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TOXICOLOGICAL REVIEW

OF

2,2,4-TRIMETHYLPENTANE

(CAS No. 540-84-1)

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

July 2007

U.S. Environmental Protection Agency
Washington, DC

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2,2,4-TRIMETHYLPENTANE (CAS No. 540-84-1)

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LIST OF ABBREVIATIONS AND ACRONYMS

ACF	atypical cell foci
ALT	alanine aminotransferase
CASRN	Chemical Abstracts Service registry number
CPN	chronic progressive nephrosis
EPA	U.S. Environmental Protection Agency
eq	equivalents
GC/MS	gas chromatography/mass spectrometry
GFR	glomerular filtration rate
hsc73	heat shock cognate protein 73
IRIS	Integrated Risk Information System
NAG	N-acetyl-β-D-glucosaminidase
NBR	NCI-black-Reiter
NZW	New Zealand white
PC	partition coefficient
po	by mouth, oral(ly)
RCT	renal cell tumor
RD_{50}	50% depression in respiratory rate
RfC	reference concentration
RfD	reference dose
SCE	sister chromatid exchange
SDH	sorbitol dehydrogenase
TK	thymidine kinase
TMP	2,2,4-trimethylpentane
UDS	unscheduled DNA synthesis
UG	unleaded gasoline
VOC	volatile organic chemical

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 2,2,4-trimethylpentane. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of 2,2,4-trimethylpentane.

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

John Stanek, Ph.D.
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

AUTHORS

Ms. Susan Goldhaber
Alpha-Gamma Technologies, Inc.
Raleigh, NC

Errol Zeiger, Ph.D.
Alpha-Gamma Technologies, Inc.
Raleigh, NC

Mr. Frank Stack
Alpha-Gamma Technologies, Inc.
Raleigh, NC

REVIEWERS

This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment 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 EPA's regional offices.

INTERNAL EPA REVIEWERS

D. Charles Thompson, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Danielle Devoney, Ph.D.
National Center for Environmental Assessment
Office of Research and Development

Marion Hoyer, Ph.D.
Environmental Scientist
Office of Transportation and Air Quality

EXTERNAL PEER REVIEWERS

Susan Borghoff, Ph.D., DABT, Panel Chair
Integrated Laboratory Systems, Inc.

Deborah Barsotti, Ph.D.
MACTEC Engineering and Consulting

Lawrence Lash, Ph.D.
Wayne State University

Brian Short, Ph.D., DVM, DACVP
Allergan, Inc.

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

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of 2,2,4-trimethylpentane. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and less-than-lifetime exposure durations, and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as 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 effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) 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). Reference values may also be derived for acute ($\leq$24 hours), short-term (up to 30 days), and subchronic (up to 10% of average lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral 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 derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is an upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water or per $\mu g/m^3$ air breathed. Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

Development of these hazard identification and dose-response assessments for 2,2,4-trimethylpentane has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and Risk Assessment Forum Technical Panel Reports that were used in the development of this assessment include the following: *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), *Science*

Policy Council Handbook: Peer Review (U.S. EPA, 1998b, 2000a, 2006), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through September 2005.

2. CHEMICAL AND PHYSICAL INFORMATION

2,2,4-Trimethylpentane is a hydrocarbon also known as isooctane, iso-octane, isobutyltrimethylmethane, and TMP. Some relevant chemical and physical properties are listed in Table 2-1.

Table 2-1. Chemical and physical properties of 2,2,4-trimethylpentane

CAS registry number	540-84-1
Empirical formula	C_8H_{18}
Molecular weight	114.22
Vapor pressure	40.6 mm Hg (at 21°C)
Vapor density	3.93
Boiling point	99.2°C
Melting point	−107.4°C
Density/specific gravity	0.69194 (at 20°C/4°C)
Solubilities	Practically insoluble in water; somewhat soluble in absolute alcohol; soluble in benzene, toluene, xylene, chloroform, ether, carbon disulfide, and carbon tetrachloride
Viscosity	<32 Saybolt Universal Seconds
ppm Conversion	1 ppm = 4.68 mg/m^3; 1 mg/m^3 = 0.21 ppm

Source: NLM (2004).

2,2,4-Trimethylpentane is a colorless liquid with the odor of gasoline (NLM, 2004). It is used primarily in the alkylation step of the reaction of isobutane and butylene in deriving high-octane fuels. 2,2,4-Trimethylpentane is synthesized from the catalytic hydrogenation of trimethylpentene with a nickel catalyst ($C_8H_{16} + H_2 = C_8H_{18}$) (API, 1985).

2,2,4-Trimethylpentane (isooctane) is one of two chemicals used in establishing the octane rating for gasoline. The octane value is a number that reflects the resistance of a gasoline mixture to knocking when used as fuel in an internal combustion engine. Pure 2,2,4-trimethylpentane has an octane value of 100, indicating that it burns without knocking, whereas n-heptane has an octane value of 0 because it burns with considerable knocking (API, 1985). When testing the knock properties of gasoline, the octane number is the percentage of 2,2,4-trimethylpentane in the 2,2,4-trimethylpentane/n-heptane mixture that matches the knock properties of the gasoline. For example, gasoline with an octane number of 89 has the same knocking properties during combustion as a mixture comprised of 89% 2,2,4-trimethylpentane and 11% n-heptane.

2,2,4-Trimethylpentane is released into the environment through the manufacture, use, and disposal of products associated with the gasoline and petroleum industry. Automotive exhaust and automotive evaporative emissions are important sources of atmospheric 2,2,4-trimethylpentane. Thus, the most probable route of exposure to the general population is

by inhalation. When 2,2,4-trimethylpentane is released into water or onto dry or moist soil surfaces, volatilization is expected to be the dominant removal process. Photolysis is not an important removal process, as 2,2,4-trimethylpentane is transparent to wavelengths available in sunlight. In subsurface soil, adsorption is expected to occur (NLM, 2004).

3. TOXICOKINETICS

There are limited published studies on the toxicokinetics of 2,2,4-trimethylpentane. The disposition of 2,2,4-trimethylpentane has been studied in rats after inhalation and oral exposures. Blood:air partition coefficients (PCs) have been estimated by using reconstituted mixtures of blood components from male rats.

3.1. ABSORPTION

3.1.1. Oral

Kloss et al. (1986) reported the disposition of radiolabeled 2,2,4-trimethylpentane in F344 rats. Male and female rats (four/group) were administered [^{14}C]-2,2,4-trimethylpentane (500 mg/kg, by mouth [po]), and expired air, urine, and feces were collected for 72 hours. Animals were sacrificed after 72 hours, and selected tissues were removed for radionuclide analysis. Approximately 5% of the radioactivity was recovered in the feces, indicating high oral absorption (approximately 95% of the administered dose). The majority of the radioactivity was recovered in the expired air (43 to 48%) and urine (49 to 67%), while less than 1% of the radioactive 2,2,4-trimethylpentane remained in the tissues of males and females.

3.1.2. Inhalation

Groups of four male F344/N rats were exposed to low (0.79 ppm or 3.7 mg/m^3) and high (385 ppm or 1800 mg/m^3) concentrations of 2,2,4-trimethylpentane via nose-only inhalation for 2 hours (Dahl, 1989). Uptake rates were 0.307 and 96.7 µg/kg-minute for the low and high 2,2,4-trimethylpentane concentrations, respectively. Based on these uptake rates, approximately 7 to 12% of the inspired 2,2,4-trimethylpentane was absorbed by the respiratory tract.

3.2. DISTRIBUTION

3.2.1. Oral

Kloss et al. (1986) reported the distribution of radiolabeled 2,2,4-trimethylpentane in F344 rats. Male and female rats were administered [^{14}C]-2,2,4-trimethylpentane (500 mg/kg, po), and expired air, urine, and feces were collected for 72 hours. Animals (four/group) were sacrificed after 72 hours and selected tissues (kidney, fat, liver, lung, heart, testis, and spleen) were removed for radionuclide analysis. The majority of the radioactive 2,2,4-trimethylpentane in male rats was found localized in the kidney, with minor amounts in peritoneal fat and liver. Analysis revealed an approximate 8- to 10-fold higher level of 2,2,4-trimethylpentane in kidneys of males than of females (1225 nmol equivalents [eq]/g wet tissue versus 157 nmol eq/g wet tissue, $p < 0.05$). The radioactivity found in the kidney was associated with the renal cortex. Statistically significant differences between genders were not noted in the peritoneal fat and

livers of males and females: 244 and 177 nmol eq/g wet tissue for males, and 336 and 193 nmol eq/g wet tissue for females, respectively. Overall, these data indicate a marked difference in the distribution of 2,2,4-trimethylpentane in male rats and, especially, in the male rat kidney, when compared to female rats.

