1,4-Dioxane (CASRN: 123-91-1)

Note: A TOXICOLOGICAL REVIEW is available for this chemical in Adobe PDF Format (XXX pp, XM). Similar documents can be found in the List of Available IRIS Toxicological Reviews.

Links to specific pages in the toxicological review are available throughout this summary. To utilize this feature, your Web browser and Adobe program must be configured properly so the PDF displays within the browser window. If your browser and Adobe program need configuration, please go to EPA's PDF page for instructions. In addition, there are hyperlinks to the reference citations throughout this document that will take you to the HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments.

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1,4-Dioxane (CASRN: 123-91-1)

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR 1,4-Dioxane

File First On-Line 08/22/1988

Category (section)	Status	Last Revised
Chronic Oral RfD Assessment (I.A.)	on-line	08/11/2010
Chronic Inhalation RfC Assessment (I.B.)	on-line	00/00/0000
Carcinogenicity Assessment (II.)	on-line	00/00/0000

_I. HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

__I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE

Substance Name – 1,4-Dioxane
CASRN – 123-91-1
Section I.A. Last Revised – 08/11/2010

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. It is expressed in units of mg/kg-day. Please refer to the guidance documents at http://www.epa.gov/iris/backgrd.html for an elaboration of these concepts. Because RfD values can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

There was no previous oral RfD for 1,4-dioxane on IRIS.

___I.A.1. CHRONIC ORAL RfD SUMMARY

Critical Effect	Point of Departure	UF	Chronic RfD
Liver and kidney toxicity Chronic oral male rat study Kociba et al. (1974)	NOAEL: 9.6 mg/kg-day	300	0.03 mg/kg-day

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Liver and kidney toxicity were the primary noncancer health effects associated with exposure to 1,4-dioxane in humans and laboratory animals. Occupational exposure to 1,4-dioxane has resulted in hemorrhagic nephritis and centrilobular necrosis of the liver (Johnstone, 1959; Barber, 1934). In animals, liver and kidney degeneration and necrosis were observed frequently in acute oral and inhalation studies (JBRC, 1998a; Drew et al., 1978; David, 1964; Kesten et al., 1939; Laug et al., 1939; Schrenk and Yant, 1936; de Navasquez, 1935; Fairley et al., 1934). Liver and kidney effects were also observed following chronic oral exposure to 1,4-dioxane in animals (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Argus et al., 1973; Argus et al., 1965) (see summary Table 4-17 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010)).

In the available chronic studies, (Kociba et al., 1974) reported the most sensitive effects in the liver and kidney based on a NOAEL of 9.6 mg/kg-day and a LOAEL of 94 mg/kg-day in male Sherman rats. Kociba et al. (1974) reported toxic effects of hepatocellular degeneration and necrosis in the liver, while liver lesions reported in other studies (JBRC, 1998b; Argus et al., 1973) appeared to be related to the carcinogenic process. Kociba et al. (1974) also reported renal tubule epithelial cell degenerative changes and necrosis in the kidney which was supported by data in NCI (1978) and Argus et al. (1973); however, kidney toxicity was observed in these studies at higher doses. For degenerative liver effects resulting from 1,4-dioxane exposure, the Kociba et al. (1974) study represents the most sensitive effect and dataset observed in a chronic bioassay. As a result, Kociba et al. (1974) was chosen as the principal study for the derivation of the RfD.

Kociba et al. (1974) conducted a 2-year study in which four groups of 6–8-week-old Sherman rats (60/sex/dose level) were administered 1,4-dioxane in drinking water at levels of 0 (controls), 0.01, 0.1, or 1.0% for up to 716 days. Based on water consumption and BW data for specific exposure groups, Kociba et al. (1974) calculated mean daily doses of 9.6, 94, and 1,015 mg/kg-day for male rats and 19, 148, and 1,599 mg/kg-day for female rats during days 114–198 for the 0.01, 0.1, and 1.0% concentration levels, respectively. Rats were observed daily for clinical signs of toxicity, and BWs were measured twice weekly during the first month, weekly during months 2–7, and biweekly thereafter. Water consumption was recorded at three different time periods during the study: days 1–113, 114–198, and 446–460. Blood samples were collected from a minimum of five male and five female control and high-dose rats during the 4th, 6th, 12th, and 18th months of the study and at termination. Each blood sample was analyzed for packed cell volume, total erythrocyte count, hemoglobin, and total and differential WBC counts. Additional endpoints evaluated included organ weights (brain, liver, kidney, testes, spleen, and heart) and gross and microscopic examination of major tissues and organs (brain, bone and bone marrow, ovaries, pituitary, uterus, mesenteric lymph nodes, heart, liver, pancreas, spleen, stomach, prostate, colon, trachea, duodenum, kidneys, esophagus, jejunum, testes, lungs, spinal cord, adrenals, thyroid, parathyroid, nasal turbinates, and urinary bladder).

Histopathological lesions were restricted to the liver and kidney from the mid- and high-dose groups and consisted of variable degrees of renal tubular epithelial and hepatocellular degeneration and necrosis (no quantitative incidence data were provided). Rats from these groups also showed evidence of hepatic regeneration, as indicated by hepatocellular hyperplastic nodule formation and evidence of renal tubular epithelial regenerative activity (observed after 2 years of exposure). These changes were not seen in controls or in low-dose rats. The authors determined a NOAEL of 9.6 mg/kg-day and a LOAEL of 94 mg/kg-day for 1,4-dioxane based on the liver and kidney effects in male rats.

Methods of Analysis. Kociba et al. (1974) did not provide quantitative incidence or severity data for liver and kidney degeneration and necrosis. Benchmark dose (BMD) modeling could not be performed for this study, and the NOAEL for liver and kidney degeneration (9.6 mg/kg-day in male rats) was used as the point of departure (POD) in deriving the RfD for 1,4-dioxane.

