Novel Approaches to Exposure Assessment and Dose Response to Contaminants in Drinking Water and Food

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Civil Engineering

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March 15, 2018
Blacksburg, VA

Keywords:
Consumer, lexicon, E. coli, beef, dose-response, risk assessment, drinking water

ABSTRACT (ACADEMIC)

Novel Approaches to Exposure Assessment and Dose Response to Contaminants in Drinking Water and Food

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The works in this dissertation focus on risk assessment and novel methods to determine exposure assessment and dose response to chemical and biological contaminants in drinking water and foods. Population susceptibility (i.e., healthy adults or children), different routes of exposures (i.e., ingestion or inhalation), carrier matrices (i.e., water or food), and intricacies of chemical and biological mixtures are major factors in risk assessment work. For water contaminants attributed to chemical spills, the complexities of chemical mixtures, such as minor and major components or geometric isomers, affect fate and transport properties, the level of human exposure, and dose response. The contribution of odorous properties to human inhalation exposure, especially during showering, can be as harmful as the effects of fate and transport properties on ingestion exposure to water contaminants. Dose response for all exposure routes can be multiple folds higher for healthy adults than for the susceptible population. Additionally, food contaminants, such as Shiga toxin producing *Escherichia coli* in the beef industry, can be minimized through a quantitative microbial risk assessment (QMRA) that follows a farm-to-fork modeling framework. Log reduction of contaminants are accomplished by using intervention strategies at slaughter plants (i.e., water washing of beef carcass), improving cooking times and temperature settings and methods to ensure maximum microbial die offs at the consumer and retail level, and assessing minimum effective dose for different population susceptibilities in order to prevent human illnesses due to foodborne pathogens. Existing public communication tools, like the Drinking Water Taste-and-Odor Wheel or Consumer Confidence Reports (better known as water quality reports), can be transformed to promote water safety. Furthermore, public health campaigns that uses social media tools or informative, layperson language websites can better educate the public on improved cooking and handling strategies for beef products. This dissertation aims to bridge the communication gap between the consumers and experts involving water and food safety.

ABSTRACT (GENERAL AUDIENCE)

Novel Approaches to Exposure Assessment and Dose Response to Contaminants in Drinking Water and Food

Katherine Phetxumphou

In the fields of water safety, food safety, and public communications, the overarching goal is to improve public health. Thus, this dissertation focuses on risk assessment and applying novel methods for exposure assessments and dose responses to contaminants in drinking water and foods. Factors that greatly impact contaminant exposures and human dose response include: population susceptibility (i.e., healthy adults or children), different routes of exposures (i.e., ingestion or inhalation), carrier matrices (i.e., water or food), and intricacies of chemical and biological mixtures. Chemical spills, such as the 2014 crude MCHM spill in Charleston, WV, revealed the complexities of both minor and major components in the chemical mixture. Slight shifts in geometric structures (isomers) can affect the fate and transport properties of the chemical mixture and as a result, the level of human exposure and dose response to each component in the chemical mixture. Odorous properties of both minor and major components can affect human inhalation exposure, especially during showering, and can be as detrimental as the ingestion route exposure and are different for healthy adults versus for children. Food contaminants, such as Shiga toxin producing *Escherichia coli* (STEC) in beef products, can be mitigated through a quantitative microbial risk assessment (QMRA) framework that follows a farm-to-fork model. Methods to ensure greatest microbial reduction include: employed intervention strategies at slaughter plants (i.e., water washing of beef carcass), improved cooking times and temperature methods at the consumer and retail level, and assessed minimum effective dose response modeling for different population susceptibilities. Current public communication tools, including the Drinking Water Taste-and-Odor Wheel or Consumer Confidence Reports (better known as water quality reports), should be redeveloped to uphold water safety. Furthermore, public health campaigns that uses social media strategies and informative websites can better educate the public on food contaminants. Ultimately, the objective is to prevent human illnesses due to water contaminants and foodborne pathogens and to bridge the communication gap between the consumers and the experts concerned with water and food safety.

DEDICATION

This dissertation is dedicated to my mother and late father…and my little Mozzie cookie.

Love always,
Katherine

ACKNOWLEDGEMENTS

First and foremost, all the honor and glory goes to God.
"And we know that all things work together for good to those who love God, to those who are the called according to *His* purpose." –Romans 8:28 (NKJV)

Thank you to my co-advisors, Dr. Andrea Dietrich and Dr. Daniel Gallagher, for their guidance and never-ending support throughout this journey. I also would like to thank my other committee members, Dr. Susan Duncan and Dr. Julia Gohlke for their efforts and support in my success.

Thank you to Mrs. Beth Lucas for her amazing administrative support and knowing the answers to literally all the questions.

Thank you to Mrs. Jody Smiley and Mrs. Julie Petruska on their invaluable guidance in the laboratory. Many of my experiments would have failed without them both!

To all of my family and friends, I appreciate all of your love and support and keeping me grounded and focused at all times. Thank you, I love you all!

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CHAPTER 3: Subtleties of Human Exposure and Response to Chemical Mixtures from Spills
K. Phetxumphou and J. Smiley prepared samples. K. Phetxumphou performed the Gas Chromatography-Olfactometry-Mass Spectrometry research after methods development in conjunction with Dr. Gallagher, Dr. Dietrich, and J. Smiley. K. Phetxumphou performed headspace analysis to determine Henry's Law constants. Dr. Gallagher performed the statistical and visual data analysis of odor threshold concentration and descriptor data and showering modeling with assistance from K. Phetxumphou. K. Phetxumphou led in writing the chapter with Dr. Gallagher and Dr. Dietrich contributing to and editing all sections of this chapter.

CHAPTER 4: Comparing Inhalation and Ingestion Exposure to Chemical Contaminants and Odorants in Mixtures
Dr. Gallagher performed the statistical and shower modeling of ingestion and inhalation exposure data with assistance from K. Phetxumphou and Dr. Dietrich. Dr. Gallagher led in writing the chapter with input and editing assistance by K. Phetxumphou and Dr. Dietrich.

CHAPTER 5: Implementing the Drinking Water Taste-and-Odor Wheel to Improve the Consumer Lexicon
K. Phetxumphou performed the odor vial descriptor research and wrote this section of the chapter. K. Phetxumphou and A. Raghuraman performed the statistical and visual data analysis of the consumer lexicon data and jointly wrote this section of the manuscript. Dr. Dietrich and A. Raghuraman assisted in writing and editing all sections of the chapter.

CHAPTER 6: Tools for Better Communication of Water Quality to Consumers
K. Phetxumphou and S. Roy equally performed the Consumer Confidence Report data collection and data analysis. K. Phetxumphou led in writing the chapter with input and editing assistance by Dr. Dietrich. S. Roy, Dr. Davy, Dr. Estabrooks, and Dr. You proofread the final version of the chapter.

CHAPTER 7: Write Consumer Confidence Reports Customers Can Understand
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K. Phetxumphou performed the literature data search and data analysis of thermal death time calculations in beef products. Additional data resources were provided by Dr. Porto-Fett and Dr. Luchansky. K. Phetxumphou developed R-code with assistance by S. Zhilyaev and Dr. Gallagher. K. Phetxumphou lead in writing the chapter with input and editing assistance by Dr. Gallagher, Dr. Gohlke, Dr. Duncan, and Dr. Porto-Fett.

CHAPTER 10: Population Susceptibility Effects on Dose-Response Modeling of STEC O157:H7 Outbreak Data
K. Phetxumphou performed the literature data search and meta-analysis of dose-response modeling and STEC foodborne outbreak data. K. Phetxumphou developed the logistic regression model with assistance by Dr. Cadavez, S. Zhilyaev, and Dr. Gallagher. K. Phetxumphou lead in writing the chapter with input and editing assistance by Dr. Barron-Gonzales, Dr. Gallagher, and S. Zhilyaev.

CHAPTER 1: Introduction

Risk management, risk assessment, and risk communication frameworks can be used to improve public health (Teaf & Kuperberg, 2004; U.S. Environmental Protection Agency, 1990). Risk management serves as the decision-making process to set regulations and policies on hazards identified during risk assessment, and then risk communication is informing the public about the potential hazards (Teaf & Kuperberg, 2004; U.S. Environmental Protection Agency, 1990).

Traditional risk assessments involve four steps: hazard identification, dose-response and exposure assessment, and which are then combined into an overall risk characterization as shown in Figure 1 (U.S. Environmental Protection Agency, 2016). Hazard identification assesses whether a stressor can cause harm to humans and/or ecological systems (U.S. Environmental Protection Agency, 2016). Exposure assessment determines frequency, timing, and contact levels with the stressor (U.S. Environmental Protection Agency, 2016). Dose-response is defined as "the relationship between the dose received and the resulting health effects" (George, Divya, & Suriyanarayanan, 2013). Dose-response entails mathematically modeling the relationship between the dose and the probability of illness in an exposed population (George et al., 2013). Different exposure assessments and dose-response models are essential for different susceptible populations (e.g., healthy adults, children, immunocompromised, elderly, etc.). Ultimately, risk characterization evaluates conclusions made about risks associated with the identified stressors so that public health officials can establish policies to improve public health and communicate any associated risks (U.S. Environmental Protection Agency, 2016). Potential hazards can be chemical (water) and/or bacterial (food).

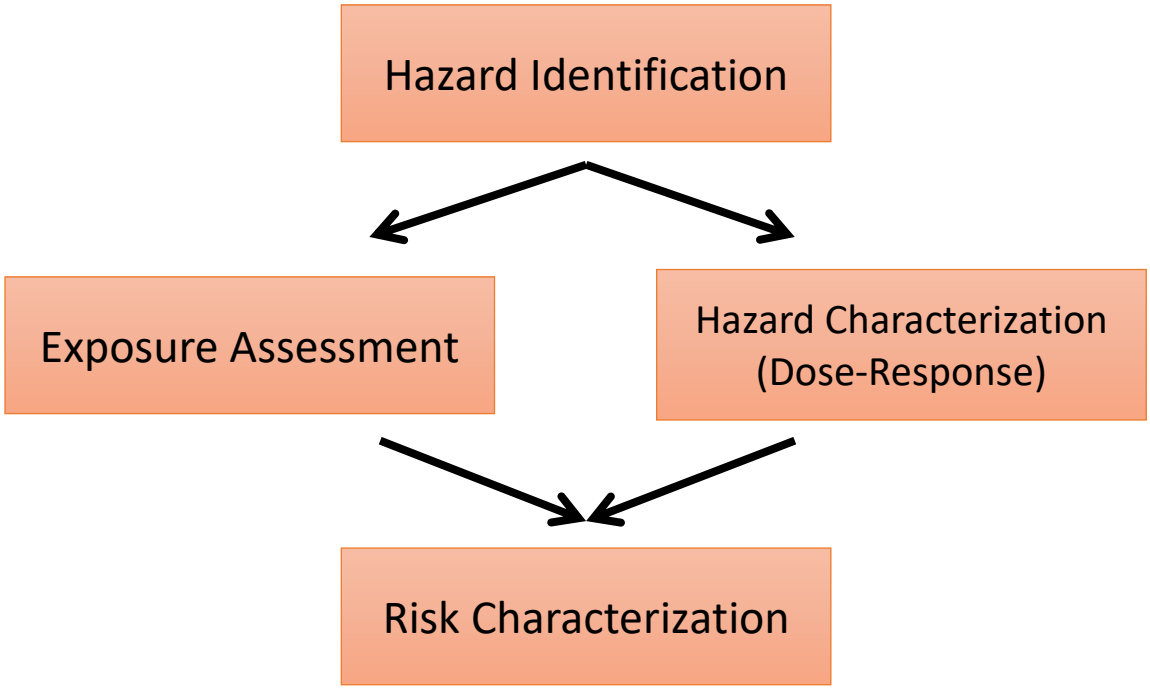

Figure 1. Traditional risk assessment framework

While food risk assessment and drinking water risk assessment professionals rarely interact, there are many similarities between the fields. During exposure assessment, both fields track changes in contaminant concentrations from a source through to consumption. An example process is depicted in Figure 2. Drinking water starts with a water source, is treated at a water treatment plant, distributed through a pipe network to the home, held in the home pipes for some time, and then consumed. Food products start on a farm, are treated at a food processing plant or slaughter facility, distributed through retail stores, then held and cooked in the home. While the specifics vary, the overall framework is almost similar. Consumption patterns leading to a dose also have similarities. Water is typically ingested at 2L/person/day for adults and 1L/child/day for children(U.S. Environmental Protection Agency, 1991). Ground beef in North America is typically consumed at 35 grams/person/day (North American Meat Institute, 2015).

Modeling the exposure through the respective pathways in Figure 2 requires the relevant chemical, physical, and biological properties of the contaminant as well as the contacted

materials and storage conditions. For water, these properties may include solubility, sorption, Henry's Law Constant, and enthalpy for chemical contaminants, and log inactivation rates, disinfection concentration, and contact time for microbial contaminants. For foods, the critical properties may include growth and die-off rates based on temperature and food matrix, including fat percentages and water content, in addition to plant intervention strategies involving water washes and chemical sprays. Thus, this dissertation aimed to create a depiction of risk assessments associated with contaminants in both water and foods.

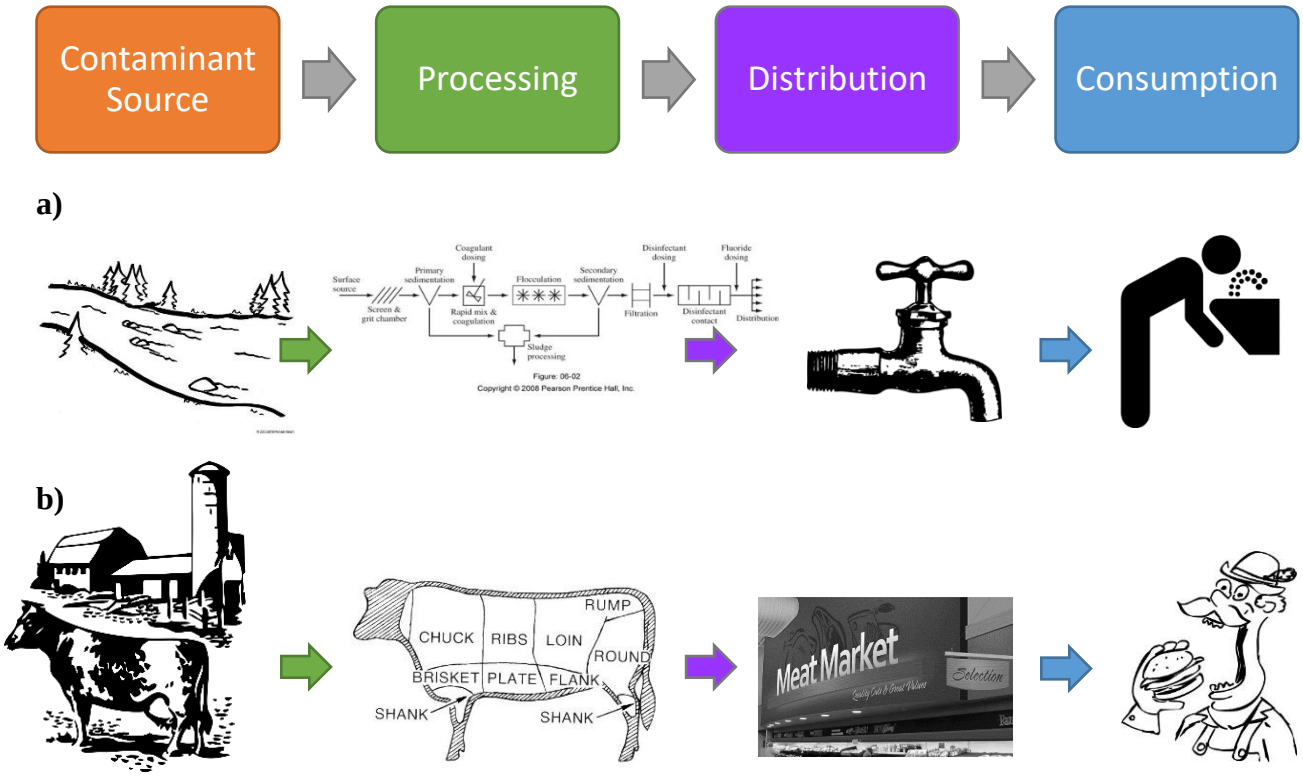

Figure 2. Processing flow patterns: a) drinking water industry; and b) beef industry

Public health is an interdisciplinary field that includes, but is not limited to environmental health, epidemiology, and prevention education, all of which are ultimately focused on prevention of human disease (Bellman, 2012). The works in this dissertation as shown in Figure

3 focus on water safety, food safety, and public communications and the impacts that these disciplines have on overall public health.

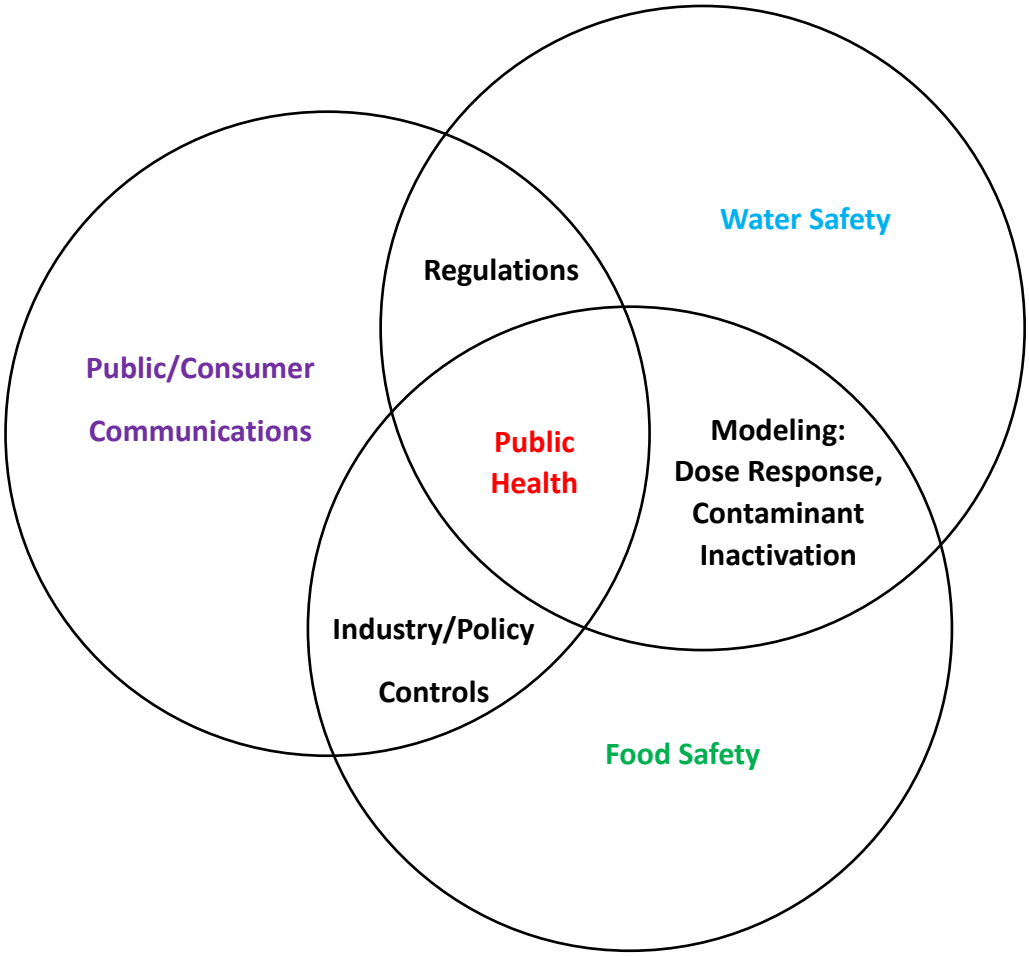

Figure 3. Multiple intersections that encompass interdisciplinary work in this dissertation

The water safety (Section I and II) research in Chapters 2-4 focuses on water contaminants from chemical spills, the complexities of chemical mixtures, such as minor and major components or geometric isomers, the effect of fate and transport properties, and the level of human exposure and resulting dose response from ingestion and inhalation. Odorous properties that affect human inhalation exposure when showering can be as harmful as the effects of ingestion to contaminants in drinking water. Dose-response for all exposure routes can be multiple folds higher for healthy adults than for the susceptible population.

Public communications concerning water are discussed in Chapters 5-7 (Section II). Communication tools for water safety include the Drinking Water Taste-and-Odor Wheel and Consumer Confidence Reports (better known as water quality reports). Furthermore, public health campaigns that uses social media tools or consumer websites to educate the public on food safety is discussed in the latter part of Chapter 11. The overall goal of this dissertation is to bridge the communication gap between drinking water and food experts and consumers.

For food safety (Section III) in Chapters 8-10, the microbial, Shiga toxin producing *Escherichia coli* (STEC), in the beef industry is the focus contaminant. It is known that STEC in the beef industry can be minimized through a quantitative microbial risk assessment (QMRA) following a farm-to-fork modeling framework. QMRA can be accomplished by using slaughter plant intervention strategies to increase log reduction of contaminants, improving cooking times and temperature methods to ensure maximum microbial die offs at the consumer and retail level, and assessing minimum effective dose for different population susceptibilities in order to prevent human illnesses due to foodborne pathogens.