The results of Kloss et al. (1986) were essentially reproduced by Charbonneau et al. (1987), who treated male and female F344 rats with 500 mg/kg (equivalent to 4.4 mmol/kg) [^{14}C]-2,2,4-trimethylpentane by gavage in a 48-hour disposition study. Tissue concentrations were determined at 4, 8, 12, 24, and 48 hours after dosing. Peak tissue concentrations in the kidney, liver, and plasma were reported to occur in males at 12 hours and in females at 8 hours after dosing. The peak concentration in the male kidneys was twice that observed in females (1252 versus 577 nmol eq/g wet weight, $p < 0.05$). The peak tissue concentrations in the liver and plasma were similar between males (1000 and 403 nmol eq/g wet weight, respectively) and females (1163 and 317 nmol eq/g wet weight, respectively). In addition, in male rats, the kidney, liver, and plasma concentrations of radiolabeled 2,2,4-trimethylpentane remained constant for 12 to 24 hours after dosing, whereas in female rats they declined rapidly 8 hours after dosing.

3.3. METABOLISM

3.3.1. Following Oral Administration

Charbonneau et al. (1987) proposed a metabolic pathway for 2,2,4-trimethylpentane (Figure 3-1). This proposed pathway is based on urinary metabolites and oxidative reactions common to hydrocarbons that are dependent on whether 2,2,4-trimethylpentane undergoes oxidation at carbon 1, 4, or 5. In this study, male and female F344 rats were treated with a single oral dose of [^{14}C]-2,2,4-trimethylpentane (500 mg/kg; 2 μCi/mmol). Levels of radiolabeled material in kidney, liver, and plasma were determined at 4, 8, 12, 24, and 48 hours after dosing. Maximum concentrations of 2,2,4-trimethylpentane-derived radioactivity in kidney, liver, and plasma of male rats were found after 12 hours (143, 114, and 46 mg eq/kg, respectively), whereas maximum concentrations in females were found after 8 hours (66, 133, and 36 mg eq/kg, respectively). The identification and quantitation of the urinary metabolites of 2,2,4-trimethylpentane showed that both male and female rats metabolized 2,2,4-trimethylpentane via the same pathway and at a similar rate. Female rats, however, excreted more conjugates of 2,4,4-trimethyl-2-pentanol in urine than males. 2,4,4-Trimethyl-2-pentanol was the major metabolite present in the male rat kidney but was absent from the female rat kidney. In male F344 rats, renal α_{2u}-globulin levels increased to 1.8 and 3.1 times control values at 24 hours after 2,2,4-trimethylpentane treatments of 50 and 500 mg/kg (0.44 and 4.4 mmol/kg), respectively. The study also reported that two metabolites, 2,2,4-trimethyl-2-pentanol and 2,4,4-trimethyl pentanoic acid, were detected in kidney homogenates of male, but not female, rats at 8 to 24 hours after an oral dose of 500 mg/kg 2,2,4-trimethylpentane.

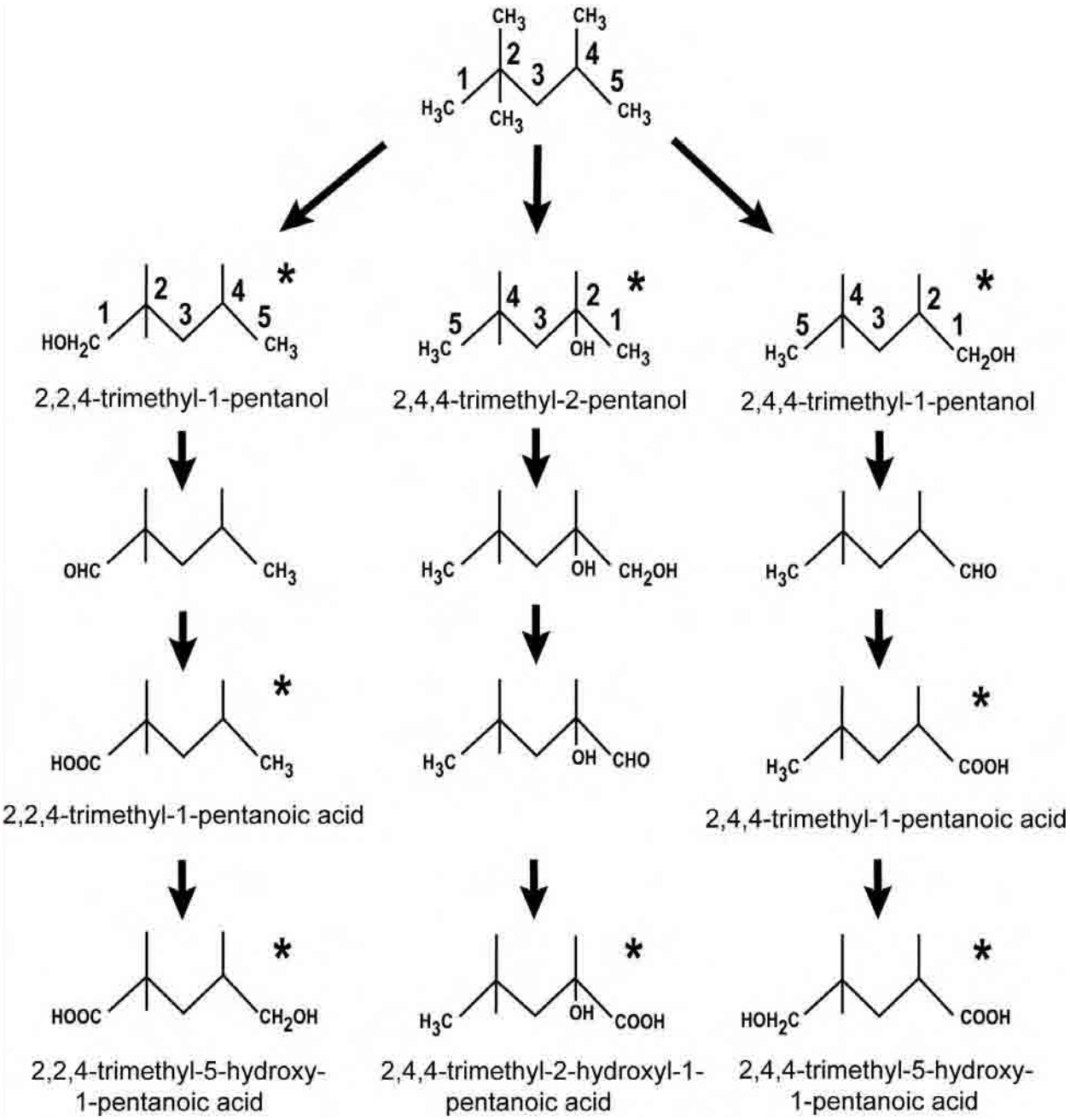

Figure 3-1. Proposed pathway for 2,2,4-trimethylpentane metabolism.

*Indicates metabolites identified in male rat urine by Olson et al. (1985).

Source: Charbonneau et al. (1987).

Olson et al. (1985) identified seven urinary products of 2,2,4-trimethylpentane metabolism in a gavage study in which 2720 mg/kg 2,2,4-trimethylpentane was administered every other day for 14 days to eight male F344 rats. Urinary metabolites were identified by using a methylene chloride extract of the urine analyzed by gas-liquid chromatography, gas chromatography/mass spectrometry (GC/MS), and thin-layer chromatography. The primary metabolites identified in this study were trimethyl pentanols (2,2,4-trimethyl-1-pentanol, 2,4,4-trimethyl-1-pentanol, and 2,4,4-trimethyl-2-pentanol), pentanoic acids (2,4,4-trimethyl-1-pentanoic acid and 2,4,4-trimethyl-2-hydroxyl-1-pentanoic acid), and hydroxypentanoic acids (2,2,4-trimethyl-5-hydroxy-1-pentanoic acid and 2,4,4-trimethyl-5-hydroxy-1-pentanoic acid), as indicated by asterisks in Figure 3-1.

3.3.2. Following Inhalation Exposure

Groups of four male F344/N rats were exposed to low (0.79 ppm or 3.7 mg/m^3) and high (385 ppm or 1800 mg/m^3) concentrations of ^{14}C-labeled 2,2,4-trimethylpentane via nose-only inhalation for 2 hours (Dahl, 1989). Urine and feces were collected at 3, 6, 9, 18, 24, 30, 42, 54, and 66 hours postexposure from rats exposed to each concentration and analyzed for the presence of metabolites. The majority (73 to 79%) of recovered radioactivity was found in the urine at each 2,2,4-trimethylpentane exposure concentration, but specific metabolites were not identified. Elimination primarily by the urinary route continued throughout the 66-hour postexposure observation period.

3.4. ELIMINATION

3.4.1. Following Oral Administration

Kloss et al. (1986) reported the disposition of radiolabeled 2,2,4-trimethylpentane in F344 rats. Male and female rats were administered [^{14}C]-2,2,4-trimethylpentane (500 mg/kg, po, four/group), and expired air, urine, and feces were collected for 72 hours. Animals were sacrificed after 72 hours and selected tissues were removed for radionuclide analysis. The total recovered radioactivity exceeded 100% for both males (115%) and females (104%); the majority of the radioactivity was recovered from urine (67% for males, 50% for females) and as expired organic material (43% for males, 49% for females). A small amount (3.5% in males, 5% in females) was recovered from the feces.

