APPENDIX A

HEALTH-BASED MAXIMUM CONTAMINANT LEVEL SUPPORT DOCUMENT 1,4-DIOXANE

(CAS # 123-91-1; Chemical Formula: $C_4H_8O_2$)

New Jersey Drinking Water Quality Institute
Health Effects Subcommittee
September 24, 2021

Subcommittee Members:
Jessie A. Gleason, M.S.P.H., Chair
Keith R. Cooper, Ph.D.
Judith B. Klotz, M.S., Dr.P.H.
Gloria B. Post, Ph.D., D.A.B.T.

Page intentionally left blank

Acknowledgements

This work would not have been possible without the ongoing support of the librarians of the NJDEP Environmental Research Library - Tonia Wu and Linda DiCicco.

Page intentionally left blank

TABLE OF CONTENTS

ABSTRACT……..……..8

EXECUTIVE SUMMARY……..…….……..9

INTRODUCTION……..……..…..……..11

BACKGROUND INFORMATION……..……..…….....13

GUIDANCE AND STANDARDS DEVELOPED BY USEPA & OTHER STATES……....14

ENVIRONMENTAL FATE, TRANSPORT, AND OCCURRENCE……..……19

HUMAN BIOMONITORING……...21

SOURCES OF HUMAN EXPOSURE……..21

TOXICOKINETICS……..22

HEALTH EFFECTS – HUMAN STUDIES…….24

HEALTH EFFECTS – ANIMAL STUDIES……25

MODE OF ACTION…….…39

REFERENCE DOSE FOR NON-CANCER EFFECTS AND CANCER SLOPE FACTOR…..44

DEVELOPMENT OF RECOMMENDED HEALTH-BASED MCL…….…….51

RECOMMENDATION…….53

REFERENCES……..54

APPENDIX 1: REVIEW OF DOURSON ET AL. (2014) AND DOURSON ET AL. (2017)…66

TABLE OF TABLES

Table 1. Levels and basis for other states' standards and guidance values for 1,4-dioxane in drinking water and ground water……..……..…..18

Table 2. New Jersey v. National Public Water System (PWS) 1,4-Dioxane Detections in UCMR3 (2013-2015) …….……....21

Table 3. Sites at which tumor incidence was increased by 1,4-dioxane in animal studies (adapted from USEPA, 2013) ……..……..34

Table 4. Incidence of liver, nasal cavity, peritoneal, and mammary gland tumors in rats and mice exposed to 1,4-dioxame in drinking water for 2 years (based on survival to 12 months) (USEPA, 2013) ……..…….....49

Table 5. Oral cancer slope factors for best-fit models for tumor incidence data for rats and mice exposed to 1,4-dioxane in drinking water for 2 years (*adapted from USEPA, 2013*) ……..52

TABLE OF FIGURES

Figure 1. Suggested metabolic pathways of 1,4-dioxane in the rat. ……...…….24

ABBREVIATIONS

ALP – alkaline phosphatase
ALT – alanine aminotransferase
AST – aspartate aminotransferase
BMD – Benchmark Dose
BMR – Benchmark Response
BW – Body weight
CDC – Centers for Disease Control and Prevention
CSF – Cancer Slope Factor
DWQI –Drinking Water Quality Institute
HED – Human Equivalent Dose
ISGWQC – Interim specific ground water quality criterion
ISGWQS – Interim Specific Ground Water Quality Standard
IRIS – Integrated Risk Information System
GWQC – Ground Water Quality Criterion
GWQS – Ground Water Quality Standard
LDH – lactate dehydrogenase
LOD – Level of detection
MAC – maximum acceptable concentration
MCLs – Maximum Contaminant Levels
MCLGs – Maximum Contaminant Level Goals
MRL – Minimum Reporting Level
NHANES – National Health and Nutrition Examination Survey
POD – Point of Departure
PQL - Practical Quantitation Level
RfD – Reference dose
SDWA – Safe Drinking Water Act
UCMR3 – Unregulated Contaminant Monitoring Rule 3
USEPA – United States Environmental Protection Agency

ABSTRACT

This document presents the Health Effects Subcommittee's recommendation for a Health-based MCL for 1,4-dioxane. The Subcommittee's review focused primarily on the carcinogenic effects and mode of action, including the evaluation presented in USEPA IRIS (2013) and additional recent relevant information.

The Health Effects Subcommittee agreed with the USEPA Integrated Risk Information System (IRIS) (2013) conclusion that 1,4-dioxane is "likely to be carcinogenic to humans" under the USEPA (2005) Guidelines for Carcinogen Risk Assessment. The Subcommittee also agreed with USEPA IRIS (2013) that the mode of action for cancer by which 1,4-dioxane causes tumors has not been established. As specified by the USEPA (2005) guidelines, when the mode of carcinogenic action is not understood, cancer risk assessment is based on low-dose linear extrapolation (i.e. a non-threshold approach based on a cancer slope factor). These conclusions were confirmed by a recent USEPA Office of Chemical Safety and Pollution Prevention (USEPA OCSPP, 2020) evaluation.

The USEPA IRIS (2013) cancer slope factor of 0.10 $(mg/kg/day)^{-1}$ was used as the basis for the Health-based MCL. This slope factor is based on the incidence of liver tumors in female mice (Kano et al., 2009), since these tumors were the most sensitive of the tumor types caused by 1,4-dioxane in several chronic studies of male and female mice and rats.

Based on the cancer slope factor of 0.10 $(mg/kg/day)^{-1}$, the one in one million (10^{-6}) cancer risk level specified in the NJ Safe Drinking Water Act, and the current USEPA default assumptions for adult body weight of 80 kg and drinking water ingestion of 2.4 L/day, a Health-based Maximum Contaminant Level (MCL) of 0.33 µg/L was recommended.

EXECUTIVE SUMMARY

This document presents the Health Effects Subcommittee's recommendation for a Health-based MCL for 1,4-dioxane. To the Subcommittee's knowledge, all current U.S. federal and state ground water and drinking water guidelines for 1,4-dioxane are based on the USEPA IRIS (2013) cancer slope factor of 0.10 $(mg/kg/day)^{-1}$. These include the USEPA (2017) Office of Water drinking water Reference Concentration, the NJDEP (2018) Ground Water Quality Standard, and the ground water and drinking guidelines developed by 13 other states.

For this reason, the Subcommittee's review focused primarily on the carcinogenicity studies and related mode of action information for 1,4-dioxane. Other non-carcinogenic effects of this chemical were also reviewed. The Subcommittee's review used the USEPA IRIS (2013) evaluation as its starting point, and it also included more recent information identified through literature searches and submissions to the DWQI.

The Health Effects Subcommittee agreed with USEPA IRIS (2013) that, based on the occurrence of several types of tumors in multiple rodent studies, 1,4-dioxane is "likely to be carcinogenic to humans" under the USEPA (2005) Guidelines for Carcinogen Risk Assessment. A recent assessment by USEPA OCSPP (2020) that considers information that became available subsequent to USEPA IRIS (2013) confirms this conclusion. According to the USEPA (2005) guidelines, risk assessment for carcinogens is based on low-dose linear extrapolation (i.e. a non-threshold approach using a cancer slope factor) when tumors occur through a mutagenic mode of action or when the mode of carcinogenic action has not been established.

The Subcommittee reviewed information relevant to 1,4-dioxane's carcinogenic mode of action including studies cited by USEPA IRIS (2013), more recent peer reviewed publications, USEPA OCSPP (2020), and the NJDEP (2015, 2018) responses to comments on the NJDEP (2010) Interim Specific Ground Water Quality Standard and promulgated NJDEP (2018) Ground Water Quality Standard.

The NJDEP (2015; 2018) responses to comments includes detailed reviews of two papers (Dourson et al., 2014; Dourson et al., 2017) suggesting that 1,4-dioxane causes liver tumors through a threshold mode of action involving cell toxicity followed by regenerative growth. NJDEP (2015, 2018) concluded that the information presented in these papers does not establish a threshold mode of action for 1,4-dioxane carcinogenicity. Furthermore, the modes of action for other types of tumors (nasal, mammary gland, peritoneal) caused by 1,4-dioxane are unknown. The Subcommittee also reviewed additional information submitted by two organizations in response to the DWQI (December 2018) request for public input that questioned a non-threshold approach for cancer risk assessment of 1,4-dioxane. The Subcommittee concluded that a mode of action for 1,4-dioxane's carcinogenicity was not established by the submitted information.

Based on its review, the Subcommittee agreed with USEPA IRIS (2013) and OCSPP (2020) that the mode of action for cancer by which 1,4-dioxane causes tumors has not been established. Therefore, the Subcommittee's cancer risk assessment for 1,4-dioxane was based on low-dose linear extrapolation (i.e. a non-threshold approach based on a cancer slope factor) as specified by the USEPA (2005) guidelines when the mode of carcinogenic action is not understood. The Health-based MCL is based on the USEPA IRIS (2013) slope factor of 0.10 $(mg/kg/day)^{-1}$. This slope factor is based on the incidence of liver tumors in female mice (Kano et al., 2009), since these tumors were the most sensitive of the tumor types caused by 1,4-dioxane in several chronic studies of male and female mice and rats. USEPA OCSPP (2020) developed a very similar, but slightly more stringent, slope factor also based on the liver tumors in female mice from Kano et al. (2009).

Based on the cancer slope factor of 0.10 $(mg/kg/day)^{-1}$, the one in one million (10^{-6}) cancer risk level specified in the NJ Safe Drinking Water Act, and the current USEPA default exposure assumptions of adult body weight of 80 kg and drinking water ingestion of 2.4 L/day, a Health-based MCL of 0.33 µg/L is recommended.

INTRODUCTION

Development of Health-based MCLs by New Jersey Drinking Water Quality Institute

The New Jersey Drinking Water Quality Institute (DWQI) was established by the 1984 amendments to the New Jersey Safe Drinking Water Act (SDWA) at N.J.S.A. 58:12A- 20. It is charged with developing standards (Maximum Contaminant Levels; MCLs) for hazardous contaminants in drinking water and for recommending those standards to the New Jersey Department of Environmental Protection (NJDEP). The Health Effects Subcommittee of the DWQI is responsible for developing health-based drinking water levels (Health-based MCLs) as part of the development of MCL recommendations (e.g., DWQI, 1987; 1994; 2009; 2015; 2017).

Health-based MCLs are based on the goals specified in the 1984 amendments to the NJ SDWA. For carcinogens, it is generally assumed that any level of exposure results in some level of cancer risk, and a one in one million (10^{-6}) risk level from lifetime exposure is specified in the statute. Health-based MCLs for carcinogens are thus set at levels that are not expected to result in cancer in more than one in one million persons ingesting the contaminant for a lifetime. For non-carcinogenic effects, it is generally assumed that exposure below a threshold level will not result in adverse effects as specified in the statue. Health-based MCLs for non-carcinogens are thus set at levels which are not expected to result in "any adverse physiological effects from ingestion" for a lifetime. The risk assessment approach used to develop Health-based MCLs is generally consistent with USEPA risk assessment guidance.

Other factors such as analytical quantitation limits and availability of treatment removal technology are also considered in the final MCL recommendation.

To support the development of an MCL recommendation by the DWQI, the Health Effects Subcommittee has developed a Health-based MCL for 1,4-dioxane. As specified in the 1984 Amendments to the NJ SDWA, this Health-based MCL is intended to be protective for chronic (lifetime) drinking water exposure.

Document Development Process

On December 19, 2018, the DWQI announced that NJDEP Commissioner Catherine McCabe requested the DWQI to recommend an MCL for 1,4-dioxane. The Health Effects Subcommittee commenced its evaluation January 2019.

The Subcommittee began its current evaluation by reviewing the basis of the USEPA IRIS (2013) 1,4-dioxane assessment. IRIS assessments represent the scientific consensus of USEPA and undergo external peer review, and IRIS is one of the sources of toxicity factors (cancer slope factors and reference doses [RfDs]) for NJ Ground Water Quality Criteria as specified in the NJ Ground Water Quality Standard (GWQS) regulations (N.J.A.C 7:9C). IRIS evaluations have

been used as the starting point for previous Health Effects Subcommittee evaluations (for example, vinyl chloride; DWQI, 2009).

Additional information evaluated by the Health Effects Subcommittee include studies identified through literature searches as well as information submitted in response to a DWQI request for public input. At the request of the Health Effects Subcommittee, the NJDEP Environmental Library conducted three literature searches of the PubMed databases relevant studies that were not cited in the USEPA IRIS (2013) 1,4-dioxane assessment. The literature searches were performed using relevant search terms including the chemical name, CASRN and common synonyms. As discussed below, the USEPA IRIS (2013) is an update of the USEPA IRIS (2010) assessment. In the USEPA (2013) update, additional information on inhalation risk assessment was added, but the information relevant to oral exposure was not revised from USEPA IRIS (2010) which includes literature through 2009. Therefore, literature searches were performed in January 2019 for relevant citations published in 2009 through 2012 (77 citations identified) and 2013 through January 2019 (160 citations identified). An additional literature search for citations published in 2019 through March 2020 (58 citations identified) was performed in March 2020. Selection of studies for inclusion in this document were based on title and abstract screening for relevance. It is noted that the large majority of citations identified in the searches were on topics that are not relevant to the information included in this document (e.g. analytical methodologies, remediation technologies).

On December 20, 2018, the DWQI posted a request for public input for 1,4 dioxane regarding data or technical information concerning toxicology, epidemiology, toxicokinetics, or other studies related to health effects for consideration in the development of the MCL. The DWQI received three submissions, and relevant health effects comments from two of these submissions were considered by the Health Effects Subcommittee. Recent publications and information submitted in response to the DWQI request for public input questioned the use of a non-threshold (e.g., slope factor) approach for cancer risk assessment of 1,4-dioxane, based on mode of action considerations.

The Subcommittee also identified ground water and drinking water standards and guidelines for 1,4-dioxane developed by NJDEP and 13 other states. All of these standards and guidelines rely on the USEPA IRIS (2013) cancer slope factor.

As such, the Subcommittee's review focused on the carcinogenic effects of 1,4-dioxane, using the USEPA IRIS (2013) assessment as a starting point. Additional information considered included reviews of 1,4-dioxane from the peer-reviewed literature and authoritative government sources, relevant information from literature searches and screening, as well as relevant information submitted in response to the call for public input.

A draft of the Health-based MCL Support Document, dated July 2020, was posted on October 21, 2020 for a 60 day public comment period that ended on December 21, 2020. The USEPA OCSPP (2020) risk evaluation of 1,4-dioxane became available in December 2020, near the end of the public comment period. Five commenters submitted comments on the Health-Based MCL Support Document. As was the case for the earlier request for public input, many of the comments questioned the use of a non-threshold approach for cancer risk assessment of 1,4-dioxane. A summary of the comments and the Health Effects Subcommittee's responses were presented at the public meeting of the DWQI on August 5, 2021.

The final Health-based MCL Support Document presented herein includes several revisions from the July 2020 draft. It discusses information that became available subsequent to development of the 2020 draft including several new publications (Lafranconi et al., 2020; Totsuka et al., 2020; Chappell et al., 2021; Charkoftaki et al., 2021) and the USEPA OCSPP (2020) risk evaluation. In response to a commenters' suggestion, discussion of several mutagenicity studies of 1,4-dioxane has been expanded, and several other minor clarifications were made. The overall conclusions and recommended Health-based MCL presented in the draft (July 2020) Health-based MCL Support Document are unchanged in the final (August 2021) document presented herein.

BACKGROUND INFORMATION

Physical and Chemical Properties (PubChem, 2019)

Chemical Name: 1,4-dioxane

Synonyms: diethylene ether, diethylene oxide, dioxyethylene ether, and dioxane

CAS #:	123-91-1
Chemical Formula:	$C_4H_80_2$

Chemical Structure:

Molecular Weight:	88.106 g/mol
Physical State:	Liquid or solid (below 53^o F)
Melting Point:	53.2 ^{o} F/ 12^o C
Boiling Point:	214 ^{o} F/ 101^o C
Vapor Pressure:	38.1 mm Hg at 25^o C
Density:	1.0337 g/cm^3 at 20^o C
Water Solubility:	>800 g/L at 25^o C
Log octanol/water partition coefficient:	-0.27
Taste threshold (water):	No data
Odor threshold (water):	No data
Odor threshold (air):	170 ppm

1,4-Dioxane is a cyclic ether that exists at room temperature as a colorless liquid with a faint, pleasant ethereal odor (NTP, 2016). It is miscible with water, oils, and most organic solvents, including aromatic hydrocarbons. When it enters the air, it exists as a vapor. 1,4-Dioxane is highly flammable and may form dangerous peroxides with prolonged exposure to air and sunlight, especially in the presence of moisture (IARC, 1976; Akron, 2009).

Production and Use

1,4 Dioxane is a synthetic chemical used as a solvent in products such as adhesives, resins, oils and waxes; and in the pulping of wood (NTP, 2016). Historically, 90% of 1,4-dioxane was used as a stabilizer for chlorinated solvents in industrial processes, particularly 1,1,1-trichloroethane (1,1,1-TCA) (NTP, 2016; Godri Pollitt et al., 2019; ITRC 2020). The production of 1,1,1-TCA was eventually phased-out as an "ozone-depleting material" pursuant to the 1987 Montreal Protocol in the United States and 1,4-dioxane production declined (ATSDR, 2012; ITRC, 2020). Therefore, use as a solvent stabilizer for 1,1,1-TCA is no longer expected to be an important use of 1,4-dioxane (USEPA, 2013).

1,4 Dioxane is used in the manufacture of pharmaceuticals, certain plastics and rubber, and other products, and it is an impurity in antifreeze (ITRC, 2020). It is found as an unintended byproduct of surfactants used in consumer products, including personal care products and cosmetics or cosmeceuticals, and it is considered to be present as a trace contaminant in these products (ATSDR, 2012). Further uses of 1,4-dioxane include as a component of inks, paints and coatings, an additive in adhesives and a component of automotive fluids (ITRC, 2020).

Large-scale commercial production of 1,4-dioxane in the United States was first reported in 1951, but small semi-commercial quantities were available in 1929 (IARC, 1976; ATSDR, 2012). During the years 1986 and 1990, the U.S. production of 1,4-dioxane reported by manufacturers was within the range of 10–50 million pounds, and during the years 1994, 1998, and 2002, production was within the range of 1– 10 million pounds with approximately 0.9 million pounds released to the environment in 2011 (USEPA, 2011a; NTP, 2016; USEPA, 2013). More recently, 700,000 pounds of total 1,4-dioxane was released off-site in 2018 (USEPA, 2018a).

GUIDANCE AND STANDARDS DEVELOPED BY USEPA, NEW JERSEY AND OTHER STATES

USEPA

USEPA does not currently have an MCL for 1,4-dioxane, and it was listed on the Drinking Water Contaminant Candidate List 3 for consideration for future regulation based on its potential for public health risk and occurrence in drinking water (USEPA, 2009). No regulatory determination as to whether to pursue MCL development for 1,4-dioxane has been made by USEPA (USEPA, 2020).

The USEPA (2018b) Table of Drinking Water Health Advisories and Standards states that a concentration of 35 ug/L 1,4-dioxane in drinking water corresponds to an excess estimated lifetime cancer risk of 1 in 10,000 (10^{-4}), based on the USEPA IRIS cancer slope factor of 0.10 $(mg/kg/day)^{-1}$. This slope factor is also the basis for the range of Reference Concentrations of 0.35 to 35 ug/L, based on risk levels of 1 in 10,000 (10^{-4}) to 1 in 1 million (10^{-6}), for evaluation of detections of 1,4-dioxane in a nationwide public water system monitoring program, the Third Unregulated Contaminant Monitoring Rule (UCMR3) (USEPA, 2017).

USEPA IRIS's initial assessment of 1,4-dioxane, posted in 1988, following the USEPA (1986) cancer risk assessment guidelines, classified the chemical as a Probable Human Carcinogen (Group B2) based on inadequate human data and sufficient evidence of carcinogenicity in animals, and an oral cancer slope factor of 0.011 $(mg/kg/day)^{-1}$ was developed (USEPA, 1988). Following the updated USEPA (2005) risk assessment guidelines, the IRIS assessment was updated in 2010. In this update, 1,4-dioxane was classified as "likely to be carcinogenic to humans," and a slope factor of 0.10 $(mg/kg/day)^{-1}$ was developed based on liver tumor data from female mice from Kano et al. (2009), which was not available when the earlier (USEPA, 1988) IRIS assessment was developed (USEPA, 2010). The USEPA draft 1,4-dioxane IRIS assessment was updated again to include additional information related to the inhalation risk assessment. The risk assessment for oral exposure, including the Reference Dose and cancer slope factor, were not revised in the updated USEPA IRIS (2013) document. The recent USEPA OCSPP (2020) evaluation of 1,4-dioxane concurred with USEPA IRIS (2013) regarding classification of 1,4-dioxane as "likely to be carcinogenic to humans" and developed a cancer slope factor of 0.12 $(mg/kg/day)^{-1}$ based on the same tumors from Kano et al. (2009) used by USEPA IRIS (2013). See Cancer slope factor derivation.