Support and funding was provided by Virginia Tech Water INTERface Interdisciplinary Graduate Education Program (IGEP), the National Science Foundation (NSF) CBET Award no. 1424234, and the Agriculture and Food Research Initiative Competitive grant 2012-68003-30155, U.S. Department of Agriculture Food, National Institute of Food and Agriculture (USDA-NIFA).

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SECTION I: Water Safety

A risk assessment framework was used to evaluate the impacts of a chemical spill. On January 9, 2014, a tank on a chemical tank farm owned by Freedom Industries had a leak that spilled over 10,000 gallons of crude MCHM into the Elk River in WV. The tank farm was located adjacent to the river, thus, the chemical flowed freely into the Elk River, which was unfortunate because the location of the spill was only 1.5 miles upstream from the drinking water intake for Charleston, WV and surrounding areas. Contaminated drinking water was processed through the water utility and distributed to people's homes. In the early morning of the spill, odor complaints from residents who lived near the tank farm were reported. However, it was not until about 10:30 AM that Freedom Industries discovered that there was a leak. It was not until later that evening around 6:00 PM that the Governor of WV declared a "DO NOT USE" water order for drinking or other uses. By midnight, President Obama declared this spill as a national disaster. The "DO NOT USE" order was not lifted until over a week in some areas, where over 300,000 residents were already affected. Bottled waters flew off the shelves and water stations were set up for residents to get water. It is estimated that there was over $61 million in economic lost within the first month alone as businesses were closed and residents could not go to work.

MCHM is commonly used in the industry for coal cleaning. However, the MSDS sheet available at the time of the spill was incomplete, with major data gaps for fate and transport properties and no toxicity information available. The research objectives were to fill these data gaps in order to perform assessment of human exposure by ingestion and inhalation for children and adults. The tasks completed included: 1) Composition of crude MCHM; 2) Odor thresholds of components; 3) Biological, physical, and chemical properties; and 4) Exposure assessment and dose-response. After significant analytical chemistry work, crude MCHM was found to

compose of 10 cyclohexane components, with *trans*- and *cis*-4-MCHM making up 60.3% and 34.5%, respectively, of the total crude MCHM mixture. These components were found to have odorous properties. *Trans*- and *cis*-MMCHC were minor components, making up 0.3% and 0.7% of the total mixture, respectively, but also had large contributing factors to the odor detected.

Chapter 2 explores the complexities of the crude MCHM chemical spill in Charleston, WV. Odorous properties and human odor detection thresholds were determined for the major components, *cis*- and *trans*-4-MCHM. Chapter 3 further evaluates the minor components, *cis*- and *trans*-MMCHC, which made up only 1% of the crude MCHM mixture, but were large contributing factors to the odors detected from the spill due to fate and transport and biological properties. Chapter 4 explores inhalation exposure from showering and ingestion exposure from drinking contaminated MCHM drinking water. In addition to different exposure routes, doses differed among children and adults.

CHAPTER 2: A Tale of Two Isomers: Complexities of Human Odor Perception for *cis*- and *trans*-4-Methylcyclohexane Methanol from the Chemical Spill in West Virginia

This chapter has been published as: Gallagher, D.L., Phetxumphou, K., Smiley, E., and Dietrich, A.M. Tale of two isomers: Complexities of human odor perception for *cis*- and *trans*-4-methylcyclohexane methanol from the chemical spill in West Virginia. *Environmental Science and Technology*, 49(3):1319–1327, 2015. DOI: 10.1021/es5049418

ABSTRACT

Application of gas chromatography with mass spectrometric and human olfactory "sniffer" detectors reveals the nature of odorous chemicals from an industrial chemical spill. Crude 4-methylcyclohexane methanol (4-MCHM) spilled in a river and then contaminated drinking water and air for over 300,000 consumers living in West Virginia. Olfactory gas chromatography allows investigators to independently measure the odor of each chemical component in a mixture. Crude 4-MCHM is comprised of several major cyclohexane components, four of which have distinct isomer pairs. The cis- and trans-4-MCHM isomers are the only components to have distinct odors at the concentrations used in this study. The trans-4-MCHM is the dominant odorant with descriptors of "licorice" and "sweet". Trans-4-MCHM has an air odor threshold concentration of 0.060 ppb-v (95% CI: 0.040 - 0.091). The odor threshold concentrations are not influenced by gender or age, but are lower by a factor of 5 for individuals with prior exposure compared to naïve subjects. Individual trans-4-MCHM odor threshold concentrations vary by more than a factor of 100. The cis-4-MCHM isomer has approximately a 2000-fold higher odor threshold concentration, different descriptors, and an even wider individual response range.

INTRODUCTION

Chemical Spill in Charleston, WV

On the morning of January 9, 2014, West Virginia (WV) residents living near the Freedom Industries tank farm complained of licorice odors in the air. This prompted the WV Department of Environmental Protection to investigate and discover that approximately 10,000 gallons (37,800 L) of crude 4-methylcyclohexane methanol (MCHM) leaked and drained into the nearby Elk River.[1, 2] The WV American Water drinking water treatment plant (DWTP) was located 2.4 km downstream of the leaking tank in the city of Charleston, which is WV's largest city and located in densely populated Kanawha County. By the afternoon of January 9, WV American Water announced that contaminated Elk River water was processed into drinking water and delivered to over 300,000 consumers in Charleston, Kanawha County, and eight other counties.[1, 3, 4]A licorice odor was acknowledged at the DWTP and complaints of strong licorice odors from drinking water were reported to government officials by residents living throughout Charleston and Kanawha County.[4] Radio, television and social media provided news about the spill and its pervasive drinking water contamination. By 6:00 PM on January 9, WV Governor Tomblin, in conjunction with WV American Water, issued "do-not-use orders"[1] and notified consumers that tap water was not safe to use for drinking, cooking, cleaning, bathing, or washing.[3] During the next day, concentrations of 4-MCHM leaving the DWTP were 750 - 2,400 ug/L (see Table S1 for concentration and timeline).[5] Schools, businesses, and government offices closed for several days. Drinking water in bottles and from trucks was provided to consumers by the government. Home-owners were instructed to flush and purge contaminated water from homes.[6] The "do-not-use orders" were lifted for some consumers four days after the spill; within a week, all "do-not-use-orders" were lifted.[1] Concentrations of 4-MCHM leaving the DWTP were below the

detection limit of 0.5 ug/L from January 14-19, but 2-5 ug/L concentrations were detected January 20-30.[7]

Even though the water was declared to meet drinking water quality, public confidence was low because of lingering licorice odors. Hundreds of people went to the hospital reporting symptoms of nausea, headaches, itching, sore throat, vomiting, eye irritation, rash, abdominal pain, and diarrhea, that may have been associated with exposure.[2, 6] Two weeks following the spill, a survey found concentrations of <10 to 420 μg/L 4-MCHM in household drinking water.[6] In February, public schools were closed again due to detection of both 4-MCHM and its corresponding licorice odor.[8, 9] The financial damage from the spill was estimated at $61 million for just the first month.[1]

After the initial spill, the Centers for Disease Control used limited toxicity data to develop an adverse effect level of 1 mg/L 4-MCHM in drinking water.[1, 10] The Eastman Chemical Company warned that at elevated temperatures, the chemical can cause irritation of the eyes and respiratory tract.[11] Jeffrey McIntyre, the President of the WV American Water DWTP, expressed his opinion that the licorice odor was "an aesthetic issue below 1 part per million. It's not a health-based issue."[12] However, the odor threshold concentration was not known. Consumers judge the quality of their water through its taste, odor, and appearance and lack confidence in the water when it does not meet sensory expectations.[13-16] Consumers are concerned about safety and quality of their drinking water and naturally avoid products that are unusual and unpleasant.[17]

Crude 4-MCHM is primarily used as an industrial foaming agent to clean impurities from coal and ores; 25% of coal plants in WV use it.[18] Annually, 5-10 million pounds are produced[19] and the manufacturer lists crude 4-MCHM as a mixture of 68-89% 4-MCHM plus four other

cyclohexane compounds.[11] Although isomers are not identified in the crude 4-MCHM by Eastman Chemical Company, pure 4-MCHM is commercially available as a mixture of cis- and trans- isomers[20] in portions of 0.6757 and 0.3243, respectively.[21] Because 4-MCHM is not regulated under the Toxic Substance Control Act,[22] little data are published on its chemical properties. Some studies report a unique licorice odor[18] although the MSDS states "no data" for odor.[11] Crude 4-MCHM is known to be composed of several chemicals, but the one associated with the licorice odor is unclear.

Human Odor Detection

Human odor detection follows a semi-logarithmic relationship, called Steven's Power law, with increased human response related to the log concentration.[23, 24] Human odor perception and individual odor threshold concentration (OTC) are affected by many factors including age, experience, genetics, health, mood, temperature, time of day, test type, and test location. OTC is often reported as a single value, but with the variability in human sensory abilities, the population odor threshold concentration can best be described by a range. Individual odor thresholds can vary by 100-fold or more.[16, 25-27]

Complex and *difficult* are often applied to describe the sense of smell. Odorant molecules interact strongly or weakly with combinations of odor receptors in the olfactory system to create neural signals that are transmitted to the brain and translated into language.[28-31] Trained assessors and experienced persons can better recognize and describe odors when they have developed "odor memories", while naïve subjects have a harder time describing an odor.[32-35] When an odor is recognized, humans will remember the smell for a long period of time[32, 36, 37] and when the odor

is encountered again, it often prompts the retrieval of previous memories and/or emotional responses.[36]

In addition to better odor memory, experienced individuals or those who with prior exposure to an odor can have higher sensitivity and lower OTC than inexperienced or naïve individuals. Subjects pre-exposed to citralva (lemon-orange odor) had lower OTCs than naïve subjects.[38] However, for benzaldehyde (cherry, almond odor), subjects did not have a change in sensitivity before and after exposure.[38] Olfactory detection and sensitivity can be improved by repetitive practice and training to discover and isolate particular odorous compounds.[35]

Gas Chromatography-Olfactometry-Mass Spectrometry (GC-O-MS) for Odorant Isomers

Since the 1980's, gas chromatography-olfactometry-mass spectrometry (GC-O-MS) has been widely applied to identify odorous components throughout the flavor and fragrance industries.[24, 39, 40] More recently, GC-O-MS is rising in prominence in environmental analyses to identify odorous components in drinking water,[41-44] waste, and reuse waters.[45, 46] GC-O-MS separates components in mixtures by GC, then direct portions of the column effluent to an MS for chemical identification and portions to a sniffer port for human assessors to detect and describe gas phase odorants. Prior chemical identity and/or purity of the odorant(s) are not required. Detection occurs if an assessor detects the odor at the known, correct retention time. Detects and non-detects over a range of concentrations leads to an OTC in air. Direct measurement of gas phase OTCs, which is how odors are perceived, is an advantage over determining the odor threshold from an aqueous phase solution. Transfer from aqueous phase would need to consider many factors such as surface area, container materials, head-space volume, and temperature.

GC-O-MS demonstrates that the human sense of smell can be very discriminating related to slight chemical structural alterations, as exemplified by stereoisomers and enantiomers. GC-O-MS resolved the different odor characteristics and a 20-fold difference in air thresholds of cis- and trans-β-methyl-γ-octalactone, key odor components of wines aged in oak barrels.[47] When applied to 20 isomers, enantiomers, or metabolites of 1,8-cineole, the main chemical in eucalyptus fragrance, GC-O-MS demonstrated that the 18 derivatives also had eucalyptus as a descriptor, usually in conjunction with other odor notes including sweet, citrus, plastic, pine, flowery, earthy, musty, rotten or fecal. The odor thresholds of the 1,8-cineole-related compounds ranged from approximately 10-fold less to 10-fold greater than 1,8-cineole.[48] To resolve drinking water odor issues, GC-O-MS successfully determined OTCs in air at pg/L and sub-pg/L concentrations for iodophenol and methyliodophenol that cause medicinal odors in bottled water.[44] Human assessors used GC-O-MS to detect odors of drinking-water associated haloanisoles at sub-pg amounts and describe them as predominantly rubbery, with additional earthy, musty, fruity, and plastic odors.[41] The origin of olive-oil and tutti-fruity odors in tap water were determined by GC-O-MS to be cis- and trans- 2-ethyl-4-methyl-1,3-dioxolane and 2-ethyl-5,5-dimethyl-1,3-dioxane spilled from a manufacturing facility.[43]

The goal of this research is to develop a strategy for determining the odorous components and their human odor threshold concentrations for individual chemicals present in complex mixture that contaminate the environment. The specific objectives of this research were to:

1) Determine the specific component(s) in crude 4-MCHM associated with the licorice odors.

2) Estimate population and individual OTCs in air for the odorous components.

3) Evaluate reported descriptors of the odorants.

4) Determine if characteristics of human subjects (prior exposure, gender, age) impact OTCs and descriptors.

MATERIALS AND METHODS

Reagents

Pure 4-methyl-1-cyclohexane methanol (CAS # 34885-03-5) was 98% pure and purchased from TCI America (USA).[20] It was a mixture of cis- and trans-isomers in portions of 0.6757 (cis) and 0.3243 (trans) based on NMR analysis[21] and confirmed by comparing GC-MS peak areas in our laboratory and assuming the same response factor for cis and trans. The MS spectra are nearly identical. This research refers to this product as pure 4-MCHM. Crude 4-MCHM was supplied courtesy of Eastman Chemical (Kingsport, Tennessee, USA). Methanol was OmniSolv methanol (CAS 67-56-1; Spectrum Chemical). Sample preparation and 4-MCHM handling occurred in a hood located in a separate room from the GC-O-MS.

Analytical Procedure for GC-O-MS

The GC-O-MS used a Focus GC-DSQII MS equipped with an autoinjector/sampler (Thermo Scientific, USA) connected to a Sniffer 9000 Olfactometer with a 10 mL glass nasal cone that is the sniffer port (Brechbühler Inc., Switzerland). Separation analysis performed on Rxi-5Sil MS of dimensions 30 m x 0.25 mm ID x 1 um film (Restek, USA). Operating parameters included injector temperature 250˚C; MS transfer line at 280˚C; Sniffer 9000 transfer line at 170˚C; split ratio was 1 part to GC and 9 parts vented; injection volume was 1 μL. Flow rate through column

was constant at 2 mL/min helium; flow rate to Sniffer 9000 was 1.5 mL/min and to mass spectrometer was 0.5 mL/min. MS operated under full scan mode from 40-200 amu.

Individual components of crude 4-MCHM were separated using a temperature program of 110˚C held 1 min, ramp 10˚C/min to 120˚C, then 1˚C/min to 124˚C, then increased to 235˚C at 15˚C/min. The methanol solvent eluted by 3.5 min and subjects began olfactory sniffing at 4 min and continued until the end of the analysis at 13.4 min. Trans-4-MCHM eluted first as determined by Foreman et al.[21] The pure cis- and trans-4-MCHM were separated with an initial temperature of 110˚C held 1 min, ramp 10˚C/min to 120˚C, then 1˚C/min to 124˚C, then increased to 184˚C at 40˚C/min. The methanol solvent eluted by 3.5 min and subjects began olfactory sniffing at 4 min and continued until the end of the analysis, 7.5 min. Elution of trans- and cis-4-MCHM occurred at approximately 4.6 and 5.9 min, respectively, for both crude and pure 4-MCHM methods. Standards were prepared in methanol and stored in amber glass vials with polyperfluoroethylene faced septa and stored at 5˚C when not in use. Calibration standards were prepared containing 0.5, 1, 2, 5, 10, 25, 50, 100, and 300 mg/L pure 4-MCHM. The regression values were R^2 = 0.999 and 0.999 for cis- and trans-4-MCHM, respectively. Detection limits were calculated based on statistical analysis[49] and yielded GC-MS detection limits of 0.03 mg/L for cis- and 0.03 mg/L for trans-4-MCHM.

Human subjects and Olfactory Analysis

The Virginia Tech Institutional Review Board (IRB) approved this study (IRB Project No. 14-174). Human subjects were required to be over 18 years old and report not having health problems or being pregnant. Thirty-four (16 female) participants, ranging from 20 to 59 years old, were recruited from Virginia Tech and Blacksburg, VA and signed consent forms. Five

subjects were experienced with smelling and describing odors. Training for each subject included a demonstration and practice for sniffing at the olfactory port and turning the sniffer dial. The sniffer port was adjusted in height and position so that subjects rested comfortably in a chair while breathing normally through their nose and exhaling through their mouth so as not to breathe into the sniffer port. The time when the dial was turned was recorded electronically by the Sniffer 9000 software. Subjects provided verbal descriptors of what they smelled.

Identifying Odorous Components of Crude 4-MCHM: In this research, five human subjects [24, 50, 51] were recruited to determine the odorous components in a 1000 mg/L crude 4-MCHM standard by olfactory analysis. These five subjects were experienced with odor description and analysis. Identities of components were determined by library matching to a NIST Mass Spectral Search Program, Version 2.0.[52]

Determining Odor Threshold Concentrations for cis- and trans-4-MCHM: OTCs were determined using GC-O detection for an ascending series of eight concentrations, the number of concentrations applied in threshold analysis by ASTM method E679-04.[53] The lowest concentration was selected to be below the concentration detected by five subjects who were familiar with the odor of 4-MCHM from working with the mixed-isomers in the laboratory. This concentration, 0.25 mg/L cis- and trans-4-MCHM, was then increased by a factor of 2.75 to obtain a series of 8 concentrations. A ninth concentration was added for cis-4-MCHM because preliminary GC-O indications were that this component had substantially less odor than trans-4-MCHM. Prepared concentrations for olfactory sniffing are provided in Table 1 and were confirmed using the calibration curves for cis- and trans-4-MCHM. The gas phase concentrations for the cis- and trans- isomers were calculated as follows. For the cis-4-MCHM, [(1 uL injection volume) * (0.1 split) * (mg/L concentration) * (0.6757 portion) * (1.5/2.0 flow to sniffer)] /0.5 L

tidal volume for one breath.[54, 55] The calculation was similar for the trans-4-MCHM but the value 0.3243 was used for the portion of trans-4-MCHM.[21] Further information is provided in Text S1.

Before commencing an individual olfactory test, a clean glass sniffer port was installed. A methanol blank was analyzed twice to ascertain that base line on the GC/MS was background level. The lowest concentration was then injected and the subject commenced orthonasal sniffing at the sniffer port from 4-7 min. Subjects waited at least five minutes before the next higher concentration was administered. For the ninth concentration of cis-4-MCHM, subjects began sniffing at the olfactory port at 5-7 min for only the cis-isomer. Subjects did not report fatigue when they sniffed for 3 min to perform each olfactory analysis. Subjects also reported that the smell of the 4-MCHM did not linger in their nose or the sniffer port and that once a smell was detected, it quickly disappeared.

Table 1. Standards and gas phase concentrations used for OTC determination

Sample Number	Standards of Pure cis- and trans-4-MCHM mg/L-methanol	cis-4-MCHM Gas phase concentration ppb-v	trans-4-MCHM Gas phase concentration ppb-v
-[a]	0.09	0.002	8.4E-4
1	0.25	0.005	0.002
2	0.69	0.013	0.006
3	1.91	0.037	0.018
4	5.25	0.102	0.049
5	14.43	0.279	0.134
6	39.67	0.768	0.369
7	109.09	2.112	1.014
8	300	5.808	2.788
9	825	15.972	7.666[b]
+[c]	2269	43.922	Not applicable

[a] This concentration was not analyzed by GC-O. It represents the next lowest cis- and trans-4-MCHM concentrations and is prescribed by ASTM Method E679-04[53] to be used when a subject detects the lowest concentration tested.

[b] This concentration was not analyzed by GC-O. It represents the next highest trans-4-MCHM concentration and is prescribed by ASTM Method E679-04[53] to be used when a subject does not detect the highest concentration tested.
[c] This concentration was not analyzed by GC-O. It represents the next highest cis-4-MCHM concentration and is prescribed by ASTM Method E679-04[53] to be used when a subject does not detect the highest concentration tested.

Statistics

Statistics were calculated using R[56] version 3.1.2 and applied to the gas phase concentrations for cis- and trans-4-MCHM. Logistic regressions were used to calculate OTCs in air for the overall population. A positive detection was considered for either of the 2 isomers when the subject recorded detecting an odor at the time the peak arrived at the sniffer ±0.11 min. The OTC was taken at an overall probability of detection of 50%.[57, 58] Individual thresholds were calculated based on geometric means according to ASTM.[53] To avoid a guess being counted as a correct response, the ASTM method calculates an individual OTC as the geometric mean of the next lowest concentration that the subject did not detect and the lowest concentration that the subject correctly identified and which was followed by correct identifications for all higher concentrations. When a subject correctly detects all eight concentrations, then the next lowest concentration using the dilution ratio (Table 1) and the lowest concentration tested are used to generate a geometric mean. When a subject failed to detect any of the eight concentrations of trans-4-MCHM or the nine concentrations for cis-4-MCHM, then the OTC is calculated as the geometric mean of the highest concentration tested and the next highest concentration using the multiplier ratio (Table 1). These assumptions were not needed for the logistic regression approach, which simply uses the fraction of subjects correctly detecting the component at each concentration. Word clouds were prepared using the wordcloud and tm (text mapper) libraries in R as described in Text S2. Descriptors shown are only for positive detections.