3.4.2. Following Inhalation Exposure

Groups of four male F344/N rats were exposed to low (0.79 ppm or 3.7 mg/m^3) and high (385 ppm or 1800 mg/m^3) concentrations of [^{14}C]-2,2,4-trimethylpentane via nose-only inhalation for 2 hours (Dahl, 1989). Approximately 90% of the total amount of 2,2,4-trimethylpentane absorbed via inhalation was eliminated by 70 hours postexposure,

approximately 60 to 70% of which was excreted via the urine at each exposure concentration. Less than 10% was eliminated via feces or exhaled as carbon dioxide.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

No physiologically based pharmacokinetic or toxicokinetic models have been reported in the literature for 2,2,4-trimethylpentane.

Beliveau and Krishnan (2000) used a water and lipid (oil) matrix as blood component surrogates to estimate and compare rat blood:air PCs of some volatile organic chemicals (VOCs). For 2,2,4-trimethylpentane, vial equilibration studies showed that the matrix:air PC for the oil+water samples (mean, 2.88; SE, 0.5; n = 7) was similar to that of rat blood (mean, 1.92; SE, 0.4; n = 7). The authors concluded that, for some VOCs, knowledge of solubility in blood components may be sufficient to determine the blood:air PC.

4. HAZARD IDENTIFICATION

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

No epidemiological studies, case reports, or clinical studies of 2,2,4-trimethylpentane in humans were identified.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.2.1. Noncancer Toxicity

4.2.1.1. *Oral Studies*

No subchronic or chronic oral studies were identified for 2,2,4-trimethylpentane. However, there have been a number of acute and short-term oral studies investigating the effects of 2,2,4-trimethylpentane on the liver and kidney (see Section 4.4.1 for a discussion of these studies).

4.2.1.2. *Inhalation Studies*

No chronic inhalation studies were identified for 2,2,4-trimethylpentane. Several acute inhalation studies for 2,2,4-trimethylpentane were identified in the literature (see Section 4.4.1). Only one subchronic inhalation study was identified.

Short et al. (1989a) exposed male and female F344 rats for 6 hours/day, 5 days/week to 0 (control) or 50 ppm (234 mg/m^3) 2,2,4-trimethylpentane via inhalation to characterize the pathogenesis of α_{2u}-globulin nephropathy. Body weight was the only other endpoint evaluated. Male rats (three/group) were exposed for 3, 10, 22, or 48 weeks and female rats (three/group) were exposed for 3, 11, 23, or 50 weeks. The presence of α_{2u}-globulin in renal tissues was measured by immunohistochemistry at each time point. Cell proliferation was determined by measuring incorporation of thymidine administered during the last exposure week of each exposure period. No significant differences were noted in body weights or in absolute or relative kidney weights in male or female rats at any time point with or without treatment. A significant increase in detectable α_{2u}-globulin in exposed males, but not females, was observed after 3 weeks of exposure, compared with controls ($p < 0.05$). This effect persisted throughout each of the exposure periods and did not vary with time. In addition, the subcellular localization of α_{2u}-globulin corresponded with hyaline droplets in serial sections. Examination of the P_2 segment of the proximal tubule of the kidney showed an increase of up to 11-fold in cell turnover in male rats, starting at 3 weeks and persisting throughout the study. This proliferative response closely paralleled the extent and severity of immunohistochemically detectable α_{2u}-globulin in the P_2 segment. Neither cytotoxicity nor α_{2u}-globulin were noted in the P_1 or P_3 segments.

Increased numbers of proximal tubules affected by chronic progressive nephrosis (CPN) were noted in male rats exposed to 2,2,4-trimethylpentane for 22 (7.7 CPN foci/section) or 44 weeks (12.3 CPN foci/section), compared with controls (0.4 and 5.0 CPN foci/section, respectively). These lesions contained highly proliferative epithelial cells. Control and exposed female rats exhibited no evidence of α_{2u}-globulin-related nephropathy, increases in cell turnover, or chronic nephrosis. In both control and exposed females, the number of CPN foci per kidney section was 0.3 at 23 weeks and 1.3 at 50 weeks.

4.2.2. Cancer Bioassays

No cancer bioassays specific for 2,2,4-trimethylpentane were identified in the literature.

4.2.2.1. *Initiation and Promotion Studies*

Short et al. (1989b) used an initiation-promotion model to evaluate the potential promoting and carcinogenic effects of both unleaded gasoline (UG) and 2,2,4-trimethylpentane. In the promotion study, 28 to 30 rats/sex/concentration were exposed to 0, 10, 70, or 300 ppm UG or 50 ppm (234 mg/m^3) 2,2,4-trimethylpentane for 6 hours/day, 5 days/week by inhalation for 59 to 61 weeks either alone (a promotion control group) or subsequent to a 2-week drinking water exposure to an initiator, N-ethyl-N-hydroxyethylnitrosoamine (experimental initiation/promotion group). In a shorter-term sequence reversal study, male rats only were exposed to UG or 2,2,4-trimethylpentane for 24 weeks, followed by initiator exposure under the same exposure conditions described above. Surviving animals for both the longer- and shorter-term studies were sacrificed at 65 to 67 weeks after the start of the experiments, and the kidneys were examined for incidence of atypical cell foci (ACF) or renal cell tumors (RCTs). No other endpoints were evaluated. No RCTs were observed in the 2,2,4-trimethylpentane promotion control group of either gender, whereas, in the initiation/promotion group, males had an increased incidence of RCTs (4/29) compared with the initiator-only control group (1/29), while females were not affected (1/29 in the initiator control group and 1/30 in the initiation/promotion group). The incidence (4/29) observed in males was approximately double the 2/27 observed in the highest exposure initiation/UG group (300 ppm). The sequence reversal study also showed promoter-like results in males for both 2,2,4-trimethylpentane and UG in that the percentage of rats affected with ACF was greater than that of concomitant controls. The incidence of ACF was not elevated over that of controls in females in any experimental group. These results indicate that, under the exposure conditions employed, UG and 2,2,4-trimethylpentane are both tumor promoters in male, but not female, rats. Information on the relative contribution of 2,2,4-trimethylpentane to the UG response could not be determined from this study.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

No reproductive or developmental studies were identified for 2,2,4-trimethylpentane.

4.4. OTHER STUDIES

4.4.1. Acute and Short-Term Studies

4.4.1.1. *Oral*

A number of acute and short-term oral studies investigating the effects of 2,2,4-trimethylpentane on the liver and kidney were found. Most of these studies involved oral gavage administration of the chemical to rats at one or two doses for study durations ranging from 1 day to 4 weeks, with subsequent examination of hepatic and renal tissues and renal function.

In a 4-week study (API, 1985), male F344 rats were administered 2,2,4-trimethylpentane via gavage in corn oil at a dose of 0.5 g/kg-day for 5 days/week. Controls were administered corn oil. Animals were evaluated after either 2 or 4 weeks of exposure. After 2 weeks of exposure, a significant decrease in glomerular filtration rate (GFR) as measured by inulin clearance was seen in the treated animals compared with controls (0.60 ± 0.13 versus 0.79 ± 0.21 mL/minute/100 g body weight, $p = 0.02$). The reduction in GFR progressed, being more significant at 4 weeks than at 2 weeks (0.41 ± 0.15 versus 0.94 ± 0.30 mL/minute/100 g body weight, $p = 0.001$). Associated with this decline in GFR was a significant increase in the urinary levels of the enzyme N-acetyl-β-D-glucosaminidase (NAG) in the treated animals compared with controls at both 2 weeks (271 mU/24 hours [treated] versus 115 mU/24 hours [controls], $p < 0.05$) and 4 weeks (231 mU/24 hours [treated] versus 139 mU/24 hours [controls], $p < 0.05$).

In another 4-week study (API, 1983), male F344 rats (10/group) were administered either 0.5 g/kg or 2.0 g/kg 2,2,4-trimethylpentane by gavage on 5 days/week. After sacrifice, the kidneys were embedded in paraffin, sectioned, stained, and examined for histopathologic evidence of hydrocarbon nephropathy, based on a grading criterion of characteristic lesions (hyaline droplet change, regenerative epithelium, and tubular dilatation with granular material). The three lesions were graded separately for each animal on a severity score of 1 to 4 and added together, and the total scores from individual animals were averaged across each treatment group. The average graded nephropathy severity scores were 7.6 (for the 0.5 g/kg dose group) and 8.5 (for the 2.0 g/kg dose group) compared with a score of 2.9 for the saline-treated controls. The scores in the treated groups were considered to be indicative of hydrocarbon nephropathy.

In a study by Short et al. (1986), groups of five male F344 rats were administered 0 (control), 50, 100, 200, or 500 mg/kg 2,2,4-trimethylpentane via gavage for 21 days, followed by light microscopic characterization of renal lesions, as well as localization and quantitation of sites of renal cell proliferation. At animal sacrifice on day 22, the livers and kidneys were

sectioned, stained, and examined. Cell proliferation was measured by thymidine incorporation. Upon examination, lesions in the proximal convoluted tubule of the kidney were found to consist of accumulated protein droplets and crystalloid bodies, degeneration, necrosis, and foci of regenerative epithelium, similar to lesions noted to be induced by other hydrocarbon compounds. In the cell proliferation studies, the most notable increase (five- to sixfold) in the labeling index was observed in the same portion of the P_2 segment of the proximal convoluted tubule that contained severe crystalloid body accumulation, degeneration, and necrosis. No dose-response relationships were noted for the various effects, except for the degree of granular cast formation in the thin limb segments, which was less in the 50 mg/kg group, compared with the three other dose groups. No histological changes were seen in the liver.

