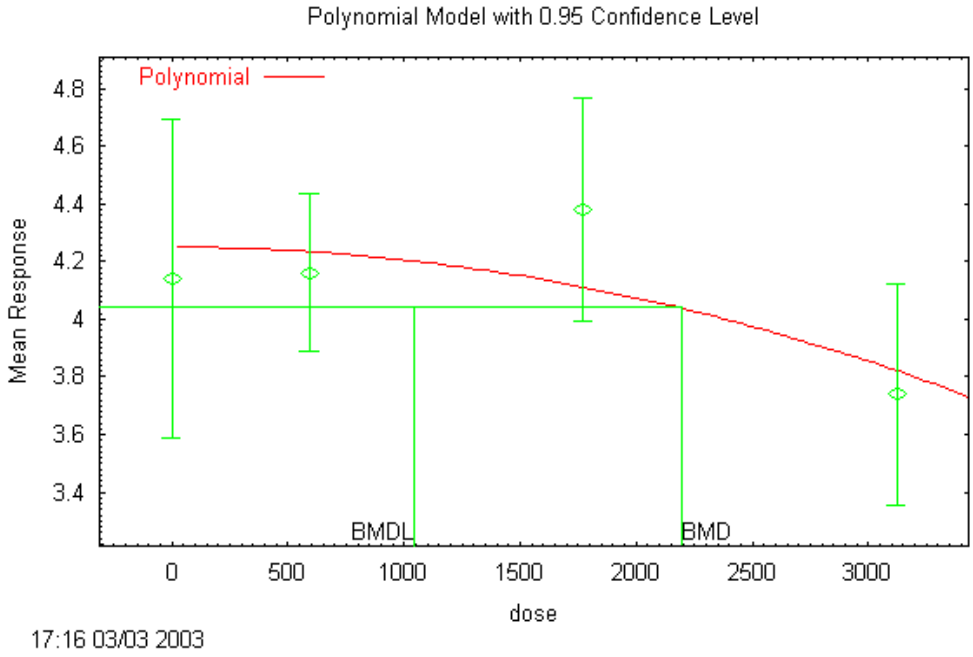
The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here

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: Cox *et al*. (1975), Reduced Fetal Body Weight in Wistar Rats, F1B Generation




```
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 |

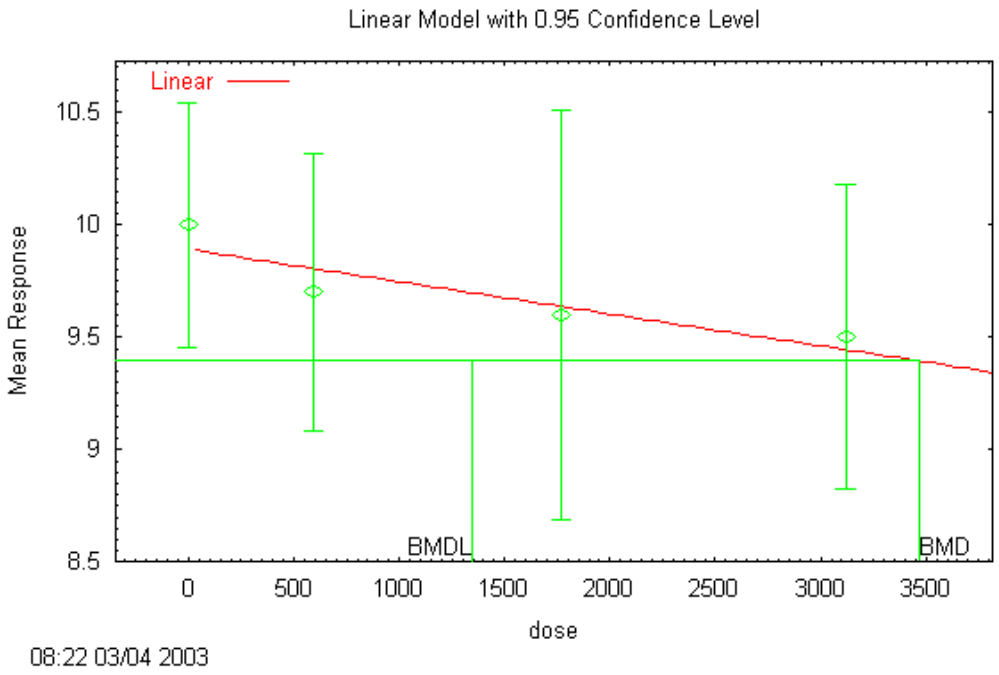
Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

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