RESULTS

The crude MCHM at 1000 mg/L was characterized by the GC-O-MS and revealed that the mixture was comprised of ten distinct cyclohexane components with four isomer pairs. A compound identified as cyclohexane methanol (probability match 59%) was the first to elute, followed by trans-4-MCHM then cis-4-MCHM. 4-MCHM was followed by two isomers of methyl-4-methylcyclohexanecarboxylate (probability match 66.8% and 63.41%), two unknown isomers of low concentration and low (<10%) probability match, two isomers of cyclohexane dimethanol (probability match 65.5% and 50.8%), and dimethyl 1,4-cyclohexanedicarboxylate (probability match 78.4%). Based on summing the total ion chromatogram for all peaks, the percent cis- and trans-4-MCHM was estimated to be approximately 86% in a 1:2 concentration ratio of cis:trans. Only the cis- and trans-4-MCHM had detectable odors with trans described as strong intensity and cis described as weak intensity.

Odor Threshold Concentrations

Logistic regression was applied to data from the 34 subjects to determine the overall threshold at which the probability of detection is 0.5.[27, 57] Plots for the cis- and trans- 4-MCHM isomers are shown in Figure 1, and corresponding OTCs and confidence intervals (CI) are given in Table 2. The general population OTCs differ by a factor of 2000 for the two isomers. The trans-isomer had a much lower OTC concentration, 0.060 ppb-v (95% CI: 0.040-0.091 ppb-v) compared to the cis-isomer, 120 ppb-v (95% CI: 1.1-14000 ppb-v). The trans-isomer regression also exhibits a much sharper rise indicating that the odor becomes detectable to most of the population for only small increases above the OTC. The cis-isomer, on the other hand, has a much shallower slope indicating a greater diversity of sensitivity among the population. Despite pretesting, the OTC for the cis-isomer had to be extrapolated beyond the tested range. These two issues, the

broad sensitivity and the need for extrapolation, help explain the wide confidence intervals.

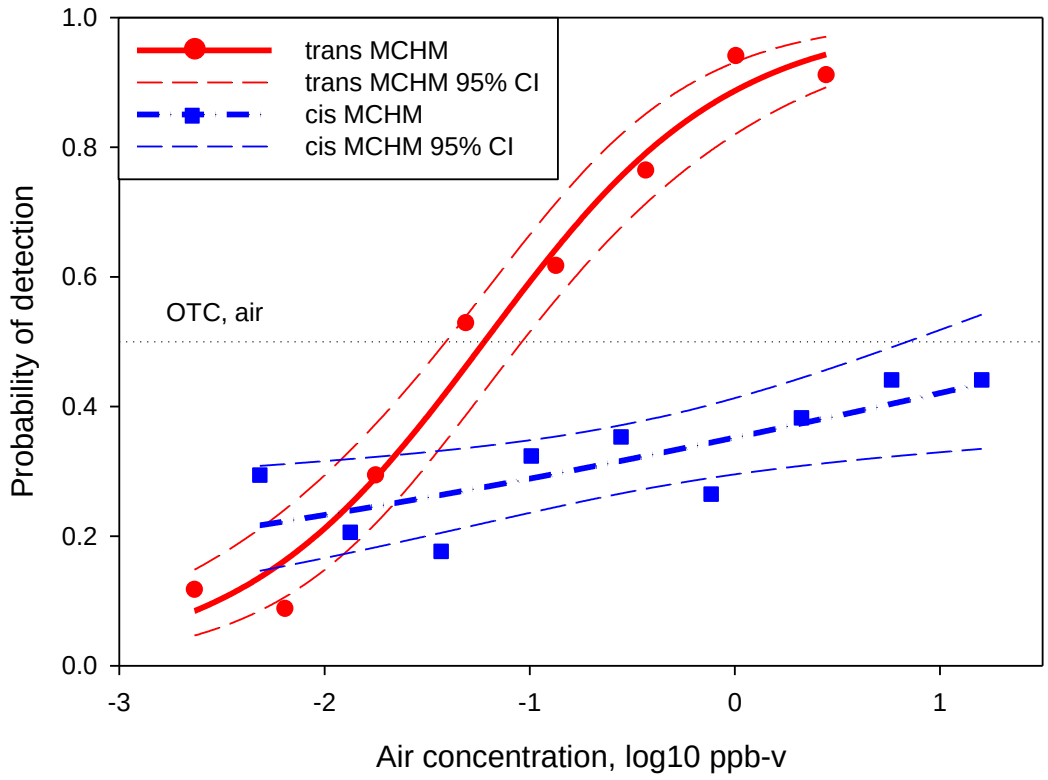


Figure 1. Odor threshold concentrations in air for cis- and trans-4-MCHM.

Differences in sub-population OTCs based on gender, age (<50 years, ≥50 years),[59] and prior exposure were tested for both isomers. Ten subjects were considered pre-exposed to 4-MCHM through 2-4 weeks of handling and smelling 4-MCHM in the laboratory before GC-O-MS testing. The remaining 24 subjects were considered naïve. The results are shown in Table 2.

Table 2. Odor threshold concentrations

Subject groups	N	trans-4-MCHM, air ppb-v OTC[a] (95% confidence interval)	cis-4-MCHM, air ppb-v OTC[a] (95% confidence interval)
All	34	0.060 (0.040 – 0.091)	1.2E+02 (1.1E+00 – 1.4E+04)
Exposed	10	0.018 (0.009 – 0.036)	1.3E-01 (2.2E-02 – 7.5E-01)
Naïve	24	0.102 (0.062 – 0.166)	1.9E+06 (4.5E-05 – 7.7E+16)
Male	18	0.092 (0.050 - 0.169)	3.4E+02 (9.7E-01 – 1.2E+05)
Female	16	0.039 (0.022 – 0.067)	9.1E+00 (3.5E-02 – 2.4E+03)
Age >50 yr	6	0.041 (0.019 - 0.086)	6.3E-01 (9.7E-02 – 4.1E+00)
Age < 50 yr	28	0.066 (0.041 – 0.107)	8.9E+03 (2.4E-02 – 3.3E+09)

a. Odor threshold concentration

No significant differences were found based on age or gender. However, a significant difference was found based on prior exposure. Subjects who had been previously exposed to 4-MCHM had an OTC for trans-4-MCHM 5.6 times lower than the naïve subjects. The difference was even greater for the cis-isomer. The logistic regression fits for these groups are shown in Figure 2. This also explains why the range-finding GC-O testing to determine the appropriate concentration range proved too low for cis-4-MCHM. The initial GC-O sniffers were all part of the exposed group, and the range was suitable, i.e., did not need extrapolation, to determine an OTC for cis-4-MCHM.

The individual geometric mean OTC analysis[53] is shown in Figure 3 and is consistent with the logistic regression results. The overall OTC for trans-4-MCHM was calculated at 0.12 ppb-v. A 6.5-fold difference was noted between naïve subjects (0.21 ppb-v) and exposed subjects (0.032 ppb-v). For the cis-isomer, the values are 5.31 ppb-v overall, with naïve at 11.4 ppb-v and exposed at 0.85 ppb-v. Because most subjects could not detect the highest concentration tested, the cis-values are highly influenced by the ASTM method assumption that the next highest concentration in the sequence would be positive. This explains why the geometric mean OTCs differ by only a factor of 44 for the isomers compared to a factor of 2000 for the logistic regression method.

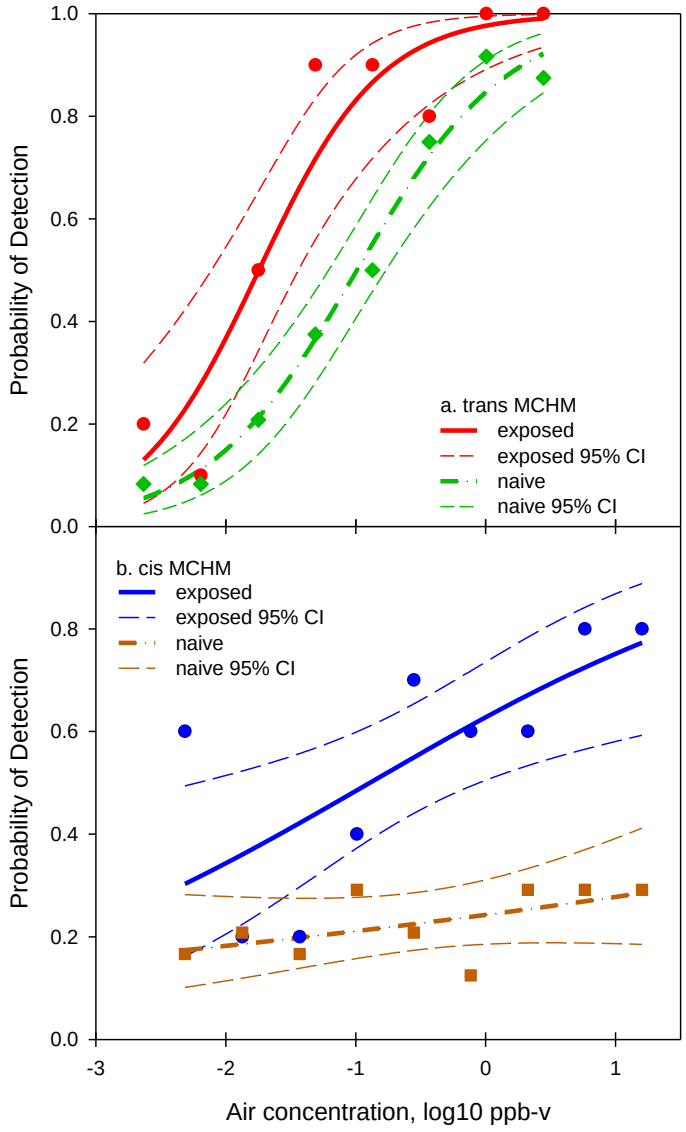


Figure 2. Comparison of exposed versus naive populations for trans-4-MCHM (a) and cis-4-MCHM (b)

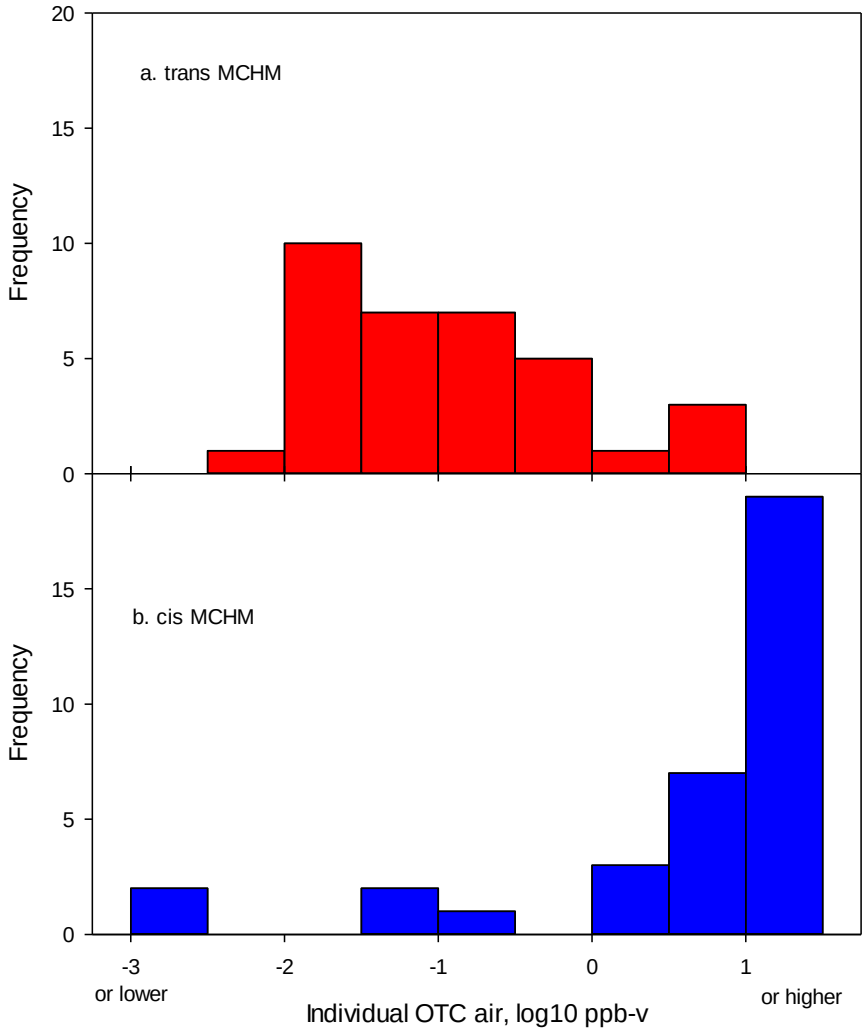


Figure 3. Histogram of individual geometric mean OTCs for trans-4-MCHM (a) and cis-4-MCHM (b).

EPI Suite v4.11[60] provided three estimates of Henry's Law constant for 4-MCHM at 25˚C: 4.9E-6 atm*m^3/mol for the vapor pressure/water solubility ratio, 6.4E-6 atm*m^3/mol for the group method[61], and 8.6E-6 for the bond method[62]. The middle value was used to estimate corresponding aqueous phase OTCs. The estimation methods cannot incorporate geometric isomer differences.

The logistic regression aqueous threshold for trans-4-MCHM was estimated as 1.20 (95% CI: 0.79-1.82) μg/L for the general population. For the exposed and naïve populations, values are 0.36 (95% CI: 0.18 – 0.72) and 2.04 (95% CI: 1.25 – 3.32) μg/L respectively. Another study reported an aqueous odor threshold for crude -4-MCHM of <0.15 ug/L; that study used only nine subjects who smelled water from plastic cups.[63] These aqueous thresholds are reported for 25˚C but would be temperature dependent. Despite the individual variation, based on the logistic regression curve, over 98% of the general public would be able to detect odors from an aqueous concentration of 1 mg/L crude 4-MCHM, as reported in the range leaving the water treatment plant during the early days of the spill.

Aqueous thresholds based on logistic regressions for cis-4-MCHM are 2.48E3 (95% CI: 22.20 – 2.77E5), 2.56 (95% CI: 0.44 – 15.08) and 3.72E7 (95% CI: 9.03E-4 – 1.53E18) μg/L for the general, exposed, and naïve populations respectively.

Descriptors

Odor descriptors for each of the positive detections of cis- and trans-4-MCHM are depicted as word clouds in Figure 4. Word clouds list each descriptor with increasing size and font weight to indicate a greater frequency of use. In general, once an odor was detected by a subject, the subject used the same descriptor for all higher concentrations. "Licorice" was the most common descriptor for the trans-isomer. An analysis of the exposed and naïve subpopulations indicates that the exposed group was very consistent, with almost all the descriptors either "licorice" or "sweet". The exposed group had read news reports of the spill where "licorice" was used extensively. They also had been exposed for several weeks to the odors of 4-MCHM in the laboratory and talked among themselves about the "licorice" odor descriptor. Naïve subjects

were generally less aware of the spill and used a much more diverse set of descriptors. “Sweet” was the most common, followed by “almonds”, “fruity”, and “licorice”.

The descriptors for the cis-isomer exhibited a different pattern. The descriptors for the exposed group were more varied. “Licorice” and “sweet” were again the most common, followed by strawberry and acetone. The naïve group descriptors had much less variability, but this is because there were relatively fewer detections. Thus, this figure is heavily influenced by the two naïve subjects who detected and described the cis-isomer at every test concentration used.

DISCUSSION

The unique abilities of GC-O-MS were essential for resolving the components in crude 4-MCHM to provide chemical identification coupled to odor identification. The ability to use this instrument to directly sniff varying concentrations of individual components was critical to determine gas phase odor threshold concentrations.

Slight changes in chemical structure of odorant molecules can profoundly affect odor qualities of detection and description principally because of alterations to odorant-receptor interactions in the olfactory system.[29] Chemical structure-biological activity effects are well established for odorants[64, 65] and confirmed in this research for 4-MCHM. Logistic regression calculated the population thresholds for trans-4-MCHM (OTC = 0.060 ppb-v) to be 2000-fold lower than for cis-4-MCHM (OTC =120 ppb-v) (Table 2).

For individual subjects, OTCs varied from 0.004 to 4.62 ppb-v for trans-4-MCHM. For cis-4-MCHM the individual OTCs varied from 0.003 ppb-v to >26.5 ppb-v, with most individuals in the upper range as shown in Figure 3. For cis-4-MCHM, two individuals detected all test concentrations and thus had OTC = 0.003 ppb-v, which is lower than their OTC values for trans-

4-MCHM. Neither individual used “licorice” to describe the cis-4-MCHM odor. The differences in cis- and trans-4-MCHM are examples of the known variability in individual subjects and an indicator of the known complexity of human odor perception.[27, 29, 30]

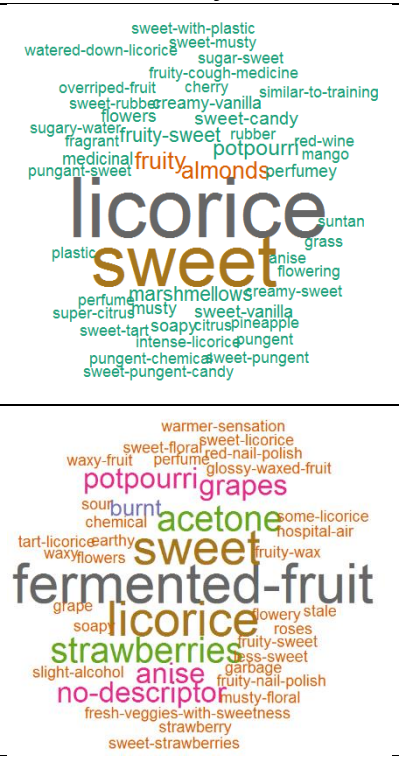
	All Subjects	Exposed Subjects	Naïve Subjects
trans-4-MCHM			
cis-4-MCHM			

Figure 4. Word clouds for 4-MCHM isomer odor descriptors.

When the tested concentration range includes concentrations that most subjects can positively detect, the OTC values calculated by logistic regression and geometric means are usually similar.[57] This was observed for trans-4-MCHM as the overall OTC values were 0.060 ppb-v for logistic regression and only a factor of 2 higher (0.12 ppb-v) for geometric mean. For cis-4-MCHM, however, these two methods provided very different OTC values as is expected when the most subjects cannot detect the odor even at the higher concentration. The value of 120 ppb-v for logistic regression required an extrapolation but is based on the actual percentages of detects and non-detects at tested concentrations. The 5.31 ppb-v value for the geometric mean is based on assuming that the subjects would detect the next highest concentration.

Cognition and sensory research establishes that prior exposure to an odor and its name produces an "odor memory" that results in improved recognition for exposed individuals compared to naïve subjects.[30, 32, 66] This phenomenon was recently demonstrated for the musty drinking water odorant 2-methylisoborneol where drinking water treatment personnel could more accurately describe the musty odor than naïve consumers.[67] In the present study, those with prior exposure to 4-MCHM consistently described the odor of the trans-4-MCHM as licorice and sweet, while naïve subjects had little consensus and many odor descriptors (Figure 4). Additionally, subjects who had prior exposure to 4-MCHM had an OTC for trans-4-MCHM 5.6 times lower than the naïve subjects. This is also consistent with previous research that indicates prior exposure can lead to increased sensitivity and lower thresholds.[35, 38, 68] Thus, prior exposure and familiarity allowed better detection and more accurate descriptions for trans-4-MCHM. Charleston, WV residents are more likely to be represented by the exposed OTCs and descriptors because of the repeated exposure and the numerous news reports using "licorice" as a descriptor.

Many consumers complained of odors even after the do-not-use ban was lifted, i.e. after the aqueous crude 4-MCHM concentration fell below the CDC guidance of 1 mg/L. This consumer feedback is consistent with an estimated aqueous OTC concentration almost 1000-fold lower, i.e. 1.2 μg/L at room temperature. Consumers could still detect the aesthetic change in the quality of their drinking water even after the ban was lifted. Because Henry's Law constant increases with temperature, hot water exposure such as showering and dishwasher use would lower the aqueous phase OTC even further.[69]

Correlating chemical structure with odor/physiological/psychological effects is an active area of study. Just as for chemical toxins and drugs, slight changes in odorant structure also cause different physiological and psychological responses.[70] Such effects have been well studied for cyclohexane-derived fragrances. Carvone, 2-methyl-5-(1-methylethenyl)-2-cyclohexenone, occurs as (+)-carvone (caraway-like odor) and (−)-carvone (spearmint-like odor). Both isomers had similar physiological effects of increased pulse rate and diastolic blood pressure. However, only (−)-carvone had a psychological effect of decreased calmness.[71] Likewise, both isomers of 1-methyl-4-(1-methylethenyl)-cyclohexene, known as limonene, had similar physiological effects but (+)-limonene (orange-like odor) had physiological effects while (−)-limonene (pine/turpentine-like odor) did not.[71]

The role of odor effects on human physiology and psychology are little studied for environmentally produced odors, which are often treated as a nuisance[72] or aesthetic-only concern.[13] One environmental-odor issue that has received in-depth attention is swine-odors from concentrated animal feedlot operations.[36, 73] Under controlled laboratory conditions, healthy subjects reported increased headaches, nausea, and eye-irritation when exposed to environmentally relevant concentrations of swine odors.[74] Residents living in neighborhoods

near swine farms and exposed to odorous hydrogen sulfide, ammonia, and volatile organic chemicals, reported that the odors interfered with activities of daily life[75] and triggered stress and negative moods.[76] These residents experienced increased systolic and diastolic blood pressure that could potentially lead to chronic hypertension.[77] The swine-odor researchers emphasized that health is not just the absence of disease, but rather a state of wellbeing that unwanted odors can negatively impact.