New Jersey Health-based Drinking Water Guidance

NJDEP Ground Water Quality Criteria (GWQC) are human health-based ground water concentrations based on drinking water exposure. As such, they are developed using the same approaches and assumptions as Maximum Contaminant Level Goals (MCLGs). The GWQC for 1,4-dioxane is based on carcinogenicity at the one-in-one million cancer risk level that is specified in the NJDEP Ground Water Quality Standard (GWQS) regulations, since carcinogenicity at this risk level is more sensitive than non-cancer effects. Also, as specified in GWQS regulations (N.J.A.C 7:9C) IRIS is one of the sources of toxicity factors that is reviewed by NJDEP in development of GWQC. NJDEP ground water quality criteria for 1,4-dioxane have been updated over time to reflect updated USEPA IRIS 1,4-dioxane assessments.

- 2008: Interim Specific Ground Water Criterion (ISGWQC) of 3 µg/L became effective in February 2008 and relied on the USEPA (1988) IRIS assessment of 1,4-dioxane.

- 2010: Revised ISGWQC of 0.35 µg/L was recommended in 2010 following NJDEP review of the USEPA IRIS (2010) updated cancer slope factor.

- 2018: NJDEP (2018) adopted a GWQS of 0.4 µg/L for 1,4-dioxane into the Ground Water Quality Standards regulations in January 2018. The earlier ISGWQS value of 0.35 µg/L was rounded to one significant figure, as specified in the NJDEP Ground Water Quality Standards regulations.

Other states' guidance values and standards

Table 1 includes information on all state standards and guidance values for 1,4-dioxane in drinking water or ground water that were identified by the Health Effects Subcommittee. Of the 14 states identified, all relied on the USEPA IRIS (2013) cancer slope factor of 0.10 $(mg/kg/day)^{-1}$ in the development of their standard or guidance value. The variations in the standards and guidance values for 1,4-dioxane are in part due to differences in the cancer risk levels (shown in Table 1) and exposure assumptions (not shown in Table 1) used by different states.

Table 1. Levels and basis for other states' standards and guidance values for 1,4-dioxane in drinking water and ground water

State	Standard or guidance value (µg/L)		Cancer slope factor	Cancer risk level
Alaska (2018)	4.6 µg/L	Groundwater cleanup	0.10 $(mg/kg/day)^{-1}$	10^{-5}
California (2018)	1 µg/L	Notification Level (NL), health-based advisory levels	0.10 $(mg/kg/day)^{-1}$	NL:3 x 10^{-6} RL: 10^{-4}
	35 µg/L	Response Level (RL), non-regulatory		
Connecticut (2011)	3 µg/L	Action Level	0.10 $(mg/kg/day)^{-1}$	10^{-5}
Indiana (2019)	4.6 µg/L	Groundwater screening level	0.10 $(mg/kg/day)^{-1}$	10^{-5}
Maine (2018)	4.6 µg/L	Remedial Action Guideline	0.10 $(mg/kg/day)^{-1}$	10^{-5}
Massachusetts (2011)	0.34 µg/L	Groundwater Standard and Non-enforceable Drinking Water Guideline	0.10 $(mg/kg/day)^{-1}$	10^{-6}
Michigan (2017)	1.0 µg/L	Health risk limit	0.10 $(mg/kg/day)^{-1}$	10^{-5}
Minnesota (2013)	1 µg/L	Health risk limit (Non-enforceable)	0.10 $(mg/kg/day)^{-1}$	10^{-5}
North Carolina (2017)	0.35 µg/L	Human health criterion – Health Advisory	0.10 $(mg/kg/day)^{-1}$	10^{-6}
New Hampshire (2018)	0.32 µg/L	Ambient Groundwater Quality Standard	0.10 $(mg/kg/day)^{-1}$	10^{-6}
New York[a] (2020)	1.0 µg/L	Maximum Contaminant Level	Not applicable – see footnote below	
Texas (2009)	9.1 µg/L	Protective concentration level – cleanup standards	0.10 $(mg/kg/day)^{-1}$	10^{-5}
Vermont (2016)	0.3 µg/L	Drinking water guidance	0.10 $(mg/kg/day)^{-1}$	10^{-6}
Washington (2019)	0.44 µg/L	MTCA (Model Toxics Cleanup Act) Hazardous Substance	0.10 $(mg/kg/day)^{-1}$	10^{-6}

[a]New York state's Drinking Water Quality Council's proposed MCL is informed by the USEPA IRIS (2013) cancer slope factor, as well as by occurrence and cost of treatment (NY DWQC, 2020).

References:

Alaska (2018). Alaska Department of Environmental Conservation. Procedures for Calculating Cleanup Levels. Accessed February 24, 2020 http://dec.alaska.gov/media/7543/20180201_pccl.pdf.

Alaska (2018). Alaska Department of Environmental Conservation. Procedures for Calculating Cumulative Risk. Accessed February 24, 2020. https://dec.alaska.gov/media/7544/20180201_pccr.pdf

California (2018). California Water Boards. State Water Resources Control Board. 1,4-Dioxane. Accessed February 24, 2020. https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/14-Dioxane.html

Connecticut (2011). State of Connecticut Department of Public Health. Documentation for Derivation of Drinking Water and Bathing/Showering Action Levels for 1,4-Dioxane. (not available online).

Indiana (2019). Indiana Department of Environmental Management. IDEM Screening and Closure Level Tables. Accessed February 24, 2020. https://www.in.gov/idem/cleanups/2392.htm;

Indiana (2016). Indiana Department of Environmental Management. Appendix A" Screening Levels. Accessed February 24, 2020. https://www.in.gov/idem/cleanups/files/risc_screening_table_2016_explanatory.pdf

Maine (2018). Maine Department of Environmental Protection. Maine Remedial Action Guidelines (RAGs) for Sites Contaminated with Hazardous Substances. Accessed February 24, 2020. https://www.maine.gov/dep/spills/publications/guidance/rags/ME-Remedial-Action-Guidelines-10-19-18cc.pdf .

Massachusetts (2011). Massachusetts Department of Environmental Protection. Office of Research and Standards. Supporting Documentation for Drinking Water Standards and Guidelines. Accessed February 24, 2020. https://www.mass.gov/files/documents/2018/05/07/contaminants.pdf

Michigan (2020). Michigan Department of Health. How Health-Based Values and Health Risk Limits are Calculated. Accessed March 1, 2020. https://www.health.state.mn.us/communities/environment/risk/rules/water/methods.html#chrls

Michigan (2015). Michigan Department of Health. Environmental Health Division. 1,4-Dioxane in Drinking Water. Accessed June 1, 2020. https://www.health.state.mn.us/communities/environment/risk/docs/guidance/dwec/dioxaneinfo.pdf

Minnesota (2013). Minnesota Department of Health. 2013 Health Risk Limits for Groundwater. Toxicological Summary for 1,4-Dioxane. Accessed February 24, 2020. https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/14dioxane.pdf

North Carolina (2020). North Carolina Division of Water Resources. 2017. 1,4-dioxane monitoring in the Cape Fear River basin of North Carolina: An ongoing screening, source identification, and abatement verification study. Raleigh,

North Carolina: North Carolina Department of Environmental Quality. Accessed February 24, 2020. https://files.nc.gov/ncdeq/Water%20Quality/Environmental%20Sciences/Dioxane/DioxaneYear2ReportWithMemo_20170222.pdf

North Carolina (2017). 15A NCAC 02B .0208. Standards for Toxic Substances and Temperature. Accessed February 24, 2020 http://reports.oah.state.nc.us/ncac/title%2015a%20-%20environmental%20quality/chapter%2002%20-%20environmental%20management/subchapter%20b/15a%20ncac%2002b%20.0208.pdf

New Hampshire (2019). New Hampshire Department of Environmental Services. 1,4-Dioxane and Drinking Water. WD-DWGB-3-24. Accessed February 24, 2020. https://www.des.nh.gov/organization/commissioner/pip/factsheets/dwgb/documents/dwgb-3-24.pdf

NY DWQC (2020). New York Drinking Water Quality Council Meeting. October webcasts https://totalwebcasting.com/view/?func=VOFF&id=nysdoh&date=2020-02-04&seq=1. Accessed February 18, 2020.

Texas (2009). Texas Commission on Environmental Quality. Chapter 350 – Texas Risk Reduction Program. Subchapter D: Development of Protective Concentration Levels. Accessed February 2020. https://www.tceq.texas.gov/assets/public/legal/rules/rules/pdflib/350d.pdf

Vermont (2019). Vermont Department of Health. Chemical Specific Guidance Values. Accessed February 24, 2020. https://www.healthvermont.gov/sites/default/files/documents/pdf/ENV_ECP_GeneralScreeningValues_Water.pdf

Washington (2019). Washington State Department of Ecology. Focus on Developing Ground Water Cleanup Standards Under the Model Toxics Control Act. https://fortress.wa.gov/ecy/publications/documents/0109049.pdf

Washington (2019). Washington State Legislature. Model Toxics Control Act – Cleanup. Chapter WAC 173-340-720 Groundwater cleanup standards. https://app.leg.wa.gov/WAC/default.aspx?cite=173-340-720&pdf=true

International drinking water guidelines

Health Canada proposed a maximum acceptable concentration (MAC) of 50 µg/L for 1,4-dioxane in drinking water in August 2018 (Health Canada, 2018). The proposed MAC is based on a Tolerable Daily Intake (equivalent to a Reference Dose) for hepatic effects in rats that are stated to occur before the development of cancer. This approach is based on the assumption that there is a threshold for carcinogenicity of 1,4-dioxane, and the proposed MAC is stated to be protective of both cancer and non-cancer health effects.

The World Health Organization (WHO, 2005) also recommends a drinking water guideline of 50 µg/L. The WHO concluded that 1,4-dioxane induces multiple tumors in various organs and used a linearized multistage model for estimating cancer risk from nasal carcinomas (NCI, 1978) and hepatic tumors (Yamazaki et al., 1994) with a 10^{-5} lifetime cancer risk. They also developed a second similar guideline value based on a non-cancer endpoint.

ENVIRONMENTAL FATE, TRANSPORT AND OCCURRENCE

1,4-Dioxane can be released into the air, water, and soil at places where it is produced or used as a stabilizer for chlorinated solvents, particularly 1,1,1-TCA, or a solvent (Abe, 1999; ATSDR, 2012). It is expected to be degraded in the atmosphere through photooxidation with hydroxyl radical and in general is not a concern in the atmosphere since it is non-volatile and has a relatively short half-life of 35 hours (Godri Pollitt et al., 2019).

In water, 1,4-dioxane is stable and breaks down to a limited extent, if at all (ATSDR 2012; Adamson et al., 2015). 1,4-Dioxane is expected to be highly mobile in soil and is expected to leach to lower soil horizons and groundwater (ATSDR, 2012). It may be more persistent in groundwater where volatilization is hindered. 1,4-Dioxane was found in groundwater samples in the United States at concentrations ranging from 1 to 109 µg/L ppb (ATSDR, 2005). A review by Adamson et al. (2017) of 1,4-dioxane occurrence data from UCMR3, a national study of unregulated contaminants in U.S. public water systems that is discussed in detail below, concludes that 1,4-dioxane was detected almost as frequently in surface water as in ground water. However, surface water sources of 1,4-dioxane are more diluted and concentrations are generally lower than in groundwater (Adamson et al., 2017). In groundwater 1,4-dioxane is persistent with a half-life of 2-5 years, and it is less persistent in surface water with an estimated half-life of 56 days (Adamson et al., 2015; Pollitt et al., 2019).

1,4-Dioxane has been detected in contaminated surface and ground water samples collected near hazardous waste sites and industrial facilities (Derosa et al., 1996; Adamson et al., 2015).1,4-Dioxane in ground water is highly associated with detections of chlorinated solvents, most notably 1,1,1-TCA, as well as 1,1-dichloroethane (a byproduct of 1,1,1-TCA) and trichloroethylene (TCE) (Adamson et al., 2015, 2017; Anderson et al. 2012; Godri Pollitt et al., 2019). It is suggested that the dominant source of 1,4-dioxane in the environment is from its use as a stabilizer for chlorinated solvents (Godri Pollitt et al., 2019).

1,4-Dioxane does not bioaccumulate or bioconcentrate to a significant extent in aquatic or marine organisms (ATSDR, 2012).

Based on its properties, 1,4-dioxane is not expected to partition to soil and will instead move with pore water or volatilize from dry surfaces (USEPA, 2018c).

Occurrence in drinking water

Data on 1,4-dioxane in public water systems in NJ and nationwide is available through the USEPA UCMR3 (USEPA, 2017). Under UCMR3, nationwide monitoring of finished water for 30 unregulated contaminants, including 1,4 dioxane, was conducted in 2013-2015 by all U.S. large public water systems (serving more than 10,000 people) and 800 representative smaller public water systems (serving population of 10,000 or less). In UCMR3 testing, 21% of public water systems detected 1,4-dioxane (USEPA, 2017). The percentage of exceedances of the health-based Reference Level (6.9%) was higher for 1,4-dioxane than for all but one other UCMR3 contaminant (chlorate) when the Reference Level of 0.35 µg/L for 10^{-6} cancer risk is used as the benchmark; there were no detections above the Reference Level of 35 µg/L for 10^{-4} cancer risk (USEPA, 2017).

In UCMR3, 174 public water systems in NJ were sampled for 1,4 dioxane including 160 large systems. Of 1433 samples analyzed, 341 (23.8%) samples from 80 different public water systems were above the Minimum Reporting Level (MRL) of 0.07 µg/L. The concentrations ranged from 0.07-5.83 µg/L, with a mean concentration of 0.41 µg/L. Of the 174 water systems tested, 27 (16%) exceeded the NJ GWQS of 0.4 µg/L.

Table 2 compares UCMR3 public water system detections above the MRL and the Health Reference Concentration in New Jersey and nationally. 1,4-Dioxane was detected above the MRL of 0.07 µg/L and the Health Reference Concentration of 0.35 µg/L (which is almost identical to the NJDEP GWQS of 0.4 µg/L) more than twice as frequently in NJ than nationally.

Table 2. New Jersey v. National Public Water System (PWS) 1,4-Dioxane Detections in UCMR3 (2013-2015)

	New Jersey PWS		National PWS (other than NJ)	
	# Detects	% Detects	# Detects	% Detects
≥ 0.07 µg/L (MRL)	80/174	45.9%	997/4741	21.0%
≥ 0.35 µg/L (Health Reference Concentration*)	30/174	17.2%	315/4741	6.6%

*USEPA (2017) UCMR3 Reference Concentration for 10^{-6} cancer risk.

HUMAN BIOMONITORING

The National Health and Nutrition Examination Survey (NHANES), a representative sample survey of the U.S. general population conducted by the U.S. Centers for Disease Control and Prevention (CDC, 2018), has monitored the blood concentration of 1,4-dioxane from 2009 through 2016. As 1,4-dioxane is quickly metabolized and excreted, it will not be detected unless the test is conducted within days after exposure (ATSDR, 2012; Godri Pollitt et al., 2019). In each of the four two-year cycles that have been undertaken since 2009, 1,4-dioxane in blood has been below the level of detection (LOD=0.5 ng/ml) in every participant, and more sensitive analytical methods may be required to detect the low levels of exposures in the general population (Godri Pollitt et al., 2019). Exposure to 1,4-dioxane may be evaluated through detection of its metabolites, and also tests may be available for detection of 1,4-dioxane in urine (Godri Pollitt et al., 2019).

SOURCES OF HUMAN EXPOSURE

The occurrence of 1,4-dioxane in the environment primarily results from the use and disposal of associated chlorinate solvents (e.g. 1,1,1-TCA) (ITRC, 2020a).

The general public is widely exposed to 1,4-dioxane as it occurs as a byproduct in consumer products containing foaming agents, including cosmetics/toiletries, household detergents, pharmaceuticals, foods, agricultural and veterinary products, and ethylene glycol-based antifreeze coolants (Godri Pollitt et al., 2019). A 2008 survey of cosmetic products by U.S. Food and Drug Administration found 6% of product contained 1,4-dioxane between 1-5 ppm, 6% between 5-10 ppm and 8% between 10-12 ppm, while 80% of products had no detection (US FDA, 2019).

Drinking Water

Drinking water is the dominant pathway of exposure to 1,4-dioxane (Godri Pollitt et al., 2019; Anderson et al., 2012). The main sources of 1,4 dioxane in drinking water are wastewater discharge, unintended spills or leaks, and historical disposal practices associated with 1,1,1-TCA (ITRC, 2020a). 1,4 -Dioxane in wastewater discharges is likely due to its widespread use in consumer products.

A two-tier multi-route exposure assessment approach concluded that exposure to 1,4- dioxane from drinking water through inhalation or dermal absorption is not significant compared to exposure from ingestion (Health Canada, 2018).

Food

1,4-Dioxane has been used as a food additive and in the formulation of pesticides and food packaging adhesives (Godri Pollitt et al., 2019; ATSDR, 2012). The maximum level of 1,4-dioxane permitted in food additives (e.g. polysorbates) by the Commission of the European Communities is 5 ppm (EU Commission Directive 2003/95/EC, 2003). 1,4-Dioxane has also

been identified in several natural products including shrimp, chicken, tomatoes, coffee and certain condiments (Hartung, 1989). A study in Japan found 1,4-dioxane present in several food groups but found dietary exposure to be low (Nishimura et al., 2004).

Consumer Products

Dermal exposure to 1,4-dioxane may occur through contact with residues in contaminated consumer products. 1,4-Dioxane may be a contaminant in ethoxylated surfactants, which are used in personal care products including cosmetics and shampoos and cleaning products including dishwashing liquids and detergents (Environment Canada and Health Canada, 2010). Exposure via products such as shampoo, body washes and hand soaps, which have been found to be quite high, is through inhalation and a lesser extent dermal absorption because of volatile nature of 1,4-dioxane causes most of it to evaporate (Health Canada, 2018; Environment Canada and Health Canada, 2010; ATSDR, 2012; EU, 2002). Because of their frequency of use of products containing 1,4-dioxane, women are the most exposed group (Environment Canada and Health Canada, 2010). Although low, infant exposure from consumer products has also been reported (Environment Canada and Health Canada, 2010; Godri Pollitt et al., 2019). The concentrations of 1,4- dioxane in cosmetic products have been declining over the past decade (ATSDR, 2012).

Taking effect on January 1, 2022, New York State has enacted legislation (S4389B) that will prohibit the sale of household cleaning products which contain more than trace amounts of 1,4-dioxane and will limit the sale of personal care products with certain levels of 1,4-dioxane (NYS, 2019). The legislation will phase-down permissible levels in cosmetics from 10 ppm in 1 ppm by the end of 2023.

Occupational

Occupational exposure to 1,4-dioxane may occur during its production and its use as a solvent (IARC, 1999).

TOXICOKINETICS

The following discussion of toxicokinetics of 1,4-dioxane is primarily based on information from USEPA (2013).

Absorption

As summarized in USEPA (2013), the oral absorption of 1,4-dioxane in humans has not been evaluated. In rats administered radiolabeled 1,4-dioxane orally, <1-2% of the radiolabel was found in the feces, indicating nearly complete oral absorption (Young et al., 1978a, b). Absorption of inhaled 1,4-dioxane in humans (Young et al., 1976, 1977) and rats (Young et al., 1978a, b) has been demonstrated by detection of the 1,4-dioxane metabolite HEAA and lower levels of unchanged 1,4-dioxane in urine after inhalation exposure. However, the fraction of 1,4-

dioxane that was absorbed was not quantitated. Limited data (reviewed by USEPA, 2013 and USEPA, 2020) suggests that dermal absorption of 1,4-dioxane is low.

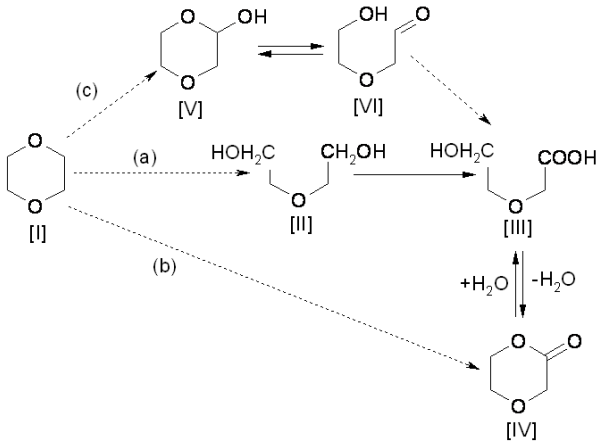
Distribution

USEPA (2013) and USEPA (2020) state that there are no available data for distribution of 1,4-dioxane in humans by any route of exposure or in animals from oral or inhalation exposure. Studies from rats injected intraperitoneally with radiolabeled 1,4-dioxane found that radiolabel was generally higher in blood than in other tissues (Woo et al., 1977; Mikheey et al., 1990).