```
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 |

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 |

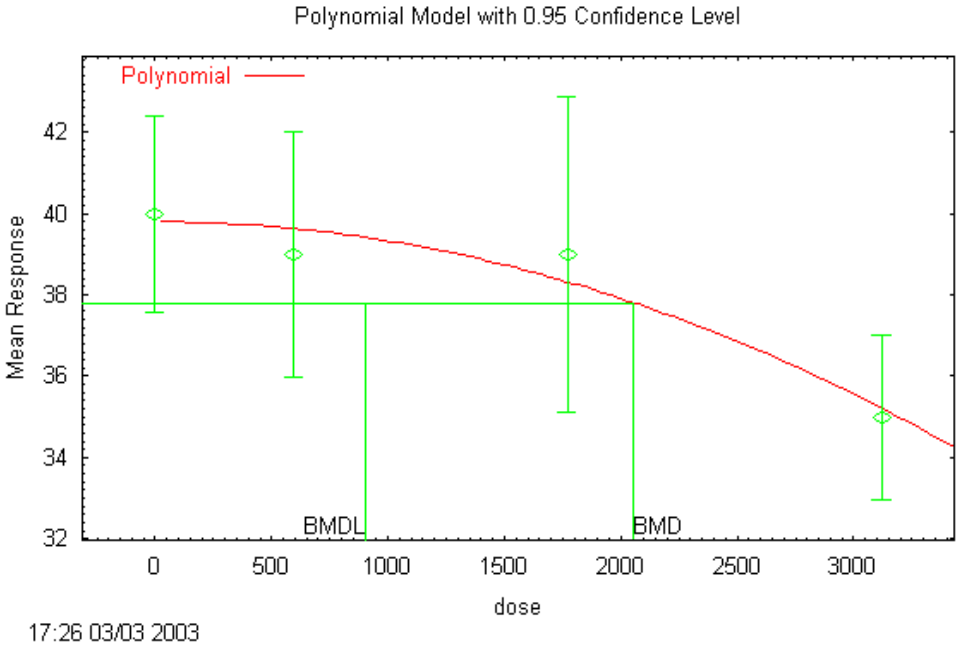
The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data

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: Cox *et al.* (1975), Reduced Pup Body Weight in Wistar Rats, F2 Generation at Postnatal Day 21**




```
 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 |

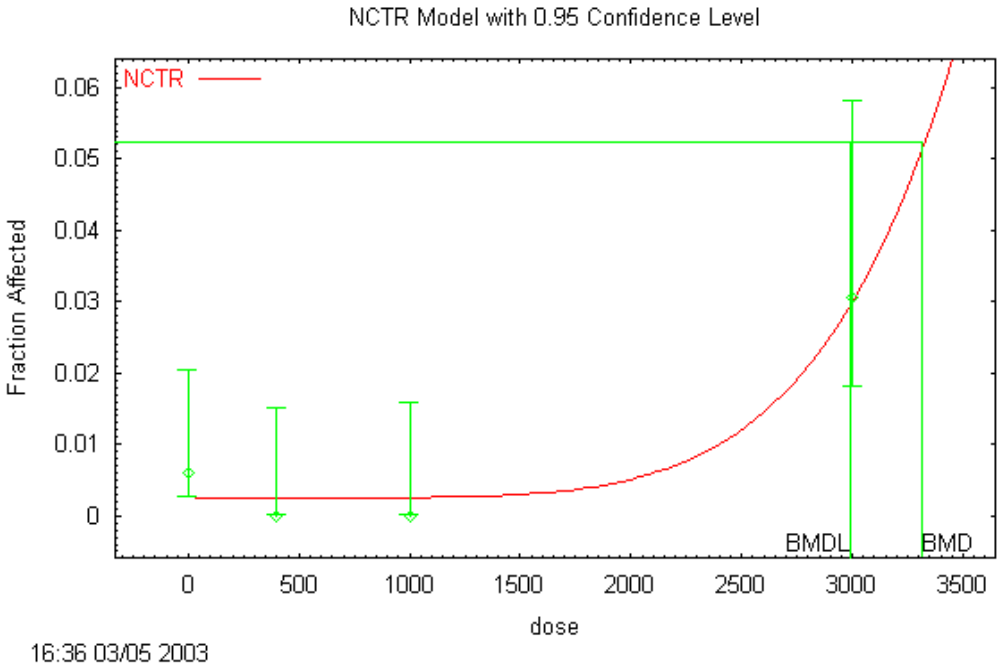
Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

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