The effect of 2,2,4-trimethylpentane administration on α_{2u}-globulin accumulation in rat kidneys was examined by Saito et al. (1992). Male Sprague-Dawley rats were treated with 50 mg/kg 2,2,4-trimethylpentane by gavage for 14 consecutive days. Control animals were administered corn oil. In treated animals, there was a marked increase (approximately 450% compared with controls) in the intensity of a protein band corresponding to α_{2u}-globulin as measured by gel electrophoresis and immunoblot analysis. Band intensity for α_{2u}-globulin was further increased in animals treated additionally with the cysteine protease inhibitors leupeptin and E-64, indicating that proteases play an important role in the degradation of α_{2u}-globulin in the male rat kidney.

Borghoff et al. (1992) administered 0.95, 3, 6, or 30 mg/kg 2,2,4-trimethylpentane by gavage for 10 consecutive days to male F344 rats (five/group) to examine α_{2u}-globulin nephropathy and renal cell proliferation. Control animals were administered corn oil. The presence of α_{2u}-globulin was determined by immunohistochemistry, and cell proliferation was measured by thymidine incorporation. Twenty-four hours after the final dose, protein droplet accumulation, α_{2u}-globulin concentration, and cell replication were measured in the kidneys of control and treated rats. Dose-related increases in protein droplet accumulation, α_{2u}-globulin concentration, and cell proliferation were detected in the kidneys of rats exposed to 2,2,4-trimethylpentane. An approximate twofold increase in each of these effects was noted at the highest dose tested, and the changes were found to be localized extensively in the P_2 segment. The magnitude of these effects was similar to that induced by exposure to UG. No significant changes in the kidney-to-body weight ratios of the exposed animals were reported.

Lock et al. (1987a) administered 12 mmol/kg (~1370 mg/kg) 2,2,4-trimethylpentane by gavage for 10 consecutive days to male and female Alderley Park rats (five/sex). Control animals were administered corn oil. Significant increases ($p < 0.05$) in liver weight and liver-to-body weight ratios in both treated males and females compared with controls and a small but significant increase in kidney weight and kidney-to-body weight ratios in treated males only were reported. The authors associated these effects with various findings, including selective

cytochrome P-450 induction, oxidation of fatty acids, and proliferation of peroxisomes. In treated males, no change in hepatic cytochrome P-450 or cytochrome b_5 content was seen, although renal cytochrome P-450 was significantly elevated compared with controls ($p < 0.05$). In treated females, increases in hepatic cytochrome P-450 and cytochrome b_5 contents were seen, but no change in renal cytochrome P-450 was noted. In addition, 2,2,4-trimethylpentane induced focal necrosis of the proximal tubules and a marked increase in the renal content of α_{2u}-globulin in male, but not female, rats.

The mechanism of uptake of α_{2u}-globulin in kidney lysosomes was investigated by Cuervo et al. (1999). Adult male Wistar rats were administered 2,2,4-trimethylpentane (1 g/kg) or corn oil vehicle via gavage for 7 consecutive days. After administration of the last dose, animals were fasted for 20 hours prior to sacrifice. Intact kidney lysosomes were isolated from treated and untreated rats, and their ability to take up α_{2u}-globulin was compared. In rats exposed to 2,2,4-trimethylpentane, the specific lysosomal transport of α_{2u}-globulin was found to increase. This direct transport of α_{2u}-globulin into lysosomes occurs in the presence of heat shock cognate protein 73 (hsc73). These results suggest that the chemically induced accumulation of cytosolic α_{2u}-globulin in lysosomes is mediated by an increased rate of direct uptake into lysosomes.

Dietrich and Swenberg (1991) investigated the hypothesis that the presence of α_{2u}-globulin is essential for development of nephropathy in male rats exposed to 2,2,4-trimethylpentane by comparing the responses observed in male NCI-black-Reiter (NBR) rats and male and female F344 rats. In other studies, NBR rats have been shown to not synthesize α_{2u}-globulin or develop renal disease when exposed to decalin, a compound known to induce α_{2u}-globulin nephropathy in other rat strains. The induction of α_{2u}-globulin nephropathy in F344 male rats with lindane was used as a positive control, and this response was contrasted to male NBR and female F344 rats treated with lindane. Five to seven 11-week-old male NBR rats were treated with TMP (500 mg/kg-day) or lindane (10 mg/kg-day), and five 11-week-old male and female F344 rats were treated with lindane (10 mg/kg-day) by oral gavage on 4 consecutive days. NBR male and male and female F344 rats gavaged with corn oil were used as vehicle controls. Perfusion-fixed kidneys were histopathologically analyzed for the presence of hyaline droplets, and the presence of α_{2u}-globulin was determined immunohistochemically. Under exposure conditions that clearly induce α_{2u}-globulin nephropathy in male F344 rats, no lesions, hyaline droplets, or α_{2u}-globulin were detectable in TMP-treated or control male NBR and female F344 rats.

A marked increase in urinary α_{2u}-globulin was noted in a study in which male Sprague-Dawley rats received 171 mg/kg 2,2,4-trimethylpentane by gavage for 7 consecutive days (Saito et al., 1996). This increase in urinary α_{2u}-globulin levels (measured on days 0, 1, 3, 5, and 7) was associated with increased concentrations of renal α_{2u}-globulin and hyaline droplet accumulation in renal proximal convoluted tubule epithelial cells.

To study renal α_{2u}-globulin accumulation, male and female F344 rats (five/sex) were orally administered 500 mg/kg 2,2,4-trimethylpentane per day for 5 days (Blumbach et al., 2000). Control rats received corn oil alone. Increases in α_{2u}-globulin content and relative kidney weight were observed in male, but not female, rats. In treated male rats, α_{2u}-globulin accounted for 70% of the total renal cytosolic protein. This increase in α_{2u}-globulin accumulation was accompanied by the formation of protein droplets in the P_2 segment of the proximal tubules.

In a study to examine the localization of α_{2u}-globulin within protein droplets of the kidney, four male F344 rats were gavaged once with 50 mg/kg 2,2,4-trimethylpentane (Burnett et al., 1989). Male and female controls were administered corn oil. For analysis, the renal tissues were embedded, sectioned, and stained, and the presence of α_{2u}-globulin was determined by immunohistochemistry. Image analysis of selected P_2 segments in treated and control rats 72 hours after treatment revealed a high correlation between subcellular localization of α_{2u}-globulin and protein droplet deposition in the cytoplasm of P_2 segment cells. Microscopic evaluation also revealed subcellular localization of α_{2u}-globulin within lysosomes of P_2 segment cells. Quantitative morphometry of proximal tubule epithelium stained for α_{2u}-globulin demonstrated a 1.5- to 2-fold increase in staining area of tubules from treated rats compared with controls. Similar increases in protein droplets were also observed.

In another study by Lock et al. (1987b), male and female F344 rats were dosed by gavage with a single dose of [^{3}H]-2,2,4-trimethylpentane (4.4 mmol/kg or ~500 mg/kg). The concentration of the radiolabeled material in various subcellular fractions of the kidney and the presence of α_{2u}-globulin were examined 24 and 72 hours after dosing by using column chromatography and immunohistochemistry. The kidneys from male rats were observed to contain more radiolabeled material compared with female rats at both time points. In addition, it was observed that covalent binding of 2,2,4-trimethylpentane to kidney proteins did not occur. However, GC/MS analysis and dialysis with and without sodium dodecyl sulfate of the low molecular weight protein fraction from male rat kidneys showed reversible binding between a metabolite of 2,2,4-trimethylpentane, identified as 2,4,4-trimethyl-2-pentanol, and α_{2u}-globulin. This was the only metabolite detected to be bound to α_{2u}-globulin in the kidney.

Hyaline droplet accumulation was stimulated in the kidney of postpubertal male Alderley Park rats 24 to 48 hours after a single oral dose of 12 or 24 mmol/kg (approximately 1370 or 2740 mg/kg) 2,2,4-trimethylpentane but had returned to normal after 7 days. No such effect was observed in female or prepubertal male animals (Stonard et al., 1986). A dose-dependent increase in the renal concentration of α_{2u}-globulin was also observed in postpubertal male rats 24 hours after single oral doses of 2,2,4-trimethylpentane over a range of 0.3 to 12 mmol/kg. In male rats treated with 12 mmol/kg, α_{2u}-globulin staining in the P_2 segment was greater compared with controls. No changes in urine volume, specific gravity, glucose, or NAG levels were

observed in treated rats, indicating that renal tubular function was not impaired. None of the effects noted in males were observed in female rats exposed to the same treatment regimen. Carruthers et al. (1987) employed a similar study paradigm, wherein a single oral gavage dose (2,2,4-trimethylpentane, 12 mmol/kg in corn oil, or vehicle alone) was administered to male CD rats (180–250 g, eight/treatment group). Liver and kidney tissues were harvested, and plasma and urine were collected 24 hours later. A significant increase in the level of α_{2u}-globulin in renal tissues, but not in liver, plasma, or urine, was found. Cycloheximide pretreatment significantly inhibited the accumulation of α_{2u}-globulin in the kidney in response to 2,2,4-trimethylpentane treatment (2.2 versus 24.0 mg/g kidney, respectively), which suggests that at least part of the increased accumulation in the kidney may be due to increased synthesis of α_{2u}-globulin in the liver. However, Carruthers et al. (1987) described the results of a single experiment in a small number of animals, and, thus, it is unclear how much significance can be attributed to the reported findings.