Metabolism

Suggested metabolic pathways of 1,4-dioxane in the rat are shown in Figure 1. Metabolism is believed to be mediated by cytochrome P-450. As summarized in USEPA (2013), the major metabolite of 1,4-dioxane in rats and humans is believed to be β-hydroxyethoxy acetic acid (HEAA). However, there is pH-dependent interconversion of HEAA with 1,4-dioxane-2-one, complicating interpretation of some of the studies in which these metabolites were measured (USEPA, 2013). Data from rats given a range of single gavage or intravenous doses of 1,4-dioxane indicate that metabolism is saturated as the dose increases (Young et al., 1978a, b). As discussed in USEPA (2013), the observation that metabolic saturation in rats occurred at lower doses in single-dose studies (Young et al., 1978a,b; Kociba et al., 1975) as compared to repeated-dose studies (Young et al., 1978a,b; Kasai et al., 2008) suggests that 1,4-dioxane induces its own metabolism. 1,4-Dioxane induced several isoforms of CYP450 in the liver microsomes, and one of these isoforms (CYP2E1) in nasal mucosa, and kidney microsomes, in male Sprague-Dawley rats dosed by with 2000 mg/kg/day by gavage for 2 days or 1.5% in drinking water for 10 days (Nannelli et al., 2005).

Figure 1. Suggested metabolic pathways of 1,4-dioxane in the rat.



Legend: I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA); IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde. Note: Metabolite [V] is a likely intermediate in pathway b as well as pathway c. The proposed pathways are based on the metabolites identified; the enzymes responsible for each reaction have not been determined. The proposed pathways do

not account for metabolite degradation to the labeled carbon dioxide (CO_2) identified in expired air after labeled 1,4-dioxane exposure. Source: USEPA (2013).

Excretion

1,4-Dioxane and its metabolites are primarily excreted in urine in humans exposed via inhalation and in rats after inhalation or oral exposure (Young et al., 1976; 1978a,b); there are no human oral exposure data. The half-life was about one hour in both species after exposure to 50 ppm in air for 6 hours (Young et al., 1977; 1978a,b). In rats dosed orally with radiolabeled 1,4-dioxane, unchanged 1,4-dioxane and smaller amounts radiolabeled CO_2 (presumably 1,4-dioxane metabolites) were also found in expired air (Young et al., 1978a,b).

HEALTH EFFECTS – HUMAN STUDIES

The following human health studies were identified and summarized from USEPA (2013) and EU (2002). The following evaluations, review reports or publications were also reviewed to identify additional human health studies: Health Canada (2018); ATSDR (2012); Health Council of the Netherlands (2015; carcinogenicity only) and Godri Pollitt et al. (2019). All of the studies are based on inhalation exposure; no oral studies were identified (EU 2002; Health Canada 2018).

As reviewed by USEPA (2013), EU (2002) and others, Barber (1934) was the first record of death caused by exposure to 1,4-dioxane; they reported the deaths of five patients following acute inhalation exposure to high concentrations five to eight days after symptom onset. Additionally, Johnstone (1959) further records the case of a 21-year old worker who died of kidney failure one week following inhalation and dermal exposure to high concentrations of 1,4-dioxane for one week. The liver and brain were also significantly affected as determined by autopsy (EU, 2002; USEPA, 2013).

USEPA (2013) and EU (2002) reviewed several studies of acute inhalation exposure in human volunteers. Studies reported nose and throat irritation, eye irritation and vertigo among the volunteers at varying exposure concentrations and durations (Yant et al., 1930; Silverman et al., 1946; Wirth and Klimmer 1936; Young et al., 1977). Two studies reported no symptoms after exposure (Fairley, 1934; Ernstgard et al., 2006).

Two occupational mortality studies described by USEPA (2013) found no effect from “low” exposure to dioxane (Thiess et al., 1979; Buffler et al., 1978). Thiess et al. (1979) presents a cross-sectional study which found no statistically significant effects between 74 German active and retired workers exposed to air concentrations ranging from 0.06-0.69 ppm of 1,4-dioxane. No pathological findings were reported for any of the workers. In a subset analysis of six actively employed workers and six controls there were no differences in percent of cells with gaps or other chromosome aberrations. Mortality statistics calculated for the 74 workers estimated an expected 14.5 deaths while only 12 were observed, and standardized mortality ratios for cancer did not significantly differ from the general German population. Buffler et al.,

(1978) conducted a retrospective mortality study of 165 Texas manufacturing (100) and processing (65) workers exposed for at least one month to "low" levels of 1,4-dioxane (< 25ppm). They found no statistically significant increase in cancer-related or all-cause related mortality. This study had small sample sizes among its cohorts and a short (<10-year) latency period. USEPA (2013) concluded that the two occupational 1,4- dioxane studies in humans found no conclusive causal link with increased risk for cancer (Thiess et al., 1976; Buffler et al., 1978).

Two additional studies described by EU (2002) that identified exposure but did not directly measure the concentration of 1,4-dioxane also found no effects on the health endpoints that were evaluated (Kramer et al., 1978; NIOSH, 1977). An epidemiology study of 151 employees in a textile factory, who were exposed for between one and six years to concentrations of up to 1,350 mg/m^3 of 1,1,1-trichloroethane blended with 4% 1,4-dioxane showed no significant differences in health including on ECG changes and liver damage, when compared to a control group (Kramer et al., 1978). Investigations on 80 men with potential exposure to 0.18 to 184 mg/m^3 1,4-dioxane showed no signs of 1,4-dioxane-related health effects (NIOSH, 1977). The complete list of health endpoints evaluated by Kramer et al. (1978) and NIOSH (1977) is not provided by EU (2002).

As described in Health Canada (2018), an additional occupational study that did not directly measure 1,4-dioxane exposure reported that workers exposed to the chemicals used in silk screening and the electronics industry (known to include 1,4-dioxane) in Russia had elevated rates of spontaneous abortion and stillbirth (NIOSH, 1988; Ailamazian, 1990).

Generally noted throughout the reviews were the low quality of study reporting, in that data were obtained from secondary sources, and that study details were missing. Also, the size of the cohorts, and thus the power of the studies, was low. The potential for increased risk of cancer from occupational exposure to 1,4-dioxane could not be adequately assessed in any of the available studies.

HEALTH EFFECTS – ANIMAL STUDIES

This section summarizes the toxicological information on 1,4-dioxane, including acute, short-term, subchronic and chronic oral and inhalation studies, as well as studies of reproductive/developmental and neurological effects. In most of the repeated-dose studies, 1,4 dioxane caused toxicity to the liver, kidney and respiratory tract. It caused liver tumors in multiple studies in rats, mice and guinea pigs, as well as nasal tumors in rats exposed through drinking water or inhalation. Increases in incidence of several other types of tumors were also reported in one or more rat studies. It should be noted that studies that evaluated the carcinogenicity of 1,4-dioxane in laboratory animals, regardless of duration (e.g. subchronic or chronic) are discussed in the section on "Chronic studies and other studies evaluating

carcinogenicity" below. The summaries of these studies also include the non-neoplastic effects that were reported.

Acute and short-term studies

LD_{50} values from a single gavage dose of 1,4-dioxane have been reported as follows: rat: 5,400-7,210 mg/kg (Laug et al., 1939; Pozzani et al., 1959; Smyth et al., 1941); mouse: 5,900 mg/kg (Laug et al., 1939); guinea pig: 3,150-4,030 mg/kg (Laug et al., 1939; Smyth et al., 1941).

Toxicological effects observed in acute (single dose) and short-term (up to 14 day) gavage and drinking water studies are summarized in USEPA (2013). Histopathological changes in the liver and kidney were reported in rats (David, 1964; Kesten et al., 1939, JBRC, 1998; Kitchin and Brown, 1990; Nelson, 1951), mice (Laug et al., 1939; JBRC, 1998), guinea pigs (Laug et al., 1939), rabbits (de Navasquez, 1935), and dogs (Schrenk and Yant, 1936). Nasal and brain lesions in rats and mice were also reported in the 14-day drinking water study conducted by the Japan Bioassay Research Center (JBRC, 1998).

Subchronic studies

Oral studies

As summarized by USEPA (2013), Fairley et al. (1934) dosed 6 rats and 6 mice with drinking water containing 1.25% (12,500 ppm) 1,4-dioxane for up to 67 days, resulting in estimated doses of 1,900 mg/kg/day in rats and 3,300 mg/kg/day in mice. Only one rat survived past day 34, while 5 mice survived until day 60. Effects associated with treatment in both species included enlarged kidneys and histopathological changes in the kidney and liver.

As summarized by USEPA (2013), Stott et al. (1981) dosed male Sprague-Dawley rats (4-6 per group) with 0, 10, or 1,000 mg/kg/day in their drinking water, 7 days/week for 11 weeks. It was noted by USEPA (2013) that the high dose was stated to be 100 mg/kg-day in the Methods section, while the Abstract, Results, and Discussion sections state that it was 1,000 mg/kg-day. As such, it was assumed to be 1000 mg/kg/day. In the high-dose group, relative liver weight was increased, histopathological changes were observed in the liver, and hepatic DNA synthesis, measured by [^{3}H]-thymidine incorporation, was increased 1.5-fold. No effects were reported in the low-dose group.

Kano et al. (2008) dosed F344/DuCrj rats (10/sex/group) and Crj:BDF1 mice (10/sex/group) with 0, 640, 1,600, 4,000, 10,000, or 25,000 ppm 1,4-dioxane in drinking water for 13 weeks. In rats, daily doses were estimated based on water consumption and body weight data as 0, 52, 126, 274, 657, and 1,554 mg/kg/day in males, and 0, 83, 185, 427, 756, and 1,614 mg/kg/day in females. One female in the high dose group died; the cause and time of death were not provided. Body weights at the end of the study were decreased at the two highest dose levels in females (12 and 21%) and males (7 and 21%), respectively. Food consumption was reduced in the highest

dose group by 13% in females and 8% in males, and water consumption was decreased in a dose-related fashion at all doses in males and starting at the second lowest dose level in females. Red blood cells, hemoglobin and hematocrit were significantly increased in high dose males but were not affected in females. The liver enzymes AST and ALT were significantly increased in high dose males, and AST was also increased in high dose females, and plasma glucose was decreased in high dose males and females. Absolute and relative kidney weights were increased in females in all but the lowest dose group. Histopathological changes were reported in respiratory, olfactory and tracheal epithelium, liver, kidneys and brain. The most sensitive effects were nuclear enlargement of the nasal cavity respiratory epithelium and hepatocyte swelling, occurring at 126 mg/kg/day in males.

In mice, daily doses were estimated based on water consumption and body weight data as 0, 86, 231, 585, 882, or 1,570 mg/kg/day in males, and 0, 170, 387, 898, 1,620, or 2,669 mg/kg/day in females. One male mouse in the high-dose group died; the cause and time of death were not provided. Body weights at the end of the study were decreased by 29% in high dose males, and by less than 10% in other dosed groups; food consumption was not affected. Water consumption was decreased in all dosed groups of males and in the highest dose group of females, with 70% and 57% decreases at the highest dose in males and females, respectively. As was the case in rats, red blood cells, hemoglobin and hematocrit were significantly increased in high dose males but were not affected in females. The liver enzymes AST and ALT were significantly increased in high dose males and females. Plasma glucose was decreased in high dose males and at the two highest doses in females. Absolute and relative lung weights were increased in high dose males and in the two highest dosed groups of females. Absolute kidney weight was increased in the two highest dosed groups of females, and relative kidney weight was also increased in the highest female dosed group. Histopathological changes were reported in respiratory, olfactory, and hepatic tissues. The most sensitive endpoint reported by the authors was nuclear enlargement in the bronchial epithelium in females at 387 mg/kg/day; USEPA (2013) notes that it does not consider nuclear enlargement to be an adverse effect.

An recent subchronic study focused on renal effects of 1,4-dioxane. Qiu et al. (2019) administered 0, 0.5 or 500 ppm 1,4-dioxane in drinking water to groups of 12 male mice for 12 weeks. Estimated doses were 0.1 and 100 mg/kg/day. While food and water consumption were not affected by treatment, body weight gain was decreased at both doses and relative kidney weight was increased at the higher dose. No histopathological changes were reported in the kidney at the low dose, while hydropic generation of the renal tubules, glomerular cell proliferation, hyperemia and slight inflammation were observed at the high dose. Other components of the study evaluated indicators of oxidative stress (superoxide dismutase and glutathione) in renal tissue, urinary protein and creatinine, transcriptomic analysis of renal tissue and metabolomic analysis of urine. Although there were no histopathological effects or changes in indicators of oxidative stress in the low dose group, transcriptomic changes in signaling pathways related to oxidative stress and other biological processes occurred, suggesting that

oxidative damage may be occurring even in the absence of more overt effects. Metabolomic and transcriptomic data also indicated effects on metabolic pathways, including those involving amino acid metabolism.

Two recent studies (Lafranconi et al., 2020; Charkoftaki et al., 2021) evaluated hepatic effects of 1,4-dioxane in drinking water in female mice, and an additional publication (Chappell et al., 2021) presents results of hepatic transcriptomic analysis from Lafranconi et al. (2020).

Lafranconi et al. (2020) evaluated hepatic effects of 1,4-dioxane in drinking water in female B6D2f1/Crl mice after exposure for of 7, 28, and 90 days. The authors state that this strain was chosen to match as closely as possible the strain of mice (Crj:BDF1) used in Kano et al. (2008; 2009). Ten mice per dose group per timepoint were exposed to 0, 40, 200, 600, 2000, or 6000 ppm in drinking water, and the estimated daily doses 0, 7.2, 37.3, 116, 364, and 979 mg/kg/day. Parameters evaluated included clinical signs, body weight, liver weight, clinical chemistry, liver histopathology, hepatocyte bromodeoxyuridine (BrDU) labeling, hepatic caspase-3 (biomarker for apoptosis), hepatic placental glutathione S-transferase (GST-P) staining (biomarker for hepatic foci), and blood levels of dioxane and its metabolite, HEAA.

The authors state that there were no treatment-related effects on clinical signs, body weight, or clinical chemistry compared to controls in any of the dosed groups. However, no data for these parameters, including parameters related to hepatic toxicity such as serum liver enzyme levels and bilirubin, are provided.

The authors state that relative liver weight is increased at 6000 ppm (the highest dose) and that there were "sporadic" increases at 2000 ppm. Relative liver weight was increased at 6000 ppm by 8.7%, 10.7%, and 8.9% at 7, 28, and 90 days, respectively. However, the data also indicate an increase in relative liver weight at all time points at 2000 ppm, with statistical significance at 28 and 90 days.

Regarding histopathological findings, the authors state that minimal to mild centrilobular vacuolation with glycogen deposition in the centrilobular region of the liver after 7 days at > 600 ppm (although hepatic glycogen was not measured), and that this effect was "largely resolved" by 28 days. However, the data show almost identical occurrence of centrilobular vacuolation at 7 and 28 days, and this effect continued to occur at 90 days. It is stated that centrilobular hypertrophy "appeared after 7 days of exposure" but the data do not show this effect until 28 days; it occurred in 1/10 animals at 2000 ppm at 90 days and in 10/10 animals at 6000 ppm (the highest dose) at 28 and 90 days.

Blood levels of the 1,4-dioxane metabolite HEAA were much higher at 7 days than 28 or 90 days, although there was little or no dioxane in blood at any dose or timepoint except at 6000 ppm at 90 days. As such, the total of HEAA plus 1,4-dioxane in blood was much lower at 28 and 90 days (except at 6000 ppm at 90 days) than at 7 days. This discrepancy is alluded to in the paper's statement that other metabolic pathways such as conjugation may come into play at 28

and 90 days. However, no data were provided to support this conclusion, and there is no discussion of the potential role of these additional metabolites in toxicity. There is no discussion of metabolism other than metabolism of 1,4-dioxane to HEAA, and there is no discussion of inhibition or induction of metabolic enzymes.

Single cell necrosis (interpreted as apoptosis) occurred at 2000 ppm in 1/10 animals at 90 days and in 8/10 and 10/10 animals at 6000 ppm at 28 and 90 days, respectively. The authors state that single cell necrosis (interpreted as apoptosis) directly correlates with the appearance of 1,4-dioxane in blood (interpreted as saturation of metabolism) after 90 days of exposure to 6000 ppm. However, the incidence of apoptosis was almost as high at 28 days (80%) as at 90 days (100%) although 1,4-dioxane blood levels were very low or non-detectable at 28 days.

The number of caspase-3 positive cells was significantly higher than in controls at the highest dose (6000 ppm) at 28 and 90 days, while GST-P positive foci were not increased compared to controls at 6000 ppm at 90 days, the only doses and timepoint evaluated.

A major conclusion of the paper is that there is a mitogenic response (as indicated by increase in % BrdU positive cells) only at the highest dose (6000 ppm) and the longest exposure duration (90 days), and that 1,4-dioxane appears in the blood at this dose and timepoint. However, the increase in the percent of BrdU positive cells at this dose and timepoint does not appear to be statistically significant, as it is stated only that there is a "pronounced increase" at this dose and timepoint, and statistical significance is not mentioned. Because the numerical data for BrdU positive cells are not presented, it is not possible to further evaluate this issue. Furthermore, while it is stated that the increase in percentage of BrdU positive cells corresponds to increased relative liver weight, relative liver weight was increased almost as much at 2000 ppm as at 6000 ppm, but percent BrdU positive cells was not increased at 2000 ppm.

The authors state that the results of this study "demonstrate a previously unreported direct mitogenic response following exposures exceeding the metabolic clearance threshold of 1,4-dioxane," and that the results support a threshold mode of action for hepatic tumors caused by 1,4-dioxane. However, as discussed above, the Health Effects Subcommittee noted multiple inconsistencies between statements in the text and the data shown in the tables and figures in this publication, as well as several conclusions that do not appear to be supported by the data. More generally, the Subcommittee concludes that a 90 day study such as this cannot establish a mode of action for tumor formation, since the occurrence of tumors cannot be evaluated in a study with this design.

Two recent molecular-based studies discussed below examined altered gene expression and modifications of connected pathways following 1,4 dioxane exposure.

Transcriptomic data for the livers of the animals from Lafranconi et al. (2020) are presented in a separate publication (Chappell et al., 2021). RNA sequencing was performed on liver samples (formalin-fixed and embedded in paraffin) from five mice from each of the six dose groups (0, 40, 200, 600, 2000, or 6000 ppm in drinking water) for each of the three exposure durations (7, 28, and 90 days). The authors reported that the number of differently expressed genes (DEGs; based on comparison to controls) was low (0-22) in the two lowest dose groups (40 and 200 ppm), that there were relatively few DEGs (20-33) at any timepoint at 2000 ppm, and that there

were more DEGs at 7 and 90 days at 600 ppm and 6000 ppm (specifically 411, 1, and 323 at 7, 28, and 90 days at 600 ppm; 415, 232, and 727 at 7, 28, and 90 days at 6000 ppm). Although Chappell et al. (2021) describe alterations in specific genes over time and dose, and the pathways that are associated with these genes, they do not provide a clear explanation as to how these pathways are related to the MOA for tumor formation. In addition, since this was a 90 day study, the relationship of increases or decreases in gene expression with the occurrence of tumors cannot be evaluated.

Charkoftaki et al. (2021) evaluated changes in hepatic immunohistochemical markers, hepatic transcriptomics, and metabolomic profiles in female BDF1 mice (6 per group) exposed to 0, 50, 500, or 5000 ppm 1,4-dioxane in drinking water for 7 or 28 days. There was no effect on body weight, drinking water consumption, or absolute and relative liver weight in any of the dosed groups. Three immunohistochemical markers were evaluated in livers from the 0, 500, and 5000 ppm groups dosed for 7 days and 0 and 5000 ppm for 28 days. 1,4-Dioxane did not affect 4-hydroxynonenal, an indicator of oxidative damage. Expression of H2AXγ, a marker of DNA double strand breaks, was evaluated separately in hepatocytes and non-hepatocytes. In hepatocytes, expression of H2AXγ was increased 2-3 fold at 5000 ppm at both 7 and 28 days. At 500 ppm, there was no effect at 7 days. In non-hepatocytes, expression was increased at 5000 ppm at 7 days but not 28 days, and it was not increased at 7 days at 500 ppm. The number of cytokeratin-7 positive cells, indicative of precursor cells (precholangiocytes) that are present when the liver repopulates itself after damage, was increased at 28 days at 5000 ppm, but not at 500 or 5000 ppm at 7 days.