Residents of WV were exposed to crude 4-MCHM through bathing, showering, and other skin contact or eating, drinking, and swallowing.[78] Hospitals reported that residents complained of adverse health effects, which included nausea, headaches, skin rash, vomiting, sore throat, cough, abdominal pain, and diarrhea during and for days after the spill. The direct role of cis- and trans-4-MCHM were not ascertained for adverse human health. Nonetheless, research on fragrance compounds confirmed that cyclohexane-based odorants can affect physiological and psychological behavior and thus 4-MCHM may have had a role in the wellness of the residents. Additionally, cognition research demonstrates that odors are strongly linked to memory, and when an odor is encountered again, it often prompts the retrieval of previous memories and/or emotional responses.[32, 36] Thus, West Virginia residents who associated the licorice odor with a chemical spill that contaminated their water supply could have an adverse reaction even if there were no known health effects at the levels of exposure.

ENVIRONMENTAL IMPLICATIONS

This research demonstrates that GC-O-MS is an essential tool for environmental analysis to identify odorous components in mixtures and to determine OTCs in air, especially when the components are unknown or not available as purified standards. The revelation that individuals

who had prior exposure to 4-MCHM had lower individual OTCs than naïve individuals has important implications for understanding at what concentrations individuals can perceive odors as well as for using published OTCs developed to predict behaviors in situations where repeated exposure occurs. This research also revealed that cis- and trans-4-MCHM had drastically different OTCs and descriptors. The spill was viewed as an "aesthetic issue" by the water utility, which overlooked that air and water aesthetics are very important to the general public. While varying biological effects of stereoisomers and enantiomers is acknowledged in the food, beverage, and fragrance industries and in toxicology, it is little discussed in environmental odor analysis. Likewise, odorants are acknowledged to have physiological and psychological effects, but they are too often reduced to an "aesthetic nuisance" in the environmental field. Future studies of environmental odorants should incorporate an interdisciplinary approach that integrates cognition, sensory, and health fields with environmental monitoring and identification to resolve issues for society.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

The authors appreciate the participation of the human subjects who contributed to the research. Ms. Phetxumphou is supported by Virginia Tech's "Water INTERface" Interdisciplinary Graduate Education Program. This research was performed in parallel with a project funded by the National Science Foundation, Division of Chemical, Biological, and Environmental Transport. CBET Award # 1424234. Opinions, findings, conclusions, and recommendations are those of the authors and do not necessarily reflect those of funding agencies.

SUPPORTING INFORMATION

This information is available free of charge via the Internet at http://pubs.acs.org/.

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CHAPTER 3: Subtleties of Human Exposure and Response to Chemical Mixtures from Spills

This chapter has been published as: Phetxumphou, K., Dietrich, A.M., Shanaiah, N., Smiley, E., and Gallagher, D.L. Subtleties of human exposure and response to chemical mixtures from spills. *Environmental Pollution, 214*: 618-626, 2016. DOI: 10.1016/j.envpol.2016.04.056

ABSTRACT

Worldwide, chemical spills degrade drinking water quality and threaten human health through ingestion and inhalation. Spills are often mixtures of chemicals; thus, understanding the interaction of chemical and biological properties of the major and minor components is critical to assessing human exposure. The crude (4-methylcyclohexyl)methanol (MCHM) spill provides an opportunity to assess such subtleties. This research determined the relative amounts, volatilization, and biological odor properties of minor components *cis*- and *trans*-methyl-4-methylcyclohexanecarboxylate (MMCHC) isomers and major *cis*- and *trans*-4-MCHM, then compared properties and human exposure differences among them. ^{1}H nuclear magnetic resonance and chromatography revealed that the minor MMCHC isomers were about 1% of the major MCHM isomers. At typical showering temperature of 40°C, Henry's law constants were 1.50×10^{-2} and 2.23×10^{-2} for *cis*- and *trans*-MMCHC, respectively, which is 20-50 fold higher than for 4-MCHM isomers. The odor thresholds were 1.83 ppb-v and 0.02 ppb-v air for *cis*- and *trans*-MMCHC, which were both described as predominantly sweet. These data are compared to the higher 120 ppb-v air and 0.06 ppb-v odor thresholds for *cis*- and *trans*-4-MCHM, for which the *trans*-isomer had a dominant licorice odor. Application of a shower model demonstrated that while MMCHC isomers are only about 1% of the MCHM isomers, during showering, the MMCHC isomers are 13.8% by volume (16.3% by mass) because of their higher volatility. *Trans*-4-MCHM contributed about 82% of the odor because of higher volatility and lower odor threshold, *trans*-MMCHC, which represents 0.3% of the mass, contributed 18% of the odor. This study, with its unique human sensory component to assess exposure, reaffirmed that hazard assessment must not be based solely on relative concentration, but also consider the chemical fate, transport, and biological properties to determine the actual levels of exposure across different media.

Capsule Abstract

Water-air partitioning and geometric isomerism made minor spill component into major human exposure chemical. *Trans*-MMCHC was only 0.3 % of mixture but contributed to 18% total odor.

Key words: chemical spill; mixtures; odors; water-atmosphere partitioning; Henry's Law

1. INTRODUCTION

Worldwide, intentional chemical releases and unintentional chemical spills contaminate water bodies and threaten ecosystems, navigation, human health, drinking water, recreation, and the economy (Jiang et al., 2012). It is common for spills to be mixtures of chemicals; individually and collectively, the chemicals have complex exposure risks due to varying fate and transport properties, monitoring capabilities, and biological effects from ingestion, inhalation or dermal sorption. When a spill directly pollutes drinking water and impacts human health, the concerns are immediate. The Sandoz Chemical spill of over 1300 metric tonnes of pesticides, dyes, and organometallic compounds into the Rhine River captured global attention in 1986. That massive spill closed drinking water treatment plants in Switzerland, Germany, France, and the Netherlands as the plume traveled 1320 km and 14 days before discharging into the North Sea (Capel et al., 1988). In 1991, 68,000 L of the pesticide metam sodium leaked into the Sacramento River, California, USA. Following the spill, residents smelled the chemical-horseradish-rotten-egg odors of metam sodium and also its degradation products methylisothiocyanate and hydrogen sulfide (Bowler et al., 1994). The known toxicity of volatilized methylisothiocyanate caused evacuation of residents as the plume travelled for days to Lake Shasta, a drinking water and recreational reservoir. In 2005, an estimated 90 metric tonnes of mostly nitrobenzene and some carcinogenic benzene spilled into the Songhua River in Jilin City, Jilin Province, China. The resulting 80 km chemical slick caused undrinkable water for millions of people during its six weeks of travel from Jilin City through Jilin and Heilongjiang Provinces and into Russia before discharging to the Sea of Okhotsk (BBC News 2005; Zhang et al., 2010). In 2014, nearly 38,000 L of sweet licorice smelling crude (4-methylcyclohexyl)methanol (MCHM) spilled into the Elk River, West Virginia, USA. Contaminated river water was processed into drinking water, distributed, and its odor and

chemical contamination disrupted lives and livelihoods of about 300,000 people for several months (Manuel, 2014; Schade et al., 2015). Crude MCHM was a mixture of at least ten cyclohexanes (Foreman et al., 2015; Dietrich et al., 2015). Furthermore, the crude MCHM plume traveled nearly 600 km through the Ohio River, causing closure of drinking water intakes in Ohio and Kentucky (Foreman et al., 2015). In 1988, prompted by the lack of fate, transport and toxicity data for chemicals in the Sandoz Chemical spill into the Rhine River, Capel et al. (1988) stated, "A data bank that stores environmentally useful information on anthropogenic chemicals should be made accessible…" Nearly a quarter century later, there was a severe lack of fate, transport, odor, and toxicity data for crude MCHM (Eastman Chemical Company, 2011). Thus, there are still large data gaps that need to be filled and many lessons to learn for managing the effects chemical mixtures from spills.

Identifying a subtle spill-like event can also be difficult because the source is not always readily apparent. In addition, the origin may be more biological than chemical. For example, an odorous black water agglomerate infiltrated the main drinking water intake for Wuxi City, China and subjected residents to weeks of odors and health concerns. The agglomerate originated from the accumulation and death of algae, primarily *Microcystis aeruginosa,* and contained high ammonium levels and strong septic/marshy odors from dimethyl trisulfide and related alkyl sulfide compounds, even though minor odors from geosmin and 2-methylisoborneol were initially suspected (Yang et al., 2008; Li et al., 2012; Ma et al., 2015). Likewise, Quintana et al. (2016) described detailed investigations required to identify the source of co-occurring odorous 1,3-dioxanes and 1,3-dioxolanes in a complex and aging urban environment with multiple water sources, multiple treatment plants and processes, and multiple potential dischargers. Low μg/L

concentrations of these resin-related contaminants from treated industrial wastewater caused closure of one water source for drinking water.

When chemicals contaminate drinking water, an initial step is to protect the public health and attempt a risk assessment that includes these four steps: hazard identification, followed by exposure assessment and dose-response assessment, which then combine to a risk characterization (USEPA, 2000). Investigating chemical effects on biological systems is usually done one chemical at a time, but ultimately chemical mixtures should be treated as a whole because in reality humans are exposed to mixtures (Carpenter et al., 2002). Some chemicals are nonadditive and behave independent of one another, while some are additive and can trigger complex interactions (Mumtaz, 1995; Carpenter et al., 2002). Estimating a dose-response from chemical mixtures is difficult because of potential biological/toxicological interactions between chemicals (Carpenter et al., 2002; Teuschler, 2007). When a chemical is also an odorant, dose-response assessment to measure odor threshold is important to investigate. Risk assessment to chemical mixtures is further complicated when there are multiple routes of exposure. For drinking water, health effects are usually focused on ingestion even though inhalation of volatile and semi-volatile chemicals can be a substantial route of exposure during showering and water use (Villanueva et al., 2014). The relative distribution of chemicals between water, air, and biological organisms also vary among individual chemicals in a mixture depending on the physicochemical partitioning properties such as aqueous solubility, volatility, and octanol-water coefficients (Jahnke et al., 2016). Thus, physicochemical partitioning properties result in varying concentrations, fate, transport, and biological properties of the individual chemicals in a mixture and can ultimately result in substantial differences in exposure for humans via ingestion and inhalation routes.

For select chemical mixtures, decades of science and experience have identified the constituent(s) of concern. For example, gasoline is a mixture of many aliphatic and aromatic hydrocarbons. While the composition of gasoline varies, it typically contains only 2% benzene by weight. However, when an underground gasoline storage tank leaks, benzene can be 15-35% by weight of the total dissolved gasoline-related compounds in the aqueous phase groundwater plume because of its higher solubility compared to other gasoline components (Hathaway and Andrews, 1990). This can lead to greater exposure to benzene in drinking water. Benzene is the primary constituent of concern for gasoline-contaminated drinking water because of its known human carcinogenicity that is acknowledged through regulation in drinking water at 1-5 μg/L benzene (SDWF, 2015). Because of the interplay of fate and transport properties and health risk (i.e., solubility and carcinogenicity), gasoline spills lead to a situation where a minor component of a mixture has the highest risk to human health.

For other mixtures of contaminants in drinking water, the science is not sufficiently developed to identify the constituent(s) causing adverse effects to humans. Chlorinated drinking water contains a mixture of various organic and inorganic components, including disinfection by-products (DBPs). Trihalomethanes (total THMs, which include usually un-equal concentrations of trichloromethane, bromodichloromethane, dibromochloromethane, and tribromomethane with differentiating physicochemical and biological properties) are DBPs from chlorine that since the 1970's have been regulated primarily based on ingestion of drinking water and concerns over possible bladder cancer (SDWF, 2015). However, epidemiological studies do not fully agree with cancer risk estimates from ingesting THMs. The lack of epidemiological support suggests the possibility that some of the other hundreds of known co-occurring chlorinated disinfection by-products (Richardson et al., 2007; Zhang et al., 2012) found at lower

concentrations but higher cancer risks may have a role in bladder cancer (Hrudey et al., 2015). Another open question for THMs and DBPs is the extent that the route of exposure plays in exposure risk. Some authors (Miles et al., 2002; Ross and Pegram, 2004) have suggested that dermal or inhalation exposures can bypass liver metabolism and lead to a higher risk than ingestion. Despite decades of study, further research is needed to identify and evaluate the constituents of concern and impact of exposure route for the mixture of chemicals called DBPs (Hrudey et al., 2015).

Establishing which chemicals result in higher human exposure for both ingestion and inhalation is challenging even when the fate, transport, and biological properties of specific chemicals in a mixture are known. Accurate, quantitative physicochemical and biological data are often lacking, however, especially for the tens of thousands low production volume specialty chemicals, including crude MCHM (Schnoor, 2014). Exposure to multiple chemicals in crude MCHM (Figure SI-1) occurred during and for months after the spill even when consumers were not drinking contaminated water. Consumers reported smelling and inhaling the licorice and sweet odor of the crude MCHM mixture especially when using heated water for washing and bathing (Gallagher et al., 2015; Schade et al., 2015). During and after the crude MCHM spill, emergency response and monitoring focused on 4-MCHM, the major chemical in the spill which was actually two chemicals, *cis* and *trans*-4-methylcyclohexyl methanol, but the isomers were not quantitatively analyzed separately (Figure SI-1). However, two isomers of methyl-4-methylcyclohexanecarboxylate (MMCHC), which together only made up about 1% of the crude 4-MCHM mixture, also revealed strong odors and high volatility (Foreman et al., 2015). It is important to consider and track all contaminants in a spill, even if minor, because their properties

such as volatility, solubility, and biological/toxicological effects can vary by orders of magnitude and affect human exposure risks (Carpenter et al., 2002; Sain et al., 2015).

Impacts of odorants in mixtures can be assessed through the odor activity value (OAV), which determines the contribution of an individual chemical to the overall odor of a food, beverage, fragrance (Grosch, 1994), or environmental contaminant (Parker et al., 2012; Chen et al., 2015). OAV is also known as the Odor Hazard Index (OHI) (Chen et al., 2015). Both OAV and OHI are the ratio of the concentration of an individual chemical to its odor threshold concentration (OTC) (i.e., the concentration at which 50% of a population detects an odor). OAV is valuable for indicating the intensity of odor contribution from an individual chemical to the overall odor/flavor and is an active area of research for odors mixtures (Du et al., 2011).

In this manuscript, the authors assess the importance of major and minor components in chemical mixtures as the fate and transport properties can vary by orders of magnitude between chemicals, biological properties and dose response can shift significantly, and interactions of mixtures are complex and require extensive environmental and toxicological data collection. The 2014 crude MCHM spill provides the basis for comparing exposure to individual chemicals in a mixture due to the availability of high quality monitoring data for the major chemical in the spill, data for actual human exposure through sensory response to the co-occurring contaminants over several months, and the spill's notable impact on the well-being of individuals and the affected community.

The objectives of this research were to: 1) determine composition and relative amounts of the *cis*- and *trans*-MMCHC in crude MCHM; 2) determine individual odor descriptors and odor threshold concentrations and estimate population thresholds in air for the odorous components of MMCHC; 3) determine Henry's Law Constants for *cis*- and *trans*-MMCHC; 4) evaluate human

exposure and odor detection during showering; and 5) compare properties and exposures differences between the minor components *cis*- and *trans*-MMCHC and major components *cis*- and *trans*-4-MCHM, which were present in the crude MCHM spill.

2. MATERIALS AND METHODS

2.1 Reagents

Pure methyl-4-methylcyclohexanecarboxylate (MMCHC; CAS no. 51181-40-9) was purchased from Sigma-Aldrich and is referred to as pure MMCHC. Pure MMCHC was a mixture of *cis*- and *trans*-isomers in portions of 0.636 and 0.363, respectively, based on NMR analysis and gas chromatography-mass spectrometry (GC-MS) peak areas. Crude MCHM was donated by Eastman Chemical (Product No. P18717ET; Batch TP14003010). The dilution solvent was OmniSolv methanol (CAS 677-56-1; Spectrum Chemical). All handling and sample preparation for MMCHC were performed in a hood and room isolated from gas chromatograph-olfactometer-mass spectrometer (GC-O-MS) instrumentation.

2.2 Nuclear Magnetic Resonance

The *cis:trans* ratio in pure MMCHC was determined by NMR at 25°C using a Bruker Avance-III 600 MHz spectrometer with a TBI probe with Z gradient. Sample of 20 μL of pure 4-MMCHC was mixed with 680 μL of $CDCl_3$ containing 0.01% tetramethylsilane (details are provided in Text SI-1).

2.3 Odor Threshold Concentration

2.3.1 Analytical Procedure for GC-O-MS. The Focus GC-DSQII MS with an autoinjector/sampler (Thermo Scientific) was coupled with the Sniffer 9000 Olfactometer that

had a 10 mL glass nasal cone as the sniffer port (Brechbühler Inc., Switzerland). GC, MS, and olfactometer conditions are described in Text SI-2 for GC methods.

2.3.2 Human Subjects and Olfactory Analysis. The Virginia Tech Institutional Review Board (IRB project no. 14-174) approved this study. All human subjects signed consent forms and were at least 18 years old and reported no health problems or current pregnancy. Eighteen subjects (10 female) from 21-60 years old participated in determining the odor threshold. Subjects were trained on sniffing and turning the olfactometer dial which electronically recorded the time and intensity of their response. Verbal odor descriptors were recorded by researchers. Subjects breathed normally through their nose and out their mouth to prevent exhaling into the sniffer port.

A sub-sample of the 18 subjects (n = 11; 8 female; 22-61 years old) evaluated the odor of the pure MMCHC standard, which was a combination of the *cis*- and *trans*-isomers. The MMCHC standard was placed in a 20 mL amber glass vial and subjects were asked to sniff and provide verbal descriptors.

2.2.3 Determining Odor Thresholds Concentrations for *cis*- and *trans*-MMCHC. ASTM method E679-04 (2011) for threshold analysis was used for an ascending series of six concentrations to determine the OTCs from GC-O detection. The lowest concentration, 0.0078 mg/L pure MMCHC, was increased by a factor of four for a total of six concentrations (Table 1). Concentrations for GC-O were confirmed using calibration curves for *cis*- and *trans*-MMCHC. The gas phase mass of odorant delivered to the sniffer port was calculated according to Gallagher et al. (2015): *cis*-MMCHC [(1 uL injection volume) x (mg/L concentration in standard) x (0.636 portion that is *cis*) x (1.5 mL/min flow to sniffer)]/0.5 L tidal volume for one

breath (Kelly, 2004). There was a similar calculation for *trans*-MMCHC, except 0.363 was used for the portion value.

Table 1. Standards and gas phase concentrations used for OTC determination

Sample Number	Standards of Pure cis- and *trans*-MMCHC mg/L-methanol	*Cis*-MMCHC Gas phase concentration ppb-v	*Trans*-MMCHC Gas phase concentration ppb-v
-[a]	0.0195	0.003	0.002
1	0.078	0.012	0.007
2	0.3125	0.047	0.027
3	1.25	0.187	0.107
4	5	0.747	0.427
5	20	2.988	1.707
6	80	11.951	6.829
+[b]	160	47.804	27.316

[a,b] These concentrations were not analyzed by GC-O. They represent the next lowest (a) and the next highest (b) *cis*- and *trans*-MMCHC concentrations. These are prescribed by ASTM Method E679-04 (ASTM 2011) to be used for calculating the geometric mean when a subject detects the lowest concentration (a) or highest concentration (b) tested.

A clean glass sniffer port was used for each subject. Subjects began sniffing 8.0 min after each injection and continued orthonasal sniffing at the sniffer port until 12.0 min. Elution times for *cis*- and *trans*-MMCHC were 9.375 and 9.700 min, respectively. A correct detection was denoted when subjects detected odors of either isomers at the time the peaks arrived at the sniffer ±0.11 min. Subjects waited at least 15 min before sniffing the next highest concentration. Subjects did not report fatigue or lingering MMCHC odors in their nose or sniffer port.

R version 3.1.2 (R Core Team, 2015) statistical software was used to determine odor thresholds for the gas phase concentrations of *cis*- and *trans*-MMCHC. Overall population odor thresholds concentrations, which signified overall probability of 50% of the population detecting an odor, was estimated through logistic regression and geometric mean methods. Individual OTC

were determined by geometric means. Word clouds were depicted using the wordcloud and text mapper (tm) packages from the R library; descriptors used were only for correct detections.

2.4 Henry's Law Constant

USEPA EPISuite™ software was used to estimate the Henry's Law Constants (HLC) for MMCHC, but the software cannot account for isomers (USEPA, 2012). Therefore, dimensionless HLCs were experimentally determined for *cis*- and *trans*-MMCHC at 22°C and 40°C. The experimental approach was previously described (Sain et al., 2015). Briefly, 10 mL of a 100 mg/L-aq solution of pure MMCHC in Nanopure® reagent water were placed in silanized 20 mL amber glass vials (Agilent, 5190-2239) with sealed PTFE lined septas (Restek, 21761). Triplicate samples were equilibrated at either room temperature of 22ºC or in a heated water bath at 40ºC. After equilibrium, the concentration of *cis*- and *trans*-MMCHC was measured in headspace and aqueous phases by GC (see Text SI-2 for GC methods). To determine HLCs, the average headspace concentration was divided by the average aqueous phase concentration for each isomer at 22°C and 40°C. The HLC for pure MMCHC was determined by the sum of the headspace concentrations divided by the sum of the aqueous phase concentrations for the two isomers. HCL values at two temperatures subsequently allowed the enthalpy of aqueous vaporization to be calculated for both *cis*- and *trans*-MMCHC using the van't Hoff equation (1).

$$\ln\left(\frac{m_1}{m_2}\right) = \left(\frac{\Delta H_{soln}}{R}\right)\left[\frac{1}{T_2} - \frac{1}{T_1}\right] \quad (1)$$

2.5 Shower Model.

The concentrations of *cis*- and *trans*-MMCHC at the air-water interface of a shower were predicted using an existing shower model (Ömür-Özbek et al., 2011; Sain et al., 2015), the experimentally determined HLCs, and liquid and gas diffusivities calculated using USEPA

EPISuite™ software (USEPA, 2012). These were respectively, 6.84 x 10^{-10} and 6.37 x 10^{-6} m^2/s for *cis*- and *trans*-MMCHC. The water flowing through a shower follows a plug flow model and the shower stall and bathroom air both follow a completely mixed flow model. The shower head flow rate and shower stall and bathroom air flow rates were assummed constant. R version 3.1.2 was used to model MMCHC concentrations in the shower with assumptions of 15 min showers, 40°C, shower volume of 1500 L, and influent 4-MCHM concentrations of 4000 μg/L-aq.