Other datasets and alternative POD values were also considered as the basis for the 1,4-dioxane RfD, including incidence data reported for cortical tubule degeneration in male and female rats (NCI, 1978) and liver hyperplasia (JBRC, 1998b). The $BMDL_{10}$ values of 22.3 mg/kg-day and 23.8 mg/kg-day from the (NCI, 1978) and (JBRC, 1998b) studies, respectively, are about double the NOAEL (9.6 mg/kg-day) observed by Kociba et al. (1974).

___I.A.3. UNCERTAINTY FACTORS

UF = 300

= 10 (UF_A) × 10 (UF_H) × 1 (UF_S) × 1 (UF_L) × 3 (UF_D)

A default interspecies UF of 10 (UF_A) was used to account for pharmacokinetic and pharmacodynamic differences between rats and humans. Existing PBPK models could not be used to derive an oral RfD for 1,4-dioxane.

A default interindividual variability UF of 10 (UF_H) was used to account for variation in sensitivity within human populations because there is limited information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of, or response to, 1,4-dioxane.

An UF to extrapolate from a subchronic to a chronic (UF_S) exposure duration was not necessary (e.g., UF_S = 1) because the RfD was derived from a study using a chronic exposure protocol.

An UF to extrapolate from a LOAEL to a NOAEL (UF_L) was not necessary (e.g., UF_L = 1) because the RfD was based on a NOAEL. Kociba et al. (1974) was a well-conducted, chronic drinking water study with an adequate number of animals. Histopathological examination was performed for many organs and tissues, but clinical chemistry analysis was not performed. NOAEL and LOAEL values were derived from the study based on liver and kidney toxicity.

An UF of 3 for database deficiencies (UF_D) was applied due to the lack of a multigeneration reproductive toxicity study. A single oral prenatal developmental toxicity study in rats was available for 1,4-dioxane (Giavini et al., 1985). This developmental study indicated that the developing fetus may be a target of toxicity.

___I.A.4. ADDITIONAL STUDIES/COMMENTS

The predominant noncancer effect of chronic oral exposure to 1,4-dioxane is degenerative effects in the liver and kidney. For degenerative liver effects resulting from 1,4-dioxane exposure, the Kociba et al. (1974) study represents the most sensitive effect and dataset observed in a chronic bioassay.

Kidney toxicity as evidenced by glomerulonephritis (Argus et al., 1973; Argus et al., 1965) and degeneration of the cortical tubule (CAA, 1990; NCI, 1978; Kociba et al., 1974) has also been observed in response to chronic exposure to 1,4-dioxane. Degenerative effects were observed in the kidney at the same dose level as effects in the liver (Kociba et al., 1974).

Rhinitis and inflammation of the nasal cavity were reported in both the NCI (1978) (mice only, dose ≥ 380 mg/kg-day) and JBRC (1998b) studies (≥ 274 mg/kg-day in rats, >278 mg/kg-day in mice). JBRC (1998b) reported nasal inflammation in rats (NOAEL 55 mg/kg-day, LOAEL 274 mg/kg-day) and mice (NOAEL 66 mg/kg-day, LOAEL 278 mg/kg-day).

Studies in experimental animals have also found that relatively high doses of 1,4-dioxane (1,000 mg/kg-day) during gestation can produce delayed ossification of the sternebrae and reduced fetal BWs (Giavini et al., 1985).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF)

___I.A.5. CONFIDENCE IN THE CHRONIC ORAL RfD

Study - Medium
Database - Medium
RfD - Medium

The overall confidence in the RfD is medium. Confidence in the principal study (Kociba et al., 1974) is medium. The 2-year drinking water study is a well-conducted, peer-reviewed study that used 3 dose groups plus a control. The study had adequate group sizes (60 rats/sex/dose group) and investigated multiple target organs.

Confidence in the oral database is medium due to the lack of a multigeneration reproductive toxicity study.

Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfD is medium.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC ORAL RfD

Source Document – (U.S. EPA, 2010)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010). **To review this appendix, exit to the Toxicological Review, Appendix A, Summary of External Peer Review and Public Comments and Disposition (PDF).**

___I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

__I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Substance Name - 1,4-Dioxane
CASRN – 123-91-1
Section I.B. Last Revised -- 00/00/0000

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. The U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation is contained in Section II of this file.

__I.B.1. INHALATION RfC SUMMARY

Critical Effect	Point of Departure	UF	Chronic RfC
Atrophy and respiratory metaplasia of the olfactory epithelium Chronic inhalation male rat study Kasai et al. (2009)	LOAEL: 50 ppm LOAEL POD_{ADJ}: 8.9 ppm LOAEL POD_{HEC}: 32.2 mg/m^3	1,000	3×10^{-2} mg/m^3

__I.B.2. PRINCIPAL AND SUPPORTING STUDIES

Four inhalation studies in animals were identified in the literature; two, 13-week subchronic studies in laboratory animals (Kasai et al., 2008; Fairley et al., 1934) and two, 2-year chronic studies in rats (Kasai et al., 2009; Torkelson et al., 1974). Nasal, liver, and kidney toxicity were the primary noncancer health effects of inhalation exposure to 1,4-dioxane in rodents (see summary Table 4-26 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013)).

The chronic Kasai et al. (2009) study was selected as the principal study for the derivation of the RfC. Groups of male 6-week-old F344/DuCrj rats (50/group) were exposed via inhalation to nominal concentrations of 0 (clean air), 50, 250, and 1,250 ppm (0, 180, 900, and 4,500 mg/m^3, respectively) of vaporized 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 104 weeks (2 years) in whole body inhalation chambers (Kasai et al., 2009). At the time of

death or at the end of the 2-years of exposure the authors examined multiple organ systems. Based on the noncancer database for 1,4-dioxane, this study demonstrated exposure concentration-related effects for histopathological lesions at a lower concentration (50 ppm) compared to the subchronic Kasai et al. study (2008). The 2-year bioassay (Kasai et al., 2009) did not observe effects in both sexes, but the use of only male rats was proposed by the study authors as justified by data illustrating the absence of induced mesotheliomas in female rats following exposure to 1,4-dioxane in drinking water (Yamazaki et al., 1994). Additionally, a similar pattern of effects was observed after oral exposure to 1,4-dioxane (Kano et al., 2009; JBRC, 1998b) as observed in the Kasai et al. (2009) 2-year inhalation study.