Transcriptomic analysis was conducted on liver samples from 3 mice each from the 28 day control and 5000 ppm groups. Based on the criteria used, there were 65 DEGs in the 5000 ppm group. By far the largest change was a 95.6-fold decrease in expression of Cyp3a16. Further analysis of affected pathways and the functions of affected genes indicated that the most significantly enriched pathways were related to xenobiotic metabolism signaling, nicotine degradation, and glutathione-mediated detoxification. Five of the DEGs are linked to activation of DNA damage response and inhibition of DNA repair, and four of the DEGs are linked to hepatocellular carcinoma.

Metabolomic analysis was performed on urine and feces samples from 3-5 mice, and liver and kidney tissues from all animals, in each of the 7 day and 28 day control and 5000 ppm groups. Untargeted metabolomic analysis did not show differences between the treated and control groups. Because the transcriptomic data indicated effects on pathways related to bile acid synthesis and bile acids are a marker for liver dysfunction, targeted metabolomic analysis for bile acid levels in liver tissue and feces was performed. There were no differences in levels of primary or secondary bile acids or their taurine and glycine conjugates in treated mice compared to controls.

Charkoftaki et al. (2021) demonstrate the complex nature of biochemical endpoints when examining 1,4 dioxane exposure. Several interesting observations can be drawn from this study: there was no indication of oxidative stress; DNA double strand break markers were increased along with a number of DEGs related to DNA damage and repair, decreased specific cytochrome P450 isozymes, enhanced glutathione detoxification pathways, DEGs related to hepatocellular

carcinoma and normal bile acid formation. The results from this study lay the groundwork for future mechanistic studies that may further inform the mode of action for 1,4-dioxane toxicity including carcinogenicity.

It is important to note that simply listing the pathways that include genes with increased or decreased expression after exposure to 1,4-dioxane provides limited information without a more detailed analysis of the relationship of these changes to potential mode of action. In order to demonstrate a mode of action for carcinogenicity, a correlation of altered gene expression and tumor formation must be shown. These types of studies must be carefully scrutinized as to how the data were generated, statistically evaluated, and interpreted as related to tissue-specific carcinogenesis. Due to the dynamic nature and the complex feedback pathways within a cell/tissue, identification of a mode of action requires plausible biochemical responses leading to formation of transformed cells in the target organ(s). While Chappell et al. (2021) and Charkoftaki et al., (2021) add to our knowledge of potential altered biochemical pathways, they fall short of establishing a definitive mechanism of action for explaining 1,4 dioxane tumorigenesis.

Inhalation studies

As summarized by USEPA (2013), Fairley et al. (1934) exposed rats, mice, guinea pigs and/or rabbits (3–6/species/group) to 1,000, 2,000, 5,000, or 10,000 ppm of 1,4-dioxane vapor for varying lengths of time, twice a day for 1.5 hours for 5 days/week and once for 1.5 hours on the sixth day of the week. At 10,000 ppm, all animals except one rat died within the first five exposures. At 5,000 ppm, two mice and one guinea pig died after 15–34 exposures while the remaining animals were sacrificed after 3 weeks or 5 weeks of exposure. At 2000 and 1000 ppm, animals were exposed for approximately 2–6 weeks and 4–12 weeks, respectively. Kidney and liver damage occurred at all doses and was more severe at higher doses.

As summarized by USEPA (2013), Kasai et al. (2008) exposed F344/DuCrj rats (10/sex/group) to 0, 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm of 1,4-dioxane for 6 hours/day, 5 days/week, for 13 weeks. At the highest dose, all rats died by the end of the first week from renal failure caused by necrosis of the renal tubules; no deaths occurred at lower concentrations. Statistically significant increases in several organ weights included lungs (≥ 1,600 ppm, males; ≥ 200 ppm, females); livers (≥ 800 ppm, both sexes), and kidneys (3,200 ppm, males; ≥ 800 ppm, females). Statistically significant changes in hematological parameters and clinical chemistry at 3,200 ppm included increased hemoglobin ALT, erythrocytes, AST, and mean corpuscular volume in both sexes; increased hematocrit in females; and decreased glucose and triglyceride in males were observed. Histopathological changes caused by 1,4-dioxane in one of both sexes were reported in the nasal respiratory, nasal olfactory, tracheal, and bronchial epithelium; kidney; and liver. Glutathione S-transferase placental form (GST-P) foci, a preneoplastic liver lesion, was found in 3/10 males and 2/10 females at 3,200 ppm and 4/10 females at 1,600 ppm. The authors identified nuclear enlargement in the respiratory epithelium of males and females at 100 ppm as the most sensitive endpoint. As noted above, USEPA (2013) stated that they do not consider this to be an adverse effect.

Studies of neurological effects

Clinical signs of CNS depression (e.g. staggered gait, narcosis, paralysis, coma, and death) were observed in some of the studies mentioned above. USEPA (2013) reviewed four rodent studies that focused on specific neurological effects of 1,4-dioxane, including one oral study (Goldberg et al., 1964) and two inhalation studies (Frantik et al., 1994; Kanada et al., 1994).

Oral studies

In male rats administered a single gavage dose of 1,050 mg/kg, dopamine and serotonin levels were reduced compared to controls in the hypothalamus, while no effects were seen in other parts of the brain (Kanada et al., 1994).

Inhalation studies

Frantik et al. (1994) evaluated the effect of inhaled 1,4-dioxane on the propagation and maintenance of an electrically-evoked seizure discharge in rats and mice. The most sensitive and reproducible effects were duration of tonic hind limb extension in rats and the velocity of tonic extension in mice. The 1,4-dioxane air concentration producing a 30% decrease in the maximal response to an electrically-evoked seizure was 1,860 ± 200 ppm in rats and 2,400 ± 420 ppm in mice, and no NOAEL was identified.

Goldberg et al. (1964) evaluated the effect of 1,4-dioxane inhalation on conditioned avoidance and escape behaviors using a pole climb methodology. A dose-related effect on conditioned avoidance behavior was observed in female rats exposed to 0, 1,500, 3,000, or 6,000 ppm of 1,4-dioxane in air for 10 days, 4 hours/day, 5 days/week. In the high dose group where the effect was greatest, a higher percentage of rats were affected during the first 2 days of exposure than on days 3-10.

Study of reproductive and developmental effects

Only one study of reproductive and developmental effects of 1,4-dioxane was identified. As summarized by USEPA (2013), Giavini et al. (1985) administered 0, 250, 500, or 1000 mg/kg/day 1,4-dioxane by gavage to pregnant female Sprague Dawley rats (18–20 per dose group) on gestations days 6–15 and sacrificed them on gestation day 21. In the high dose group, fetal weight was decreased by 5% ($p < 0.01$) and ossification of the sternebrae was reduced ($p < 0.05$), while maternal weight gain was decreased by 10% in this dose group. Numbers of corpora lutea, implantations, resorptions, and live fetuses and external, visceral, and skeletal fetal malformations were not affected by treatment. The study authors suggested that delayed ossification and decreased fetal weight indicate a developmental delay at 1000 mg/kg/day.

Chronic studies and other studies evaluating carcinogenicity

As shown in Table 3, 1,4-dioxane caused tumors in multiple organs in studies of rats, mice and guinea pigs. These studies are summarized below.

Table 3. Sites at which tumor incidence was increased by 1,4-dioxane in animal studies (adapted from USEPA, 2013)

Study Details				Tumor Sites							
Study	***Exposure Route***	***Species***	***Sex***	***Liver***	***Nasal Cavity***	***Mammary Gland***	***Peritoneal Meso-thelioma***	***Testis/ Epididymis Meso-thelioma***	***Kidney***	***Zymbal Gland***	***Subcutis Fibroma***
Argus et al. (1965)											
	Drinking Water	Rat	M	+	-	-	-	-	-	-	-
Hoch-Ligeti et al. (1969) / Argus et al. (1973)											
	Drinking Water	Rat	M	+	+	NR*	NR	NR	NR	NR	-
Hoch-Ligeti and Argus (1970)											
	Drinking Water	Guinea Pig	M	+	-	-	-	-	-	-	-
Kociba et al. (1974)											
	Drinking Water	Rat	M/F	+	+	- **	-	-	-	-	-
NCI (1978)											
	Drinking Water	Rat	M	-	+	-	-	+***	-	-	-
			F	+	+	-	+	NA	-	-	-
		Mouse	M	+	-	-	-	-	-	-	-
			F	+	-	-	-	NA	-	-	-
Kano et al. (2009)											
	Drinking Water	Rat	M	+	+	+	-	-	-	-	-
			F	+	+	+	+	NA	-	-	-
		Mouse	M	+	-	-	-	-	-	-	-
			F	+	-	-	-	NA	-	-	-
Torkelson et al. (1974)											
	Inhalation	Rat	M	-	NR	-	-	-	-	-	-
			F	-		-	-	-	-	-	-
Kasai et al. (2009)											
	Inhalation	Rat	M	+	+	+	-	-	+	+	+

*NR –not reported.
**Some organs marked "-" may not have been evaluated.
***Increase was noted as not statistically significant. not reported.

Oral studies

Argus et al. (1965) administered drinking water containing 1% (10,000 ppm) 1,4-dioxane to 26 adult male Wistar rats for 63 weeks, resulting in a dose estimated by USEPA (2013) of 640 mg/kg/day. There were 9 rats in the control group. Liver tumors occurred in 6/26 treated rats, while there were no liver tumors in the control group. Histopathological changes in the liver were observed in rats that died before the end of the dosing period, beginning at 2.5 weeks after

dosing began. Extensive histopathological changes in the kidney also occurred in "many" treated rats (incidence not provided).

Stoner et al. (1986) included 1,4-dioxane in a study of 19 chemicals that compared the induction of lung tumors in A/J mice from exposure via gavage versus intraperitoneal injection. Groups of 16 male and female mice were dosed 3 times per week for 8 weeks with average daily doses that were estimated by USEPA (2013) as 430 mg/kg/day for gavage dosing and 86, 210, or 430 mg/kg/day for intraperitoneal dosing. The mice were sacrificed 24 weeks after dosing began. It was concluded that 1,4-dioxane did not induce lung tumors by either route of exposure in this subchronic study.

Hoch-Ligeti et al. (1969) and Argus et al. (1973) reported on a study in which groups of 28-32 male Charles River CD rats (two to three months old) were administered drinking water containing 0, 0.75, 1.00, 1.40 or 1.80 % of 1,4-dioxane to for 13 months. Doses were estimated by USEPA (2013) as 0, 430, 574, 803, and 1032 mg/kg/day. Animals were sacrificed at 16 months, or earlier if nasal cavity tumors were observed. An additional group of 10 rats was exposed to 1% 1,4-dioxane for electron microscopy studies of the liver after exposure for 5 months (5 rats) or 13 months (5 rats). Tumor data were reported only for the nasal cavity (Hoch-Ligeti et al., 1969) and the liver (Argus et al., 1973). Only nasal tumors visible from gross examination were reported, and histological examination of the nasal cavity was not performed on rats without visible nasal tumors. The number of nasal cavity tumors per group (28-32 rats) was: 0 mg/kg/day – 0; 430 mg/kg/day – 1; 574 mg/kg/day – 1; 803 mg/kg/day – 2; 1032 mg/kg/day – 2. These tumors were observed between approximately 11-16 months after dosing began. In the liver, the incidence of "incipient tumors" (nodules showing all of the histological characteristics of fully developed hepatomas) and hepatomas was reported in the treated groups, but liver tumor data were not provided for the control group. The number of liver tumors increased with dose as follows: 430 mg/kg/day – 4 incipient tumors, 0 hepatomas, 4 total tumors; 574 mg/kg/day – 9 incipient tumors, 0 hepatomas, 9 total tumors; 803 mg/kg/day – 13 incipient tumors, 3 hepatomas, 16 total tumors; 1032 mg/kg/day – 11 incipient tumors, 12 hepatomas, 23 total tumors. Two other types of nodules (one consisting of large cells with reduced cytoplasmic basophilia, the other consisting of large cells filled with fat) often occurred in the livers that had tumors. The electron microscopy studies showed changes in the liver cell ultrastructure comparable to those caused by other hepatic carcinogens (aflatoxin B1; dialkylnitrosamines, and others), with effects progressing between 8 and 13 months of exposure. In addition to the nasal cavity and liver tumors, all dose levels of 1,4-dioxane caused notable histopathological changes in the kidney (incidence not reported).

Hoch-Ligeti and Argus (1970), as summarized by USEPA (2013), provide a "brief account" of a study in which 22 male guinea pigs were exposed to 1,4-dioxane in drinking water for 23-28 months at concentrations varying from 0.5 – 2% over time. USEPA (2013) estimated the dose to 944 – 1019 mg/kg/day. The control group consisted of 10 guinea pigs. In the treated group, two

guinea pigs had carcinoma of the gallbladder, three had early hepatomas, and one had a renal adenoma. The incidence of histopathological changes in the lungs was increased in the dosed group.

Kociba et al. (1974) administered 0, 0.01, 0.1 or 1.0% 1,4-dioxane in drinking water to groups of 60 male and female 6 to 8-week-old Sherman rats for up to 716 days (102 weeks). Based on measured water consumption and body weight data, mean daily doses were calculated as 0, 9.6, 94 and 1015 mg/kg/day in males and 0, 19, 148 and 1599 mg/kg/day in females. Body weight was decreased in the high dose group throughout the study. Mortality in high dose males and females was increase significantly in the first 4 months of the study, with less than 60% of rats surviving, and the rats that died showed degenerative changes in the liver and kidney. The rate of mortality did not differ substantially between control and treated groups starting at month 5, but due to the high early mortality, only 1 male and a small number of females survived until the end of the study. There were no effects on hematological parameters evaluated at 4, 6, 12, 18, and 24 months. The only significant organ weight change was increased absolute and relative liver weight in the few high dose rats that survived until the end of the study. Non-neoplastic histopathological changes were reported in the kidneys (renal tubular epithelial degeneration and regenerative activity) and livers (hepatocellular degeneration and necrosis; hyperplastic nodules) in the mid- and high-dose groups, but not in the low dose groups (incidence not reported). Hepatocellular carcinomas occurred in 1 control, no low dose, 1 mid-dose, and 10 (6 male, 4 female) high dose rats, and nasal carcinomas occurred only in 3 (1 male, 2 female) high-dose rats. The statistical analysis of tumor incidence presented by the authors is based on the number of rats surviving at 12 months, since almost all of the tumors (including all hepatic tumors) were noted at 12 months or later. Total hepatic tumors ($p=0.00022$), hepatocellular carcinomas ($p=0.00033$), and nasal carcinomas ($p=0.05491$) were significantly increased in high-dose rats (males and females combined).

The National Cancer Institute (NCI, 1978) conducted a chronic study in which 0, 0.5 or 1% 1,4-dioxane in drinking water was administered to groups of 35 male and female Osborne-Mendel rats were dosed for 110 weeks and groups of 50 male and female mice B6C3F1 mice for 90 weeks (approximately 4 weeks old). Dosing of the control and high-dose male rats started 1 year after the study began, due to death of the original groups due to an air-conditioning failure. Therefore, the study of the control and high dose males took place at a different time than the study of the females and low dose males.

In rats, mean daily doses were calculated as 0, 240 and 530 mg/kg/day in males, and 0, 350 and 640 mg/kg/day in females, based on measured water consumption and body weight data. There was a statistically significant dose-related increase in mortality in both males and females. Non-neoplastic lesions that were significantly increased in treated groups included renal cortical tubular degeneration (low- and high-dose males; high-dose females), hepatocytomegaly (high-dose females), gastric ulcers (low- and high-dose males), and pneumonia (high-dose females). In

treated rats, there was an increased incidence of tumors of the nasal cavity (squamous cell carcinomas, adenocarcinomas, and one rhabdomyoma) in males and females, liver (hepatocellular adenomas) in females, and testis/epididymis (mesotheliomas; not statistically significant) in males. The first tumors were observed at week 52 in males and week 66 in females. Of these tumor types, the increases in nasal cavity squamous cell carcinomas (0/33, 12/33, 16/34 in control, low-dose, and high-dose females; 0/34, 10/35, 8/35 in control, low-dose, and high-dose males) were statistically significant, as was the increase in hepatocellular adenomas in females (0/31, 10/33, 11/32 in control, low-dose, and high-dose).

In mice, mean daily doses were calculated as 0, 720 and 830 mg/kg/day in males, and 0, 380 and 860 mg/kg/day in females, based on measured water consumption and body weight data. There was a statistically significant dose-related increase in mortality in females beginning at about week 80, while mortality was not affected by treatment in males. The authors stated that differences in body weight between controls and dosed groups in the second year may have been due to fluctuations within the smaller numbers of remaining mice surviving until this time. Non-neoplastic lesions that were significantly increased in treated groups included pneumonia in males and females, and rhinitis in females. In a statistical analysis performed by USEPA (2013), the incidence of pneumonia and rhinitis in low-dose and high-dose females compared to controls was significantly increased at $p < 0.001$. In treated mice, the incidence of hepatocellular carcinomas and hepatocellular adenomas or carcinomas was significantly increased in a dose-related manner in all treated groups. In males, the incidence of carcinomas was 2/49, 18/50, and 24/27, and the incidence of adenomas or carcinomas was 8/49, 19/50, and 28/47, in the control, low-, and high-dose groups, respectively. In females, the incidence of carcinomas was 0/50, 12/48, and 29/37, and the incidence of adenomas or carcinomas was 0/50, 21/48, and 35/37, in the control, low-dose, and high-dose groups, respectively.

Kano et al. (2009) reported on a study in which groups of 50 male and female F344/DuCrj rats and groups of 50 male and female Crj:BDF1 mice were exposed to drinking water containing 0, 500, 2000 or 8000 ppm 1,4-dioxane for 2 years. This study was also reported in a Japan Bioassay Research Center report (JBRC, 1998) and in conference proceedings by Yamazaki et al. (1994).

In rats, mean daily doses were calculated as 0, 11, 55, and 274 mg/kg/day in males, and 0, 18, 83, and 429 mg/kg/day in females, based on measured water consumption and body weight data. Growth rates and terminal body weights were significantly lower in male and female high-dose rats than controls, although food consumption was not affected by treatment. No mortality occurred in controls or treated rats during the first 12 months of the study. At the end of the two-year study, only about 50% of high-dose males and females survived, and survival in this group was significantly lower than in the controls. Kano et al. (2009) attributed the lower survival in the high-dose groups to deaths due to nasal tumors and peritoneal mesotheliomas in males, and nasal and hepatic tumors in females.

USEPA (2013) summarized the hematology and clinical chemistry parameters that were evaluated at the end of the two-year study and reported by JRBC (1998). Decreases in red blood cells, mean corpuscular volume, hemoglobin and hematocrit, and increases in platelets, occurred in high-dose males and females; all of these effects except increased mean corpuscular volume also occurred in mid-dose males. There were significant changes in serum chemistry parameters in the high dose groups. In males, these included increased phospholipids, AST, ALT, LDH, ALP, GGT, CPK, potassium and inorganic phosphorus, and decreased total protein, albumin, and glucose. In females, changes included increased total bilirubin, cholesterol, phospholipids, AST, ALT, LDH, GGT, ALP, CPK and potassium, and decreased blood glucose. Serum enzyme activity was increased by <2 to 17-fold compared to controls, with the largest increases for ALT, AST and GGT. Relative liver weight was increased in mid-dose males and in high-dose males and females.

Data on non-neoplastic histopathological lesions presented by JBRC (1998) and Kano et al. (2009) are summarized in USEPA (2013). Effects occurred in the nasal cavity, liver and kidneys, primarily in the high-dose groups with some effects also seen in the mid-dose groups. Nasal cavity lesions in high-dose males included nuclear enlargement and metaplasia of the olfactory and respiratory epithelia, atrophy of the olfactory epithelium, hydropic changes and sclerosis of the lamina propria, adhesion, and inflammation. In female rats, nuclear enlargement and metaplasia of the respiratory epithelium, squamous cell hyperplasia, respiratory metaplasia of the olfactory epithelium, hydropic changes and sclerosis of the lamina propria, adhesion, inflammation, and proliferation of the nasal gland occurred in the high-dose group, and nuclear enlargement of the olfactory epithelium occurred in both the mid- and high-dose groups. In the liver, spongiosis hepatis and clear and mixed cell foci occurred in mid- and high-dose males, and spongiosis hepatis, cyst formation and mixed cell foci occurred in high-dose females.
In the kidney, nuclear enlargement of the renal proximal tubule occurred in high-dose males and mid- and high-dose females.

Tumors of the liver, nasal cavity and mammary gland were significantly increased in high-dose males and females, and peritoneal mesotheliomas were also increased in high-dose males. Liver tumors were observed beginning at earlier time points in high-dose males and females than in lower dose groups and controls. In high-dose males, the incidence of hepatocellular adenomas and either adenoma or carcinoma were 32/50 and 39/50, respectively, compared to 3/50 for both parameters in controls. Nasal cavity squamous cell carcinomas occurred in 3/50 and 7/50 high-dose males and females, while the incidence in male and female controls was 0/50. Peritoneal mesotheliomas occurred in 28/50 high-dose males and 2/50 controls, and these tumors were the most frequent cause of death in high-dose males. In high dose males, mammary gland fibroadenomas (4/50) and either fibroadenoma or adenoma (6/50) were increased compared to controls (1/50 for both parameters). Similarly, in high-dose females, mammary gland adenomas (16/50) and adenomas or fibroadenomas (18/50) were increased compared to controls (3/50 and 8/50, respectively).