Fowlie et al. (1987) exposed nine male Wistar rats to 2 mL/kg (~1.4 g/kg) 2,2,4-trimethylpentane by gavage for 2 to 3 days and examined liver and kidney effects. The animals were examined on each day of exposure for changes in body weight, food and water consumption, and urinary parameters. There were significant decreases in food and water consumption and considerable weight loss in treated animals by day 2 that led to an early cessation of the experiment. In the urine of treated rats, NAG activity was significantly increased ($p < 0.01$) after day 2 and alkaline phosphatase activity was significantly increased on day 1 ($p < 0.01$) and on day 2 ($p < 0.05$) compared with control animals. Urinary creatinine levels were significantly decreased ($p < 0.001$) after day 1 compared with controls. These changes in urinary parameters are consistent with renal toxicity. Six rats were sacrificed on the second day and the remainder on the third day. At necropsy, there was a significant increase ($p < 0.001$) in the relative weights of both the liver and kidneys of treated animals compared with controls. Microscopic examination of the liver demonstrated centrilobular necrosis and hydrophobic degeneration of hepatocytes. Microscopic examination of the kidneys revealed hyaline droplet accumulation in the proximal tubules as well as tubule degeneration and dilatation.

In a study by Loury et al. (1986), replicative DNA synthesis was significantly increased ($p < 0.05$) in hepatocytes isolated from male F344 rats and from male and female B6C3F$_1$ mice treated with 500 mg 2,2,4-trimethylpentane/kg by gavage. This effect was seen in rats at 24 hours after exposure but not at shorter (2 and 12 hours) or longer (48 hours) times postexposure. Mice were sampled only at 24 hours postexposure, and no other effects were measured or reported in mice. The 500 mg/kg dose was based on pilot experiments that showed this dose caused a maximum accumulation of hyaline droplets in the rat kidney. A significant increase in mg DNA/gram liver but not in total liver DNA or liver weight was reported in rats following administration of 2,2,4-trimethylpentane (100 mg/kg) by gavage for 11 days.

Similar results were observed in mice in a study conducted by Standeven and Goldsworthy (1994). Five to six female $B6C3F_1$ mice were treated with 1000 mg/kg-day 2,2,4-trimethylpentane by intragastric intubation for 3 days (days 2–4) after implantation of a bromodeoxyuridine osmotic pump on day 1. Control animals were administered 5 mL/kg corn oil vehicle. Animals were sacrificed on day 5. Treatment with 2,2,4-trimethylpentane produced a significant increase in the hepatocyte-labeling index and an increase in relative liver weight, indicating a mitogenic effect on the liver. No changes in serum alanine aminotransferase (ALT) or sorbitol dehydrogenase (SDH) activities were noted.

4.4.1.2. *Inhalation*

Swann et al. (1974) exposed Swiss mice (four/group, sex not reported) to 2,2,4-trimethylpentane for 5 minutes via inhalation at concentrations of 1000, 2000, 4000, 8000, 16,000, 32,000, 64,000, and 128,000 ppm (approximately 4700 to 600,000 mg/m^3). At 16,000 ppm, sensory and motor irritation were observed throughout the exposure, and 1/4 of the mice had sudden respiratory arrest. At 32,000 ppm, all of the mice stopped breathing within 4 minutes of the onset of exposure. No apparent anesthesia was noted at any exposure concentration.

In another inhalation study (Exxon, 1987), male and female Sprague-Dawley rats, CD-1 mice, and Hartley guinea pigs (10/group) were exposed to 39,630 mg/m^3 (8322 ppm) 2,2,4-trimethylpentane for up to 4 hours. No abnormal signs were seen in the rats after 15 minutes of exposure; however, after 20 minutes, convulsions were seen in most animals and two rats died within 30 minutes of the onset of exposure. Convulsions, excessive lacrimation and salivation, and labored breathing were reported in the surviving rats, and all rats were dead within 55 minutes of the start of the exposure. In the mice, one animal died after 20 minutes and all the mice were dead within 75 minutes of exposure. Death occurred in 8/10 guinea pigs between 60 and 120 minutes of the onset of exposure. At necropsy, lung discoloration was observed in all of the animals, and liver and kidney discoloration was observed in 2/10 of the animals.

In a study of the potential toxicity of volatile emissions from indoor carpeting, Stadler and Kennedy (1996) evaluated the sensory irritation potential of a number of VOCs that were identified in carpet emissions, including 2,2,4-trimethylpentane. (The authors indicate 2,2,4-trimethylpentane to be known by the U.S. Environmental Protection Agency (EPA) to be frequently detectable in carpet volatiles but do not otherwise elaborate on the specific reason for its being present in carpets.) Toxicity was assessed by measuring the airborne concentration required to elicit a 50% depression in respiratory rate (RD_{50}) in Swiss-Webster mice, both for carpet emission mixtures and for pure chemical vapors. The mice were first exposed to a chemical vapor mixture emitted from a heated carpet sample for two exposures per day (each exposure 1 hour in duration) for 2 days. Total VOCs emitted were analyzed, and general signs

of toxicity, respiratory rate decreases, and breathing patterns of respiratory irritation in the mice were noted. In a second set of experiments, 2,2,4-trimethylpentane was one of 11 identified VOCs that were tested as pure chemical vapors (single 30-minute exposures). 2,2,4-Trimethylpentane, along with four other VOCs, exhibited RD_{50} values that were each greater than 1000 ppm, indicating that these VOCs are nonirritating chemicals when present alone.

4.4.1.3. *Dermal*

In a dermal study (Exxon, 1987), doses of 0.2 g/kg and 3.15 g/kg 2,2,4-trimethylpentane were applied to the abdominal area of New Zealand white (NZW) rabbits (four/group) for 24 hours with no mortality reported. At necropsy, in the low-dose group, one animal appeared to be normal, three had dark livers, and two had mottled livers. In the high-dose group, four animals had dark livers, two animals had mottled livers, and one had a pale kidney.

4.4.1.4. *Ocular*

Exxon (1987) also conducted an eye irritation study in NZW rabbits. 2,2,4-Trimethylpentane (0.1 mL, or ~70 mg) was instilled into the conjunctival sac of one eye of six rabbits. The ocular reactions were graded at 1 and 4 hours and at 1, 2, 3, 4, and 7 days after instillation. The results showed that 2,2,4-trimethylpentane was nonirritating to the eye.

4.4.2. Genotoxicity

There are a few reports on testing of 2,2,4-trimethylpentane for genetic toxicity. There are no available reports of testing for mutagenic activity in bacterial cells (e.g., the Ames salmonella test) or for chromosome breaking activity in vitro or in vivo.

4.4.2.1. *Mutation and Chromosome Effects*

A human lymphoblastoid cell line, TK6, was treated with a saturated (5% v/v) solution of 2,2,4-trimethylpentane in cell culture medium for 3 hours in the presence and absence of rat liver S9 fraction (Richardson et al., 1986). There were no detected increases in gene mutations at the thymidine kinase (TK) locus or in sister chromatid exchanges (SCEs).

4.4.2.2. *DNA Damage*

McLaren et al. (1994) investigated the induction of DNA double-strand breaks and poly-ADP-ribosylation in the renal cortex of male Wistar rats administered 12 mmol/kg (~1370 mg/kg) of 2,2,4-trimethylpentane via gavage for 5 consecutive days. Treatment failed to induce poly-ADP-ribosylation or a significant increase in DNA double-strand breaks in the renal cortex. Unscheduled DNA synthesis (UDS) was not induced in isolated male F344 hepatocytes

exposed to 2,2,4-trimethylpentane at final media concentrations of 0.33, 1.00, or 3.33% (high dose may be cytotoxic) (Loury et al., 1986).

4.4.3. Cytotoxicity

Two- to three-day HeLa cell cultures containing 0.1 to 7.5% 2,2,4-trimethylpentane did not exhibit altered cell growth or changes in viability or adenosine triphosphate/ADP content (Forman et al., 1999). However, Loury et al. (1986) observed cytotoxicity in isolated male rat hepatocytes exposed to a media concentration of 3.33% 2,2,4-trimethylpentane.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS

4.5.1. Oral

A number of acute and short-term studies were identified in the literature. Overall, these studies provide focused or limited information, as either they were designed to investigate only endpoints specific to α_{2u}-globulin-associated nephropathy in male rats or found no other significant 2,2,4-trimethylpentane-induced effects. The majority of noncancer effects induced by 2,2,4-trimethylpentane exposure were found to occur primarily in the kidneys of male rats, as the majority of the studies examined only the kidney. The effects reported included altered renal function, an increase in α_{2u}-globulin protein and hyaline droplet accumulation in the proximal tubules of male rats, necrosis of the tubule epithelium, increased cell turnover, and foci of regenerative epithelium (Blumbach et al., 2000; Saito et al., 1996, 1992; Borghoff et al., 1992; Burnett et al., 1989; Lock et al., 1987a,b; Short et al., 1986; Stonard et al., 1986; API, 1985, 1983). No increases in α_{2u}-globulin protein and hyaline droplet accumulation in the proximal tubules or in necrosis of the tubule epithelium were noted to occur in female rats (Blumbach et al., 2000; Lock et al., 1987a,b).