```
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. |
|---|---|---|
| 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 |

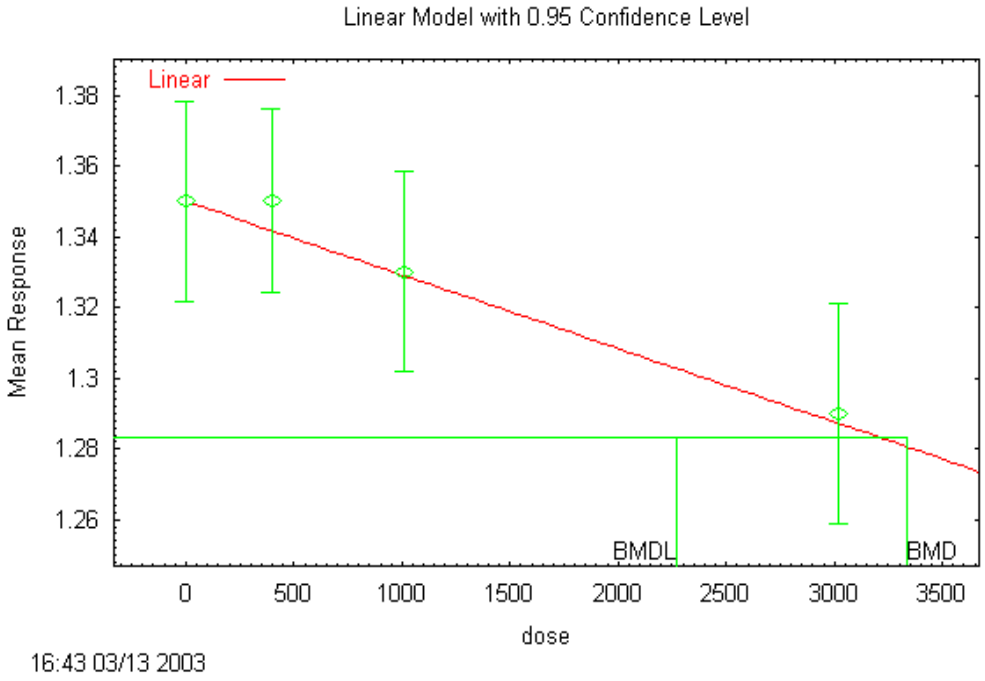
| | | | | |
|---|---|---|---|---|
| 1000.0000 | 9 | 0.003 | 0.023 | 0 |
| 1000.0000 | 10 | 0.003 | 0.051 | 0 |
| 1000.0000 | 11 | 0.003 | 0.113 | 0 |
| 1000.0000 | 12 | 0.003 | 0.031 | 0 |
| 1000.0000 | 13 | 0.003 | 0.067 | 0 |
| 1000.0000 | 14 | 0.003 | 0.072 | 0 |
| 1000.0000 | 15 | 0.003 | 0.154 | 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