In mice, mean daily doses were calculated as 0, 49, 191, and 677 mg/kg/day in males, and 0, 66, 278, and 964 mg/kg/day in females, based on measured water consumption and body weight data. Growth rates and terminal body weights were significantly lower than control in mid-dose and high-dose males and females, with decreases of 43% and 45%, respectively, in the high-dose males and females. However, food consumption was not significantly affected by treatment. Survival was not affected by treatment in males, but survival in mid- and high-dose females was significantly lower than in controls. Almost all of the deaths in the treated groups occurred during the second year of the study, and most of these deaths were attributed to hepatic tumors.

USEPA (2013) summarized the hematology and clinical chemistry parameters that were evaluated at the end of the two-year study and reported by JRBC (1998). Red blood cell numbers, hemoglobin, and hematocrit were increased in males, and platelets were decreased in mid- and high-dose males and females. Clinical chemistry changes included, among others, increased AST, ALT, LDH, and ALP activities in mid- and high-dose females, and increased CPK activity in high-dose females.

Absolute and relative lung weights were increased in high-dose males and in mid- and high-dose females (JBRC, 1998, reported in USEPA, 2013). Non-neoplastic histopathological changes were observed in the epithelium of the respiratory tract in high-dose and some mid-dose mice and in the proximal tubule of the kidney in high-dose males, as well as and angiectasis (dilation of blood vessels) in the liver in high-dose males.

An increased incidence of liver tumors (adenomas and carcinomas) occurred in treated male and female mice. In males, the incidence of hepatocellular carcinoma and adenoma or carcinoma) was significantly increased in all dose groups, and the incidence of adenoma was statistically increased only in the mid-dose group only. In female mice, the incidence of hepatocellular carcinoma was significantly increased in all dosed groups, and hepatocellular adenoma was increased in the low- and mid-dose groups.

Inhalation studies

Torkelson et al. (1974) exposed male and female Wistar rats (288 per sex) to 111 ppm 1,4-dioxane in whole body inhalation chambers for 7 hours/day, 5 day/week for 2 years. There were 192 controls per sex. 1,4-Dioxane exposure did not affect mortality, body weight gain or organ weights. Slight, but statistically significant, changes in hematology and clinical chemistry parameters were within normal limits and were not considered to be toxicologically relevant by the investigators. No non-neoplastic histopathological changes were associated with treatment. The incidence of various types of tumors did not differ significantly between the control and treated groups. It is noted that no nasal cavity tumors were observed. However, nasal tissues were not examined microscopically, and tumors that are not identified during the gross pathology examination may be identified through the histopathology evaluation.

Kasai et al. (2009) exposed 6-week-old male F344/DuCrj rats (50 per group) to 0, 50, 250 or 1250 ppm 1,4-dioxane in whole body inhalation chambers for 6 hours/day, 5 day/week for 2 years. 1,4-Dioxane did not significantly affect growth rates during the first 5 months of the study, but growth was decreased in all treated groups during the second year of the study, while food consumption was not affected. Survival was significantly decreased following 91 weeks of exposure to 1,250 ppm of 1,4-dioxane, and these deaths were attributed primarily to increased incidences of peritoneal mesotheliomas, with nasal tumors also contributing. Statistically significant changes in hematology and clinical chemistry parameters in the high-dose group at the end of the two-year study included decreased hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin, and increased AST, ALT, ALP and γ-GTP.

Non-tumor histopathological changes occurred at an increased incidence in all dose groups in the nasal cavity, in the mid- and high-dose groups in the kidney, and in the high-dose group in the liver. There was a significant increase in multiple types of tumors. These included squamous cell carcinomas of the nasal cavity, hepatocellular adenomas, renal cell carcinomas, mammary gland fibroadenomas, peritoneal mesotheliomas, and Zymbal gland adenomas. The tumor types with the highest incidence in the high-dose group were peritoneal mesotheliomas (41/50 compared to 2/50 in controls) and hepatocellular adenomas (21/50 compared to 1/50 in controls).

MODE OF ACTION

Genotoxicity

The genotoxicity of 1,4-dioxane has been evaluated in numerous *in vitro* and *in vivo* studies. USEPA (2013) provides a detailed review of the genotoxicity data for 1,4-dioxane that were available at the time that it was written. Two more recent *in vivo* studies, Gi et al. (2018) and Itoh and Hattori (2019), were identified by the Health Effects Subcommittee.

As summarized by USEPA (2013), the majority of *in vitro* genotoxicity tests gave negative results. A number of bacterial mutagenicity assays, with and without metabolic activation, were negative. However, it should be noted that a recent *in vivo* study (Gi et al., 2018 – discussed below) provides evidence that 1,4-dioxane causes mutations in rat liver. Other negative genotoxicity studies include an evaluation of induction of aneuploidy in yeast, a sex-linked recessive lethal test in *Drosophila melanogaster* (fruit flies), a mouse lymphoma forward mutation assay, a study of chromosomal aberrations and micronucleus formation in Chinese hamster ovary (CHO) cells, a study of sister chromatic exchange in CHO cells, and a study of *in vitro* covalent binding to DNA.

Positive *in vitro* genotoxicity studies reported increased meiotic non-disjunction in Drosophila oocytes (Munoz and Barnett, 2002), increased single strand DNA breaks in rat hepatocytes at concentrations that decreased cell viability (Sina et al., 1983), increased sister chromatid

exchange in CHO cells only at the highest dose tested without metabolic activation and not at any dose with metabolic activation (Galloway et al., 1987), and increased transformation of BALB/3T3 cells accompanied by toxicity (Sheu et al, 1988).

Data on micronucleus formation in peripheral blood and bone marrow are mixed. 1,4-Dioxane did not cause micronucleus formation in the bone marrow of B6C3F1, BALB/c, CBA, or C57BL6 mice (McFee et al., 1994; Mirkova, 1994; Tinwell and Ashby, 1994) and male F344/DuCrlCrlj rats (Itoh and Hattori, 2019; summarized below), or in peripheral blood of CD1 mice (Morita and Hayashi, 1998; Morita, 1994). In contrast, dose-related increases in bone marrow micronuclei were reported by in male and female C57BL6 mice (Mirkova, 1994) at the same test conditions that gave negative results in this strain in Tinwell and Ashby (1994), and in CD1 mice (Roy et al., 2005).

All four studies of micronucleus formation in the liver, which is a target tissue for 1,4-dioxane carcinogenicity, were positive (Roy et al., 2005; Morita and Hayashi, 1998; Gi et al., 2018; Itoh and Hattori, 2019). Micronucleus formation in male CD1 mice was increased at doses $\geq$ 2500 mg/kg/day (Roy et al., 2005) and at > 2000 mg/kg/day following partial hepatectomy to induce cellular mitosis (Morita and Hayashi, 1998). Roy et al. (2005) further investigated the origin of the micronuclei in bone marrow and liver. They concluded that 1,4-dioxane causes micronuclei formation primarily through chromosomal breakage, and that the compound can interfere with cell proliferation in both the liver and bone marrow. Itoh and Hattori (2019), summarized below, also found a dose-related increased in hepatic micronuclei in partially hepatectomized rats at all doses tested ($\geq$1000 mg/kg)

Itoh and Hattori (2019) recently reported on a study of hepatic and bone marrow genotoxicity of 1,4-dioxane in male F344/DuCrlCrlj rats (4 per dose group for each of the three partial hepatectomy studies; 5 per dose group for the bone marrow micronuclei and *Pig-a* assay studies); this recent publication was not included in USEPA IRIS (2013). . The effects of 1,4-dioxane on the following endpoints were evaluated: relative liver weight and hepatic micronuclei in partially hepatectomized rats; micronuclei in bone marrow; and the *Pig-a* gene mutation assay in peripheral red blood cells. Dose levels were 0, 1000, 2000, and 3000 mg/kg. In the partial hepatectomy studies, three different methods were used as follows: juvenile method with dosing of 4-week-old rats on two consecutive days four and five days before hepatocytes were collected; dosing of 8 week old rats once on the day before hepatectomy with hepatocytes collected four days after partial hepatectomy; dosing of 8 week old rats once on the day after hepatectomy with hepatocytes collected four days after partial hepatectomy . In the bone marrow micronucleus studies, bone marrow was examined one or two days after a single dose of 1,4-dioxane. For the *Pig-a* assay, effects were evaluated 14 and 29 days after a single dose of 1,4-dioxane.

With all three methods of partial hepatectomy, there was a dose-dependent increase in micronucleated hepatocytes, with statistical significance ($p<0.05$) at all doses except the low

dose (1000 mg/kg) in rats dosed after partial hepatectomy. The incidence of binucleated hepatocytes was increased in the high dose (3000 mg/kg) group dosed before partial hepatectomy; no other effects on cell classification occurred in the partial hepatectomy studies. Relative liver weight was increased only at the low (1000 mg/kg) and mid (2000 mg/kg) doses in the rats dosed after partial hepatectomy. In contrast, 1,4-dioxane did not increase bone marrow micronuclei in this study, and the *Pig-a* assay, which detects inactivating mutations in an X-linked reporter gene in peripheral blood cells, was also negative. The authors concluded the 1,4-dioxane is a hepatic clastogen.

Gi et al. (2018) evaluated mutagenicity and other endpoints related to the mechanism of hepatic carcinogenicity of 1,4-dioxane in *gpt* delta transgenic rats, a model for detection of *in vivo* mutations; this recent publication is not included in USEPA IRIS (2013). Their overall conclusions from the studies, described below, were that "1,4-dioxane is a genotoxic hepatocarcinogen and induces hepatocarcinogenesis through a mutagenic mode of action rats." Male *gpt* delta transgenic F344 rats were given 0, 200, 1000, and 5000 ppm and wild type (WT) F344 rats were given 0, 2, 20, 200, 2000, and 5000 ppm in their drinking water for 16 weeks, and daily doses in mg/kg/day were calculated from body weight and water consumption data. At the highest dose (5000 ppm - 440 mg/kg/day in transgenic rats, 562 mg/kg/day in WT rats), body weight was significantly decreased in WT and transgenic rats, and relative liver weight was slightly increased in transgenic rats. Histopathological changes including hypertrophy, swelling, necrosis, apoptosis or fatty changes were not seen in the liver at any dose in either strain. Glutathione S-transferase placental form (GST-P) foci, a preneoplastic lesion in rat liver, were increased at 2000 ppm (222 mg/kg/day) and 5000 ppm (562 mg/kg/day) in WT rats, and at 5000 ppm (440 mg/kg/day) but not at lower doses ($\leq$ 92 mg/kg/day) in transgenic rats. The BrdU labeling index, an indicator of proliferating cells, was significantly evaluated in livers of WT rats at 5000 ppm, but not at lower doses; it was not evaluated in transgenic rats. The effect of 1,4-dioxane on mutation frequency and the types of mutations in the *gpt* gene was evaluated in the livers of transgenic mice. Mutation frequency, and A:T – G:C transition and A:T – T:A transversion mutations, were increased at 5000 ppm (440 mg/kg/day), and A:T – T:A transversion mutations were also increased at 1000 ppm (92 mg/kg/day). In gene expression studies in livers from transgenic rats, expression of genes involved in cell proliferation (proliferating cell nuclear antigen) and DNA damage repair (O-6-methylguanine-DNA methyltransferase; MGMT) were increased at 5000 ppm (440 mg/kg/day).

Based on the above, Gi et al. (2018) conclude that 1,4-dioxane is mutagenic to liver, its target organ in rats, and that the discrepancy between the negative *in vitro* mutagenicity studies and their positive *in vivo* study may arise from "organ-specific pathways of xenobiotic metabolism and DNA repair *in vivo*." They further conclude that the increased in mutations at 1000 ppm (92 mg/kg/day) and 5000 ppm (440 mg/kg/day), and the increase in cell proliferation and MGMT at 5000 ppm (440 mg/kg/day), indicate that 1,4-dioxane is "a genotoxic carcinogen" that causes

liver tumors through a mutagenic mode of action in rats, and that their data indicate that 1,4-dioxane is mutagenic and carcinogenic in rat liver only above a point of departure.

In a follow-up study (Totsuka et al., 2020), DNA from the livers from the wild type F344 rats in the 0, 20, 200, and 500 ppm groups in the Gi et al. (2018) study were evaluated for the presence of DNA adducts. An increase in DNA adducts compared to the control group was observed in the 200 and 500 ppm groups, but not in the 20 ppm group. Three adducts were associated with 1,4-dioxane treatment. One of these adducts was identified as 8-oxo-2'-deoxyguanosine (8-oxo-dG), which is formed from reactive oxygen species and is associated with oxidative stress. In contrast to the increase in 8-oxo-dG in the wild type rats observed in this study, Gi et al. (2018) reported no differences in 8-oxo-dG levels in 1,4-dioxane treated transgenic rats compared to controls. The other two adducts were not definitively identified but were found to contain thymine or cytosine/uracil groups.

Kitchin and Brown (1990) evaluated biochemical and histopathological changes in female Sprague-Dawley rats dosed with 1,4-dioxane by gavage. Single-strand DNA breaks in hepatocytes were increased at a dose that did not cause histopathological changes. At the doses that caused DNA damage in this study, ornithine decarboxylase and cytochrome P450 were also elevated. These two parameters were stated to be associated with tumor promotion, and the authors suggested that promotion may be involved with 1,4-dioxane carcinogenicity (Kitchin and Brown, 1990).

Hepatocyte DNA synthesis, indicative of cell proliferation, was also increased in several *in vivo* studies (Miyagawa et al., 1999; Uno et al., 1994; Goldsworthy et al., 1991; Stott et al., 1981). However, DNA repair in hepatocytes after *in vitro* or *in vivo* exposure and DNA repair in the nasal cavity after *in vivo* exposure were not affected by 1,4-dioxane (Goldsworthy et al., 1991; Stott et al., 1981), although, as discussed above, Gi et al. (2018) reported increased hepatic expression of a gene indicative of DNA repair.

Additional studies reported that 1,4-dioxane caused transient inhibition of RNA polymerase A and B in rat liver (Kurl et al., 1981), and that DNA alkylation was not detected in the liver of Sprague Dawley rats after gavage exposure (Stott et al., 1981).

Finally, Furihata et al. (2018) compared effects of 1,4-dioxane, two genotoxic hepatic carcinogens (N-nitrosodiethylamine; 3,3’-dimethylbenzidine), and a non-genotoxic hepatic carcinogen (di[2-ethylhexyl]phthalate) on hepatic expression of 11 “marker genes” stated to discriminate genotoxic and non-genotoxic hepatic carcinogens; this recent study was not included in USEPA IRIS (2013). In this study, male F344 rats were dosed with 0.5% (5000 ppm) 1,4-dioxane in drinking water for 4 weeks. Based on drinking water intake for male F344 rats dosed with 4000 or 5000 ppm in drinking water from Kano et al. (2008) and Gi et al. (2018), the daily dose in this study is estimated as 340 – 440 mg/kg/day. The gene expression profile of 1,4-

dioxane differed from the other carcinogens tested, and the authors stated that the gene expression profile of 1,4-dioxane was "intermediate" between that of the "typical" genotoxic and non-genotoxic to which it was compared.

In summary, while many of the genotoxicity studies reviewed above were negative, others provide evidence for mutagenicity and chromosomal damage, including some *in vivo* studies. Notably, a recent *in vivo* study (Gi et al., 2018 – discussed above provides evidence that 1,4-dioxane causes mutations in rat liver. Totsuka et al., 2020 also identified specific DNA adducts. Additionally, 1,4-dioxane induced micronuclei in the liver in all three studies where this effect was evaluated.

Tumor initiation and promotion studies

Bull et al. (1986) reported that 1,4-dioxane was negative for cancer initiation in an initiator/promoter test in female SENCAR mice (6–8 weeks old). The mice were administered a single dose of 1,000 mg/kg by gavage, subcutaneous injection or topical application, followed by dermal application of a tumor promoter (1 µg of 12-O-tetradecanoylphorbol-13-acetate; TPA) or acetone (control) 3 times per week for 20 weeks. At 24 weeks, the formation of papillomas was not increased in mice treated with 1,4-dioxane and the promoter, and no tumors occurred in mice treated only with only 1,4-dioxane.

In a study in mice by King et al. (1973), 1,4-dioxane was negative as a complete carcinogen and was positive as a tumor promoter. Swiss Webster mice (30 per sex per treatment group) were dosed dermally with: 0.2 ml of a solution of 1,4-dioxane (concentration not provided) 3 times/week for 78 weeks; 0.2 ml of a solution of 1,4-dioxane 3 times/week for 78 weeks and with a tumor initiator (50 µg of dimethylbenzanthracene) one week before 1,4-dioxane dosing began; or only with the tumor initiator. In mice dosed with both the initiator and 1,4-dioxane, only 4 male and 5 female mice survived for 60 weeks (compared to 22 males and 25 females dosed only with 1,4-dioxane, and 20 males and 26 females dosed only with the initiator). 1,4-dioxane did not cause skin tumors in the absence of the initiator. In contrast, the incidence and multiplicity of skin tumors was higher in mice treated with both 1,4-dioxane and the initiator than in mice treated only with the initiator. Tumors of the lung and kidney also occurred in mice treated with 1,4-dioxane and the initiator.

Lundberg et al. (1987) reported that 1,4-dioxane is a promoter of liver tumors in rats. Partially hepatectomized male Sprague Dawley rats (9-11 per group) were dosed with: a tumor initiator (diethylnitrosamine, 30 mg/kg by intraperitoneal injection); 1,4-dioxane at 100 or 1,000 mg/kg/day by gavage, 5 days per week for 7 weeks; the initiator and 1,4-dioxane; or neither compound (controls). When evaluated 10 days after the last dose of 1,4-dioxane, the number and volume of hepatic GGT-foci were increased in rats dosed with both the initiator and 1,000 mg/kg/day 1,4-dioxane as compared to the rats dosed with only the initiator. Histopathological changes including enlarged, foamy hepatocytes containing numerous fat-containing cytoplasmic

vacuoles were observed in the livers of rats dosed with 1,000 mg/kg/day 1,4-dioxane, regardless of whether or not they had been treated with the initiator.

Finally, as mentioned above, Kitchin and Brown (1990) reported that hepatic ornithine decarboxylase and cytochrome P450, which were stated to be associated with tumor promotion, were elevated in female Sprague-Dawley rats at a 1,4-dioxane dose that caused single-strand DNA breaks but did not cause histopathological changes in hepatocytes (Kitchin and Brown, 1990).

REFERENCE DOSE FOR NON-CANCER EFFECTS AND CANCER SLOPE FACTOR

USEPA (2013) Reference Dose for non-carcinogenic effects

As mentioned above, USEPA (2018) and numerous states have used the USEPA (2013) cancer slope factor as the basis for their 1,4-dioxane drinking water guidelines, and the Health Effects Subcommittee agrees that the MCLG should be based on this approach. The USEPA (2013) Reference Dose for non-carcinogenic effects of oral exposure to 1,4-dioxane is presented here for the sake of completeness.

USEPA (2013) identified histopathological lesions of the liver and kidney (renal tubular epithelial and hepatocellular degeneration and necrosis) in rats in the Kociba et al. (1974) drinking water study as the most sensitive non-carcinogenic effect of oral exposure to 1,4-dioxane. Kociba et al. (1974) reported that these effects occurred in the mid- and high-dose groups but not in the control or low-dose groups, but they did not provide incidence data for the mid- and high-dose groups. The USEPA (2013) Reference Dose is based on the mean daily doses in males (0, 9.6, 94, and 1,015 mg/kg/day) reported by Kociba et al. (1974), since it is assumed these lesions occurred in both sexes and that the doses in males were lower than the doses in females (0, 19, 148, and 1,599 mg/kg/day).

Because the incidence of the lesions was not reported, Benchmark Dose (BMD) modeling could not be used in RfD development. Therefore, the NOAEL of 9.6 mg/kg/day was used as the point of departure (POD) for the Reference Dose. A total uncertainty factor of 300 (10 for interindividual variation; 10 for animal-to-human extrapolation; 3 for database deficiencies including lack of a multigeneration reproductive toxicity study) was applied to the NOAEL of 9.6 mg/kg/day to derive the RfD of 0.03 mg/kg/day.