Of the studies with sufficient 2,2,4-trimethylpentane-specific dose-effect information (see Section 4.4.1), only two studies reported effects in organs other than kidney: Fowlie et al. (1987) observed centrilobular necrosis and hydrophobic degeneration of hepatocytes induced by 2,2,4-trimethylpentane, and Lock et al. (1987a) observed increases in liver weight and liver-to-body weight ratios in both treated males and females. The effects noted by Lock et al. (1987a) were thought to result from an induction in cytochrome P-450 and peroxisome proliferation. However, Short et al. (1986) found no significant histological changes in the liver.

4.5.2. Inhalation

Only one subchronic inhalation study was identified for 2,2,4-trimethylpentane (Short et al., 1989a). In this study, male and female F344 rats were exposed for 3 to 50 weeks to 50 ppm (234 mg/m^3) 2,2,4-trimethylpentane to characterize the pathogenesis of α_{2u}-globulin nephropathy. Body weight was the only other endpoint to be evaluated. As observed in the oral

studies, the notable effects in this study were limited to the male rat kidney and consisted of an increase in α_{2u}-globulin protein and hyaline droplet accumulation in the P_2 segment of the proximal tubules, necrosis of the tubule epithelium, sustained regenerative tubule cell proliferation, and enhancement of CPN in male rats. Control and exposed female rats exhibited no evidence of α_{2u}-globulin-nephropathy, increases in cell turnover, or chronic nephrosis.

Otherwise, only three other inhalation studies were identified (Stadler and Kennedy, 1996; Exxon, 1987; Swann et al., 1974), and, as discussed above, these studies were designed to assess only acute toxicity endpoints. Results from such studies are not amenable to use in the development of chronic inhalation RfC values.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

4.6.1. Summary of Overall Weight of Evidence

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is "inadequate information to assess carcinogenic potential" for 2,2,4-trimethylpentane. No epidemiological studies in humans and no chronic bioassay studies are available that assess the carcinogenic effects of 2,2,4-trimethylpentane. The majority of the reported studies contribute information specifically related to the histopathological sequence of α_{2u}-globulin-associated nephropathy. Thus, these studies did not examine any other tissues/organs except the kidney. In comparing the tumor-promoting capability between 2,2,4-trimethylpentane and UG (a mixture), Short et al. (1989b) showed that both agents had promoting potential in male but not female rats. However, the results were not sufficiently descriptive to ascribe the portion of the promoting potential of UG that could be attributable to 2,2,4-trimethylpentane. The few studies available on its genotoxic potential were negative, as 2,2,4-trimethylpentane did not increase mutations at the TK locus (Richardson et al., 1986), induce DNA double-strand breaks (McLaren et al., 1994), or stimulate UDS (Loury et al., 1986).

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

No other studies or supportive information are available on the carcinogenic effects of 2,2,4-trimethylpentane.

4.7. MODE-OF-ACTION INFORMATION

A limited number of studies evaluating the effects of exposure to 2,2,4-trimethylpentane by the oral or inhalation routes have been conducted, and no human studies are available. The majority of these studies have been designed to specifically address and characterize the involvement of α_{2u}-globulin in the renal toxicity observed in the male rat, with little or no evaluation of other endpoints in tissues/organs other than the kidney or of other potential modes of action.

4.7.1. General Issues Concerning the Determination of α_{2u}-Globulin-Associated Nephropathy

α_{2u}-Globulin is a member of a large superfamily of low-molecular-weight proteins and was first characterized in male rat urine. It has been detected in various tissues and fluids of most mammals, including humans. However, the particular isoform of α_{2u}-globulin commonly detected in male rat urine appears to be largely specific for the male rat; moreover, the urine and kidney concentrations detected in the mature male rat are several orders of magnitude greater than in any other age, sex, or species tested (U.S. EPA, 1991).

The mode of action ascribed to α_{2u}-globulin-associated nephropathy is defined by a progressive sequence of effects in the male rat kidney, often culminating in renal tumors. The involvement of hyaline droplet accumulation in the early stages of nephropathy associated with α_{2u}-globulin-binding chemicals is an important difference from the sequence of events observed with classical carcinogens. The pathological changes that precede the proliferative sequence for classical renal carcinogens also include early nephrotoxicity (e.g., cytotoxicity and cellular necrosis) but no apparent hyaline droplet accumulation. Furthermore, the nephrotoxicity that can ensue from hyaline droplet accumulation is novel because it is associated with excessive α_{2u}-globulin accumulation. This α_{2u}-globulin accumulation is proposed to result from reduced renal catabolism of the α_{2u}-globulin chemical complex and is thought to initiate a sequence of events leading to chronic proliferation of the renal tubule epithelium, as well as an exacerbation of CPN. The histopathological sequence in mature male rats consists of the following (see Table 4-1 for a summary of this sequence specific for 2,2,4-trimethylpentane):

- Excessive accumulation of hyaline droplets containing α_{2u}-globulin in renal proximal tubules
- Subsequent cytotoxicity and single-cell necrosis of the tubule epithelium
- Sustained regenerative tubule cell proliferation
- Development of intralumenal granular casts from sloughed cellular debris associated with tubule dilatation and papillary mineralization
- Foci of tubule hyperplasia in the convoluted proximal tubules
- Renal tubule tumors

Table 4-1. Summary of renal effects specific to male rats reported in 2,2,4-trimethylpentane studies

Study (route, dose, duration)	Accumulation of α_{2u}-globulin hyaline droplets	Cytotoxicity, necrosis of tubule epithelium	Sustained regenerative tubule cell proliferation	Intralumenal granular casts and papillary mineralization	Foci of tubule hyperplasia
Short et al. (1989a) (inhalation, 50 ppm [234 mg/m^3] 6 h/d, 5 d/w, 3–50 w)	X	X	X		
API (1983) (oral, 0.5 or 2.0 g/kg-d, 5 d/w, 4 w)	X	X		X	
Short et al. (1986) (oral, 50–500 mg/kg-d, 21 d)	X	X	X	X	X
Saito et al. (1992) (oral, 50 mg/kg-d, 14 d)	X				
Borghoff et al. (1992) (oral, 0.95–30 mg/kg-d, 10 d)	X		X		
Lock et al. (1987a) (oral, 1370 mg/kg-d, 10 d)	X				
Saito et al. (1996) (oral, 171 mg/kg-d, 7 d)	X				
Blumbach et al. (2000) (oral, 500 mg/kg-d, 5 d)	X				
Burnett et al. (1989) (oral, 50 mg/kg, 1 d)	X				
Lock et al. (1987b) (oral, 500 mg/kg, 1 d)	X				
Stonard et al. (1986) (oral, 34–2740 mg/kg, 1 d)	X				

In addition to this histopathological sequence, EPA (1991) provides more specific guidance for evaluating chemically induced male rat renal tubule tumors for the purpose of risk assessment. To determine the appropriateness of the data for use in risk assessment, chemicals inducing renal tubule tumors in the male rat are examined in terms of three categories:

- The α_{2u}-globulin sequence of events accounts for the renal tumors.
- Other potential carcinogenic processes account for the renal tumors.
- The α_{2u}-globulin-associated events occur in the presence of other potential carcinogenic processes, both of which result in renal tumors.

Therefore, it is important to determine whether the α_{2u}-globulin process is involved and, if so, to what extent α_{2u}-globulin-associated events, rather than other processes, account for the tumor increase.

Determination of these elements requires a substantial database of bioassay data not only from male rats but also from female rats and mice, and such toxicity studies must demonstrate whether or not α_{2u}-globulin processes are operative. In the absence of minimum information demonstrating the involvement of α_{2u}-globulin processes, it should be assumed that any male rat renal toxicity/tumors are relevant for risk assessment purposes. A technical report available from EPA (1991) outlines the data necessary to determine the involvement of α_{2u}-globulin.

As outlined in the EPA Risk Assessment Forum Technical Panel report (U.S. EPA, 1991), the following information from adequately conducted studies of male rats is used for demonstrating that the α_{2u}-globulin process may be a factor in any observed renal effects—an affirmative response in each of the three categories is required. If data are lacking for any of the criteria in any one category, the available renal toxicity data should be analyzed in accordance with standard risk assessment principles. The three categories of information and criteria are as follows:

- *Increased number and size of hyaline droplets in the renal proximal tubule cells of treated male rats*. The abnormal accumulation of hyaline droplets in the P_2 segment helps differentiate α_{2u}-globulin inducers from chemicals that produce renal tubule tumors by other modes of action.
- *Accumulating protein in the hyaline droplets is α_{2u}-globulin*. Hyaline droplet accumulation is a nonspecific response to protein overload, and, thus, it is necessary to demonstrate that the protein in the droplet is, in fact, α_{2u}-globulin.
- *Additional aspects of the pathological sequence of lesions associated with α_{2u}-globulin nephropathy are present*. Typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules, and tubule hyperplasia. If the response is mild, not all of these lesions may be observed. However, some elements consistent with the pathological sequence must be demonstrated to be present. This pathological sequence is

outlined above and in Table 4-1 for effects specifically demonstrated for 2,2,4-trimethylpentane.