3. RESULTS

3.1 Composition of MMCHC in Pure and Crude MCHM

As demonstrated in Figure SI-2, ^{1}H NMR of pure MMCHC shows two upfield doublets at δ 0.83 and 0.82 (J ~ 6.4 and 6.6 Hz) assigned to 4-CH_3 protons and two downfield singlets at δ 3.61 and 3.59, assigned OCH_3 protons of carboxylate group, respectively, indicating the presence of two isomers. Signals in the 1.2−2.5 ppm region could be attributed to cyclohexane ring protons. For 1,4-di-substituted cyclohexane derivatives downfield signals are assigned to the *cis*-isomer and upfield signals to the *trans*-isomer (Kitching et al., 1981). The proton-decoupled ^{13}C NMR for pure MMCHC (Figure SI-3) showed 14 singlets spread over the range of 19−178 ppm (seven singlets at δ 176.02, 51.39, 40.39, 32.01, 30.49, 26.09, and 20.92 for the *cis*-isomer and seven singlets at δ 176.72, 51.43, 43.14, 34.30, 31.37, 29.08 and 22.46 for the *trans*-isomer), confirming the presence of *cis*- and *trans*-isomers. The ^{13}C chemical shift values closely agree with the reported values (Kitching et al., 1981).

NMR analysis determined that the fraction of each isomer in pure MMCHC was 0.636 *cis* and 0.363 *trans* and that the ratio of the two isomers was 1.75:1 *cis:trans.* The fraction of MMCHC isomers in crude MCHM were determined by NMR to be 0.674 *cis*-MMCHC and

0.326 *trans*-MMCHC with a ratio of 2.07:1 *cis:trans*. Previous research determined that crude MCHM contained 91.3 % cyclohexanes with the remainder being methanol and water (Figure SI-1). The ratio of 4-MCHM:MMCHC was 94.8:1 in crude MCHM. The fractions of 4-MCHM isomers in crude MCHM were 0.363 *cis*-4-MCHM and 0.637 *trans*-4-MCHM with a ratio of 1.75:1 *cis:trans* (Dietrich et al., 2015).

3.2 Odor Thresholds and Descriptors for *cis*- and *trans*-MMCHC

3.2.1 MMCHC Thresholds by Logistic Regression. Both *cis*- and *trans*-MMCHC had detectable odors with *trans*- possessing a lower OTC and stronger intensity and *cis*- having a higher OTC (Figure 1). Logistic regression was used to calculate overall OTC at 50% detection of 0.021 ppb-v, air (95% CI: 0.011-0.040 ppb-v, air) for *trans*-MMCHC and 1.828 ppb-v, air (95% CI: 0.636-5.257 ppb-v, air) for *cis*-MMCHC, which had an OTC 87 times higher than the *trans*-MMCHC. As shown in Figure 1, the logistic regression for *cis*-MMCHC has a shallower, steady-rising slope, meaning there is more variability among the subjects than compared to *trans*-MMCHC, which has a sharper rising curve with most subjects detecting the odor at small increasing concentrations immediately after the 50% OTC.

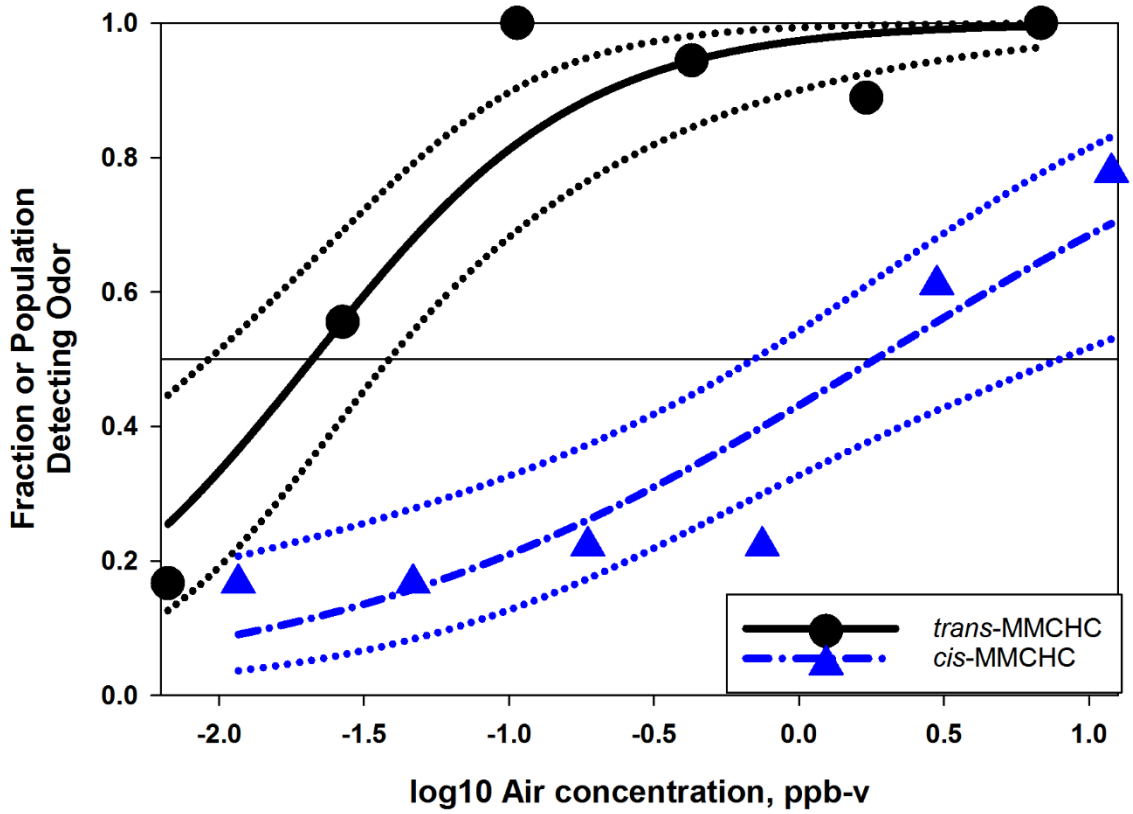
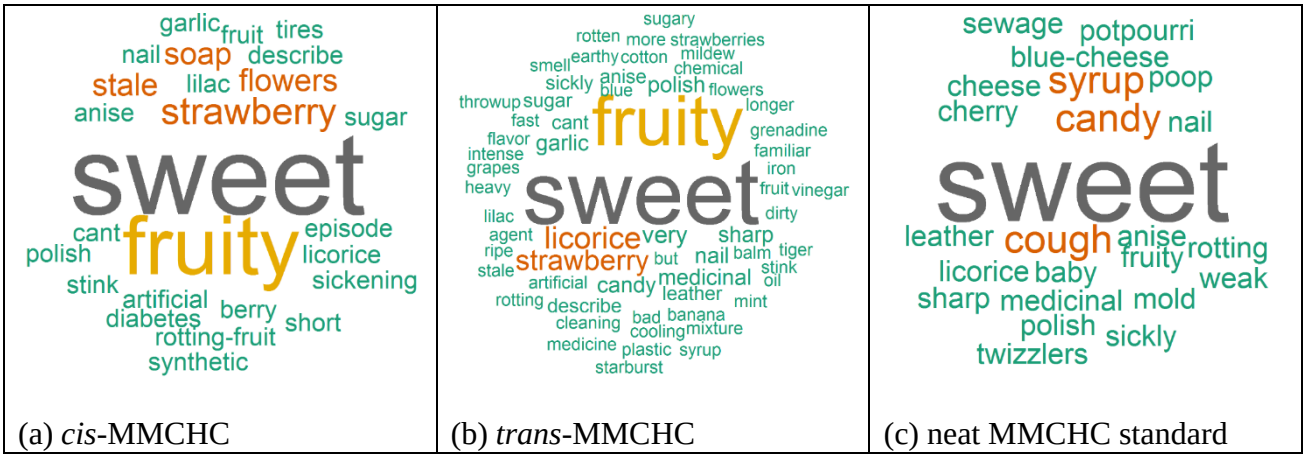


Figure 1. Air odor threshold concentrations for *cis*- and *trans*-MMCHC.

3.2.2 MMCHC Threshold by Geometric Mean. Analysis of individual geometric mean OTCs as shown in Figure SI-4 are consistent with logistic regression OTC estimates. The overall geometric mean OTC for *trans*-MMCHC is 0.046 ppb-v, air and was about 48-fold more odorous than *cis*-MMCHC at 2.196 ppb-v, air.

3.2.3 Odor Descriptors for Individual Isomers: *cis*- and *trans*-MMCHC. Both MMCHC isomers have distinct sweet and fruity odors as depicted in word clouds in Figure 2. Word clouds are visual tools that plot descriptors with increasing size and font weight for frequency. The descriptors used to generate the word clouds were those provided by human subjects for samples where the subject detected the odorant at the appropriate retention time on the GC-O. Subjects tend to use the same descriptor for concentrations after their initial positive

detection. Descriptors for *cis*-MMCHC and *trans*-MMCHC (Figure 2a and 2b), however, were predominantly sweet and fruity, but also include strawberry, soap, rubbery, garlic, and nail polish. The diversity of descriptors from human subjects is consistent with previous research that indicates that un-trained consumers demonstrate high variability in describing odors associated with drinking water contamination (Dietrich et al., 2014).



(a) *cis*-MMCHC (b) *trans*-MMCHC (c) neat MMCHC standard

Figure 2. Word clouds for odor descriptors of (a) *cis*-MMCHC, (b) *trans*-MMCHC, and (c) MMCHC standard.

3.2.4 Odor Descriptors for Pure MMCHC. When subjects smelled the odor of the neat *cis*- and *trans*-MMCHC combined standard, the verbal odor descriptors were predominantly sweet with individual subjects having variable secondary descriptor that ranged from fruit and candy to sewage and blue-cheese (Figure 2c). Only one subject described the odor as weak licorice. This is consistent with previous research that stated that the MMCHC standard had a strong but not licorice odor (Foreman et al., 2015).

3.2.5 Comparing MMCHC and 4-MCHM thresholds. Figure SI-5 and Table 2 provide odor threshold values and descriptors for *cis*- and *trans*-MMCHC and *cis*- and *trans*-4-MCHM. For each isomer pair, the *trans*-isomer was more odorous and had a lower odor

threshold concentration. In both cases, the MMCHC isomers had lower OTCs, meaning they were more odorous than their corresponding MCHM isomers. The *cis*- and *trans*-MMCHC had similar fruity, sweet descriptors, while the *cis*- and *trans*-4-MCHM had dissimilar descriptors of fermented fruit and licorice, respectively (Gallagher et al., 2015).

3.3 Henry's Law Constants

EPISuite™ v4.11 (USEPA, 2012) estimated HLC for MMCHC using the bond method: 1.73×10^{-2} at 25°C and 3.98×10^{-2} at 40°C. Unfortunately, EPISuite™ v4.11 cannot estimate the HLCs for individual isomers. Thus, the dimensionless HLCs were experimentally determined to be 1.00×10^{-2} and 1.38×10^{-2}, respectively, for *cis*- and *trans*-MMCHC at 22°C (Table 2). With increasing temperature to 40°C, the HLC for *cis*-MMCHC is 1.50×10^{-2} and 2.23×10^{-2} for *trans*-MMCHC. Using the van't Hoff equation and experimentally determined HLCs at 22°C and 40°C, enthalpies of aqueous vaporization were calculated to be 17313 J/mol and 20599 J/mol for *cis*- and *trans*-MMCHC, respectively. At 22°C, the HLC for *trans*-MMCHC is 28 times higher than for *trans*-4-MCHM, and HLC for *cis*-MMCHC is 50 times higher than *cis*-4-MCHM. The ratios are lower at 40°C (24 for *cis*- and 15 for *trans*-), but the MMCHC isomers are still more volatile than the corresponding 4-MCHM isomers.

3.4 Modeling Human Exposure and Odor Detection of MMCHC and 4-MCHM during Showering

Human exposure to *cis*- and *trans*-MMCHC during showering was assessed using the shower model and concentrations of *cis*- and *trans*-MMCHC predicted based on 4-MCHM concentration measured in the drinking water during and after the crude MCHM spill. Within

days of the crude MCHC spill in the Elk River, concentrations near 4000 μg/L-aq 4-MCHM (combined *cis*- and *trans*- isomers) were measured in drinking water (WVDHSEM, 2014). During the spill situation, only 4-MCHM was quantitatively monitored in drinking water, although chromatograms presented by Foreman et al. (2015) demonstrate that MMCHC was also present in drinking water samples. The 4-MCHM:MMCHC ratio by mass was 94.8:1 in the crude MCHM mixture, as determined by ^{1}H NMR and chromatography (see section 3.1). This ratio can be applied to estimate a drinking water concentration of 42 μg/L-aq MMCHC when the 4-MCHM concentration was 4000 μg/L-aq. Once concentrations of total MMCHC and MCHM are obtained, then the amount of the individual *cis*- and *trans*- isomers in drinking water can be calculated using the previously determine ratios for the crude chemical of 2.07:1 *cis:trans* MMCHC and 1.75:1 *cis:trans* 4-MCHM. These concentrations for individual isomers in influent drinking water were entered in the shower model to determine concentrations of individual isomers in the air during showering. The four isomer concentrations in air over showering time are shown in Figure SI-6. As reported in Table 2, the OAVs for both *cis*-MMCHC (OAV = 26.8) and *cis-4*-MCHM (OAV = 0.8) show that these two components provided minimal odor contributions compared to OAVs of 1546 for *trans*-MMCHC and 6934 for *trans*-4-MCHM. While *trans*-4-MCHM always contributed the most to the odor, *trans*-MMCHC was a substantial fraction as shown in Figure SI-6.

Figure SI-7 models the fraction of the population detecting *trans*-MCHM and *trans*-MMCHC odors in the shower after 15 minutes at 40°C, with the assumption that 4-MCHM:MMCHC ratio was 94.8:1 in the crude MCHM that contaminated drinking water used for showering.

Table 2. Physical, chemical, and sensory properties for *cis*- and *trans*-MMCHC and *cis*- and *trans-4*-MCHM

Compound	OTC ppb-v, air	Dominant Descriptor	Dimensionless Henry's Law Constant		Aqueous solubility mg/L	Enthalpy (J/mol)	Diffusivity[a] m^2/s		Shower[b] ppb-v, air	Odor Activity Value[b]
			22°C	40°C			Liquid	Gas		
cis-MMCHC	1.828	Sweet, Fruity	1.00×10^{-2}	1.50×10^{-2}	173[a]	17313	6.84×10^{-10}	6.37×10^{-6}	49	26.8
trans-MMCHC	0.021	Sweet, Fruity	1.38×10^{-2}	2.23×10^{-2}	173[a]	20599			32	1546
c*is-4*-MCHM	120[b]	Fermented fruit[c]	2.00×10^{-4}[d]	6.38×10^{-4}[d]	1300[e]	40600[c]	7.36×10^{-10}	6.71×10^{-6}	94	0.8
t*rans-4*-MCHM	0.06[b]	Licorice[c]	4.85×10^{-4}[d]	1.52×10^{-3}[d]	1010[e]	27200[c]			416	6934

a. Estimated using EPISuite™ software (USEPA 2012); this software cannot estimate properties for individual isomers
b. Shower conditions: 40°C, 15 min of showering, influent drinking water concentration of 4000 µg/L-aq total 4-MCHM with a 94.8:1 ratio of 4-MCHM:MMCHC in crude MCHM. The odor activity value for exposure during showering was then calculated as concentration in shower air divided by OTC.
c. Gallagher et al., 2015
d. Sain et al., 2015
e. Dietrich et al., 2015

4-MCHM concentration ranging from 1 to 40 μg/L were reported almost 4 weeks after the initial spill (Foreman et al., 2015). At an influent concentration of 40 μg/L, approximately 95% of the population could detect the resulting *trans*-4-MCHM in the shower air and 93% could still detect the *trans*-MMCHC. Even when the influent 4-MCHM concentration dropped to 1 μg/L, 60% and 30% of the population could detect *trans*-4-MCHM and *trans*-MMCHC, respectively (Figure SI-7). This indicates that the subtle impact of the minor MMCHC components lingered for weeks after the spill and detecting the odor was not confined to the early spill timeframe.

3.5 Mixture Complexity

The complexity of mixtures and the interaction of chemical and biological properties is illustrated in Figure 3. Note all scales are log. Figure 3a depicts the aqueous concentrations for the four isomers assuming a 4000 μg/L total 4-MCHM concentration (The concentrations scale proportionally for lower spill concentrations of 4-MCHM.). The concentrations are dominated by the 4-MCHM isomers, which constitute approximately 95% of the crude MCHM mass. The MMCHC isomers are only about 1% of the crude MCHM; the ratio is 94.8:1 for 4-MCHM:MMCHC. Figure 3b presents the HLCs, with the MMCHC isomers having over an order of magnitude higher values. The resulting higher volatility is depicted in the shower air concentrations after 15 minutes at 40°C in Figure 3c. Here, the MMCHC isomers are 13.8% by volume (16.3% by mass) of the four compounds. The biological parameters for the four isomers are shown in Figure 3d as OTCs. Recall that lower OTCs represent more odorous compounds. *Trans*-MMCHC is approximately three times more odorous than *trans*-4-MCHM. The *cis*-isomers are much less odorous than their corresponding *trans*-isomers. The resulting biological endpoint measured as OAV is shown in Figure 3e. *Trans*-4-MCHM still represents the bulk

(~82%) of the odor because of its high concentration and low OTC. *Trans*-4-MCHM accounts for the dominant licorice descriptor used by West Virginia residents during the spill. The mixture complexity, however, is well illustrated by the *trans*-MMCHC results. Through a combination of high volatility and even lower OTC, a compound which represents 0.3% of the mass of the spill contributes over 18% of the odor. Because of the slight isomeric difference, *cis*-MMCHC has a much higher OTC. While more than twice as much *cis*-MMCHC was present than *trans*-MMCHC, the *cis*-isomer contributes almost nothing (0.01%) to the overall odor.

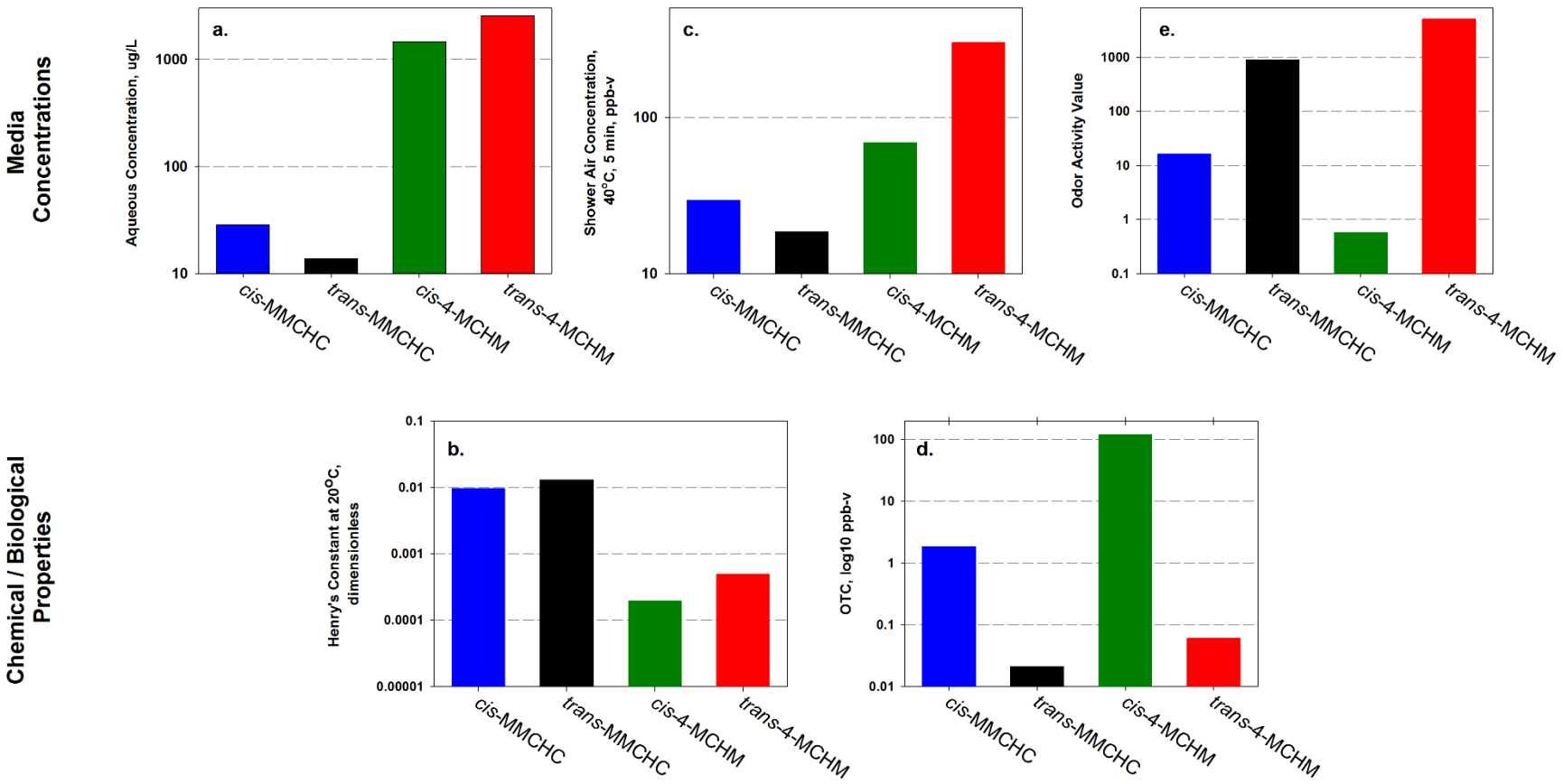


Figure 3. Interaction of chemical and biological properties on the media concentrations and human impacts for the mixture of MMCHC and MCHM isomers.