Weight of evidence for carcinogenicity

Data relevant to carcinogenicity of 1,4-dioxane (summarized above) come from seven studies in rats (five drinking water; two inhalation), two drinking water studies in mice, and one drinking water study in guinea pigs. Sites at which tumors were increased in at least one dosed group in these studies are shown in Table 3 above. In summary, liver tumors were increased in the studies of rats, mice and guinea pigs, with the exception of female rats in the NCI (1978) drinking water

study and both sexes of rats in the Torkelson et al. (1974) inhalation study. Additionally, nasal cavity tumors in rats were increased in four drinking water studies and one inhalation study. Torkelson et al. (1974) state that nasal tumors were not observed in any animals. However, as noted by USEPA (2013), histopathological examination was not performed on nasal tissue in this study, so that nasal tumors that were not detected during the gross pathology examination would not have been identified through histological evaluation. Increases of tumors at several other sites (mammary gland, mesothelioma of the peritoneum or testis/epididymis, kidney, zymbal gland, subcutis fibroma) were also reported in one or more rat studies.

1,4-Dioxane is described by USEPA IRIS (2013) and USEPA OCSPP (2020) as "likely to be carcinogenic to humans" as defined in the USEPA (2005) Guidelines for Carcinogen Risk Assessment. The Health Effects Subcommittee agrees with this USEPA (2013) conclusion, and it notes that the data shown in Table 3 clearly show that 1,4-dioxane fulfills the following USEPA (2005) criterion for this descriptor: "…tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans." The Health Effects Subcommittee also agrees with USEPA (2013) and USEPA (2020) that the "likely to be carcinogenic to humans" descriptor applies to oral and inhalation exposure, since 1,4-dioxane caused tumors at sites remote from the portal of entry/site of absorption in oral and inhalation studies. Similarly, the International Agency for Research on Cancer (IARC, 1999) classified 1,4-dioxane as Group 2B (possibly carcinogenic to humans) based on inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals, and the National Toxicology Program (NTP, 2011) concluded that 1,4-dioxane is "reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies of experimental animals."

Selection of dose-response approach for cancer risk assessment

According to the USEPA (2005) Guidelines for Carcinogen Risk Assessment, an approach based on a non-threshold dose-response relationship (i.e. linear low-dose extrapolation; cancer slope factor) is used when the mode of action for carcinogenicity has not been conclusively established, or if a mutagenic mode of action has been established. An approach based on a threshold dose-response relationship (i.e. a Reference Dose) is used when a mode of action for carcinogenicity that is not linear at low doses (i.e. a threshold for carcinogenicity exists) has been clearly established. Information relevant to the choice of non-threshold or threshold approach for cancer risk assessment of 1,4-dioxane is discussed below.

USEPA IRIS (2013), USEPA OCSPP; 2020), NJDEP (2015, 2018), and the other states listed in Table 1 all base their risk assessments for 1,4-dioxane on a non-threshold dose-response (i.e., a slope factor). In contrast, Health Canada (2018) concluded that "analysis supports a non-genotoxic mode of action involving cytotoxicity followed by regenerative hyperplasia and stimulation of endogenously formed mutations. Since 1,4-dioxane acts through a non-genotoxic mode of action and is known to operate via non-linear kinetics, a non-linear (threshold) risk

assessment is considered appropriate." It is noted that Health Canada's decision relied heavily on the conclusions of Dourson et al. (2014, 2017), which are discussed in detail below.

The Health Effects Subcommittee reviewed two publications by Dourson et al. (2014, 2017), as well as the recent Lafranconi et al. (2020) publication, that conclude that 1,4-dioxane is carcinogenic through a threshold mode of action (MOA) and that the default non-threshold (linear low-dose extrapolation) approach for cancer risk assessment is therefore not appropriate. NJDEP (2015, 2018) responded to the conclusions of the two Dourson et al. (2020) publications when they were submitted in comments on the draft Interim Ground Water Quality Standard for 1,4-dioxane in 2015 (Dourson et al., 2014) and the proposed Ground Water Quality Standard for 1,4-dioxane in 2018 (Dourson et al., 2017). NJDEP (2015, 2018) concluded that the mode of action of 1,4-dioxane carcinogenicity remains unknown, and that the analyses presented in Dourson et al. (2014; 2017) do not conclusively establish a threshold mode of action. Similarly, USEPA OCSPP (2020) also reviewed Dourson et al. (2014, 2017), as well as unpublished data from the study reported in Lafranconi et al. (2020), and other relevant data. USEPA OCSPP (2020; Appendix J) presents a detailed mode of action evaluation that considers all of the data that was reviewed and concludes that a threshold mode of action has not been established and that a non-threshold (slope factor) approach should be used for cancer risk assessment of 1,4-dioxane. The Health Effect Subcommittee agrees with NJDEP (2018) that "the data and explanations provided by Dourson et al. (2017) do not establish a firm or unique link to the proposed mode of action, and they do not indicate that a threshold approach is appropriate for risk assessment for [1,4-dioxane]." Health Effects Subcommittees conclusions about specific points presented by Dourson et al. (2014, 2017) are discussed in Appendix 1. Additionally, as discussed in the section on subchronic animal studies above, the Subcommittee concludes that Lafranconi et al. (2020) does not demonstrate a threshold mode of action for liver tumors caused by drinking water exposure to 1,4-dioxane in female mice.

As shown in Table 3 above, 1,4-dioxane increased the incidence of nasal tumors in rats exposed orally and through inhalation, suggesting that these tumors do not occur only as a point of contact effect. Kasai et al. (2009) suggested a mode of action involving both induction of metabolic enzymes, cytotoxicity, and regenerative cell proliferation and genotoxicity. However, as noted by USEPA (2013), Kasai et al. (2009) did not observe cytotoxicity in the nasal cavity. USEPA (2013) further notes that "nasal lesions, including inflammation, hyperplasia, and metaplasia, were frequently seen in inhalation studies conducted by the NTP with no evidence of nasal carcinogenicity…)." OCSPP (2020) further notes that 1,4-dioxane caused several types of rare nasal tumors that were not reported in historical control data in both rats and mice, and that these rare tumors are unlikely to occur through a cytotoxic mode of action. The Health Effects Subcommittee agrees with the USEPA IRIS (2013) and USEPA OCSPP (2020) conclusions that, based on the information discussed above, the mode of action for the nasal tumors has not been established. Similarly, the mode of action for other tumors reported to have been caused by 1,4-

dioxane (kidney, lung, peritoneal mesothelioma, mammary gland, Zymbal gland, subcutis tumors) has not been established.

In summary, the mode of action for 1,4-dioxane carcinogenicity has not been conclusively established, and the USEPA (2005) Guidelines for Carcinogen Risk Assessment specify that a non-threshold approach (i.e., linear low-dose extrapolation; cancer slope factor) is to be used in such cases. Therefore, the Health Effects Subcommittee agrees with the USEPA (2103, 2020) decision to use linear low-dose extrapolation (a cancer slope factor) for cancer risk assessment of 1,4-dioxane.

Cancer slope factor derivation

Table 4 provides incidence data for liver tumors in mice and rats, and for nasal cavity, peritoneal, and mammary gland tumors in rats, from the Kociba et al. (1974), NCI (1978), and Kano et al. (2009) studies of exposure to 1,4-dioxane in drinking water.

Table 4: Incidence of liver, nasal cavity, peritoneal, and mammary gland tumors in rats and mice exposed to 1,4-dioxame in drinking water for 2 years (based on survival to 12 months) (USEPA, 2013)

			Tumor Incidence			
Study	***Species/strain/sex***	***Dose (mg/kg/day)***	***Liver***	***Nasal Cavity***	***Peritoneal***	***Mammary gland***
Kociba et al. (1974)	Sherman rats, male and female combined [a,b]	0	1/106[h]	0/106[h]	NA	NA
		14	0/110	0/110	NA	NA
		121	1/106	0/106	NA	NA
		1307	10/66[i]	3/66	NA	NA
NCI (1978)	Male Osborne-Mendel rats[b]	0	NA	0/33[h]	NA	NA
		240	NA	12/26	NA	NA
		530	NA	16/33[i]	NA	NA
	Female Osborne-Mendel rats[b,c]	0	0/31[h]	0/34[h]	NA	NA
		350	10/30	10/30[i]	NA	NA
		640	11/29	8/29[i]	NA	NA
	Male B6C3F1 mice[d]	0	8/49[h]	NA	NA	NA
		720	19/50[i]	NA	NA	NA
		830	28/47[i]	NA	NA	NA
	Female B6C3F1 mice[d]	0	0/50[h]	NA	NA	NA
		380	21/48[i]	NA	NA	NA
		860	35/37[i]	NA	NA	NA
Kano et al. (2009)	Male F344/DuCrj rats[d,e,f,g]	0	3/50	0/50	1/50	8/50
		11	4/50	0/50	0/50	8/50
		55	7/50	0/50	0/50	11/50
		274	39/50[j,k]	7/50[j,k]	0/50	18/50[j,k]
	Female F344/DuCrj rats[d,e,f,g]	0	23/50	0/50	NA	NA
		18	31/50	0/50	NA	NA
		83	37/50	0/50	NA	NA
		429	40/50	8/50	NA	NA
	Male Crj:BDF1 mice[d]	0	5/50	0/50	NA	NA
		49	35/50	0/50	NA	NA
		191	37/50[i]	0/50	NA	NA
		677	40/50[j,k]	1/50	NA	NA
	Female Crj:BDF1 mice[d]	0	5/50	0/50	NA	NA
		66	35/50[j]	0/50	NA	NA
		278	41/50[j]	0/50	NA	NA
		964	46/50[j,k]	1/50	NA	NA

[a] Incidence of hepatocellular carcinoma.
[b] Incidence of nasal squamous cell carcinoma.
[c] Incidence of hepatocellular adenoma.
[d] Incidence of hepatocellular adenoma or carcinoma.
[e] Incidence of all types of nasal tumors combined.
[f] Incidence of peritoneal mesotheliomas.
[g] Incidence of mammary gland fibroadenomas or carcinomas.
[h] $p < 0.05$; positive dose-related trend (Cochran-Armitage or Peto's test).
[i] Significantly different from control at $p < 0.05$ by Fisher's exact test.
[j] Significantly different from control at $p < 0.01$ by Fisher's exact test.
[k] $p < 0.01$; positive dose-related trend (Peto test).
NA = data not available for modeling.

Benchmark Dose (BMD) modeling was performed by USEPA (2013) using the dichotomous models included in Benchmark Dose Software (BMDS, version 2.1.1) for the data on the incidence of liver tumors (hepatocellular carcinoma or adenoma) in rats and mice, and nasal

tumors, peritoneal mesotheliomas, and mammary gland tumors in rats from Kano et al. (2009), NCI (1978), and Kociba et al. (1974). When deriving a cancer slope factor, the point of departure is the BMDL, which is the 95% lower confidence limit on the dose associated with a benchmark response (BMR) near the lower end of the observed data from the study. For 1,4-dioxane, modeling was performed using a BMR of 10%, and, as discussed below, additional modeling based on BMRs of 30% and 50% was conducted for the female mouse hepatic tumor data from Kano et al. (2009). The BMD (dose associated with the BMR) and BMDLs were first calculated based on the doses administered to the animals. The BMDs and BMDLs based on the animal doses were then converted by USEPA (2013) to the BMD_{HED} and $BMDL_{HED}$, which are the BMDs and BMDLs based on Human Equivalent Doses, using the default body weight (BW) scaling factor of $BW^{0.75}$ (U.S. EPA, 2011b), time-weighted average animal body weight data, and an assumed human body weight of 70 kg, as follows:

$$HED = \text{animal dose (mg/kg)} \times (\text{animal BW [kg]}/\text{human BW [kg]})^{0.25}$$

The dose-response data (Table 4-above) and cancer slope factors (Table 5-below) indicate that liver tumors in female mice (observed in both Kano et al., 2009 and NCI, 1978) are more sensitive to 1,4-dioxane than the other tumor types observed in rats and mice in Kano et al. (2009), NCI (1978) and Kociba et al. (1974). USEPA (2013) selected the hepatic tumors in female mice in the drinking water study conducted by Kano et al. (2009) as the basis for the cancer slope factor. NCI (1978) was not selected by USEPA (2013) because it included only two dose levels while Kano et al. (2009) used three dose levels, and because the lowest dose in NCI (1978) was much higher than in Kano et al. (2009). Kociba et al. (1974) was not selected by USEPA (2013) because it did not include mice and reported only hepatocellular carcinomas but not adenomas. In regard to the use of the Kano et al. (2009) mouse liver tumor data as the basis for risk assessment, USEPA (2013) notes that the background incidence of liver tumors is similar in the BDF1 strain of mice used by Kano et al. (2009) as in the B6C3F1 strain used by the National Toxicology Program and concludes that "the BDF1 mouse is not particularly sensitive compared to the commonly used B6C3F1 strain" (USEPA, 2013).

The dose-response curve for the female mouse hepatic tumor data from Kano et al. (2009) is very steep at the low dose and plateaus at a very high tumor incidences in the two higher doses; control, low-, mid-, and high-dose incidence are 10%, 70%, 82%, and 92% respectively. The log-logistic model was the only model that provided an adequate fit to these data (USEPA, 2013). Since the response level (70%) at the lowest dose in the study (Kano et al., 2009) was much higher than the initial BMR of 10%, modeling was also performed using the log-logistic model for BMRs of 30 and 50%. USEPA (2013) selected the human equivalent dose BMDL for a BMR of 50% ($BMDL_{50\text{-}HED}$ of 4.95 mg/kg/day) for female mouse liver tumors in Kano et al., 2009 as the point of departure for deriving for the cancer slope factor. The slope factor was calculated as follows:

$$CSF = BMR/BMDL_{50\text{-}HED} = 0.5 / 4.95 \text{ mg/kg/day} = 0.10 \text{ (mg/kg/day)}^{-1}$$

Similarly, USEPA OCSPP (2020) stated that the hepatic tumors in female mice from Kano et al. (2009) could not be modeled using the standard multistage models because of the steep dose-response curve which was followed by an apparent plateau at higher doses. USEPA OCSPP (2020) obtained individual animal data from the study authors, and they used time-to-tumor modeling with the Multistage Weibull Model and a BMR of 50%. The resulting slope factor of 0.12 $(mg/kg/day)^{-1}$ is very close to, but slightly more stringent, than the USEPA IRIS (2013) slope factor of 0.10 $(mg/kg/day)^{-1}$.

Since USEPA IRIS risk assessments represent the consensus of multiple USEPA programs and have been established as a source of toxicity factors for risk assessments developed by New Jersey, the USEPA IRIS slope factor of 0.10 $(mg/kg/day)^{-1}$ is used as the basis for the recommended Health-based MCL.

Table 5. Oral cancer slope factors for best-fit models for tumor incidence data for rats and mice exposed to 1,4-dioxane in drinking water for 2 years (*adapted from USEPA, 2013*)

Study	*Gender/strain/species*	*Tumor type*	*BMR*	*Oral Cancer Slope Factor (mg/kg/day)$^{-1}$*	*Model*
Kano et al. (2009)	Male F344/DuCrj rats	Hepatocellular adenoma or carcinoma	0.1	0.007	Probit, slope parameter not restricted
	Female F344/DuCrj rats		0.1	0.0069	Multistage, degree of polynomial=2
	Male Crj:BDF1 mice		0.1	0.037	Log-logistic, slope restricted $\geq$ 1
	Female Crj:BDF1 mice		0.1	0.18	
			0.3	0.14	
			0.5	0.10	
	Female F344/DuCrj rats	Nasal squamous cell carcinoma	0.1	0.0014	Multistage, degree of polynomial=3
	Male F344/DuCrj rats		0.1	0.0015	
	Male F344/DuCrj rats	Peritoneal mesothelioma	0.1	0.0047	Probit, slope parameter not restricted
	Female F344/DuCrj rats	Mammary gland adenoma	0.1	0.0049	Log-logistic, slope restricted $\geq$ 1
Kociba et al. (1974)	Male and female combined Sherman rats	Nasal squamous cell carcinoma	0.1	0.00029	Multistage, degree of polynomial=3
		Hepatocellular carcinoma	0.1	0.00042	Probit, slope parameter not restricted
NCI (1978)	Male Osborne-Mendel rats	Nasal squamous cell carcinoma	0.1	0.0094	Log-logistic, slope restricted $\geq$ 1
	Female Osborne-Mendel rats		0.1	0.0039	
	Female Osborne-Mendel rats	Hepatocellular adenoma	0.1	0.0054	
	Female B6C3F1 mice	Hepatocellular adenoma or carcinoma	0.1	0.01	Multistage, degree of polynomial=2
	Male B6C3F1 mice		0.1	0.0028	Gamma

DEVELOPMENT OF RECOMMENDED HEALTH-BASED MAXIMUM CONTAMINANT LEVEL

Updated drinking water exposure assumptions

The USEPA (2015) has updated its default assumptions for body weight and drinking water consumption used in calculation of health-based water values. USEPA (2015) updated the default adult body weight from 70 kg to 80.0 kg based on the mean body weight for adults age

21 and older in National Health and Nutrition Examination Survey (NHANES) from 1999-2006 (USEPA, 2011). The previous value of 70 kg was stated by USEPA (2015) to have been based on the mean adult body weight from NHANES III (1988-1994). USEPA (2015) also updated the default drinking water consumption rate to 2.4 L/day based on the estimated 90th percentile of community water ingestion for adults ages 21 and older in NHANES 2003-2006 (USEPA, 2011b). The previous value of 2 L/day was stated by USEPA (2015) to have been based on the 86th percentile of community water ingestion for adults from the US Department of Agriculture's 1994-1996 Continuing Survey of Food Intake by Individuals (CSFII) analysis and the 88th percentile of adults in the National Cancer Institute study of the 1977-1978 Nationwide Food Consumption Survey. These updated values were used by the Health Effects Subcommittee to develop the Health-based MCL for 1,4-dioxane, and they will also be used when Health-based MCLs for other contaminants are developed in the future.

Health-based MCL based on non-cancer effects

As discussed above, it is well established that non-carcinogenic effects are less sensitive endpoints than carcinogenicity for 1,4-dioxane. The health-based MCL based on the Reference Dose for non-cancer effects (0.03 mg/kg/day; 30 µg/kg/day) and default exposure assumptions is presented here for comparison purposes.

$$\frac{30\ \mu g/kg/day \times 80.0\ kg \times 0.2}{2.4\ L/day} = 200\ \mu g/L$$

Where: 80.0 kg is the assumed body weight of an adult, 2.4 L/day is the default value for daily water consumption of an adult, and 0.2 (20%) is the default Relative Source Contribution factor.

Health-based MCL based on carcinogenicity

The Health-based MCL for 1,4-dioxane is based on the one-in-one million (10^{-6}) risk of cancer from lifetime exposure to carcinogens specified in the 1984 Amendments to the New Jersey Safe Drinking Water Act (SDWA) at N.J.S.A. 58:12A-20.

The daily dose of 1,4-dioxane predicted to result in a one-in-one-million lifetime cancer risk is calculated from the slope factor of 0.10 $(mg/kg/day)^{-1}$ as:

$$10^{-6} / 0.10\ (mg/kg/day)^{-1} = 1 \times 10^{-5}\ mg/kg/day = 0.01\ \mu g/kg/day$$

The Health-based Maximum Contaminant Level for 1,4-dioxane based on this daily dose is:

$$\frac{0.01\ \mu g/kg/day \times 80\ kg}{2.4\ L/day} = 0.33\ \mu g/L$$

Where: 80 kg is the assumed body weight of an adult and 2.4 L/day is the default value for daily water consumption of an adult.

This Health-based MCL is far below the Health-based MCL based on non-carcinogenic effects of 200 µg/L.

RECOMMENDATION:

The recommended Health-based Maximum Contaminant Level for 1,4-dioxane is 0.33 µg/L.

REFERENCES

Abe A. (1999). Distribution of 1,4-dioxane in relation to possible sources in the water environment. Sci Total Environ. 227: 41-47.

Adamson, DT, Anderson, RH, Mahendra, S. and Newell, CJ (2015). Evidence of 1,4-dioxane attenuation at groundwater sites contaminated with chlorinated solvents and 1,4-dioxane. Environ Sci Technol 49: 6510-6518.

Adamson DT, Piña EA, Cartwright AE, et al. (2017). 1,4-Dioxane drinking water occurrence data from the third unregulated contaminant monitoring rule. Sci Total Environ. 596-597: 236–245.

Aĭlamazian, EK (1990). Effect of ecological factors on the course of pregnancy. Vestnik Akademii Medicinskih Nauk S.S.S.R. 7: 23–25, cited in Health Canada (2018).

Anderson, RH, Anderson, JK, and Bower, PA (2012). Co-occurrence of 1,4-dioxane with trichloroethylene in chlorinated solvent groundwater plumes at US Air Force installations: Fact or fiction. Integr Environ Assess Manag 8: 731-737.

Akron (2009). The Chemical Database. The Department of Chemistry at the University of Akron. http://ull.chemistry.uakron.edu/erd and search on CAS number. Last accessed: 3/23/19.

Argus, MF; Arcos, JC; Hoch-Ligeti, C. (1965). Studies on the carcinogenic activity of protein-denaturing agents: Hepatocarcinogenicity of dioxane. J Natl Cancer Inst 35: 949-958.