Nonneoplastic lesions from the Kasai et al. (2009) study that were statistically increased as compared to control were considered candidates for the critical effect. The candidate endpoints included centrilobular necrosis of the liver, squamous cell metaplasia of nasal respiratory epithelium, squamous cell hyperplasia of nasal respiratory epithelium, respiratory metaplasia of nasal olfactory epithelium, sclerosis in lamina propria of nasal cavity, and two degenerative nasal lesions, that is, atrophy of nasal olfactory epithelium and hydropic change in the lamina propria. Despite statistical increases at the low- and mid exposure concentrations (50 and 250 ppm, respectively), incidences of nuclear enlargement of respiratory epithelium (nasal cavity), olfactory epithelium (nasal cavity), and proximal tubule (kidney) were not considered candidates for the critical effect given that the toxicological significance of nuclear enlargement is uncertain.

Methods of Analysis. Benchmark dose (BMD) modeling methodology (U.S. EPA, 2012) was used to analyze the candidate endpoints identified for 1,4-dioxane. BMDs and BMDLs were able to be determined for centrilobular necrosis, squamous cell metaplasia and hyperplasia of the respiratory epithelium, and hydropic change of lamina propria. Due to poor fit or substantial model uncertainty, BMD model results were inadequate for the following nasal lesions: atrophy (olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). Consequently, the NOAEL/LOAEL approach was used to determine potential PODs for these endpoints.

Other endpoints in Kasai et al. (2009) were considered as alternative POD values in the derivation of the RfC, including incidence data reported for centrilobular necrosis in the liver and other respiratory effects. Alternative PODs are shown in Table 5-4 and Figure 5-5 of the *Toxicological Review for 1,4-Dioxane* (2013).

__I.B.3. UNCERTAINTY FACTORS

UF = 1,000

$= 3\ (UF_A) \times 10\ (UF_H) \times 1\ (UF_S) \times 10\ (UF_L) \times 3\ (UF_D)$

An interspecies UF of 3 (UF_A) was used for animal-to-human extrapolation to account for pharmacodynamic differences between species. This uncertainty factor is comprised of two separate areas of uncertainty to account for differences in the toxicokinetics and toxicodynamics of animals and humans. In this assessment, the toxicokinetic uncertainty was accounted for by the calculation of a HEC and application of a dosimetric adjustment factor as outlined in the RfC methodology (U.S. EPA, 1994). As the toxicokinetic differences are thus accounted for, only the toxicodynamic uncertainties remain, and an UF_A of 3 is retained to account for this uncertainty.

A default interindividual variability UF of 10 (UF_H) was used to account for variation in sensitivity within human populations because there is limited information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of,

or response to, 1,4-dioxane.

An UF to extrapolate from a subchronic to a chronic (UF_S) exposure duration was not necessary (e.g., UFS = 1) because the RfC was derived from a study using a chronic exposure protocol.

An UF of 10 (UF_L) was used to extrapolate from a LOAEL to a NOAEL because a LOAEL was used as the POD. A NOAEL for atrophy and respiratory metaplasia of the olfactory epithelium was not identified in the study by Kasai et al. (2009).

An UF of 3 for database deficiencies (UF_D) was applied due to the lack of a multigeneration reproductive toxicity study. The oral toxicity database included a single prenatal developmental study that indicated the developing fetus may be a target of toxicity (Giavini et al., 1985).

__I.B.4. ADDITIONAL STUDIES/COMMENTS

Prior to the Kasai et al. (Kasai et al., 2009; Kasai et al., 2008) studies, two other studies were available for consideration in the derivation of inhalation toxicity values: one subchronic study (Fairley et al., 1934) and one chronic inhalation study (Torkelson et al., 1974). In the subchronic study, rabbits, guinea pigs, rats, and mice (3–6/species/group) were exposed to 1,000, 2,000, 5,000, or 10,000 ppm of 1,4-dioxane vapor for 1.5 hours two times a day for 5 days, 1.5 hours for one day, and no exposure on the seventh day. Animals were exposed until death occurred or were sacrificed after various durations of exposure (3-202.5 hours). Detailed dose-response information was not provided; however, severe liver and kidney damage and acute vascular congestion of the lungs were observed at concentrations ≥ 1,000 ppm. Kidney damage was described as patchy degeneration of cortical tubules with vascular congestion and hemorrhage. Liver lesions varied from cloudy hepatocyte swelling to large areas of necrosis.

Torkelson et al. (1974) performed a chronic inhalation study in which male and female Wistar rats (288/sex) were exposed to 111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week for 2 years. Control rats (192/sex) were exposed to filtered air. No significant effects were observed on BWs, survival, organ weights, hematology, clinical chemistry, or histopathology. Because Fairely et al. (1934) identified a free-standing LOAEL only, and Torkelson et al. (1974) identified a free-standing NOAEL only, neither study was sufficient to characterize the inhalation risks of 1,4-dioxane.

__I.B.5. CONFIDENCE IN THE INHALATION RfC

Study — Medium
Database — Medium
RfC —Medium

The overall confidence in the RfC is medium. Confidence in the principal study (Kasai et al., 2009) is medium. The 2-year inhalation study is a well-conducted, peer-reviewed study that used 3 dose groups plus a control. The study had adequate group sizes (50 rats/dose group) and investigated multiple target organs; however, the study did only used male rats and did not investigate chronic effects in females.

Confidence in the database is medium due to the lack of supporting studies and a multigeneration reproductive toxicity study.

Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

__I.B.6. EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC

Source Document - U.S. EPA (2013)

This document has been reviewed by EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). ***To review this appendix, exit to the toxicological review, Appendix A, Summary Of External Peer Review And Public Comments And Disposition (PDF)***.