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




```
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 |

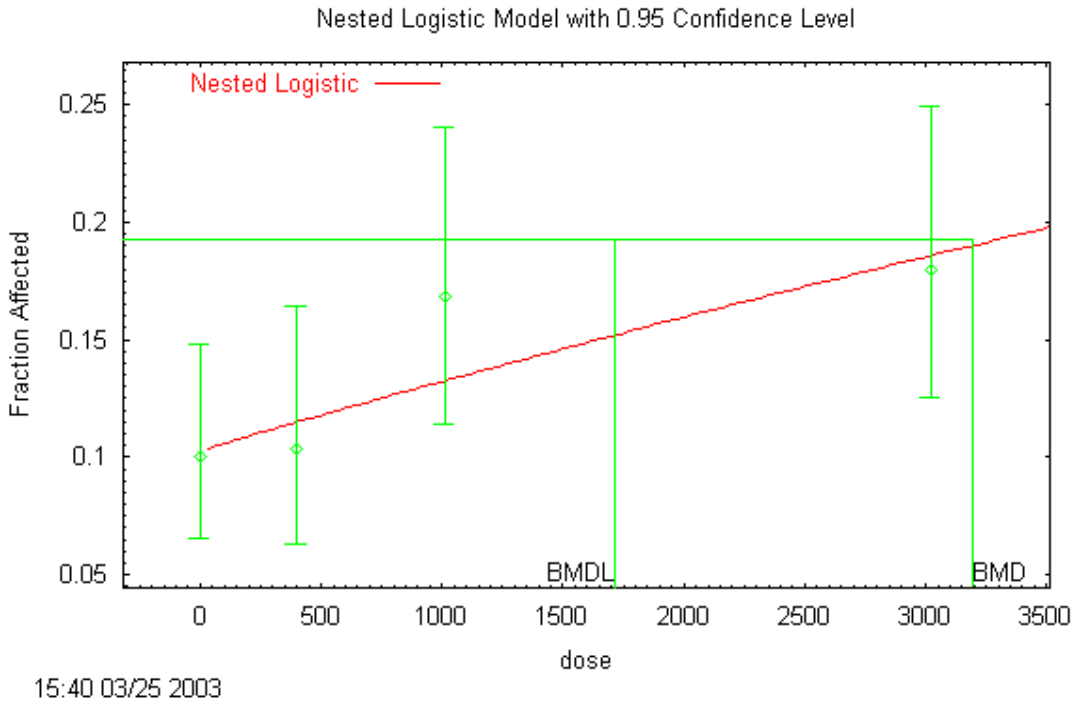
Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are Variances Homogeneous (A1 vs A2)
Test 3: Does the Model for the Mean Fit (A1 vs. fitted)

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




```
 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 | |

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

| Dose | Lit.-Spec. Cov. | Est._Prob. | Litter Size | Expected | Observed | chi-squared 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 |
| 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 |

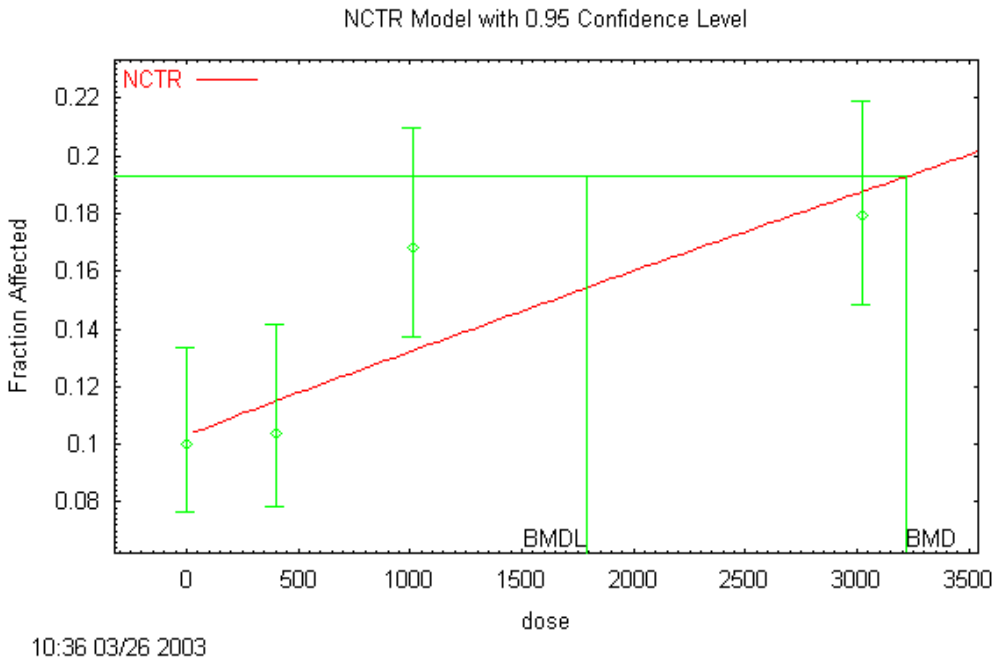
| | | | | | | |
|---|---|---|---|---|---|---|
| 1010.0000 | 12.0000 | 0.133 | 12 | 1.600 | 0 | -0.9191 |
| 1010.0000 | 12.0000 | 0.133 | 12 | 1.600 | 1 | -0.3448 |
| 1010.0000 | 13.0000 | 0.133 | 13 | 1.734 | 5 | 1.7594 |
| 1010.0000 | 13.0000 | 0.133 | 13 | 1.734 | 2 | 0.1435 |
| 1010.0000 | 14.0000 | 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