4. DISCUSSION

4.1 Hazard assessment of chemical mixtures.

When determining exposure and risk for chemical mixtures, it is important to acknowledge minor components because even when minor, can have significant effects (Carpenter et al., 2002). The initial hazard assessment of the WV spill focused only on the major component in the crude-mixture, which was 4-MCHM and the other cyclohexanes with lower concentrations were generally ignored. Despite the odorous nature of the mixture, sampling focused exclusively on water quality monitoring (WVDHSEM, 2014) and ignored any crude MCHM contaminants in air (Sain et al., 2015). As a result, investigations of chemical and biological effects and interactions are hindered because there were no real-time analysis and data collection for the other chemicals or air concentrations.

4.2 OTC and descriptors.

Numerous studies comparing OTCs for single chemicals and their mixtures found that to some extent, the components in mixtures worked cooperatively and gained odor detectability, meaning multiple constituents increased the probability of odor detection (Baker, 1963; Laska and Hudson, 1991; Patterson et al., 1993; Cometto-Muñiz et al., 2005). Intensity and quality are two odor parameters, with intensity following a logarithmic function of concentration, also known as the Weber-Fechner Law (Leonardos et al., 1969). Odor threshold concentration is the intensity that elicits a response; a concentration below that point would be considered not odorous (Leonardos et al., 1969; Dalton, 1996). The quality parameter involves both odor descriptors as well as hedonics, which is liking or disliking the odor; quality can be for a single chemical or an integrated response to a chemical mixture (Leonardos et al., 1969). It is important to recognize the impact of odor intensity and quality during a chemical spill. West Virginia

consumers exposed to crude MCHM in their homes and drinking water reported smelling both licorice and sweet (Whelton et al., 2015). From this study, the OTCs were 0.02 ppb-v air and 1.8 ppb-v air for *trans*-MMCHC and *cis*-MMCHC, respectively, and both isomers have sweet as the dominant odor descriptor. Comparison of MMCHC odor properties to *trans*-4-MCHM, with its OTCs of 0.06 ppb-v air and dominant descriptor of licorice with some sweet (Gallagher et al., 2015), shows that MMCHC may have had a larger role in the odors detected than previously understood.

OVA can be a useful tool to indicate intensity of odor contribution by an individual chemical to the overall odor/flavor. While mostly used the fragrant and food industries (Grosch, 1994), and currently underused for environmental contamination (Parker et al., 2012; Chen et al., 2015), it is the only tool available for odor comparisons starting at the same baseline. The OAV values in Table 2 show that the odorous fraction of the *trans*-MMCHC (OAV = 1546) isomer to *trans*-4-MCHM (OAV = 6934) is over 18%, signaling that *trans*-MMCHC most likely had a substantial contribution to the odor. Chen et al. (2105) indicate that when the OAV (or OHI) values exceed 1, then human exposure to odors may cause risk of discomfort and health effects. Certainly, in the case of *trans*-4-MCHM and *trans*-MMCHC, OVA values greatly exceeded 1 and humans in the exposed population acknowledged smelling the contaminants and discomfort (Gallagher et al., 2015; Schade et al., 2015). Additionally, individuals are known to have varying sensitivities to environmental odorants and with some individuals more sensitive to exposures and possibly exhibiting more discomfort (Yu et al., 2014; Dietrich and Burlingame, 2015).

4.3 Isomers: MMCHC vs. MCHM.

NMR proved to be a valuable tool to distinguish between isomers of the same chemicals, which is important because this and previous research have shown a slight shift in chemical

structure and orientation, such as *cis*- and *trans*- isomers, can have different chemical and physical properties and effects. Limonene, for example, is a widely studied chemical which has two major isomers. The d-limonene isomer has a fresh, citrus, orange-like odor with an OTC of 200 ppb-v air, while l-limonene has a piney-harsh, turpentine-like odor with a higher OTC of 500 ppb-v air (Friedman and Miller, 1971; Boelens et al., 1993). Isomers may have similar odor descriptors and dissimilar OTCs, such as geosmin, which contaminates drinking water. Both enantiometric isomers smell earthy-musty but the odor threshold is 0.86 μg/L-aq for (-) geosmin, which is a factor of ten lower than the OTC of (+)geosmin (Piriou et al., 2009). The odor study of crude MCHM by Gallagher et al. (2015) found that *trans*-4-MCHM had a 2000-fold lower OTC than *cis*-4-MCHM with odor descriptors of licorice and fermented-fruit, respectively. This present study found similar isomeric differences with the *trans*-MMCHC being more odorous than *cis*-MMCHC, although both MMCHC isomers had similar sweet and fruity descriptors. Likewise, other physicochemical properties differed between isomers (Table 2). Dietrich et al. (2015) attributed isomeric differences for *cis*- and *trans*-4-MCHM in solubility, octanol-water partition coefficient, and carbon loading for 4-MCHM to differences in the dipole moments with *trans*-4-MCHM being less polar.

During a chemical spill risk assessment, identifying isomeric differences using NMR and understanding the implications of isomeric structures for physical chemical and biological properties can assist the risk assessment experts, laboratory toxicologists, and regulatory agencies to determine which components of a chemical mixture are the causes for concern.

4.4 Limitations.

No air sampling was conducted during the crude MCHM spill or anytime thereafter, even though West Virginia consumers reported smelling licorice and sweet odors for months. Total MCHM monitoring failed to detect MCHM in tap water during this time, mainly because the analytical detection limits were well above the human odor detection limits. While the predicted shower concentrations for MCHM and MMCHC isomers are estimates, they are based on valid analytical measurement in drinking water and in depth chemical analysis of crude MCHM by chromatography and NMR. Given the much higher volatility of the MMCHC isomers to MCHM, relatively more MMCHC may have volatilized as the crude mixture traveled the 2.4 km downstream to the water treatment plant. Thus, less MMCHC may have been present during showering. However, Foreman et al. (2015) found suspected MMCHC, along with *cis*- and *trans*-4-MCHC, in tap water samples in West Virginia and some river water samples as far as 600 km downstream from the spill, which indicates that MMCHC and 4-MCHM co-occurred during and after the spill.

5. CONCLUSIONS

This study assessed human exposure related to a spill that disrupted the well-being of about 300,000 residents for many months. A novelty of our study is the thorough integration of field monitoring data for the major components, human exposure based on odor detection of major and minor components, and recently determined fate, transport, and biological properties from our research for the major and minor odorous components. Although *trans*-4-MCHM was the major component and approximately present at a 200-fold higher concentration than *trans*-MMCHC, the minor component *trans*-MMCHC was about 30-fold more volatile and 3-fold more odorous. While GC-MS data from field monitoring often revealed non-detects, the human sense of smell, which has a lower detection value, revealed the presence of crude MCHM

components in drinking water and breathing air. Application of an established model to predict air-concentrations during showering confirmed that many people could detect the odors of inhaled *trans*-4-MCHM and *trans*-MMCHC during and for months after the spill. This study reaffirms the need to address the complexity of mixtures when chemical spills co-contaminate water to impact human well-being. Hazard assessment must not be based solely on relative concentration, but also consider the chemical fate and transport properties that determine the actual levels of exposure across different media and their resulting biological effects. Thus, minor components should be recognized and investigated during chemical spills to water; associated air samples should be collected when water is contaminated, and human reports of odors or other sensory effects should be recognized as valuable exposure data.

6. AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

7. ACKNOWLEDGEMENTS

Ms. Phetxumphou is supported by Virginia Tech's "Water INTERface" Interdisciplinary Graduate Education Program. This project was funded by the USA National Science Foundation, Division of Chemical, Biological, and Environmental Transport; CBET- 1424234. The authors extend great appreciation to the human subject participants in this research study.

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CHAPTER 4: Comparing Inhalation and Ingestion Exposure to Chemical Contaminants and Odorants in Mixtures

Short title: **Assessing Inhalation and Ingestion Exposure to Chemical Mixtures**

This chapter has been published as: Gallagher, D.L., Phetxumphou, K., and Dietrich, A.M. Comparing Inhalation and Ingestion Exposure to Chemical Contaminants and Odorants in Mixtures. *Water Science and Technology*, 2017. DOI: 10.2166/ws.2017.234

ABSTRACT

Chemical spills polluting drinking water are often mixtures with each chemical having unique characteristics for partitioning, toxicity, and odour leading to significant differences to human risk exposures. A 2014 chemical spill of crude (4-methylcyclohexyl)methanol (MCHM) resulted in a $126 million USD fine to the water utility. The spill consisted of at least ten chemicals including 34% *cis*- and 60% *trans*-4-MCHM and 0.7% *cis*- and 0.3% *trans*-methyl-4-methylcyclohexanecarboxylate (MMCHC). While very minor component, *trans*-MMCHC contributed substantially to odour because of its high Henry's Law Constant, $2.23x10^{-2}$ at 40°C showering, and low odour threshold concentration (OTC), 0.02 ppb-v, air. Using USEPA risk assessment parameters in a 15-minute shower model with influent concentration of 42 ppb-aq *cis*- and *trans*-4-MMCHC, representative of initial spill concentrations in the distribution system, adult ingestion and inhalation for *trans*-MMCHC were almost equal, $4.00x10^{-4}$ and $4.26x10^{-4}$ mg/kg/d, respectively. For children, inhalation doses exceeded ingestion dose: $1.72x10^{-3}$ mg/kg/d versus $0.93x10^{-3}$ mg/kg/day *trans*-MMCHC. This exposure assessment with varying OTC for crude MCHM chemicals reinforces considering chemical, physical, and biological properties of all chemicals in the spill. Consumers aware of their exposure to chemicals in drinking water lost confidence; the water utility was required to compensate individuals and businesses for financial losses.

Keywords: chemical spill, exposure, ingestion, inhalation, odour, mixture

INTRODUCTION

The rupture of a poorly maintained chemical storage tank owned by Freedom Industries allowed nearly 38,000 L of licorice-smelling crude (4-methylcyclohexyl)methanol (MCHM) to enter the Elk River, which was the drinking water supply for over 300,000 people in Charleston, West Virginia and surrounding counties. Contaminated Elk River water was processed through a conventional drinking water plant. Attempts to treat and remove the chemical contaminants with addition of activated carbon and potassium permanganate were ineffective (Weidhaas et al. 2017). Licorice-smelling drinking water was distributed to consumers who noticed the odorous drinking water early in the morning of January 9, 2014. By 6 PM that evening, a "do-not-use the water order" was issued by government officials. Consumers were told not to drink, wash, or use water; the "do-not-use the water order" remained in place for up to a week and a half for some sections of the distribution system (Gallagher et al. 2015; Schade et al. 2015). Communication through public notification and social media was effective. In a post spill survey, 89% of affected consumers reported knowing about the spill the day that it occurred (Savoia et al. 2015).

Minimizing exposure was critical as there was little technical and/or toxicological data for crude MCHM, which is an unregulated industrial chemical consisting of a mixture of predominantly ten substituted cyclohexanes (Figure 1) (Gallagher et al. 2015). Even through at the time of the spill it was known that crude MCHM was a mixture of chemicals, the liquid-liquid extraction GC/MS monitoring method used at the water company only measured and reported the two major isomers of crude MCHM (*cis*- and *trans*-4-MCHM) as total 4-MCHM rather than the individual species. Monitoring during and for months after the spill by the United States Geological Survey (USGS), which used a purge-and-trap-GC-MS method, demonstrated

that both the 4-MCHM isomers and minor components, methyl-4-methylcyclohexanecarboxylate (MMCHC) isomers, had high volatility, occurred in tap water, and could cause odours (Foreman et al. 2015).

Immediately and for days after the spill, many people reported illnesses and sought medical help. It required about a week for the US Centers for Disease Control and Prevention (US CDC) to issue an ingestion health advisory of 1 mg/L-aqueous for the crude MCHM mixture (CDC 2014). Even though exposure through smelling the chemicals was a main complaint of consumers, and respiratory illness was one of the three main reasons people sought medical help (Thomasson et al. 2017). An inhalation advisory of 0.01 ppm-v of inhalable air would not be issued until approximately 6 months after the spill (US EPA 2014). While a consumer survey indicated that 69% of the affected population complied with instructions to not drink the tap water, few consumers reported complying with other behaviour modifications like not using contaminated water for washing (Savoia et al. 2015). In another survey, 37% of affected consumers reported continual use of tap water for any household purpose during the time of the spill, with showering being the most common use (Burrer et al. 2017). Thus, many consumers subjected themselves to inhalation exposure. For months after the "do-not-use the water order" was lifted, consumers were reluctant to use the tap water. Approximately three months after the spill, only 34% of consumers reported using the tap water for drinking, and only 36% believed the water was safe (Burrer et al. 2017).

A maximum reported concentration of 3800 μg/L-aq combined *cis*- and *trans*-4-MCHM (total 4-MCHM) was measured in the distribution system during the early days of the spill. Recent data from court records suggest that aqueous concentrations in drinking water during the early hours of the spill may have ranged from 13,000-100,000 μg/L-aq (Ward 2016c). Days and

weeks later, limited monitoring data demonstrated that water concentrations subsequently fell below the US CDC health advisory of 1 mg/L in water for combined *cis*- and *trans*-4-MCHM. Even so, consumers living in the affected area were occasionally plagued by licorice-odours when using drinking water in residential homes, schools, and local buildings. Odours were especially noticeable when warm and hot water were used (Sain et al. 2015; Schade et al. 2015). Eventually, the saturated carbon filters at the water treatment plant were confirmed as one source of continued contamination and lingering odour; replacement of the carbon began about 3 months after the initial spill (Constantino 2014). Subsequent research confirmed that activated carbon could both remove and release odorous MCHM (Ahart et al. 2016). The US EPA short-term air screening level of 0.01 ppm-v for inhalable air (US EPA 2014) issued in July 2014 was more than a factor of 150 higher than the odour threshold for individual components of the crude MCHM mixture (Gallagher et al. 2015; Sain et al. 2015). While residents of Charleston, West Virginia repeatedly complained of odours even when not drinking the water, inhalation exposure was not investigated by water utility personnel or governmental agencies during or after the spill.

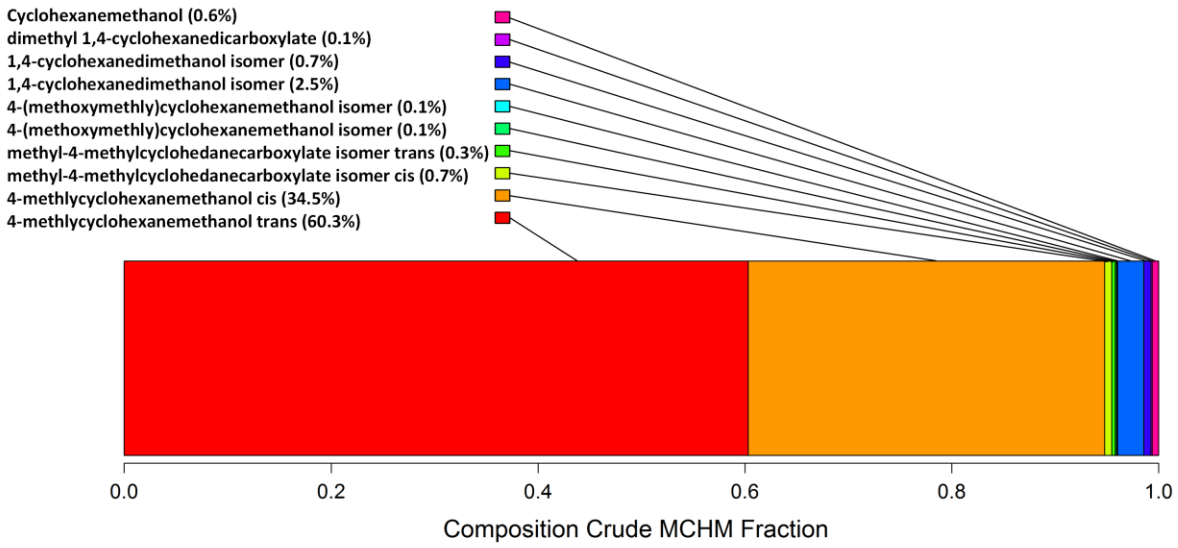

Figure 1. Chemical composition of crude MCHM that spilled into the Elk River and contaminated drinking water in West Virginia (Eastman Chemical Company 2011; Gallagher et al. 2015).

A year after the spill, residents of West Virginia were still reluctant to drink water distributed by the water treatment plant and not all their questions and concerns had been answered (Ward 2015). The legal ramifications of the spill took longer than two years to settle and are summarized below.

- Freedom Industries (owner of ruptured crude MCHM tank): This company declared bankruptcy soon after the spill. In February 2016, a US Federal Court fined the company a $900,000 USD “symbolic” fine because a bankrupt company would not pay (Ward 2016a). The Company’s president, Gary Southern, received a one month jail sentence and $20,000 USD fine as did the former co-owner, Dennis Farrell (Ward 2016a, b). Four other employees were charged with violations of the Clean Water Act, the Refuse Act, and permitting violations (Ward 2016a).

- Eastman Chemical Company (manufacturer of crude MCHM): Eastman Chemical will be required to pay $25 million USD because they failed to properly caution Freedom Industries about the potential dangers of MCHM and did not take appropriate actions when information pertaining to the poor state of the chemical storage tanks were made known (Ward 2016b).
- West Virginia American Water (the water utility): In the class action settlement of Crystal Good vs. American Water, West Virginia American Water will have to provide $126 million USD to compensate over 224,000 residents, 7,300 businesses, and an undetermined figure of hourly wage workers (Ward 2016b). The lawsuits found that American Water failed to store treated water that would have allowed for temporary closing of the water intake. The water utility also failed to properly maintain or even destroyed water samples taken early after the spill (Ward 2016c). Additionally, the water utility did not have a suitable plan in place to respond to such chemical spills (Ward 2016c). Within the settlement, American Water will not be able to increase water rates to cover the cost of the settlement; the money should come from investors, stockholders, and insurance policies (Ward 2016b).

A 2017 American Water Works Associate survey found that the water industry's most important regulatory concern is chemical spills (AWWA 2017). While chemicals spills occur worldwide and disrupt drinking water supplies, distribution systems, and consumers' lives and livelihoods (Zhang et al. 2011; Jiang et al. 2012), this chemical spill and drinking water contamination in West Virginia highlights many important issues related to drinking water protection, consumer protection, and consumer satisfaction (Weidhaas et al. 2016), including:

1. Lack of source water protection policies;

2. Lack of monitoring methods for industrial chemicals in water;
3. Lack of detailed exposure assessment for both inhalation and ingestion;
4. Lack of human toxicity data;
5. Lack of attention to consumer communication and perception.

While all of these issues need to be addressed to provide safe and sustainable drinking water, the focus of this document is on Item *3: Lack of detailed exposure assessment for both inhalation and ingestion.* The aim is to compare inhalation and ingestion exposure for adults and children under conditions that occurred during the crude MCHM spill.

Methods

The biological and physicochemical data used to develop a comparison of human inhalation during showering versus ingestion exposure are summarized in Tables 1 and 2. Inhalation and ingestion doses were estimated by calculation of a daily dose (mg/kg/d) based on US EPA risk assessment parameters (US EPA 2014). Risk parameters included adults weighing 70 kg, ingesting 2 L/d of water, and inhaling 20 m^3/d of air. For children, risk parameters were weighing 15 kg, ingesting 1 L/d of water, and inhaling 17.3 m^3/d of air. A previously published shower model, as described by Sain et al. (2015), was applied to assess exposure to chemicals with a concentration of 4000 ppb-aq combined *cis*- and *trans*-4-MCHM, which represents a high concentration detected in the distribution system during the spill. Since the MMCHC isomers represent about 1% of the concentration of the 4-MCHM isomers, 42 μg/L-aq combined *cis*- and *trans*-4-MMCHC was used as the influent MMCHC concentration in the shower model. Inhalation doses were calculated by integrating the area under the predicted shower concentration curves versus time data. Odour activity values (OAV) represent the ratio of a

chemical's concentration in a mixture to its odour threshold concentration (OTC) (Grosch 1994). Statistical analyses and modelling were performed in R (R Core Team 2015).