4.7.2. 2,2,4-Trimethylpentane and α_{2u}-Globulin-Associated Nephropathy

A number of studies (see Sections 4.2.1.2 and 4.4.1) have demonstrated that oral or inhalation exposures to 2,2,4-trimethylpentane result in renal effects in male rats in dosing regimens ranging from a single dose to daily dosing for periods as long as 50 weeks (Blumbach et al., 2000; Saito et al., 1996, 1992; Borghoff et al., 1992; Burnett et al., 1989; Short et al., 1989a, 1986; Lock et al., 1987a,b; Stonard et al., 1986; API, 1983). The effects observed in male rat kidney include an increase in protein in the proximal tubules, an increase in hyaline droplets, the confirmed presence of α_{2u}-globulin, necrosis and degeneration of the tubule epithelium, increased cell turnover/proliferation, foci of regenerative epithelium, and exacerbation of chronic nephrosis. The significant effects noted in these studies provide evidence that corresponds to the histopathological sequence for α_{2u}-globulin-associated nephropathy in male rats (see Table 4-1), as well as the additional categorical criteria outlined above. These effects have not been observed in female rats, mice, guinea pigs, dogs, or monkeys (Alden, 1986). Taken together, this information provides strong evidence that the renal effects induced by 2,2,4-trimethylpentane result from a mode of action associated with α_{2u}-globulin accumulation.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

No studies are available on possible childhood susceptibility to 2,2,4-trimethylpentane.

4.8.2. Possible Gender Differences

No studies are available on possible gender differences in humans regarding 2,2,4-trimethylpentane. Overall, the available animal studies provide focused or limited information on gender-specific differences, as either they were designed to investigate only endpoints specific to α_{2u}-globulin-associated nephropathy in male rats or found no other significant 2,2,4-trimethylpentane-induced effects (Blumbach et al., 2000; Saito et al., 1996, 1992; Borghoff et al., 1992; Burnett et al., 1989; Short et al., 1989a, 1986; Lock et al., 1987a,b; Stonard et al., 1986; API, 1983). These α_{2u}-globulin-associated renal effects have not been shown to occur in female rats (Blumbach et al., 2000; Lock et al., 1987a,b). These renal effects, specific to the male rat, are not thought to be relevant to humans. No other studies are available that indicate gender-specific effects.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

No subchronic or chronic oral studies were identified that demonstrated a dose-response effect that could be used to determine the noncarcinogenic risk for 2,2,4-trimethylpentane.

A number of acute and short-term studies were identified in the literature. Overall, these studies provide focused or limited information, as either they were designed to investigate only endpoints specific to α_{2u}-globulin-associated nephropathy in male rats or found no other significant 2,2,4-trimethylpentane-induced effects. The majority of noncancer effects induced by 2,2,4-trimethylpentane exposure were found to occur primarily in the kidney of male rats as the majority of the studies examined only the kidney. The effects reported include altered renal function, an increase in α_{2u}-globulin protein and hyaline droplet accumulation in the proximal tubules, necrosis of the tubule epithelium, increased cell turnover, and foci of regenerative epithelium (Blumbach et al., 2000; Saito et al., 1996, 1992; Borghoff et al., 1992; Burnett et al., 1989; Lock et al., 1987a,b; Short et al., 1986; Stonard et al., 1986; API, 1983). No increases in α_{2u}-globulin protein and hyaline droplet accumulation in the proximal tubules or necrosis of the tubule epithelium were noted to occur in female rats (Blumbach et al., 2000; Lock et al., 1987a,b).

Detailed studies that contain sufficient dose-response and duration information on 2,2,4-trimethylpentane for endpoints other than nephropathy are currently lacking. Liver effects were noted in two acute or short-term oral studies. Fowlie et al. (1987) observed centrilobular necrosis and hydrophobic degeneration of hepatocytes induced by 2,2,4-trimethylpentane and Lock et al. (1987a) observed increases in liver weight and liver-to-body weight ratios in both treated males and females. The effects noted by Lock et al. (1987a) were thought to result from an induction in cytochrome P-450 and peroxisome proliferation. Short et al. (1986) found no significant histological changes in the liver.

As discussed above (see Sections 4.4.1, 4.5.1, and 4.7), the available studies provide evidence that the kidney toxicity induced by 2,2,4-trimethylpentane in male rats is related to α_{2u}-globulin accumulation in the proximal tubules. Because this response is specific to male rats, as a matter of science policy, EPA (1991) has concluded that "if a chemical induces α_{2u}-globulin accumulation in male rats, the associated nephropathy is not used as an endpoint for determining noncarcinogenic hazard. Estimates of noncarcinogenic risk are based on other endpoints." No other studies were considered suitable for the derivation of an RfD. Therefore, an oral RfD was not derived. The previous IRIS assessment (dated 11/01/1991) did not include an RfD derivation.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No subchronic or chronic inhalation studies were identified that demonstrate a dose-response effect that could be used to determine the noncarcinogenic risk for 2,2,4-trimethylpentane.

Only one subchronic inhalation study was identified for 2,2,4-trimethylpentane (Short et al., 1989a). In this study, male and female F344 rats were exposed for 3 to 50 weeks to 50 ppm 2,2,4-trimethylpentane to characterize the pathogenesis of α_{2u}-globulin-associated nephropathy. Body weight was the only other endpoint evaluated. As observed in the oral studies, the notable effects in this study were limited to the male rat kidney and consisted of an increase in α_{2u}-globulin protein and hyaline droplet accumulation in the P_2 segment of the proximal tubules, necrosis of the tubule epithelium, sustained regenerative tubule cell proliferation, and enhancement of CPN in male rats. Control and exposed female rats exhibited no evidence of α_{2u}-globulin-associated nephropathy, increases in cell turnover, or chronic nephrosis.

In addition, three short-term inhalation studies (Stadler and Kennedy, 1996; Exxon, 1987; Swann et al., 1974) were identified that addressed only limited endpoints (e.g., lethality and irritancy) at high-exposure concentrations ($\geq$4600 mg/m^3) and acute exposure durations (<4 hrs) and, thus, are of limited value for chronic reference concentration assessment purposes.

As discussed above (see Sections 4.2.2, 4.5.2, and 4.7), the available studies provide evidence that the kidney toxicity induced by 2,2,4-trimethylpentane in male rats is related to α_{2u}-globulin accumulation in the proximal tubules. Because this response is specific to male rats, as a matter of science policy, EPA (1991) has concluded that "if a chemical induces α_{2u}-globulin accumulation in male rats, the associated nephropathy is not used as an endpoint for determining noncarcinogenic hazard. Estimates of noncarcinogenic risk are based on other endpoints." No other studies were considered suitable for the derivation of the RfC. Therefore, an inhalation RfC was not derived. The previous IRIS assessment (dated 11/01/1991) did not include an RfC derivation.

5.3. CANCER ASSESSMENT

No studies are available on the carcinogenic effects of 2,2,4-trimethylpentane on which to base a cancer assessment. This overall lack of information represents a data gap and does not allow for a quantitative assessment of the carcinogenicity of 2,2,4-trimethylpentane.

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

6.1. HUMAN HAZARD POTENTIAL

2,2,4-Trimethylpentane is a colorless liquid with the odor of gasoline (NLM, 2004). It is used primarily in the alkylation step of the reaction of isobutane and butylene in deriving high-octane fuels. 2,2,4-Trimethylpentane is synthesized from the catalytic hydrogenation of trimethylpentene with a nickel catalyst (API, 1985). 2,2,4-Trimethylpentane is released to the environment through the use and disposal of gasoline-associated products, and inhalation appears to be the major route of exposure (NLM, 2004).

Epidemiological or poisoning case studies of 2,2,4-trimethylpentane in humans are not available. Exposure of laboratory animals, including mice, rats, and guinea pigs, to high levels (≥8322 ppm) of 2,2,4-trimethylpentane via inhalation has resulted in death. There are few reliable oral or inhalation studies that have evaluated the toxicity of 2,2,4-trimethylpentane administered at lower, nonlethal levels in any species other than rats, the database being dominated by studies in male rats characterizing a species- and gender-specific renal effect that is not relevant to humans. Toxicity studies in other species and studies examining effects in other tissues/organs except the kidney are lacking, which is considered a major deficiency in the hazard identification process for 2,2,4-trimethylpentane.