| Dose | Mean Lit.-Spec. Cov. | Expected | Observed | chi-squared 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

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
```




```
 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 |
| 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

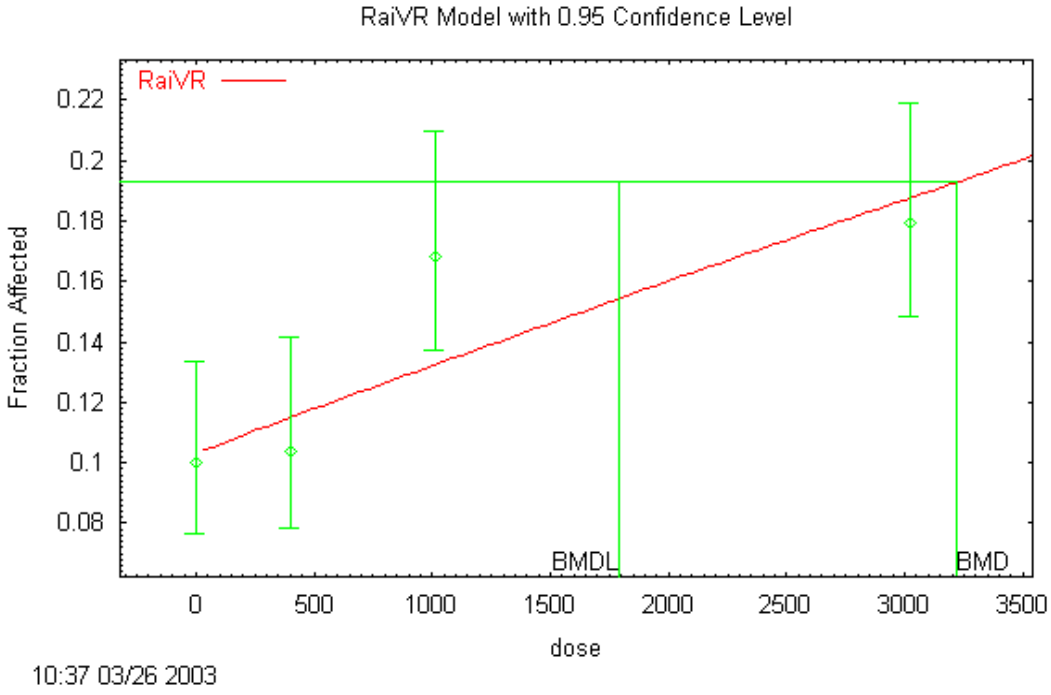
| Dose | Litter_Size | Est._Prob. | Expected | Observed |
|---|---|---|---|---|
| 0.0000 | 5 | 0.104 | 0.518 | 0 |
| 0.0000 | 7 | 0.104 | 0.725 | 0 |
| 0.0000 | 10 | 0.104 | 1.035 | 1 |
| 0.0000 | 11 | 0.104 | 9.110 | 12 |
| 0.0000 | 12 | 0.104 | 7.453 | 5 |
| 0.0000 | 13 | 0.104 | 2.692 | 1 |
| 0.0000 | 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




```
 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

****  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 |

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 |
| 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