Agency Completion Date -- 00/00/2013

__I.B.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name - 1,4-Dioxane
CASRN – 123-91-1
Section II. Last Revised -- 00/00/0000

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The 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 a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is a plausible upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated concentration of the chemical substance in drinking water when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

A cancer assessment for 1,4-dioxane via the oral route of exposure was posted on the IRIS databse in 2010. At that time, 1,4-dioxane was classified as a likely human carcinogen, based on the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). This update adds an inhalation unit risk (IUR) to that assessment, and the weight-of-evidence cancer classification remains the same.

A previous cancer assessment for 1,4-dioxane was posted on the IRIS database in 1988. At that time, 1,4-dioxane was classified as a B2 carcinogen (probable human carcinogen), based on inadequate human data and sufficient evidence of carcinogenicity in animals (induction of nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea pigs). An oral cancer slope factor (CSF) of 1.1×10^{-2} $(mg/kg\text{-}day)^{-1}$ was derived from the tumor incidence data for nasal squamous cell carcinoma in male rats exposed to 1,4-dioxane in drinking water for 2 years (NCI, 1978). The linearized multistage extra risk procedure was used for linear low dose extrapolation. An inhalation unit risk (IUR) was not previously derived.

__II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

___II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,4-dioxane is characterized as "likely to be carcinogenic to humans." This characterization is based on the following findings: (1) inadequate evidence of carcinogenicity in humans, and (2) sufficient evidence in animals (i.e., hepatic tumors in multiple species [three strains of rats, two strains of mouse, and in guinea pigs]; mesotheliomas of the peritoneum, mammary, and nasal tumors have also been observed in rats following 2 years of oral exposure to 1,4- dioxane).

There is adequate evidence of liver carcinogenicity in several 2-year bioassays conducted in three strains of rats, two strains of mice, and in guinea pigs (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Argus et al., 1973; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Argus et al., 1965). Additionally, tumors of the peritoneum (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994), mammary (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994), and nasal cavitiy (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Argus et al., 1973; Hoch-Ligeti et al., 1970), as well as kidney, Zymbal gland, and subcutaneous tissue (Kasai et al., 2009) have been observed in rats due to exposure to 1,4-dioxane. Studies in humans are inconclusive regarding evidence for a causal link between occupational exposure to 1,4-dioxane and increased risk for cancer; however, only two studies were available and these were limited by small cohort size and a small number of reported cancer cases (Buffler et al., 1978; Thiess et al., 1976).

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing information (e.g., toxicokinetic data) that absorption does not occur by other routes. Information available on the carcinogenic effects of 1,4-dioxane via the oral route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of 1,4-dioxane via the dermal route in humans and animals is absent. Based on the observance of systemic tumors following

oral and inhalation exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, 1,4-dioxane is "likely to be carcinogenic to humans" by all routes of exposure.

A MOA hypothesis involving sustained proliferation of spontaneously transformed liver cells has some support from data indicating that 1,4-dioxane acts as a tumor promoter in mouse skin and rat liver bioassays (Lundberg et al., 1987; King et al., 1973). Dose-response and temporal data support the occurrence of cell proliferation and hyperplasia prior to the development of liver tumors (JBRC, 1998b; Kociba et al., 1974) in the rat model. However, the dose-response relationship for induction of hepatic cell proliferation has not been characterized, and it is unknown if it would reflect the dose-response relationship for liver tumors in the 2-year rat and mouse studies. Conflicting data from rat and mouse bioassays (JBRC, 1998b; Kociba et al., 1974) suggest that cytotoxicity may not be a required precursor event for 1,4-dioxane-induced cell proliferation. Liver tumors were observed in female rats and female mice in the absence of lesions indicative of cytotoxicity (Kano et al., 2009; JBRC, 1998b; NCI, 1978). Thus, data regarding a plausible dose response and temporal progression from cytotoxicity and cell proliferation to eventual liver tumor formation are not available. The MOA by which 1,4-dioxane produces liver, nasal, peritoneal (mesotheliomas), and mammary gland tumors is unknown, and the available data do not support any hypothesized carcinogenic MOA for 1,4-dioxane.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF).

___II.A.2. HUMAN CARCINOGENICITY DATA

Human studies of occupational exposure to 1,4-dioxane were inconclusive to assess the evidence of carcinogenicity of 1,4-dioxane (see Section 4.1 in the *Toxicological Review of 1,4-Dioxane,* (U.S. EPA, 2010). In each case, the cohort size and number of reported cases were of limited size (Buffler et al., 1978; Thiess et al., 1976).

___II.A.3. ANIMAL CARCINOGENICITY DATA

Three chronic drinking water bioassays provided incidence data for liver tumors in rats and mice, and nasal cavity, peritoneal, and mammary gland tumors in rats only (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974). With the exception of the NCI (1978) study, the incidence of nasal cavity tumors was generally lower than the incidence of liver tumors in exposed rats. The Kano et al. (2009) drinking water study was chosen as the principal study for derivation of an oral cancer slope factor (CSF) for 1,4-dioxane. This study used three dose groups in addition to controls and characterized the dose-response relationship at lower exposure levels, as compared to the high doses employed in the NCI (1978) bioassay. The Kociba et al. (1974) study also used three low dose exposure groups; however, the study authors only reported the incidence of hepatocellular carcinoma, which may underestimate the combined incidence of rats with adenoma or carcinoma. In addition to increased incidence of liver tumors, chosen as the most sensitive target organ for tumor formation, the Kano et al.

(2009) study also noted increased incidence of peritoneal and mammary gland tumors. Nasal cavity tumors were also seen in high-dose male and female rats; however, the incidence of nasal tumors was much lower than the incidence of liver tumors in both rats and mice.