Table 1. Descriptors and odour threshold concentrations (OTC) for major odorous chemicals in crude MCHM.

Compound	Dominant Odour Descriptors	OTC ppb-v, air
c*is-4*-MCHM	Fermented fruit[a]	120[a]
trans-4-MCHM	Licorice[a]	0.06[a]
cis-MMCHC	Sweet, Fruity[b]	1.828[b]
trans-MMCHC	Sweet, Fruity[b]	0.021[b]

a. Gallagher et al. 2015

b. Phetxumphou et al. 2016

Table 2. Physical and chemical properties for *cis*- and *trans-4*-MCHM and for *cis*- and *trans*-MMCHC.

Compound	Aqueous solubility mg/L	Dimensionless, air/water Henry's Law Constant		Δ H Aqueous Vapor (J/mol)	Diffusivity m^2/s	
		22°C	40°C		Liquid	Gas
c*is-4*-MCHM	1300[a]	$2.00x10^{-4}$[b]	$6.38x10^{-4}$[b]	40600[b]	$7.36x10^{-10}$[c]	$6.71x10^{-6}$[c]
trans-4-MCHM	1010[a]	$4.85\ x10^{-4}$[b]	$1.52x10^{-3}$[b]	27200[b]		
cis-MMCHC	173[c]	$1.00x10^{-2}$[d]	$1.50x10^{-2}$[d]	17313[d]	$6.84x10^{-10}$[c]	$6.37x10^{-6}$[c]
trans-MMCHC	173[c]	$1.38x10^{-2}$[d]	$2.23x10^{-2}$[d]	20599[d]		

a. Dietrich et al. 2015
b. Sain et al. 2015
c. US EPA 2012
d. Phetxumphou et al. 2016

Results and Discussion

Establishing which chemicals resulted in higher human exposure for both ingestion and inhalation is challenging because the fate, transport, and odour properties of specific chemical compounds in crude MCHM varied, as shown in Tables 1 and 2. For odorous chemicals in

drinking water, the human sense of smell confirms exposure even when quantitative chemical monitoring data are lacking (Dietrich & Burlingame 2015).

The interaction of the physical-chemical properties (Henry's Law Constant) and biological properties (odour thresholds) results in the media concentrations shown in Figure 2. Influent aqueous concentrations were 4000 μg/L-aq combined *cis*- and *trans*-4-MCHM with 1% or 42 μg/L-aq combined *cis*- and *trans*-MMCHC. The 4-MCHM isomers dominate with concentrations of 2545 and 1455 μg/L-aq for the *trans*- and *cis*- isomers, respectively. MMCHC isomers' concentrations are approximately two orders of magnitudes lower, with *trans*-MMCHC having the lowest concentration.

MMCHC isomers have more than an order of magnitude higher Henry's Law Constant than the 4-MCHM isomers, which results in a relative increase in shower air concentrations. MMCHC isomers are also more odorous than their 4-MCHM counterparts, resulting in even greater human detection as measured by odour activity values. The *trans*- isomers are much more odorous than the *cis*- isomers. *Trans*-MMCHC is three times more odorous (i.e., three times lower OTC) than *trans*-4-MCHM. *Trans*-MMCHC is only 0.3% of the aqueous mass but leads to approximately 20% of the odour due to its higher volatility and lower OTC. The odour percentage for *trans*-MMCHC increases slightly as shower time increases, reaching approximately 15% after 2 minutes and 19% after 15 minutes with a concomitant decrease in the *trans*-4-MCHM percentages.

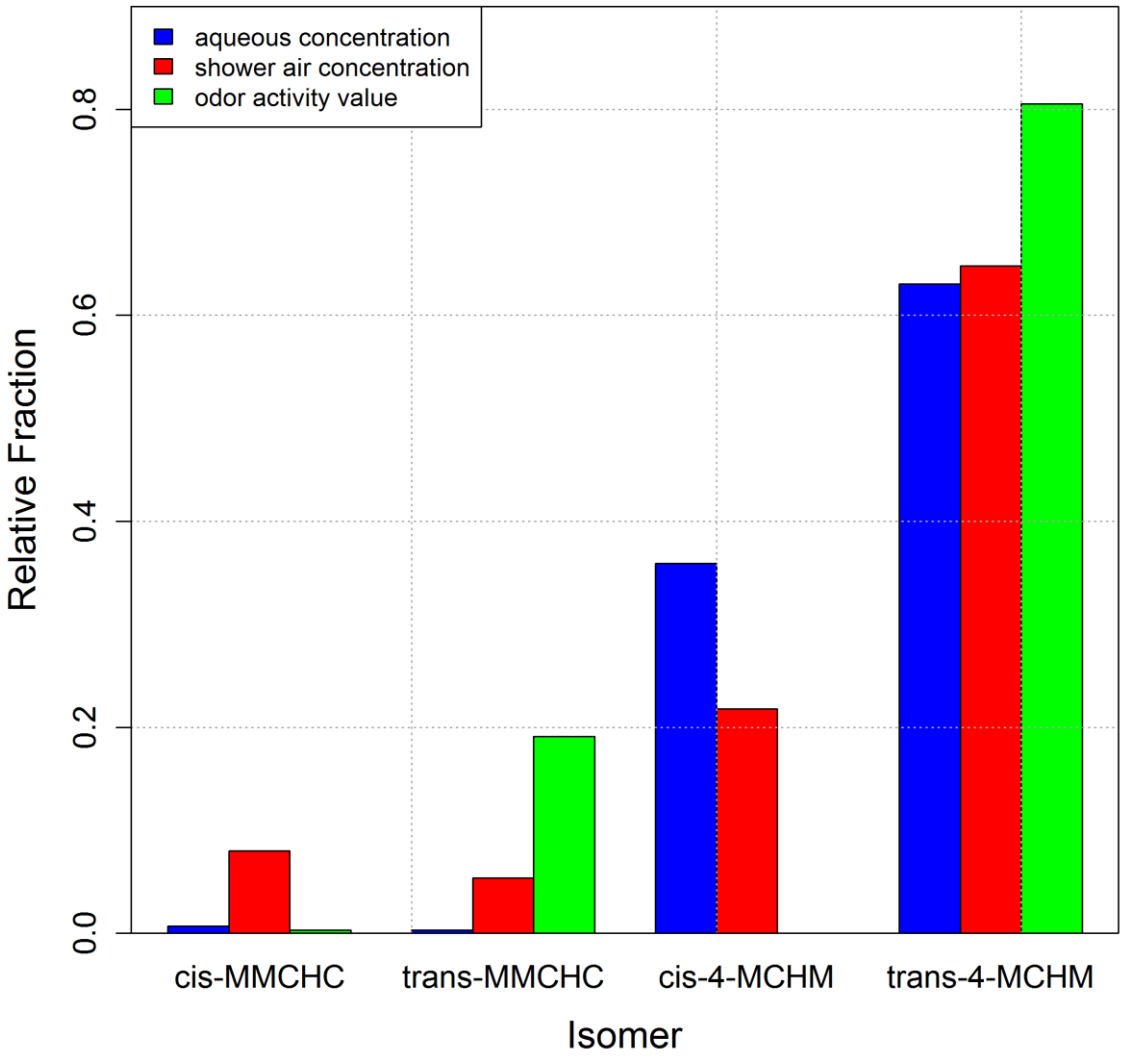

Figure 2. Changing distributions of components of crude MCHM in water (based on relative µg/L-aq), in shower air after 15 minutes of showering at 40°C (based in relative ppb-v), and in odour activity value for human perception during showering.

Figure 3 shows the comparison of inhalation versus ingestion doses for adults (Figure 3a) and children over time during showering (Figure 3b). The high volatility of MMCHC isomers leads to relatively high inhalation doses. At approximately 15 minutes, *trans*-MMCHC doses for adult ingestion and inhalation are approximately equal. After a 10-12 minute shower, children's inhalation exposures exceed their ingestion doses for both isomers.

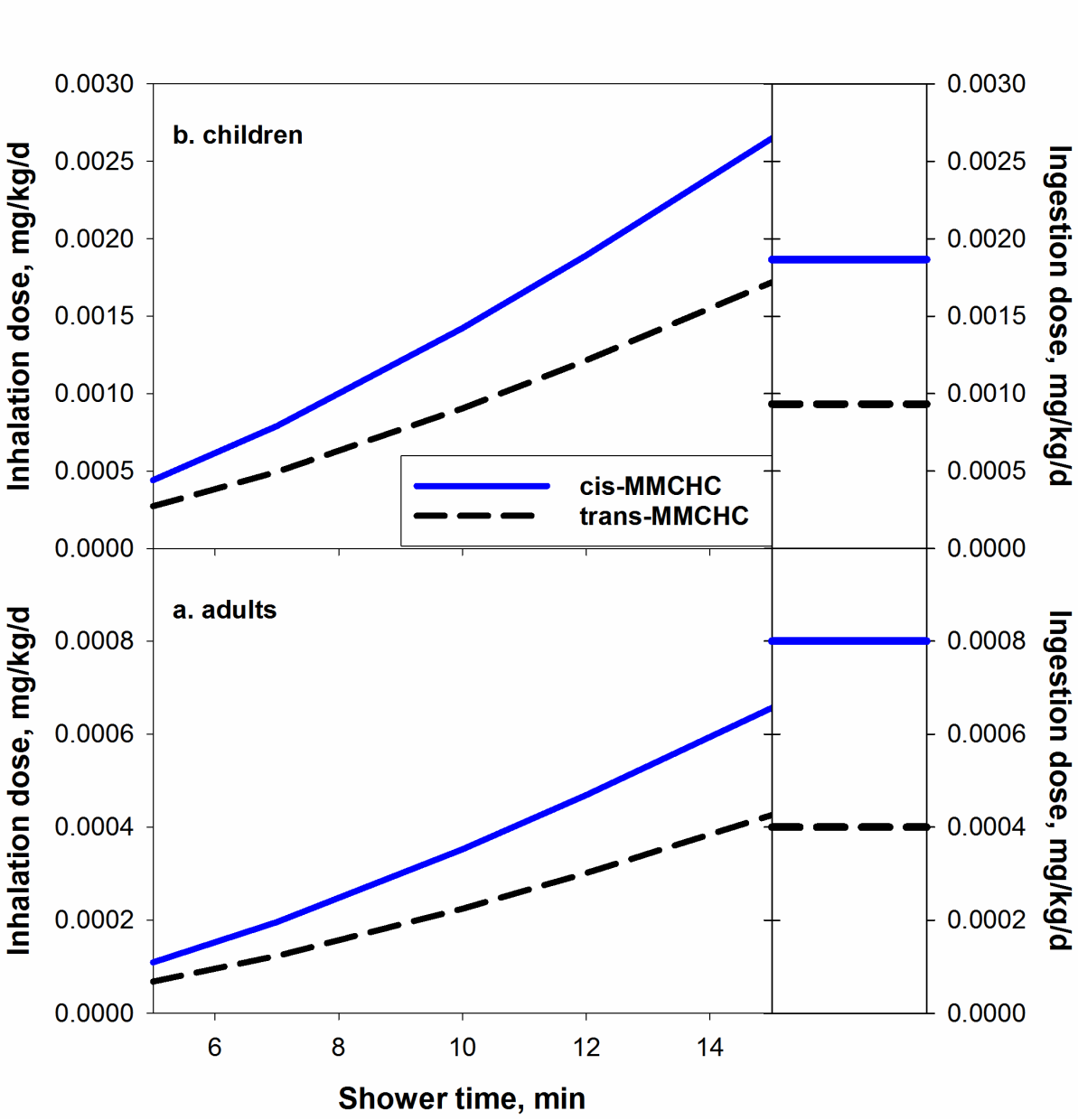

Figure 3. Inhalation during showering versus ingestion doses for the MMCHC isomers: a. adult doses; b. children doses.

A comparison of the inhalation and ingestion exposures for individual isomers appears in Table 3. Because of their much higher aqueous concentrations and moderate volatilities, the 4-MCHM isomers have corresponding higher doses for both exposure pathways compared to the MMCHC isomers. In general, approximately 90% of the dose for the 4-MCHM isomers was from

ingestion. Although the MMCHC isomers had lower aqueous concentrations, their higher volatilities potentially allowed inhalation exposure to be a more significant route than ingestion. This was always true for children—children had higher exposures to MMCHC isomers through showering than through drinking water. While this research focuses on exposure to individual chemicals in the spill, it is important to acknowledge that consumers are simultaneously exposed to all chemicals, as shown with the total dose entry in Table 3.

Table 3. Doses of crude MCHM contaminants from showering (15 minute shower at 40°C) and drinking for adults and children for an aqueous concentration of 4000 µg/L-aq combined *cis*- and *trans*- 4-MCHM and 42 µg/L-aq combined *cis*- and *trans*-MMCHC.

	Adult			Child		
Contaminant	**Inhalation in Shower (mg/kg/d)**	**Ingestion of Drinking Water (mg/kg/d)**	**Ratio[a]**	**Inhalation in Shower (mg/kg/d)**	**Ingestion of Drinking Water (mg/kg/d)**	**Ratio[a]**
trans-MMCHC	0.426×10^{-3}	0.4×10^{-3}	0.532	1.72×10^{-3}	0.93×10^{-3}	1.84
cis-MMCHC	0.656×10^{-3}	0.8×10^{-3}	1.64	2.65×10^{-3}	1.87×10^{-3}	1.42
trans-4-MCHM	4.82×10^{-3}	72.7×10^{-3}	0.067	19.5×10^{-3}	170.0×10^{-3}	0.115
cis-4-MCHM	1.63×10^{-3}	41.6×10^{-3}	0.039	6.56×10^{-3}	97.0×10^{-3}	0.067
Total Dose, 4 isomers	7.53×10^{-3}	116×10^{-3}	-	30.4×10^{-3}	270×10^{-3}	-

[a]Inhalation dose divided by ingestion dose

Figure 4 shows the development of OAV over time for the two MMCHC isomers during showering. For most people, the odour from the *trans*-MMCHC isomer is readily apparent within seconds and dominates throughout the shower. While the dose for the *cis*-MMCHC isomer was always higher because of its higher aqueous concentration and near equal volatility, the *trans*-MMCHC isomer completely dominates the odour because of its almost 100x lower OTC.

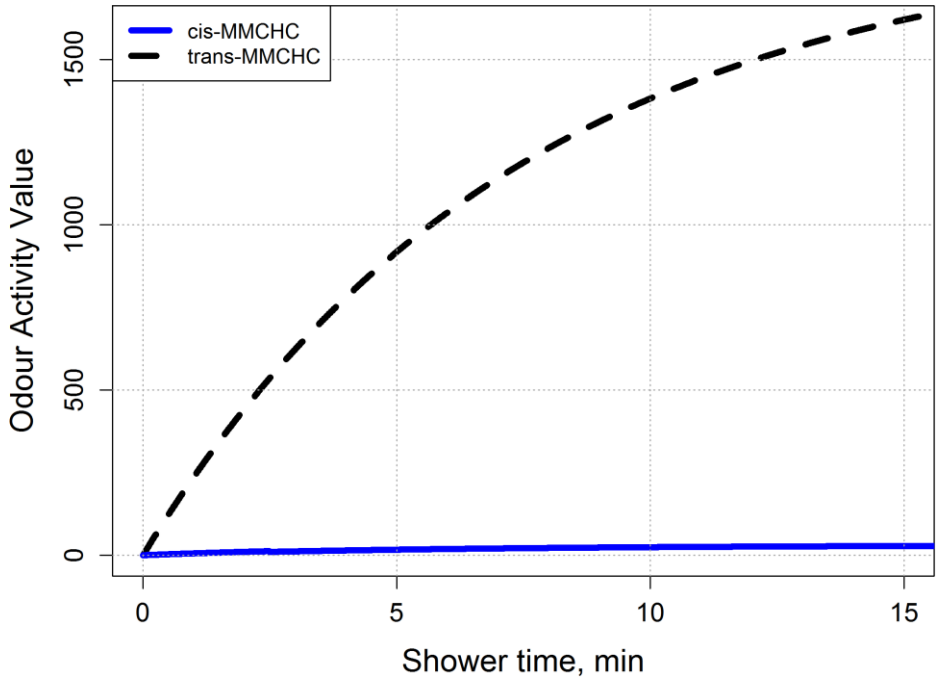

Figure 4. Odour activity value (OAV) during showering time for the MMCHC isomers.

This research confirms that both inhalation and ingestion are substantial routes of exposure to aqueous contaminants. In-depth research on mixtures of trihalomethanes, that included monitoring exposure and human blood and breath concentrations, demonstrated that water-use activities such as showering, bathing, and washing clothes or dishes result in human inhalation exposure to drinking water contaminants (Gordon et al. 2006). Inhalation is a more important route of exposure for the two more volatile trihalomethanes, trichloromethane and bromodichloromethane (Gordon et al. 2006).

In the study of the chemical mixture, crude MCHM, odour detection was a marker for human exposure (McGuire et al. 2014a, b). Odour, as an indicator of human exposure, is readily implemented and reported without expensive and potentially invasive monitoring of human body fluids. While not all chemicals are odorous, many are. Depending on the concentration present in the water and the chemical's odour threshold concentration, it may be readily detected by the

sense of smell. If consumers are smelling chemicals in their water, then they are exposed. The drinking water industry recently ranked chemical spills as its most important current regulatory concern, followed by point source pollution (AWWA 2017). Attention focused on inhalation exposure and interpreting consumer complaints related to taste and odour can be an asset for identifying and controlling drinking water contamination as well as maintaining and improving consumer confidence. Not heeding consumer responses to contamination, as was done in the crude MCHM contamination of the Elk River and West Virginia American Water Company, resulted in loss of consumer confidence (Burrer et al. 2017) in the drinking water and costly legal settlements (Ward 2016b, c) for the water utility.

Conclusions

Preventing chemical spills through adequate source water protection should be a water industry priority. When spills occur, utilities need to be prepared for such events to limit drinking water contamination and public health concerns. To protect individual and the public health, there is a vital need to know essential fate and transport properties (such as solubility, Henry's Law Constants, enthalpy), biological properties (toxicity and odour), and exposure routes for each chemical in the spill. For different chemicals, these properties vary by orders of magnitude and affect human exposure risks, even among isomers. Chemicals that are considered minor components because of their percent composition should always be investigated as they can have major human impacts. Additionally, air samples are as vital as water samples and should be collected and examined. The ramifications for not protecting the public from inhalable and ingestible waterborne contaminants can result in loss of consumer confidence and costly litigation.

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Section II: Water Safety - Public Communication Tools

Risk communication is a critical component of risk analysis. This section explores risk communication tools that help bridge the communication gap between water experts and consumers.

Chapter 5 explores the Drinking Water Taste and Odor Wheel, which is a reference tool that depicts common odorants in drinking water and their respective descriptors. It is not readily available to the public, in comparison to other taste and odor wheels such as the beer or coffee wheels. The research revealed that this improved the consumer's ability to describe odors in drinking water. After research involving an odor-sniffing panel, 92% of the participants preferred to have access to the Drinking Water Taste and Odor Wheel when attempting to identify aesthetic problems in drinking water. There are potentials to improvements to this tool by adding words from the Odor Atlas (a reference tool for chemical odorants) and changing descriptors that entail common lay-terms, such as dirt for earthy.

Consumer Confidence Reports (CCRs) are also known water quality reports. These reports are mandated by the EPA to be released every year by water utilities to their constituents to inform consumers about the safety and quality of their drinking water. The main message question explored by research was "Are CCRs written in a way that is clear and understandable to consumers?" A total of 30 CCRs were selected for review and discussed in Chapters 6 and 7. Unfortunately, the grade level for these CCRs ranged from grade 11.1 to 14.3, which is far higher than the NIH's target of 6-7th grade reading level for public communication tools. Additionally, the reading ease scores were comparable to the Harvard Law Review, which is far more difficult for the lay-consumer to comprehend. Furthermore, these CCRs were not

effectively communicating that the water was safe to drink according to all state and federal regulations.

CHAPTER 5: Implementing the Drinking Water Taste-and-Odor Wheel to Improve the Consumer Lexicon

This chapter has been published as: Phetxumphou, K., Raghuraman, A., Dietrich, A.M. Implementing the Drinking Water Taste-and-Odor Wheel to Improve the Consumer Lexicon. *Journal of American Water Works Association, 109*(11): E453-E463, 2017. DOI: https://doi.org/10.5942/jawwa.2017.109.0122

ABSTRACT

Flavor lexicons and associated Taste-and-Odor (T&O) Wheels are professionally recognized descriptors for sensory qualities of beverages that can also be used by consumers. Human subject responses to known odorants were evaluated before and after instruction on using the Drinking Water T&O Wheel lexicon. Fifty-one naïve human subjects separately sniffed and recorded descriptors for 2-methylisoborneol (musty), geosmin (earthy), orange extract (orange, citrus, lemon), and dimethyltrisulfide (sulfurous, rotten egg, swampy, garlic, onion). Two months later, subjects were instructed before sniffing and describing these odorants for a second time. McNemar's Test statistically assessed the number of correct answers "pre-" and "post-instruction." Instructed subjects showed significant improvements in correct descriptors for 2-methylisoborneol, geosmin, and orange. Instruction increased accuracy of describing dimethyltrisulfide, but was not significant. Ninety-four percent of subjects stated that the T&O Wheel was helpful in identifying odorants, and 92% preferred to have the T&O Wheel when identifying their drinking water odors.