Limited data are available on the absorption of 2,2,4-trimethylpentane. The available studies in rats suggest that the chemical has high oral absorption and is distributed to the kidney, fat, and liver, with higher concentrations detected in the kidneys of males compared with females (Kloss et al., 1986). Based on the results from the inhalation study conducted by Dahl (1989), absorption via the respiratory tract may be quite low (7 to 12% of inspired concentration). The major metabolites identified in rats are trimethyl pentanols, pentanoic acids, and hydroxypentanoic acids (Olson et al., 1985), with one study (Charbonneau et al., 1987) reporting two metabolites, 2,2,4-trimethyl-2-pentanol and 2,2,4-trimethyl pentanoic acid, detected in the liver of male, but not female, rats. Elimination of 2,2,4-trimethylpentane in rats occurs primarily via the urine (60 to 70%), and less than 10% is eliminated via the feces (Dahl, 1989; Kloss et al., 1986).

In addition, no subchronic or chronic oral animal studies are available for the chemical. However, a number of acute and short-term oral studies were identified in the literature. The majority of noncancer effects induced by 2,2,4-trimethylpentane exposure were found to occur in the kidney of male rats and included altered renal function, an increase in α_{2u}-globulin protein and hyaline droplet accumulation in the proximal tubules, necrosis of the tubule epithelium, increased cell turnover, and foci of regenerative epithelium (Blumbach et al., 2000; Saito et al., 1996, 1992; Borghoff et al., 1992; Burnett et al., 1989; Lock et al., 1987a,b; Short et al., 1986; Stonard et al., 1986; API, 1983). No increases in α_{2u}-globulin protein and hyaline droplet

accumulation in the proximal tubules, or necrosis of the tubule epithelium, were noted to occur in female rats (Blumbach et al., 2000; Lock et al., 1987a,b). One acute study also reported effects on the liver (Fowlie et al., 1987).

Chronic inhalation studies for 2,2,4-trimethylpentane are lacking. Only one subchronic inhalation study was identified for 2,2,4-trimethylpentane (Short et al., 1989a). In this study, male and female F344 rats were exposed for 3 to 50 weeks to 50 ppm (234 mg/m^3) 2,2,4-trimethylpentane. As observed in the oral studies, the notable effects in this study were limited to the male rat kidney and consisted of an increase in α_{2u}-globulin protein and hyaline droplet accumulation in the P_2 segment of the proximal tubules, necrosis of the tubule epithelium, sustained regenerative tubule cell proliferation, and enhancement of CPN in male rats. Control and exposed female rats exhibited no evidence of α_{2u}-globulin nephropathy, increases in cell turnover, or increase in the extent of chronic nephrosis.

No studies are available on the reproductive or developmental effects of 2,2,4-trimethylpentane. Limited genotoxicity data are available. The few studies available on its genotoxic potential were negative, as 2,2,4-trimethylpentane did not increase mutations at the TK locus, did not induce SCEs in a human lymphoblastoid cell line (Richardson et al., 1986) or DNA double-strand breaks in male rat kidney (McLaren et al., 1994), and did not stimulate UDS in isolated male rat or mouse hepatocytes (Loury et al., 1986).

One study (U.S. EPA, 1991) has reviewed the available data on α_{2u}-globulin-associated nephropathy in rats and has concluded that this endpoint is not relevant to humans and, therefore, should not be used to determine the noncarcinogenic hazard. In addition, most of the animal studies of 2,2,4-trimethylpentane were designed to investigate only the renal effects and α_{2u}-globulin nephropathy in male rats. Consequently, the limited focus of studies that have been conducted with 2,2,4-trimethylpentane identifies a need for additional studies that examine a more comprehensive set of toxicological endpoints.

6.2. DOSE RESPONSE

In the case of 2,2,4-trimethylpentane, there is currently a lack of studies presenting sufficient dose-response and duration information from which to derive quantitative estimates of noncancer or cancer risks. As described above, the available information demonstrates that the renal effects induced by 2,2,4-trimethylpentane are primarily attributable to processes involving α_{2u}-globulin. In addition, the available data are concordant with the histopathological sequence and criteria outlined in the EPA (1991) guidance developed to address the issues concerning α_{2u}-globulin-associated renal toxicity (nephropathy) and neoplasia. Consequently, in agreement with EPA science policy, no quantitative estimates of noncancer risk were developed for 2,2,4-trimethylpentane.

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

The *Toxicological Review of 2,2,4-Trimethylpentane* has undergone internal review by scientists within EPA, an interagency review, and an external panel peer review. The external review was conducted in February 2007 in accordance with EPA guidance on peer review (U.S. EPA, 2006). Comments made by the internal as well as interagency reviewers were addressed prior to submitting the document for external review and are not part of this appendix. For the external peer review, the reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific charge questions, addressing key scientific issues of the assessment. The charge questions, summary of reviewer comments, and EPA's disposition of the comments are provided below. Editorial comments were considered and incorporated into the document as appropriate and are not discussed further. EPA also received comments from the public. A summary of public comments and EPA's responses are also included.

Charge to External Reviewers

Question 1: Are there additional key published studies or publicly available scientific reports that are missing from the draft document that might be useful for the discussion of the hazards of 2,2,4-trimethylpentane?

Question 2: No oral RfD has been derived in the current draft assessment. Has the rationale and justification for not deriving an RfD been transparently described? Is the rationale scientifically justified and appropriate?

Question 3: No inhalation RfC has been derived in the current draft assessment. Has the rationale and justification for not deriving an RfC been transparently described? Is the rationale scientifically justified and appropriate?

Question 4: Does the *Toxicological Review* provide sufficient information to support a conclusion that there is a causal relationship between accumulation of α2u-globulin and the pathology observed exclusively in the male rat kidney in response to 2,2,4-trimethylpentane exposure?

Question 5: The majority of the studies available for 2,2,4-trimethylpentane were designed only to investigate various aspects of α2u-globulin-induced nephropathy. Thus, data and information on effects in target organ systems other than the kidney are limited in quantity and quality (e.g., liver). Has the available information on effects unrelated to α2u-globulin-associated nephropathy been adequately and appropriately described?

Question 6: Has the appropriate cancer descriptor been chosen? Has the rationale and justification for not deriving a quantitative cancer assessment been transparently described? Do you agree with EPA's rationale, justification, and conclusion?

Comments and Response

Question 1: Two reviewers thought the literature database was complete and could not identify any additional literature. Two reviewers suggested additional studies from the literature that would add support to the conclusions made and would perhaps add value but not change the conclusions reached in the assessment.

Response: The suggested references (Cuervo et al., 1999; Standeven and Goldsworthy, 1994; Dietrich and Swenberg, 1991) were reviewed and incorporated into the assessment in section 4.4.1.1, Other Studies, Acute and Short-term Studies, Oral. Specifically, these references added information to support the mode-of-action information for TMP, and TMP-/TMP metabolite-renal protein binding.

Question 2: All four reviewers felt that the studies available were not sufficient for use in deriving an RfD, as they were mainly designed to address specific mode-of-action questions. The reasoning, justification, and discussion presented for not deriving an RfD were transparently described and scientifically appropriate.

Response: No response required.

Question 3: All four reviewers felt that the studies available were not sufficient for use in deriving an RfC, as they were mainly designed to address specific mode-of-action questions. The reasoning, justification, and discussion presented for not deriving an RfC were transparently described and scientifically appropriate.

Response: No response required.

Question 4: All four reviewers felt that sufficient information was provided, documented, and described to support the causal relationship between accumulation of α_{2u}-globulin and the pathology observed exclusively in the male rat kidney in response to 2,2,4-trimethylpentane exposure. In addition, one reviewer provided an additional reference on chemical-α_{2u}-globulin binding to support the mode of action described.

Response: The reference cited was incorporated into the assessment in section 4.4.1.1, Other Studies, Acute and Short-term Studies, Oral.

Question 5: All reviewers agreed that the limited information for the effects of TMP on other organs was adequately described except for a paper by Standeven and Goldsworthy (1994) describing liver mitogenic effects in mice. However, the inclusion of this study would not change the conclusions reached in the assessment.

Response: Reference to this study was added to the assessment in section 4.4.1.1, Other Studies, Acute and Short-term Studies, Oral, as part of the response to Question 1 to complete the database.

Question 6: All reviewers agreed that the appropriate cancer descriptor was used and the analysis and rationale to justify not assessing the cancer risk for TMP were transparently described.

Response: No response required.

Comments from the Public

Comment: One reviewer agreed with and endorsed the assessment's conclusion on the renal effects characterized for TMP. This reviewer also offered a few literature citations of studies conducted for several isoparaffinic and hydrocarbon solvents that the reviewer believes could be used to qualitatively inform the database and enhance the hazard characterization of TMP.

Response: While we agree that other information on similar chemicals could be used to enhance the database and findings as a whole, those studies identified examined solvent mixtures that may or may not be related to or contain TMP. An attempt was made in the assessment to include some information on the mixture, unleaded gasoline, of which TMP is a component but only in those studies in which TMP was examined separately. Even in those studies, there is great difficulty in specifically ascribing the effects that directly result from TMP alone and not from the mixture.

Comment: One reviewer had general comments concerning risk assessment practices, the period of time to comment, animal testing, and a reference to thalidomide.

Response: No response required as no comments pertaining specifically to this assessment were made.