As described in detail in Section 4.2.1.2.6 and Appendix E of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010), the Japanese Bioassay Research Center conducted a 2-year drinking water study on the effects of 1,4-dioxane in both sexes of rats and mice. The results from that study were reported several times, once as conference proceedings (Yamazaki et al., 1994), once as a detailed laboratory report (JBRC, 1998b), and once as a published manuscript (Kano et al., 2009). As a result of the most recent publication (Kano et al., 2009), the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010) was updated and the data in the new publication was considered. Although the data contained in the reports varied, the differences were minor and did not affect the conclusions of this assessment. The variations included: (1) the level of detail on dose information reported; (2) categories for incidence data reported (e.g., all animals or sacrificed animals); and (3) analysis of non- and neoplastic lesions.

A chronic bioassay of 1,4-dioxane by the inhalation route reported by Kasai et al. (2009) provides data adequate for dose-response modeling and was subsequently chosen as the study for the derivation of an IUR for 1,4-dioxane. In this bioassay, groups of 50 male F344 rats were exposed to either 0, 50, 250 or 1,250 ppm 1,4-dioxane, 6 hours/day, 5 days/week, for 2 years (104-weeks). In male F344 rats, 1,4-dioxane produced a statistically significant increase in incidence and/or a statistically significant dose-response trend for the following tumor types: hepatomas, nasal squamous cell carcinomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, Zymbal gland adenomas, and subcutis fibromas (Kasai et al., 2009).

___II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea pigs (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Torkelson et al., 1974; Argus et al., 1973; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Argus et al., 1965). Liver tumors have been observed following drinking water exposure in male Wistar rats (Argus et al., 1965), male guinea pigs (Hoch-Ligeti and Argus, 1970), male Sprague Dawley rats (Argus et al., 1973; Hoch-Ligeti et al., 1970), male and female Sherman rats (Kociba et al., 1974), female Osborne-Mendel rats (NCI, 1978), male and female F344/DuCrj rats (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994), male and female B6C3F$_1$ mice (NCI, 1978), and male and female Crj:BDF$_1$ mice (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994). In the earliest cancer bioassays, the liver tumors were described as hepatomas (Argus et al., 1973; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Argus et al., 1965); however, later studies made a distinction between hepatocellular carcinoma and hepatocellular adenoma (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978). Both tumor types have been seen in rats and mice exposed to 1,4-dioxane. Kociba et al. (1974) noted evidence of liver toxicity at or below the dose levels that produced liver tumors but did not report incidence data for these effects. Hepatocellular degeneration and necrosis were observed in the mid- and high-dose groups of male and female Sherman rats exposed to 1,4-dioxane, while tumors were only observed at the highest dose. Hepatic regeneration was indicated in the mid- and high-dose groups by the formation of hepatocellular hyperplastic nodules. Findings from JBRC (1998b) also provided

evidence of liver hyperplasia in male F344/DuCrj rats at a dose level below the dose that induced a statistically significant increase in tumor formation.

Nasal cavity tumors were also observed in Sprague Dawley rats (Argus et al., 1973; Hoch-Ligeti et al., 1970), Osborne-Mendel rats (NCI, 1978), Sherman rats (Kociba et al., 1974), and F344/DuCrj rats (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994). Most tumors were characterized as squamous cell carcinomas. Nasal tumors were not elevated in $B6C3F_1$ or Crj:BDF_1 mice. JBRC (1998b) was the only study that evaluated nonneoplastic changes in nasal cavity tissue following prolonged exposure to 1,4-dioxane in the drinking water. Histopathological lesions in female F344/DuCrj rats were suggestive of toxicity and regeneration in this tissue (i.e., atrophy, adhesion, inflammation, nuclear enlargement, and hyperplasia and metaplasia of respiratory and olfactory epithelium). Some of these effects occurred at a lower dose (83 mg/kg-day) than that shown to produce nasal cavity tumors (429 mg/kg-day) in female rats. Reexamination of tissue sections from the NCI (1978) bioassay suggested that the majority of nasal tumors were located in the dorsal nasal septum or the nasoturbinate of the anterior portion of the dorsal meatus. Nasal tumors were not observed in an inhalation study in Wistar rats exposed to 111 ppm for 5 days/week for 2 years (Torkelson et al., 1974).

Tumor initiation and promotion studies in mouse skin and rat liver suggested that 1,4-dioxane does not initiate the carcinogenic process, but instead acts as a tumor promoter (Lundberg et al., 1987; Bull et al., 1986; King et al., 1973) (see Section 4.2.3 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010)).

In addition to the liver and nasal tumors observed in several studies, a statistically significant increase in mesotheliomas of the peritoneum was seen in male rats from the Kano et al. (2009) (also (JBRC, 1998b; Yamazaki et al., 1994)) and Kasai et al. (2009). Female rats dosed with 429 mg/kg-day in drinking water for 2 years also showed a statistically significant increase in mammary gland adenomas (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994) A significant increase in the incidence of these tumors was not observed in other chronic oral bioassays of 1,4-dioxane (NCI, 1978; Kociba et al., 1974). Additional statistically significant increases in other tumor types were observed in male F344 rats exposed to 0, 50, 250 or 1,250 ppm 1,4-dioxane, 6 hours/day, 5 days/week, for 2 years (104-weeks) including renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, Zymbal gland adenomas, and subcutis fibromas (Kasai et al., 2009).

__II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

___II.B.1. SUMMARY OF RISK ESTIMATES

____II.B.1.1. Oral Slope Factor: 1×10^{-1} per mg/kg-day

The derivation of the oral slope factor 1×10^{-1} per mg/kg-day is based on the incidence of hepatocellular adenomas and carcinomas in female mice exposed to 1,4-dioxane in drinking water for 2 years (Kano et al., 2009). The dose metric used in the current estimate of the human equivalent dose (HED) is the applied or external dose because a PBPK model was determined not to be suitable for species extrapolation (see Appendix B of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010). The rat $BMDL_{50}$ of 32.93 mg/kg-day represents the POD used to calculate the $BMDL_{50HED}$ of 4.95 mg/kg-day.