Argus, MF; Sohal, RS; Bryant, GM; Hoch-Ligeti, C; Arcos, JC. (1973). Dose-response and ultrastructural alterations in dioxane carcinogenesis. Influence of methylcholanthrene on acute toxicity. Eur J Cancer 9: 237-243.

ATSDR (2005). Health Consultation. 1,4-Dioxane in Private Drinking Water Near Naval Air Station Whidbey Island, Ault Field. Agency for Toxic Substances and Disease Registry. Cited by NTP (2016). National Toxicology Program. Department of Health and Human Services. Report on Carcinogens, Fourteenth Edition. 1,4-Dioxane Cas No. 123-91-1. Accessed June 7, 2020. https://ntp.niehs.nih.gov/ntp/roc/content/profiles//dioxane.pdf

ATSDR (2012). Toxicological Profile for 1,4-Dioxane. Agency for Toxic Substances and Disease Registry. Accessed January 30, 2019. https://www.atsdr.cdc.gov/toxprofiles/tp187.pdf.

Barber, H. (1934). Haemorrhagic nephritis and necrosis of the liver from dioxane poisoning. Guy's Hosp Rep 84:267-280, cited in USEPA (2013).

Buffler, PA; Wood, SM; Suarez, L; Kilian, DJ. (1978). Mortality follow-up of workers exposed to 1,4-dioxane. J Occup Environ Med 20: 255-259, cited in USEPA (2013).

Bull, RJ; Robinson, M; Laurie, RD. (1986). Association of carcinoma yield with early papilloma development in SENCAR mice. Environ Health Perspect 68: 11-17.

CDC (2018). Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. 2015-2016 Data Documentation, Codebook, and Frequencies. Volatile Organic Compounds and Trihalomethanes. Accessed February 24, 2020. https://wwwn.cdc.gov/Nchs/Nhanes/2015-2016/VOCWB_I.htm

Chappell, G.A., Heintz, M.M., Haws, L.C. (2021). Transcriptomic analyses of livers from mice exposed to 1,4-dioxane for up to 90 days to assess potential mode(s) of action underlying liver tumor development. Current Research in Toxicology 2: 30-41.

Charkoftaki, G., Golla, J. P., Santos-Neto, A., Orlicky, D. J., Garcia-Milian, R., Chen, Y., Rattray, N., Cai, Y., Wang, Y., Shern, C. T., Mironova, V., Wang, Y., Johnson, C. H., Thompson, D. C., & Vasiliou, V. (2021). Identification of dose-dependent DNA damage and repair responses from subchronic exposure to 1,4-dioxane in mice using a systems analysis approach. Toxicological Sciences. Advance online publication, https://doi.org/10.1093/toxsci/kfab030.

David, H. (1964). Electron-microscopic findings in dioxan-dependent nephrosis in rat kidneys. Beitr Pathol Anat 130: 187-212. Cited in USEPA (2013).

de Navasquez, S. (1935). Experimental tubular necrosis of the kidneys accompanied by liver changes due to dioxane poisoning. J Hyg 35: 540-548. Cited in USEPA (2013).

DeRosa CT, Wilbur S, Holler J, Richter P, Stevens YW. Health evaluation of 1,4-dioxane. Toxicol Ind Health. 1996 Jan-Feb;12(1):1-43. Cited in ATSDR (2012).
Dourson, M, Reichard, J, Nance, P, Burleigh-Flayer, H, Parker, A, Vincent, M, and McConnell, EE. (2014). Mode of action analysis for liver tumors from oral 1,4-dioxane exposures and evidence-based dose response assessment. Reg Toxicol Pharmacol 68: 387–401.

Dourson, ML., Higginbotham, J, Crum, J, Burleigh-Flayer, H, Nance, P, Forsberg, ND, Lafranconi, M, & Reichard, J. (2017). Update: Mode of action (MOA) for liver tumors induced by oral exposure to 1,4-dioxane. Reg Toxicol Pharmacol 88: 45–55.

DWQI (1987). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. March 26, 1987.

DWQI (1994). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. March 26, 1987.

DWQI (2009). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Hazardous Contaminants in Drinking Water. March, 2009a.

DWQI (2015). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Perfluorononanoic Acid in Drinking Water. July 1, 2015. http://www.nj.gov/dep/watersupply/pdf/pfna-recommend-final.pdf

DWQI (2017). New Jersey Drinking Water Quality Institute. Maximum Contaminant Level Recommendations for Perfluorooctanoic Acid in Drinking Water. March 2017. https://www.state.nj.us/dep/watersupply/pdf/pfoa-recommend.pdf

Environment Canada and Health Canada (2010). Screening Assessment for the Challenge 1,4-Dioxane. March 2010. Accessed February 2020. https://ec.gc.ca/ese-ees/default.asp?lang=En&n=789BC96E-1

Ernstgard, L; Iregren, A; Sjogren, B; Johanson, G. (2006). Acute effects of exposure to vapours of dioxane in humans. Hum Exp Toxicol 25: 723-729.

Ernstgård L, Bottai M, Johanson G, Sjögren B. (2019). Down-regulation of the inflammatory response after short-term exposure to low levels of chemical vapours. Occup Environ Med. 76: 482-487.

EU Commission Directive 2003/95/EC (2003). Accessed February 24, 2020. https://op.europa.eu/en/publication-detail/-/publication/d16dea9d-1ce7-4c21-86d4-39cefc9a987f

EU (2002). European Union Risk Assessment Report - 1,4-Dioxane. European Chemicals Bureau. Institute for Health and Consumer Protection. Accessed February 24, 2020 https://echa.europa.eu/documents/10162/a4e83a6a-c421-4243-a8df-3e84893082aa

Fairley, A; Linton, EC; Ford-Moore, AH. (1934). The toxicity to animals of 1:4 dioxan. J Hyg 34: 486-501. Cited in USEPA (2013).

Frantik, E, Hornychova, M, Horvath, M. (1994). Relative acute neurotoxicity of solvents: Isoeffective air concentrations of 48 compounds evaluated in rats and mice. Environ Res 66: 173-185. Cited in USEPA (2013).

Furihata C, Toyoda T, Ogawa K, Suzuki T. (2018). Using RNA-Seq with 11 marker genes to evaluate 1,4-dioxane compared with typical genotoxic and non-genotoxic rat hepatocarcinogens. Mutat Res Genet Toxicol Environ Mutagen. 834:51-55.

Gi M, Fujioka M, Kakehashi A, Okuno T, Masumura K, Nohmi T, Matsumoto M, Omori M, Wanibuchi H, Fukushima S. (2018). In vivo positive mutagenicity of 1,4-dioxane and quantitative analysis of its mutagenicity and carcinogenicity in rats. Arch Toxicol. 92: 3207-3221.

Giavini, E, Vismara, C, Broccia, ML. (1985). Teratogenesis study of dioxane in rats. Toxicol Lett 26: 85-88. Cited in USEPA (2013).

Godri Pollitt KJ, Kim JH, Peccia J, et al. (2019). 1,4-Dioxane as an emerging water contaminant: State of the science and evaluation of research needs. Sci Total Environ. 690:853-866.

Goldberg, ME, Johnson, HE, Pozzani, UC, Smyth, H Jr. (1964). Effect of repeated inhalation of vapors of industrial solvents on animal behavior: I. Evaluation of nine solvent vapors on pole-climb performance in rats. Am Ind Hyg Assoc J 25: 369-375. Cited in USEPA (2013).

Goldsworthy, TL, Monticello, TM, Morgan, KT, Bermudez, E, Wilson, DM, Jäckh, R,; BE, B. (1991). Examination of potential mechanisms of carcinogenicity of 1,4-dioxane in rat nasal epithelial cells and hepatocytes. Arch Toxicol 65: 1-9.

Hartung, R (1989) Health and environmental effects assessment for 1,4-dioxane. Michigan, USA, Gelman Sciences Inc. Cited in European Union (2002).
Health Canada (2018). 1,4-Dioxane in Drinking Water - Guideline Technical Document for Public Consultation. https://www.canada.ca/content/dam/hc-sc/documents/programs/consultation-1-4-dioxane-drinking-water/pub-eng.pdf. Accessed April 10, 2019.

Health Council of the Netherlands (2015). 1,4-Dioxane: Re-evaluation of the carcinogenicity and genotoxicity. Subcommittee on the Classification of Carcinogenic Substances of the Dutch Expert Committee of the Health Council of the Netherlands. No. 2015/26, The Hague, November 13, 2015. Accessed February 24, 2020. https://www.healthcouncil.nl/documents/advisory-reports/2015/11/13/14-dioxane-re-evaluation-of-the-carcinogenicity-and-genotoxicity

Hoch-Ligeti, C, Argus, MF. (1970). Effect of carcinogens on the lung of guinea pigs. In P Nettlesheim; MG HannaJr; JW DeatherageJr (Eds.), Morphology of Experimental Respiratory Carcinogenesis: Proceedings of a Biology Division, Oak Ridge National Laboratory, Conference

held in Gatlinburg, Tennessee, May 13-16, 1970 (pp. 267-279). Oak Ridge, TN: United States Atomic Energy Comission, Division of Technical Information.

Hoch-Ligeti, C, Argus, MF, Arcos, JC. (1970). Induction of carcinomas in the nasal cavity of rats by dioxane. Br J Cancer 24: 164-167.

IARC (1976). International Agency for Research on Cancer. 1,4-Dioxane. In *Cadmium, Nickel, Some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 11. Lyon, France. pp. 247-256.

IARC (1991). International Agency for Research on Cancer. 1,4-Dioxane. *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans*, vol. 71. Lyon, France. pp. 589-602

IARC (1999). International Agency for Research on Cancer. 1,4-Dioxane. In: Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. Lyon, France. pp. 589-602.

Itoh, S, Hattori, C. (2019). In vivo genotoxicity of 1,4-dioxane evaluated by liver and bone marrow micronucleus tests and Pig-a assay in rats. Mutat Res Genet Toxicol Environ Mutagen 837: 8-14.

ITRC (2020). Interstate Technology Regulatory Council. History of Use and Potential Sources 1,4-Dioxane. Accessed May 18, 2020. https://14dx-1.itrcweb.org/wp-content/uploads/2020/03/14DX-History-of-Use-and-Potential-Sources-1.pdf

JBRC (1998). Japan Bioassay Research Center. Two-year studies of 1,4-dioxane in F344 rats and BDF1 mice (drinking water). Kanagawa, Japan.

Johnstone, RT. (1959). Death due to dioxane? AMA Arch Ind Health 20: 445-447, cited in USEPA (2013).

Kanada, M, Miyagawa, M, Sato, M, Hasegawa, H, Honma, T. (1994). Neurochemical profile of effects of 28 neurotoxic chemicals on the central nervous system in rats (1) Effects of oral administration on brain contents of biogenic amines and metabolites. Ind Health 32: 145-164.

Kano, H, Umeda, Y, Kasai, T, Sasaki, T, Matsumoto, M;,Yamazaki, K;,Nagano, K, Arito, H, Fukushima, S. (2009). Carcinogenicity studies of 1,4-dioxane administered in drinking-water to rats and mice for 2 years. Food Chem Toxicol 47: 2776-2784.

Kano, H, Umeda, Y, Saito, M, Senoh, H, Ohbayashi, H, Aiso, S, Yamazaki, K, Nagano, K, Fukushima, S. (2008). Thirteen-week oral toxicity of 1,4-dioxane in rats and mice. J Toxicol Sci 33: 141-153.

Kasai, T, Kano, H, Umeda, Y, Sasaki, T, Ikawa, N, Nishizawa, T, Nagano, K, Arito, H, Nagashima, H, Fukushima, S. (2009). Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol 21: 889-897.

Kasai, T, Saito, M,;Senoh, H, Umeda, Y, Aiso, S, Ohbayashi, H, Nishizawa, T, Nagano, K, Fukushima, S. (2008). Thirteen-week inhalation toxicity of 1,4-dioxane in rats. Inhal Toxicol 20: 961-971.

Kesten, HD, Mulinos, MG, Pomerantz, L. (1939). Pathologic effects of certain glycols and related compounds. Arch Pathol 27: 447-465.

King, ME, Shefner, AM, Bates, RR. (1973). Carcinogenesis bioassay of chlorinated dibenzodioxins and related chemicals. Environ Health Perspect 5: 163-170.

Kitchin, KT, Brown, JL. (1990). Is 1,4-dioxane a genotoxic carcinogen? Cancer Lett 53: 67-71.

Kociba, RJ, McCollister, SB, Park, C, Torkelson, TR, Gehring, PJ. (1974). 1,4-dioxane. I. Results of a 2-year ingestion study in rats. Toxicol Appl Pharmacol 30: 275-286.

Kociba, RJ, Torkelson, TR; Young, JD, Gehring, PJ. (1975). 1,4-Dioxane: Correlation of the results of chronic ingestion and inhalation studies with its dose-dependent fate in rats. In Proceedings of the 6th Annual Conference on Environmental Toxicology. Wright-Patterson Air Force Base, OH: Wright-Patterson Air Force Base, Air Force Systems Command, Aerospace Medical Division, Aerospace Medical Research Laboratory.

Kramer CG, Ott MG, Fulkerson JE, Hicks N and Imbus HR (1978). Health of workers exposed to 1,1,1trichloroethane: a matched-pair study. Arch. Environ. Health 33, 331-342, cited in EU 2002.

Kurl, RN, Poellinger, L, Lund, J, Gustafsson, JA. (1981). Effects of dioxane on RNA synthesis in the rat liver. Arch Toxicol 49: 29-33.

Lafranconi, M., Budinsky, R., Corey, L., Klapacz, J., Crissman, J., LeBaron, M., Golden, R., & Pleus, R. (2021). A 90-day drinking water study in mice to characterize early events in the cancer mode of action of 1,4-dioxane. Regulatory toxicology and pharmacology : RTP 119: 104819.

Laug, EP, Calvery, HO, Morris, HJ, Woodard, G. (1939). The toxicology of some glycols and derivatives. J Ind Hyg Toxicol 21: 173-201.

Lundberg, I, Hogberg, J, Kronevi, T, Holmberg, B. (1987). Three industrial solvents investigated for tumor promoting activity in the rat liver. Cancer Lett 36: 29-33.

McFee, AF, Abbott, MG, Gulati, DK,,Shelby, MD. (1994). Results of mouse bone marrow micronucleus studies on 1,4-dioxane. Mutat Res 322: 145-148.

Mikheev, MI, Gorlinskaya Ye, P, Solovyova, TV. (1990). The body distribution and biological action of xenobiotics. J Hyg Epidemiol Microbiol Immunol 34: 329-336. Cited in USEPA (2013).

Mirkova, ET. (1994). Activity of the rodent carcinogen 1,4-dioxane in the mouse bone marrow micronucleus assay. Mutat Res 322: 142-144.

Miyagawa, M, Shirotori, T, Tsuchitani, M, Yoshikawa, K. (1999). Repeat-assessment of 1,4-dioxane in a rathepatocyte replicative DNA synthesis (RDS) test: Evidence for stimulus of hepatocyte proliferation. Exp Toxicol Pathol 51: 555-558.

Morita, T. (1994). No clastogenicity of 1,4 dioxane as examined in the mouse peripheral blood micronucleus test. Mammalian Mutagenicity Study Group Communications 2: 7-8.

Morita, T, Hayashi, M. (1998). 1,4-Dioxane is not mutagenic in five in vitro assays and mouse peripheral blood micronucleus assay, but is in mouse liver micronucleus assay. Environ Mol Mutagen 32: 269-280.

Munoz, ER, Barnett, BM. (2002). The rodent carcinogens 1,4-dioxane and thiourea induce meiotic nondisjunction in Drosophila melanogaster females. Mutat Res 517: 231-238.

Nannelli, A, De Rubertis, A, Longo, V, Gervasi, PG. (2005). Effects of dioxane on cytochrome P450 enzymes in liver, kidney, lung and nasal mucosa of rat. Arch Toxicol 79: 74-82.

NCI (1978). National Cancer Institute. Bioassay of 1,4-dioxane for possible carcinogenicity. Bethesda, MD, Department of Health, Education and Welfare, National Institutes of Health, National Cancer Institute (Technical Report Series 80; DHEW Publication No. 78-1330).

Nelson, N. (1951). Solvent toxicity with particular reference to certain octyl alcohols and dioxanes. Med Bull 11: 226-238. Cited in USEPA (2013).

NIOSH (1977). National Institute for Occupational Safety and Health. Criteria for a recommended standard ...: Occupational Exposure to Dioxane. U.S. Department of Health, Education and Welfare, Center for Disease Control, National Institute for Occupational Safety and Health, Washington D.C. DHEW (NIOSH) Publication No. 77-226. Cited in EU (2002).

NIOSH (1987). National Institute for Occupational Safety and Health. Guide to industrial respiratory protection. U.S. Department of Health, Education and Welfare, Center for Disease Control, National Institute for Occupational Safety and Health. DHHS (NIOSH) Publication No. 87-116, cited in EU (2002).

Nishimura, T, Iizuka, S, Kibune, N, Ando, M. (2004). Study of 1,4-dioxane intake in the total diet using the market-basket method. J. Health Science 50: 101-107.

NJDEP (2010). New Jersey Department of Environmental Protection. Memorandum. Recommendation of revised interim specific ground water criterion for 1,4-dioxane. Accessed March 12, 2019. https://www.nj.gov/dep/dsr/supportdocs/1,4-Dioxane_TSD.pdf

NJDEP (2015). New Jersey Department of Environmental Protection. Response to Public Input on Draft Interim Ground Water Quality Criteria and Draft Interim Practical Quantitation Levels for Eleven Chemicals. https://www.nj.gov/dep/dsr/supportdocs/11%20Chemicals_Response.pdf

NJDEP (2018). New Jersey Department of Environmental Protection. Ground Water Quality Standards; Discharges of Petroleum and Other Hazardous Substances Rules. Adopted Amendments: N.J.A.C. 7:1E Appendix A and 7:9C-1.7 and 7:9C Appendix; January 16, 2018. https://www.nj.gov/dep/rules/adoptions/adopt_20180116c.pdf

NTP. National Toxicology Program. (2011). 1,4-dioxane. In Report on carcinogens, twelfth edition (pp. 176-178). U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.

NTP (2016). National Toxicology Program. 1,4-dioxane factsheet 14th RoC. Accessed February 2020. https://ntp.niehs.nih.gov/ntp/roc/content/profiles/dioxane.pdf

NYS (2018). New York State Department of Health. 2018 Press Releases. Drinking Water Quality Council Recommends Nation's Most Protective Maximum Contaminant Levels for Three Unregulated Contaminants in Drinking Water. Accessed February 8, 2019 https://www.health.ny.gov/press/releases/2018/2018-12-18_drinking_water_quality_council_recommendations.htm

NYS (2019). New York State Senate Bill S4389B/A6295A. Accessed June 7, 2020. https://www.nysenate.gov/legislation/bills/2019/s4389

NY DWQC (2020). New York Drinking Water Quality Council. Webcasts of October 2019 meetings. https://www.health.ny.gov/environmental/water/drinking/dwqc/#f . Accessed February 18, 2020.

Pozzani, UC; Weil, CS; Carpenter, CP. (1959). The toxicological basis of threshold limit values. 5: The experimental inhalation of vapor mixtures by rats, with notes upon the relationship between single dose inhalation and single dose oral data. Am Ind Hyg Assoc J 20: 364-369.

PubChem (2019). 1,4-Dioxane. Accessed January 30, 2019

Qiu J, Cheng J, Xie Y, Jiang L, Shi P, Li X, Swanda RV, Zhou J, Wang Y. (2019). 1,4-Dioxane exposure induces kidney damage in mice by perturbing specific renal metabolic pathways: An integrated omics insight into the underlying mechanisms. Chemosphere 228:149-158.

Roy, SK; Thilagar, AK; Eastmond, DA. (2005). Chromosome breakage is primarily responsible for the micronuclei induced by 1,4-dioxane in the bone marrow and liver of young CD-1 mice. Mutat Res 586: 28-37.

Schrenk, HH; Yant, WP. (1936). Toxicity of dioxan. J Ind Hyg Toxicol 18: 448-460.
Sheu, CW; Moreland, FM; Lee, JK; Dunkel, VC. (1988). In vitro BALB/3T3 cell transformation assay of nonoxynol-9 and 1,4-dioxane. Environ Mol Mutagen 11: 41-48.

Silverman, L; Schulte, HF; First, MW. (1946). Further studies on sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 28: 262-266.

Sina, JF; Bean, CL; Dysart, GR; Taylor, VI; Bradley, MO. (1983). Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res Environ Mutagen Relat Subj 113: 357-391.

Smyth, HF, Jr; Seaton, J; Fischer, L. (1941). The single dose toxicity of some glycols and derivatives. J Ind Hyg Toxicol 23: 259-268.Cited in USEPA (2013a).