The oral slope factor is derived from the $BMDL_{50HED}$, the 95% lower bound on the exposure associated with a 50% extra cancer risk, by dividing the risk (as a fraction) by the $BMDL_{50HED}$, and represents an upper bound, continuous lifetime exposure risk estimate:

$BMDL_{50HED}$, lower 95% bound on exposure at 50% extra risk – 4.95 mg/kg-day
BMD_{50HED}, central estimate of exposure at 50% extra risk – 7.51 mg/kg-day

The slope of the linear extrapolation from the central estimate is 0.5/ (7.51 mg/kg-day) = 7×10^{-2} per mg/kg-day

The slope factor for 1,4-dioxane should not be used with exposures exceeding the point of departure ($BMDL_{50HED}$ = 4.95 mg/kg-day), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 1,4-dioxane.

____II.B.1.2. Drinking Water Unit Risk*: 2.9×10^{-6} per µg/L

Drinking Water Concentrations at Specified Risk Levels

Risk Level	Lower Bound on Concentration Estimate*
E-4 (1 in 10,000)	35 µg/L
E-5 (1 in 100,000)	3.5 µg/L
E-6 (1 in 1,000,000)	0.35 µg/L

* The unit risk and concentration estimates assume water consumption of 2 L/day by a 70 kg human.

____II.B.1.3. Extrapolation Method

Log-logistic model with linear extrapolation from the POD ($BMDL_{50HED}$) associated with 50% extra cancer risk.

The log-logistic model provided the best-fit to the female mouse liver tumor data Kano et al. (2009) female data as indicated by the AIC and *p*-value as was chosen as the best-fitting model to carry forward in the analysis; however, this model resulted in a $BMDL_{10}$ much lower than the response level at the lowest dose in the study (Kano et al., 2009). Thus, the log-logistic model was also run for BMR values of 30 and 50%. Using a higher BMR value resulted in BMDL values closer to the lowest observed response data, and a BMR of 50% was chosen to carry forward in the analysis.

___II.B.2. DOSE-RESPONSE DATA

Tumor Type – hepatocellular adenoma and carcinoma
Test Species – female BDF1 mouse
Route – Oral, drinking water
References – Kano et al. (2009)

Incidence of liver tumors in female BDF1 female mice exposed to 1,4-dioxane in drinking water for 2 years

	Dose (mg/kg-day)			
Tumor	**0**	**66**	**278**	**964**
Hepatocellular adenoma or carcinoma	5/50	35/50[a]	41/50[a]	46/50[a,b]

[a]Significantly different from control by Fisher's exact test ($p < 0.01$).
[b]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

Source : Kano et al. (2009)

Oral Cancer Slope Factor (CSF) using linear low-dose extrapolation approach and interspecies extrapolation

Tumor	**Dose groups modeled**	**BMD_{50} mg/kg-day**	**$BMDL_{50}$ mg/kg-day**	**BMD_{HED} mg/kg-day**	**$BMDL_{HED}$ mg/kg-day**	**Oral SF $(mg/kg\text{-}day)^{-1}$**
Female mouse hepatocellular adenoma or carcinoma	0, 66, 278, 964 mg/kg-day	49.88	32.93	7.51	4.95	0.10

___II.B.3. ADDITIONAL COMMENTS

Supplementary information not required.

___II.B.4. DISCUSSION OF CONFIDENCE

Relevance to humans. The oral CSF was derived using the tumor incidence in the liver of female mice. A thorough review of the available toxicological data available for 1,4-dioxane provides no scientific justification to propose that the liver adenomas and carcinomas observed in animal models following exposure to 1,4-dioxane are not plausible in humans. Liver adenomas and carcinomas were considered plausible outcomes in humans due to exposure to 1,4-dioxane.

Choice of low-dose extrapolation approach. The range of possibilities for the low-dose extrapolation of tumor risk from exposure to 1,4-dioxane, or any chemical, ranges from linear to nonlinear, but is dependent upon a plausible MOA(s) for the observed tumors. The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. Exposure to 1,4-dioxane has been observed in animal models to induce multiple tumor types, including liver adenomas and carcinomas, nasal carcinomas, mammary adenomas and fibroadenomas, and mesotheliomas of the peritoneal cavity (Kano et al., 2009). MOA information that is available for the carcinogenicity of 1,4-dioxane has largely focused on liver adenomas and carcinomas, with little or no MOA information available for the remaining tumor types. In Section 4.7.3 of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010), hypothesized MOAs, other than a mutagenic MOA, were explored due to the lack of mutagenicity observed in genetic toxicology tests performed for 1,4-dioxane. Data were not available to support a carcinogenic MOA for 1,4-dioxane. In the absence of a MOA(s) for the observed tumor types associated with exposure to 1,4-dioxane, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,4-dioxane exposure.

In the studies evaluated (Kano et al., 2009; NCI, 1978; Kociba et al., 1974), the multistage model provided good descriptions of the incidence of a few tumor types in male (nasal cavity) and female (hepatocellular and nasal cavity) rats and in male mice (hepatocellular) exposed to 1,4-dioxane via ingestion (see Appendix D of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010) for additional details). However, the multistage model did not provide an adequate fit for female mouse liver tumor dataset based upon the following (U.S. EPA, 2012):

- Goodness-of-fit *p*-value was less than 0.10 indicating statistically significant lack of fit;
- AIC was larger than other acceptable models;
- Observed data deviated substantially from the fitted model, as measured by their standardized χ^2 residuals (i.e., residuals with values greater than an absolute value of one).

By default, the BMDS software imposes constraints on the values of certain parameters of the models. When these constraints were imposed, the multistage model and most other models did not fit the incidence data for female mouse liver adenomas or carcinomas, even after dropping the highest dose group.

The log-logistic model was selected because it was the only model that provided an adequate fit to the female mouse liver tumor data (Kano et al., 2009). A BMR of 50% was used because it is proximate to the response at the lowest dose tested and the $BMDL_{50}$ was estimated by applying appropriate parameter constraints to the selected model, consistent with the BMD

Technical Guidance Document (U.S. EPA, 2012).

The human equivalent oral CSF estimated from liver tumor datasets with statistically significant increases ranged from 4.2×10^{-4} to 1.0×10^{-1} per mg/kg-day, a range of about three orders of magnitude, with the upper and lower extremes coming from the combined male and female data for hepatocellular carcinomas (Kociba et al., 1974) and the female mouse liver adenoma and carcinoma dataset (Kano et al., 2009).

Dose metric. 1,4-Dioxane is known to be metabolized in vivo. However, it is unknown whether a metabolite or the parent compound, or some combination of parent compound and metabolites, is responsible for the observed carcinogenicity. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric is the least biased choice. On the other hand, if this is not the correct dose metric, then the impact on the CSF and IUR is unknown.

Interspecies extrapolation. An adjustment for cross-species scaling ($BW^{0.75}$) was applied to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the U.S. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). It is assumed that equal risks result from equivalent constant lifetime exposures.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-logistic model applied to the female mouse data, there is a reasonably small degree of uncertainty at the 50% excess incidence level (the POD for linear low-dose extrapolation), as indicated by the proximity of the $BMDL_{HED}$ (4.95 mg/kg-day) to the BMD_{HED} (7.51 mg/kg-day).

Bioassay selection. The study by Kano et al. (2009) was used for development of an oral CSF. This was a well-designed study, conducted in both sexes in two species with a sufficient number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was adequate, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Alternative bioassays (Kociba et al., 1974) are available and were fully considered for the derivation of the oral CSF.

Choice of species/gender. The oral CSF for 1,4-dioxane was derived using the tumor incidence data for the female mouse, which was thought to be more sensitive than male mice or either sex of rats to the carcinogenicity of 1,4-dioxane. While all data, from both species and sexes reported from the Kano et al. (2009) study, were suitable for deriving an oral CSF, the female mouse data represented the most sensitive indicator of carcinogenicity in the rodent model. The lowest exposure level (66 mg/kg-day [animal dose] or 10 mg/kg-day [HED]) observed a considerable and significant increase in combined liver adenomas and carcinomas. Additional testing of doses within the range of control and the lowest dose (66 mg/kg-day [animal dose] or 10 mg/kg-day [HED]) could refine and reduce uncertainty for the oral CSF.

Human population variability. The extent of inter-individual variability in 1,4-dioxane metabolism has not been characterized. A separate issue is that the human variability in response to 1,4-dioxane is also unknown. Data exploring whether there is differential sensitivity to 1,4-dioxane carcinogenicity across life stages is unavailable. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. Also, the lack of information linking a MOA for 1,4-dioxane to the observed carcinogenicity is a source of uncertainty.

____II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

___II.C.1. SUMMARY OF RISK ESTIMATES

____II.C.1.1. Inhalation Unit Risk: 5×10^{-6} $(\mu g/m^3)^{-1}$

The derivation of the inhalation unit risk 5×10^{-6} per $\mu g/m^3$ is based on combined tumor incidence in male rats exposed to 1,4-dioxane in via inhalation for 2 years (Kasai et al., 2009). The dose metric used in the current estimate of the human equivalent concentration (HEC) is the applied or inhaled concentration because a PBPK model was determined not to be suitable for species extrapolation (see Appendix B of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). The rat multitumor $BMCL_{10}$ of 30.3 ppm represents the POD used to calculate the $BMCL_{HEC}$ of 19.5 mg/m^3.

The inhalation unit risk is derived from the $BMCL_{HEC}$, the 95% lower bound on the exposure associated with a 10% extra cancer risk, by dividing the risk (as a fraction) by the $BMCL_{HEC}$, and represents an upper bound, continuous lifetime exposure risk estimate:

$BMCL_{10HEC}$, lower 95% bound on exposure at 10% extra risk – 19.5 mg/m^3
BMC_{10HEC}, central estimate of exposure at 10% extra risk – 26.0 mg/m^3

The slope of the linear extrapolation from the central estimate is 0.1/ (26.0 mg/m^3) = 4×10^{-6} per $\mu g/m^3$

The inhalation unit risk for 1,4-dioxane should not be used with exposures exceeding the point of departure ($BMCL_{10HEC}$ = 19.5 mg/m^3), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 1,4-dioxane.

____II.C.1.2. Extrapolation Method

Multi-tumor dose-response model with linear extrapolation from the POD ($BMCL_{10HEC}$) associated with 10% extra cancer risk. Statistically significant dose-response trends for the increase in tumors with increasing dose was observed for the nasal cavity squamous cell carcinomas, hepatomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, and Zymbal gland adenomas. All of these tumors were considered to be of independent origin and included in the multi-tumor analysis.

___II.C.2. DOSE-RESPONSE DATA

Tumor Type – multiple (nasal, liver, kidney, peritoneal, mammary gland, and Zymbal gland)
Test Species – male F344 rats
Route – Inhalation
References – Kasai et al. (2009)

Incidence of tumors in F344 male rats exposed to 1,4-dioxane for 104 weeks (6 hours/day, 5 days/week)

Tumor Type	Animal Exposure (ppm)			
	0	**50**	**250**	**1,250**
Nasal cavity squamous cell carcinoma	0/50	0/50	1/50	6/50[a,b]
Hepatocellular adenoma or carcinoma[e]	1/50	2/50	4/50	22/50[a,c]
Renal cell carcinoma	0/50	0/50	0/50	4/50[a]
Peritoneal mesothelioma	2/50	4/50	14/50[c]	41/50[a,c]
Mammary gland fibroadenoma	1/50	2/50	3/50	5/50[d]
Mammary gland adenoma	0/50	0/50	0/50	1/50
Zymbal gland adenoma	0/50	0/50	0/50	4/50[a]
Subcutis fibroma	1/50	4/50	9/50[c]	5/50

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[b]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.05$).
[c]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.01$).
[d]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.05$).
[e]Provided via personal communication from Dr. Tatsuya Kasai to Dr. Reeder Sams on 12/23/2008 (2008). Statistics were not reported for these data by study authors, so statistical analyses were conducted by EPA.