Stoner, GD; Conran, PB; Greisiger, EA; Stober, J; Morgan, M; Pereira, MA. (1986). Comparison of two routes of chemical administration on the lung adenoma response in strain A/J mice. Toxicol Appl Pharmacol 82: 19- 31.

Stott, WT; Quast, JF; Watanabe, PG. (1981). Differentiation of the mechanisms of oncogenicity of 1,4-dioxane and 1,3-hexachlorobutadiene in the rat. Toxicol Appl Pharmacol 60: 287-300.

Thiess, AM; Tress, E; Fleig, I. (1976). Arbeitsmedizinische Untersuchungsergebnisse von Dioxan-exponierten Mitarbeitern [Industrial-medical investigation results in the case of workers exposed to dioxane]. Arbeitsmedizin, Sozialmedizin, Umweltmedizin 11: 35-46.

Tinwell, H; Ashby, J. (1994). Activity of 1,4-dioxane in mouse bone marrow micronucleus assays. Mutat Res 322: 148-150.

Torkelson, TR; Leong, BKJ; Kociba, RJ; Richter, WA; Gehring, PJ. (1974). 1,4-Dioxane. II. Results of a 2-year inhalation study in rats. Toxicol Appl Pharmacol 30: 287-298Uno et al. (1994).

Totsuka, Y., Maesako, Y., Ono, H., Nagai, M., Kato, M., Gi, M., Wanibuchi, H., Fukushima, S., Shiizaki, K., & Nakagama, H. (2020). Comprehensive analysis of DNA adducts (DNA adductome analysis) in the liver of rats treated with 1,4-dioxane. Proceedings of the Japan Academy. Series B, Physical and biological sciences, 96: 180–187.

USEPA (1986). United States Environmental Protection Agency. The Risk Assessment Guidelines of 1986. Washington, DC. EPA/600/8-87/045. August 1987.

USEPA IRIS (1988). Integrated Risk Information System. 1,4-Dioxane (CASRN 123-91-1). Integrated Risk Information System. Cited in NJDEP (2010).

USEPA (2005). United States Environmental Protection Agency. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, USEPA, Washington, DC. EPA/630/P03/001F, March 2005.

USEPA (2009). Drinking Water Contaminant Candidate List. Accessed February 8, 2019 https://www.epa.gov/ccl

USEPA IRIS (2010). Toxicological Review of 1,4-Dioxane. Accessed February 13, 2019 https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P100FHIM.txt. August 2010.

USEPA (2011a). TRI (Toxic Release Inventory) National Analysis Basic Data Files. https://www.epa.gov/toxics-release-inventory-tri-program/2011-tri-national-analysis-basic-data-files. Accessed January 31, 2019.

USEPA (2011b). Exposure Factors Handbook: 2011 Edition. EPA-600-R-09-052F. Office of Research and Development, Washington, DC. http://www.epa.gov/ncea/efh/pdfs/efhcomplete.pdf

USEPA IRIS (2013). United States Environmental Protection Agency. Toxicological Review of 1,4-Dioxane (with Inhalation Update). In Support of Summary Information on the Integrated Risk Information System (IRIS). September 2013, Washington, DC. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0326tr.pdf

USEPA (2015). United States Environmental Protection Agency. Human Health Ambient Water Quality Criteria: 2015 Update. Office of Water. EPA 820-F-15-00. June 2015. https://www.epa.gov/sites/production/files/2015-10/documents/human-health-2015-update-factsheet.pdf

USEPA (2017). United States Environmental Protection Agency. UCMR3 (2013-2015) Occurrence Data. January 2017. https://www.epa.gov/sites/production/files/2017-02/documents/ucmr3-data-summary-january-2017.pdf. Accessed June 4, 2020.

USEPA (2018a) TRI. Toxic Release Inventory. TRI Basic Data Files: Calendar Years 1987-2018. https://www.epa.gov/toxics-release-inventory-tri-program/tri-basic-data-files-calendar-years-1987-2018. Accessed February 18, 2020.

USEPA (2018b). United States Environmental Protection Agency. 2018 Edition of the Drinking Water Standards and Health Advisories. EPA 822-F-18-001. Office of Water, Washington, DC. https://www.epa.gov/sites/production/files/2018-03/documents/dwtable2018.pdf

USEPA (2018c). Problem Formulation of the Risk Evaluation for 1,4-Dioxane. Office of Chemical Safety and Environmental Pollution Prevention. EPA Document# EPA-740-R1-7012. May 2018.

USEPA (2020). United States Environmental Protection Agency. Final Risk Evaluation for 1,4-Dioxane. EPA-740-R1-8007. United States Office of Chemical Safety and Pollution Prevention. December 2020. https://www.epa.gov/sites/production/files/2020-12/documents/1._risk_evaluation_for_14-dioxane_casrn_123-91-1.pdf.

USEPA (2020). Announcement of Preliminary Regulatory Determinations for Contaminants on the Fourth Drinking Water Contaminant Candidate List. Federal Register 85 (47): 14098-14142. March 10, 2020.

USFDA (2019). United States Food and Drug Administration. 1,4-Dioxane in Cosmetics: A Manufacturing Byproduct. Accessed June 7, 2020. https://www.fda.gov/cosmetics/potential-contaminants-cosmetics/14-dioxane-cosmetics-manufacturing-byproduct

WHO (2005). World Health Organization. 1,4-Dioxane in Drinking-water. Background document for development of WHO Guidelines for Drinking-water quality. Accessed February 22, 2020. https://www.who.int/water_sanitation_health/dwq/chemicals/14dioxane0505.pdf

Wirth, W; Klimmer, O. (1936). [On the toxicology of organic solvents. 1,4 dioxane (diethylene dioxide)]. Archiv fuer Gewerbepathologie und Gewerbehygiene 17: 192-206.

Woo, YT; Arcos, JC; Argus, MF; Griffin, GW; K, N. (1977). Structural identification of p-dioxane-2-one as the major urinary metabolite of p-dioxane. Naunyn Schmiedebergs Arch Pharmacol 299: 283-287 Cited in USEPA (2013).

Yamazaki K et al. (1994) Two-year toxicological and carcinogenesis studies of 1,4-dioxane in F344 rats and BDF1 mice. In: Proceedings of the Second Asia-Pacific Symposium on Environmental and Occupational Health. Kobe, Japan, Kobe University, pp. 193–198.

Yant, WP; Schrenk, HH; Waite, CP; Patty, FA. (1930). Acute response of guinea pigs to vapors of some new commercial organic compounds: VI. Dioxan. Public Health Rep 45: 2023-2032.
Young, JD; Braun, WH; Gehring, PJ. (1978a). The dose-dependent fate of 1,4-dioxane in rats. J Environ Pathol Toxicol 2: 263-282. Cited in USEPA (2013).

Young, JD; Braun, WH; Gehring, PJ. (1978b). Dose-dependent fate of 1,4-dioxane in rats(b). J Toxicol Environ Health A 4: 709-726. Cited in USEPA (2013).

Young, JD; Braun, WH; Gehring, PJ; Horvath, BS; Daniel, RL. (1976). 1,4-Dioxane and betahydroxyethoxyacetic acid excretion in urine of humans exposed to dioxane vapors. Toxicol Appl Pharmacol 38: 643-646. Cited in USEPA (2013).

Young, JD; Braun, WH; Rampy, LW; Chenoweth, MB; Blau, GE. (1977). Pharmacokinetics of 1,4-dioxane in humans. J Toxicol Environ Health 3: 507-520. Cited in USEPA (2013).

APPENDIX 1: REVIEW OF DOURSON ET AL. (2014) AND DOURSON ET AL. (2017)

The Health Effects Subcommittee reviewed two publications by Dourson et al. (2014, 2017) that conclude that 1,4-dioxane is carcinogenic through a threshold mode of action (MOA) and that the default non-threshold (linear low-dose extrapolation) approach for cancer risk assessment is therefore not appropriate. NJDEP (2015, 2018) responded to the conclusions of these two publications when they were submitted in comments on the draft Interim Ground Water Quality Standard for 1,4-dioxane in 2015 (Dourson et al., 2014) and the proposed Ground Water Quality Standard for 1,4-dioxane in 2018 (Dourson et al., 2017). NJDEP (2015, 2018) concluded that the mode of action of 1,4-dioxane carcinogenicity remains unknown, and that the analyses presented in Dourson et al. (2014; 2017) do not conclusively establish a threshold mode of action. Similarly, USEPA (2019) also reviewed Dourson et al. (2014, 2017) and other relevant data and concluded that a threshold mode of action has not been established and that a non-threshold (slope factor) approach should be used for cancer risk assessment of 1,4-dioxane.

Health Effects Subcommittee conclusions about specific points made by Dourson et al. (2014, 2017) are presented below:

- **Dourson et al. (2014, 2017)** conclude that 1,4-dioxane causes tumors through a threshold mode of action involving cytotoxicity, necrosis, and regenerative hyperplasia followed by tumor formation.

  The Health Effects Subcommittee finds that the Dourson et al. (2014, 2017) analyses do not conclusively establish a threshold mode of action for 1,4-dioxane carcinogenicity. The Subcommittee's conclusions are consistent with those of NJDEP (2015b, 2018) and USEPA (2019). Relevant to this issue, as summarized in Table 4, liver tumors in several rodent studies occurred at doses at which there were no lesions indicative of cytotoxicity and/or regeneration (Kano et al., 2009; NCI, 1978). These data indicate that 1,4-dioxane can cause liver tumors in the absence of cytotoxicity followed by cell proliferation.

Table 4. Temporal sequence and dose-response relationship for possible key events and liver tumors in rats and mice. Adapted from USEPA, 2013.

	Key event (time →)			
Dose (mg/kg-day) or Exposure (ppm)	**Liver damage**	**Cell proliferation**	**Hyperplasia**	**Adenomas and/or carcinomas**
Kociba et al., (1974)—Sherman rats (male and female combined)				
0 mg/kg-day	__[a]	__[a]	__[a]	__[a]
14 mg/kg-day	__[a]	__[a]	__[a]	__[a]
121 mg/kg-day	+[c]	__[a]	+[c]	__[a]
1,307 mg/kg-day	+[c]	__[a]	+[c]	+[c]
NCI, (1978)—female Osborne-Mendel rats				
0 mg/kg-day	__[a]	__[a]	__[a]	__[a]

350 mg/kg-day	—[a]	—[a]	—[a]	+[c]
640 mg/kg-day	—[a]	—[a]	—[a]	+[c]
NCI, (1978)—female Osborne-Mendel rats				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
720 mg/kg-day	—[a]	—[a]	—[a]	+[c]
830 mg/kg-day	—[a]	—[a]	—[a]	+[c]
NCI, (1978)—male B6C3F$_1$ mice				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
720 mg/kg-day	—[a]	—[a]	—[a]	+[c]
830 mg/kg-day	—[a]	—[a]	—[a]	+[c]
NCI, (1978)—female B6C3F$_1$ mice				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
380 mg/kg-day	—[a]	—[a]	—[a]	+[c]
860 mg/kg-day	—[a]	—[a]	—[a]	+[c]
Kano et al., (2009) —male F344/DuCrj rats				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
11 mg/kg-day	—[a]	—[a]	—[a]	—[a]
55 mg/kg-day	—[a]	—[a]	—[a]	—[a]
274 mg/kg-day	+[c,d]	—[a]	—[a]	+[c,e]
Kano et al., (2009)—female F344/DuCrj rats				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
18 mg/kg-day	—[a]	—[a]	—[a]	—[a]
83 mg/kg-day	—[a]	—[a]	—[a]	—[a]
429 mg/kg-day	—[a]	—[a]	—[a]	+[c,e]
Kano et al., (2009)—male Crj:BDF1 mice				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
49 mg/kg-day	—[a]	—[a]	—[a]	+[c,e]
191 mg/kg-day	—[a]	—[a]	—[a]	+[c,e]
677 mg/kg-day	+[c,d]	—[a]	—[a]	+[c,e]
Kano et al., (2009)—female Crj:BDF1 mice				
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]
66 mg/kg-day	—[a]	—[a]	—[a]	+[c,e]
278 mg/kg-day	—[a]	—[a]	—[a]	+[c,e]
964 mg/kg-day	+[c,d]	—[a]	—[a]	+[c,e]
Kasai et al., (2009)—male F344 rats				
0 ppm	—[a]	—[a]	—[a]	—[a]
50 ppm	—[a]	—[a]	—[a]	—[a]
250 ppm	—[a]	—[a]	—[a]	—[a]
1,250 ppm	+[h]	—[a]	—[a]	+[h]

[a]— No evidence demonstrating key event.

[b]+ 1,4-dioxane metabolism was not evaluated as part of the chronic bioassays. Data from pharmacokinetic studies suggest that metabolism of 1,4-dioxane by CYP2E1 and CYP2B2 occurs immediately and continues throughout the duration of exposure at all exposure levels.

[c]+ Statistically significant increase noted.

[d]+ Single cell necrosis was observed in a 13 week bioassay for male rats (274 mg/kg-day), male mice (585 mg/kg-day), and female mice (898 mg/kg-day) exposed to 1,4-dioxane in drinking water (Kano et al., 2008).

[e]+ Kano et al. (2009) reported incidence rates for hepatocellular adenomas and carcinomas.

[f]+ Kasai et al. (2008) reported significant incidence rates for single cell necrosis in female rats only (3,200 ppm) following a 2 year bioassay.

[g]— All rats died during the first week of the 13-week bioassay (Kasai et al., 2008).

[h]+ Kasai et al. (2009) reported incidence rates for centrilobular necrosis and hepatocellular adenomas in male rats (1,250 ppm).

- **Dourson et al. (2014)** conducted a pathology review of the mouse liver slides from the chronic oral study conducted by NCI (1978). In NCI (1978), there was a dose-related increase in the incidence of liver tumors in both male and female mice, as follows:

  Males: Control-16%, 720 mg/kg/day-38%, 830 mg/kg/day-60%.
  Females: Control-0%, 380 mg/kg/day- 44%, 860 mg/kg/day-95%.

  Dourson et al. (2014) state that, at the time that the NCI (1978) study was conducted, only the most severe response seen on a slide was recorded, so that if a tumor was observed, non-neoplastic changes on the same slide would not have been noted. They suggest that non-neoplastic changes such as glycogen depletion, hypertrophy, necrosis, inflammation, and Kupffer cell hyperplasia preceded and were causative to tumor formation.

  The Health Effects Subcommittee notes that, in the Dourson et al. (2014) pathology review, a higher incidence and/or greater severity for these non-neoplastic effects were observed in both the high and low dose male mice than in controls. However, the incidence and/or severity of the non-neoplastic changes in female mice was similar or greater in controls than in the low dose group, while the incidence of liver tumors in the control and low dose female mice were 0 and 44% respectively. Therefore, the Health Effects Subcommittee agrees with the NJDEP (2015) conclusion that these data suggest that such non-neoplastic changes are not part of the sequence of events leading to tumor formation in the low dose female mice.

- **Dourson et al. (2014)** that the incidence of non-neoplastic effects does not correlate with the tumor incidence in control and low-dose females in NCI (1978). That state that the lower incidence of non-neoplastic changes in low-dose females than in low-dose males may be due to the fact that the low dose in females was about half of the low dose in males.

  The Health Effects Subcommittee agrees with the NJDEP (2015) conclusion that Dourson et al. (2014) do not provide a logical explanation since the incidence of liver tumors in low dose females (44%, as compared to 0% in controls) is greater than in low dose males (38%, as compared to 16% in controls) at a dose almost 2-fold higher.

The Health Effects Subcommittee as notes that, as discussed in more detail below, the USEPA IRIS (2013) and USEPA OCSPP (2020) oral slope factor is based on female mouse liver tumors from Kano et al. (2009), not data from NCI (1978) study. In this study, a different strain of mice (Crj:BDF1) were used. In Kano et al. (2009), the low dose in female mice (66 mg/kg/day) was almost 6-fold lower than the low dose in NCI (1978) (380 mg/kg/day), and non-neoplastic changes such as necrosis were not observed in the liver. However, the tumor incidence in the low dose females (70% compared to 10% in controls) in Kano et al. (2009) was higher than at the much higher dose (44% at 380 mg/kg/day compared to 0% in controls) in the low dose females in NCI (1978). These data further support the conclusion that non-neoplastic changes do not necessarily precede hepatic tumors caused by 1,4-dioxane.

- **Dourson et al. (2014)** state that the incidence of non-neoplastic changes in the female controls may have been due to a viral infection that "was known to occur in all mice at the time of the bioassay." They attribute this statement to E.E. McConnell, who conducted the pathology review and is a co-author of Dourson et al. (2014).

  The Health Effects Subcommittee notes that no citation about the presence of the viral infection is provided, and this issue is not mentioned in either NCI (1978) or the pathology review report by Dr. McConnell. Additionally, this explanation is not logical, since it was not stated that the control females were specifically infected with the virus, as compared with other groups of male and female mice included in the study. The Health Effects Subcommittee agrees with the NJDEP (2015) conclusion that, if the pathway hypothesized by Dourson et al. (2014), in fact, resulted in tumors, then the presence of the elements of this pathway in control females, with incidence of necrosis and inflammation greater than in the low dose group, would also have been expected, regardless of its etiology, to result in tumors. The absence of tumors in the control females is thus inconsistent with the hypothesized link between the non-neoplastic changes observed in both control and treated mice and the observed tumors.

- **Dourson et al. (2014)** made the general conclusion, based on the points above, that non-neoplastic changes occur both more frequently at higher doses and are necessary precursors to tumor formation.

  The Health Effects Subcommittee agrees with the NJDEP (2015) conclusion that, when considered as a whole, the information presented by Dourson et al. (2014) does not support the conclusions that non-neoplastic changes occur both more frequently at higher doses and are necessary precursors to tumor formation. In general, the data and explanation provided by Dourson et al. (2014) do not establish a firm or unique link to the proposed MOA of cytotoxicity followed by regenerative hyperplasia, and does not indicate that a threshold approach is appropriate for risk assessment for this compound. As such, the information provided by Dourson et al. (2014) does not invalidate the conclusion made by USEPA IRIS

(2013) that the available information does not establish a plausible mode of action for 1,4-dioxane, and that the available data are not sufficient to establish significant biological support for a non-linear (threshold) mode of action. Furthermore, USEPA OCSPP (2020) also reviewed Dourson et al. (2014) and concluded that it does not demonstrate a threshold mode of action. For these reasons, the approach used by USEPA IRIS (2013) and USEPA OCSPP (2020) which is based on a linear low dose extrapolation to develop an oral cancer slope factor for 1,4-dioxane is appropriate.

- **Dourson et al. (2017)** revisited the results of the Kano et al. (2008, 2009) 13 week and two-year drinking water studies in rats and mice and reviewed English translations of the original Japanese laboratory reports of these studies (JBRC, 1990a, b). Some of the analyses presented by Dourson et al. (2017) are based on pooled data from male and female rats and mice (i.e. dose-response analyses are based on data points from male groups combined with data points from female groups). Furthermore, Dourson et al. (2017) adjusted doses from the 13-week studies, dividing them by a factor of 3, for comparison with the doses at which effects occurred in the two-year study. The rationale for this adjustment was that when point of departure (NOAEL, LOAEL, BMDL) from a subchronic study is used as the basis for a chronic Reference Dose, an uncertainty factor of 3 or 10 is applied to account for potential effects at lower doses with longer exposure durations.

  The Health Effects Subcommittee agrees with the NJDEP (2018) conclusion that this adjustment is not appropriate for quantitative comparison of dose-response relationships from studies of different durations as was done by Dourson et al. (2017), and USEPA OCSPP (2020) also agreed with this conclusion. Therefore, conclusions from Dourson et al. (2017) based on such comparisons (e.g. that centrilobular swelling and single cell liver necrosis in the 13-week rat study occurred at lower doses than tumors in the two-year rat study) are not scientifically supportable. Additionally, the Subcommittee notes that, as acknowledged by Dourson et al. (2017), even with the subchronic dose adjusted downward by a factor of 3, liver tumors occurred in mice in the chronic studies at doses below those that caused liver swelling and necrosis in the subchronic studies.

- **Dourson et al. (2017)** also conclude that the toxicity pathway for 1,4-dioxane is dependent on saturation kinetics, with decreased metabolism of the parent compound leading to increased toxicity.

  The Health Effects Subcommittee concluded that, in the chronic mouse study, liver tumors were increased in females at doses that did not cause the postulated "key events" including saturation of metabolism, as well as, increased liver weight/hypertrophy and necrosis/inflammation, that are required for the proposed threshold mode of action.

In summary, the Health Effect Subcommittee agrees with NJDEP (2018) that "the data and explanations provided by Dourson et al. (2017) do not establish a firm or unique link to the proposed mode of action, and they do not indicate that a threshold approach is appropriate for risk assessment for [1,4-dioxane]." USEPA OCSPP (2020) also concluded that Dourson et al. (2017) does not demonstrate that a threshold approach is appropriate.