Source: Kasai et al. (2009) and Kasai personal communication (2008)

Inhalation Unit Risk (IUR) using linear low-dose extrapolation approach and interspecies extrapolation

Tumor	**Dose groups modeled**	**BMC_{10} mg/m^3**	**$BMCL_{10}$ mg/m^3**	**BMC_{HEC} mg/m^3**	**$BMCL_{HEC}$ mg/m^3**	**Inhalation Unit Risk (µg/m^3)$^{-1}$**
Multiple – F344 male rats	0, 50, 250, 1,250 ppm (0, 180, 900, or 4,500 mg/m^3)	40.4	30.3	26.0	19.5	5.0×10^{-6}

___II.C.3. ADDITIONAL COMMENTS

Supplementary information not required.

___II.C.4. DISCUSSION OF CONFIDENCE

Relevance to humans. The derivation of the inhalation unit risk is based on the tumor

incidence at multiple sites in male rats. There is no information on 1,4-dioxane to indicate that the observed rodent tumors are irrelevant to humans. Further, no data exist to guide quantitative adjustment for differences in sensitivity among rodents and humans. In the absence of information to indicate otherwise and considering similar cell types are prevalent throughout the respiratory tract of rats and humans, the nasal, liver, renal, peritoneal, mammary gland, Zymbal gland and subcutis tumors were considered relevant to humans.

Choice of low-dose extrapolation approach. The range of possibilities for the low-dose extrapolation of tumor risk from exposure to 1,4-dioxane, or any chemical, ranges from linear to nonlinear, but is dependent upon a plausible MOA(s) for the observed tumors. The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. Exposure to 1,4-dioxane has been observed in animal models to induce multiple tumor types, including liver adenomas and carcinomas, nasal carcinomas, mammary adenomas and fibroadenomas, and mesotheliomas of the peritoneal cavity (Kano et al., 2009). MOA information that is available for the carcinogenicity of 1,4-dioxane has largely focused on liver adenomas and carcinomas, with little or no MOA information available for the remaining tumor types. In Section 4.7.3 of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010), hypothesized MOAs, other than a mutagenic MOA, were explored due to the lack of mutagenicity observed in genetic toxicology tests performed for 1,4-dioxane. Data were not available to support any of the hypothesized carcinogenic MOAs for 1,4-dioxane. In the absence of sufficient information to support a MOA(s) for the observed tumor types associated with exposure to 1,4-dioxane, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,4-dioxane exposure.

The BMDS multistage cancer model provided adequate fits for the tumor incidence data following a 2-year inhalation exposure to 1,4-dioxane by male rats (Kasai et al., 2009), thus the BMDS MS_Combo multi-tumor model was used to determine a $BMCL_{10}$.

Interspecies extrapolation. Differences in the anatomy of the upper respiratory tract and resulting differences in absorption or in local respiratory system effects are sources of uncertainty in the inhalation cancer assessment. However, since similar cell types are prevalent throughout the respiratory tract of both rats and humans, the tumors are considered biologically plausible and relevant to humans.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the multistage, multi-tumor model applied for the male rat inhalation dataset, there is a reasonably small degree of uncertainty at the 10% extra risk level (the POD for linear low-dose extrapolation).

Bioassay selection. The study by Kasai et al. (2009) was used for derivation of an inhalation unit risk. This was a well-designed study, conducted in male rats with a sufficient number (N=50) of animals per dose group. Three dose levels plus an untreated control group were examined following exposure to 1,4-dioxane via inhalation for 2 years.

Choice of species/gender. Male F344 rat data were used to estimate risk following inhalation of 1,4-dioxane. Kano et al. (2009) showed that male rats were more sensitive than female rats to the effects of 1,4-dioxane following oral administration; therefore, male rats were chosen to be studies in the 2-year bioassay conducted by the same laboratory (Kasai et al., 2009).

The sensitivity and tumorigenic response of female rats or male or female mice following inhalation of 1,4-dioxane is unknown. Since female mice were the most sensitive gender and species examined in the Kano et al. (2009) oral study, female mice may also be more sensitive to the inhalation of 1,4-dioxane which would result in a greater risk.

Human population variability. The extent of inter-individual variability in 1,4-dioxane metabolism has not been characterized. A separate issue is that the human variability in response to 1,4-dioxane is also unknown. Data exploring whether there is differential sensitivity to 1,4-dioxane carcinogenicity across life stages is unavailable. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. Also, the lack of information linking a MOA for 1,4-dioxane to the observed carcinogenicity is a source of uncertainty.

__II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

___II.D.1. EPA DOCUMENTATION

Source Document – (U.S. EPA, 2013)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010). **To review this appendix, exit to the Toxicological Review, Appendix A, Summary of External Peer Review and Public Comments and Disposition (PDF).**

___II.D.2. EPA REVIEW

Agency Completion Date Oral -- 08/11/2010
Agency Completion Date Inhalation – 00/00/0000

___II.D.3. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_III. [reserved]
_IV. [reserved]
_V. [reserved]

_VI. Bibliography

Substance Name – 1,4-Dioxane
CASRN – 123-91-1
Section VI. Last Revised – 00/00/0000

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VII. Revision History

1,4-Dioxane
CASRN – 123-91-1
File First On-Line 08/22/1988

Date	Section	Description
08/22/1988	II.	Carcinogen summary on-line
08/11/2010	I., II., VI.	RfD and cancer assessment updated; RfC discussion added.
08/12/2010	NA	Archived review drafts and comments from the development of this assessment are available.
00/00/0000		Added RfC and IUR assessment.

_VIII. Synonyms

Substance Name – 1,4-Dioxane
CASRN – 123-91-1
Section VIII. Last Revised – 00/00/0000

- 123-91-1
- diethylene dioxide
- diethylene oxide
- dioxane, 1,4-
- p-dioxane
- dioxane
- dioxyethylene ether
- diethylene ether
- 1,4-diethylene dioxide