KEYWORDS: consumers, drinking water, lexicon, odor, taste

INTRODUCTION

Flavor lexicons and associated Taste-and-Odor (T&O) Wheels are a standardized vocabulary with descriptors for the sensory qualities of foods and beverages (Drake & Civille 2003; Lawless & Civille 2013). They are widely used by industry professionals to describe desirable organoleptic characteristics and taints in products. Early beverage lexicons include beer (Meilgaard et al. 1979), scotch whiskey (Shortread et al. 1979), wine (Noble et al. 1984), and drinking water (Mallevialle & Suffet 1987). The drinking water lexicon is unique among beverage lexicons in its focus on off-flavors, as all other lexicons balance descriptors for both desirable organoleptic characteristics and undesirable off-flavors. The off-flavor emphasis is because many people expect drinking water to have no noticeable taste or smell (Dietrich 2006; Burlingame & Doty 2015); thus, off-flavors in water are readily noticed. The drinking water industry recognizes eight major odor categories, which are earthy-musty, chlorinous, grassy, sulfurous/septic, fragrant/vegetable, fishy/rancid, medicinal, and chemical (Figure 1) (Suffet et al. 1999; Khiari et al. 2002; APHA et al. 2012). As shown in Figure 1, the inner wheel indicates eight *general categories* of odors, four tastes, and mouth feel. The next layer of the Wheel indicates *specific descriptors* for each odor category. Another version has a third outer concentric ring with chemical names of reference standards (APHA et al. 2012).

Professionals develop descriptive beverage lexicons to evaluate and compare products. Consumers also benefit from using descriptive T&O Wheels developed by professionals, such as wine and coffee T&O wheels that are commercially available on the web and in stores (Noble 2016; Boone 2005; Fegan 1989; Caspersen 2012; Kubota 2016; SCAA 2016). Consumers judge foods and beverages, including water, by first assessing tastes, odors, and appearances, then search their memory to identify and recognize the sensory properties, and finally apply language to describe the sensory properties. It is a challenging task for consumers to verbalize language

with appropriate descriptors, which is why flavor wheels and checklists are used by both consumers and professionals for guidance (Stone & Sidel 1993; Etievant et al. 2016; Lawless 2013).

The drinking water industry has a well-developed T&O Wheel. If the T&O wheel were made readily available to consumers, it could be beneficial for improving the dialogue with consumers concerning aesthetic water quality issues. Consumers are quite capable of recognizing aesthetic changes in their drinking water and often complain to their drinking water professionals that the water is "different". This can be frustrating to water professionals who desire reliable sensory descriptors and feedback to identify issues and promptly treat and respond to the problem. Consumers often lack the experience and the vocabulary to accurately describe aesthetic problems, especially for odors that consumers do not expect to encounter in their water (Köster et al. 2002; Köster 2005; Teixeirs et al. 2013). A study of describing common odors in drinking water found that drinking water personnel without formal taste-and-odor instruction were superior to consumers from the general public in describing the odors in samples containing either 2-methylisoborneol (2-MIB) or dimethyltrisulfide (DMTS) (Dietrich et al. 2014). Although the drinking water personnel did not have formal training, they did have both the experience and the expectation that 2-MIB, an earthy-musty odorant, or DMTS, a sulfurous odorant, could occur in drinking water. The water treatment personnel were better able to describe 2-MIB as earthy-musty compared to DMTS, which they described using many sulfur-related descriptors such as sulfur, rotten egg, garlic, onion, propane, and methane.

The drinking water industry has an increasing interest in responding to consumer complaints of undesirable tastes and odors. Simultaneously, there is a need to understand and utilize consumers as "sensors" who can monitor drinking water quality throughout the

distribution system to provide valuable feedback for water quality issues. The water quality issues can be nuisance tastes and odors that are unlikely to have health effects (Burlingame & Mackey 2007; Dietrich & Burlingame 2015), or aesthetic problems from chemicals with possible health effects (Hrudey & Hrudey 2007; Phetxumphou et al. 2016; Weidhaas et al. 2016). Historically, and in contemporary times, the drinking water T&O Wheel (Figure 1) and flavor profile analysis (Standard Method 2170, APHA et al. 2012) are important tools utilized to characterize tastes and odors issues in drinking water. Select problems identified and solved with these sensory tools include cucumber odors (Burlingame et al. 1992; Burlingame 2015); earthy-musty odors (Rashash et al. 1997; Piriou et al. 2009; Yu et al. 2014); odors from materials to transport water such as plastic/citrus odors (Khiari et al. 2002; Devesa et al. 2004; Heim & Dietrich 2007), and sulfurous odors (Devesa et al. 2004; Watson & Jüttner 2016).

Figure 1. Drinking Water Taste-and-Odor (T&O) Wheel (Suffet et al. 1999; Khiari et al. 2002; APHA et al. 2012). The inner ring is the *general category;* the outer ring presents *specific descriptors*. Note: A third ring that contained specific reference chemical names associated with the *specific category* is not shown.

In the 21st century, the dominant aesthetic problems in drinking water, in order of world-wide occurrence, are: earthy-musty, chlorinous, rotten egg-sulfurous, metallic, and chemical (Ömür-Özbek 2012). This research is focused on musty, earthy, orange, and rotten egg-sulfurous which are all descriptors on the T&O Wheel (Figure 1). Common globally occurring odorous terpenoids that occur in drinking water are 2-MIB and geosmin (Watson et al. 2016). 2-MIB, with focused odor descriptors of earthy-musty-moldy-woody-medicinal odors (Dravnieks 1992), is generally associated with musty odors in drinking water. Geosmin, described in the food and fragrance industries as having earthy-musty-moldy-woody-grassy odors (APHA et al. 2012; Pepper et. al. 2014), is generally associated with earthy odors in drinking water. The orange-like

odor is consistent with citrus odors that occur in drinking water after contact with high density polyethylene pipes (Heim & Dietrich 2007) or orange-fruity odors associated with aliphatic aldehydes produced through ozonation (Mallevialle & Suffet 1987). Orange flavor is associated with the chemical d-limonene, which is described as citrus-orange-lemon by the food and fragrance industries (Dravnieks 1992). Aqueous rotten egg-sulfurous odors are associated with a cluster of methylated sulfur oligiomers (Franzmann et al. 2001; Heitz et al. 2000; Watson & Jüttner 2016) with DMTS being a major contributor in drinking water odor episodes (Yang et al. 2008; Zhang et al. 2013; Ma et al. 2015). DMTS is described by many diverse descriptors, including sulfur-septic-rotten egg-swampy-vegetable-decayed odors (Dravnieks 1992). K

The objectives of this research are to:

1. Compare descriptors of odorants with and without instruction by:
    a. First, allowing naïve subjects to smell common odorants known to cause aesthetic problems in drinking water and then express in their own words descriptors for the odorants
    b. Second, instructing subjects to use the Drinking Water T&O Wheel (Figure 1) and then asking them to smell and describe the same odorants with the aid of the T&O Wheel
    c. Assessing the ability of the T&O Wheel to improve consumer descriptions

2. Obtain feedback from the subjects on the value of the Drinking Water T&O Wheel for describing odors

MATERIALS AND METHODS

Human subjects. The Virginia Tech Institutional Review Board approved this study (IRB 12-710). Informed consent was obtained verbally in accordance with the IRB. Subjects reported being 18 or older, of any health status, not pregnant, and not consuming food or beverages, or smoking one hour before the sensory evaluation. A total of 51 subjects (17 females) from the Virginia Tech community participated; subjects' age ranged from 19 to 39 years old with median age of 21. Subjects indicated that their continent of origin was predominantly North America (96%) with one each indicated in Asia and South America. All

subjects lived in the United States and spoke and read English. Subjects were not screened for their sensory abilities and had no prior sensory training, but reported that they normally drank water.

Odorants. Four odorants were selected based on common odors detected in drinking water (Ömür-Özbek 2012) and present on the T&O wheel (APHA et al. 2012): 2-MIB (98% pure, Supelco, Belefonte, PA, USA), which is a common musty odorant (APHA et al. 2012); geosmin (97% pure, Sigma-Aldrich, USA), which is an earthy odorant (APHA et al. 2012); orange, which was purchased at a local grocery store (Orange extract, Simply Organic, Frontier Natural Products, Iowa, USA); and DMTS (98% pure, Sigma-Aldrich Chemical Company, USA), which is associated with sulfur and rotten egg descriptors (Dravnieks 1992; Dietrich et al. 2014).

Odorants were prepared a day prior to the human sensory tests in 40 mL amber glass vials (VOA, volatile organic analysis vials). Each vial contained a cotton ball absorbing the odorant. Odor intensity of odorants were designed as moderate to strong to be above odor threshold concentrations; the intensities were confirmed by research personnel and office staff prior to the sensory testing.

Subjects were given the vials in random order at room temperature. They were instructed to hold the vial with a KimTech® wipe tissue to prevent confusing linger odors on their hands with the tested odorant. They then were instructed to open a vial, sniff lightly, and record their odorant descriptor(s). Subjects waited at least 2 minutes before sniffing a new odorant.

Use of Taste-and-Odor Wheel: Pre-instruction & Post-instruction.

Pre-instruction. During "pre-instruction," four odor vials were presented to naïve human subjects who were asked to separately sniff each vial and then use their own words to

describe each odor. Neither instruction nor the T&O Wheel (Figure 1) was provided to the subjects. The "pre-instruction" response form collected subject's demographic information as well as their odor descriptors for each odorant.

Post-instruction. Two months after the "pre-instruction" session, the same subjects were provided instructions on how to read the T&O Wheel (Figure 1). This instruction period consisted of a straightforward 10-minute explanation of the eight *general categories* and associated *specific descriptors*, plus the importance of consumer identification of odors to assist water utilities in solving taste-and-odor problems.

After instructions were provided, a "post-instruction" sensory test was performed. Human subjects were again asked to separately sniff each of the four vials and provide descriptors with the aid of the T&O Wheel (Figure 1). Subjects were free to use either descriptors from the wheel or their own words. Subjects were also asked to complete a detailed "post-instruction" questionnaire that explored their opinions of the helpfulness of the wheel for describing odors. The questions included:

1-4) Was the T&O Wheel helpful to you for identifying the odor in each of the four vials (A, B, C, D)? (yes or no)

5) If you were a consumer who noticed a change in your tap water's taste-and-odor, how helpful would the T&O Wheel for describing the *general category* of the odor? (**Circle one**: very helpful, helpful, neutral, slightly not helpful, very not helpful)

6) If you were a consumer who noticed a change in your tap water's taste-and-odor, how helpful would the T&O Wheel be for describing the *specific odor*? (**Circle one**: very helpful, helpful, neutral, slightly not helpful, very not helpful)

7) Think back to the first time you did the odor activity and compare your ability to identify odors during that experience to today's activity. How helpful was having a copy of the Taste- and-Odor Wheel to improve your ability to identify odors? (**Circle one**: very helpful, helpful, neutral, slightly not helpful, very not helpful)

8) Would you prefer to have a copy of the Taste-and-Odor Wheel when attempting to identify aesthetic problems in drinking water? (**Circle one**: yes or no)

Interpreting Human Subject Responses. A subject's response was considered correct if their descriptor was present on the list of reference descriptors presented in Table 1. Most reference descriptors were taken from the T&O Wheel; a few were from the Atlas of Odors Character Profiles (referred to as Odor Atlas in this study), published by the American Society for Testing and Materials (ASTM DS61; Dravnieks 1992). The Odor Atlas is a compilation of odor descriptors provided by trained sensory evaluators for pure chemicals. The descriptors are ranked by the percentage of evaluators who used the term and by the percentage of descriptor applicability to the odorant. An Odor Atlas descriptor that had more than 3 stars (*) and was applicable to the specified odorant was considered significant and was included in this research. The additional descriptors from the Odor Atlas are included in the Extended Descriptor list (Table 1). The correctness of subject responses was assessed using two scales: Odor Comparison 1 and Odor Comparison 2.

Odor Comparison 1. Subjects provided descriptors for each of the four odorants. Odor Comparison 1 measures the improvement of number of correct responses from subjects for "pre-instruction" to "post-instruction" considering only the descriptors present in the "T&O Wheel Descriptors" column of Table 1.

Odor Comparison 2. In Odor Comparison 2, responses from the "pre-instruction" were considered correct using only T&O Wheel Descriptors and were compared to the "post-instruction" results where responses were considered correct using the T&O Wheel Descriptors combined with the Extended Descriptors (Table 1). Odor Comparison 2 measures the improvement of subject descriptors with both instruction and use of a more comprehensive descriptor list that included descriptors commonly used in the food and beverage industries but not currently used in the water industry.

No Extended Descriptors were included for 2-MIB and geosmin. However, descriptors from the Odor Atlas for d-limonene and DMTS were added as Extended Descriptors because they were not present on the T&O Wheel (Table 1). Lemon and citrus descriptors were added for orange, and garlic and onion were added to the Extended Descriptors list for DMTS (Dravnieks 1992).

Statistical analysis. The McNemar's Test evaluates a significant statistical difference between two related groups with respect to a dichotomous dependent variable (Salkind 2010). In this study, McNemar's Tests assessed statistical differences between the responses (correct/incorrect) without the T&O Wheel ("pre-instruction") and with the aid of the T&O Wheel ("post-instruction").

Table 1. Reference descriptors used to determine if subjects' response was correct

Odorant	T&O Wheel Descriptors[1]	Extended Descriptors[2]
2-Methylisoborneol[3]	Earthy, musty, moldy, woody, cork, potato	-
Geosmin[4]	Earthy, musty, moldy, potato, cork, grassy	-
Orange[5]	Fruity, orange-like	lemon, citrus
Dimethyltrisulfide[6]	Sulfurous, septic, rotten-egg, swampy, decaying vegetation, rubbery	garlic, onion

1) APHA et al. 2012. 2) Additional descriptors (Dravnieks 1992) in this research that were included with the T&O Wheel descriptors list. 3) Descriptors include the specific descriptors from the earthy/musty/moldy *general category* of the T&O Wheel augmented with "woody" which is associated with 2-methylisoborneol (Dravnieks 1992) and present on the T&O Wheel under the grassy/hay/straw/woody *general category.* 4) Descriptors include the specific descriptors from the earthy/musty/moldy *general category* of the T&O Wheel augmented with "grassy" which is associated with geosmin (Pepper et al. 2014) and present on the T&O Wheel under the grassy/hay/straw/woody *general category.* 5) T&O Wheel Descriptors include the specific descriptors from the fragrant/vegetable/fruity/flowery *general category*. "Lemon, citrus" were taken from the Odor Atlas (Dravnieks 1992). 6) T&O Wheel Descriptors include the specific descriptors from the moldy/swampy/septic/sulfurous *general category*. "Garlic, onions" were taken from the Odor Atlas (Dravnieks 1992).

For Odor Comparison 1, McNemar's Test measured the descriptors recorded before providing the subjects with the T&O Wheel ("pre-instruction") as correct if the descriptor was present on the T&O Wheel reference list (Table 1) and compared it to the number of responses in

the “post-instruction” sensory test that was marked as correct if the descriptor was present on the T&O Wheel.

A second McNemar Test, Odor Comparison 2, evaluated if adding the Extended Descriptors significantly improved the subjects’ performance. Responses received before providing the subjects with the T&O Wheel (“pre-instruction”) were marked correct if the descriptor was on the T&O Wheel list. Then, these responses were compared to recorded descriptors in the “post-instruction” study and were marked correct if the descriptor given was on either the T&O Wheel or Extended Descriptor lists.

A McNemar’s Test was conducted on each odorant and the resultant p-values were tested against $\alpha = 0.05$ to prove statistical significance (Dalgaard 2008). After assigning data as correct/incorrect, a contingency table was developed in R, version 3.2.5 (R Development Core Team 2016) to perform McNemar’s Test. In this study, the contingency table is a frequency distribution of the number of correct and incorrect responses for “pre-instruction” and “post-instruction” with the T&O Wheel.

Furthermore, to have a visual picture of the descriptors frequently described by the subjects, wordclouds were developed using the R package, wordcloud; a color palette package, RColorBrewer; and a text mining R package, tm (R Development Core Team 2016). The text mining package was used to remove any punctuations, conjunctions, prepositions, and interjections from subjects’ responses and to create a (m x n) binary frequency matrix where (m) is the total number of unique descriptors used by the subjects and (n) is the total number of descriptors used by the subjects. The binary frequency table was then used to create the frequency table for the wordcloud generator function from the wordcloud package.

RESULTS

Human subjects reported that describing the odorants was challenging in the pre-instruction session. In the post-instruction session, two months later, the subjects reported the odors were still unfamiliar, but the T&O Wheel was helpful in identifying odors. The results are presented in Figure 2. The wordclouds for each odorant under the "pre-instruction" column depicts subjects' responses without the presence of the T&O Wheel. The wordclouds in the "post-instruction" column are descriptors recorded with the use of the T&O Wheel. There were two sets of descriptors tested for each odorant, thus, there were two McNemar's Tests per odorant. The first McNemar's Test compared odor studies one and two by including only T&O Wheel descriptors, Odor Comparison 1. The second McNemar's Test associated the "pre-instruction" results using only T&O Wheel descriptors to "post-instruction" responses using both the T&O Wheel and Extended Descriptors lists (Table 1).

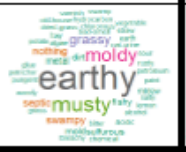
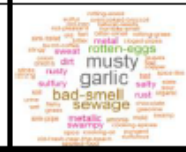
	2-Methylisoborneol (2-MIB)			Geosmin			Orange			Dimethyltrisulfide (DMTS)		
	Pre-instructing	Post-instructing		Pre-instructing	Post-instructing		Pre-instructing	Post-instructing		Pre-instructing	Post-instructing	
Descriptors provided by Subjects												
Taste-and-Odor (T&O) Wheel Descriptors	earthy, musty, moldy, woody, cork, potato			earthy, musty, moldy, potato, cork, grassy			fruity, orange-like			sulfurous, septic, rotten-egg, swampy, decaying vegetation, rubbery		
Contigency Matrix (Only descriptors on Wheel)		Pre-	Post-		Pre-	Post-		Pre-	Post-		Pre-	Post-
	Incorrect	40	19	Incorrect	5	24	Incorrect	17	21	Incorrect	6	6
	Correct	11	32	Correct	4	18	Correct	2	11	Correct	5	34
Odor Comparison 1 McNemar's Test: p-value	3.18×10^{-4}			3.30×10^{-4}			1.75×10^{-4}			1		
Odor Comparison 1 Interpretation	Descriptors on T&O Wheel improved consumer description			Descriptors on T&O Wheel improved consumer description			Descriptors on T&O Wheel improved consumer description			T&O Wheel had no effect		
Extended and T & O Wheel Descriptors	earthy, musty, moldy, woody, cork, potato			earthy, musty, moldy, potato, cork, grassy			fruity, orange-like, **lemon, citrus**			sulfurous, septic, rotten-egg, swampy, decaying vegetation, rubbery, **garlic, onion**		
Contigency Matrix (Pre-instructing - Only descriptors on Wheel vs. Post-instructing - Both Descriptors from Wheel and Extended list)		Pre-	Post-		Pre-	Post-		Pre-	Post-		Pre-	Post-
	Incorrect	40	19	Incorrect	5	24	Incorrect	19	29	Incorrect	6	11
	Correct	11	32	Correct	4	18	Correct	0	3	Correct	5	29
Odor Comparison 2 McNemar's Test: p-value	3.18×10^{-4}			3.30×10^{-4}			7.00×10^{-7}			2.11×10^{-1}		
Odor Comparison 2 Interpretation	Same as Odor Comparison 1 because no extended decsriptors added			Same as Odor Comparison 1 because no extended decsriptors added			Adding extended descriptors to the T&O Wheel can improve consumer description			Adding extended descriptors to the T&O Wheel improved consumer description as seen with substantially lower, although not significant, p-value		

Figure 2. Subject responses and comparison of the number of correct responses before and after instruction on how to read the Drinking Water T&O Wheel. Since 51 subjects participated, the sum of correct and incorrect responses sum to 51 in each contingency table.

Methylisoborneol (2-MIB). Without instruction, naïve subjects struggled to narrow down the odor descriptors for 2-MIB. Their "pre-instruction" responses were predominantly cleaning-supplies and chemicals to less frequent descriptors of earthy, hospital, clean-bathroom, dirt, and musty. The descriptors recorded "post-instruction" with the T&O Wheel were more consistent and accurate descriptors that included: earthy, moldy, and musty. By glancing at the "pre-" and "post-instruction" 2-MIB wordclouds (Figure 2), the use of the T&O Wheel (Figure 1) indicates that the descriptors available on the T&O Wheel improved descriptions of 2-MIB. McNemar's Tests confirmed this observation with significant p-value = 0.00318. Since no additional descriptors were included for 2-MIB on the Extended Descriptors list (Table 1), the p-values were the same for Odor Comparison 1 and Odor Comparison 2.

Geosmin. The wordclouds in Figure 2 for geosmin clearly depict an improvement to subject descriptors "pre-" and "post-instruction" with the use of the T&O Wheel. Initially, geosmin was described mostly as dirt, earthy, nothing, soil, bad-smell, body-odor, and grass. However, with the T&O Wheel tool, their responses were more consistent, predominantly, grassy, earthy, and musty. Similar to the results of 2-MIB, where no new descriptors were added in the Extended Descriptor list, Odor Comparison 1 and 2 returned significant p-values of 0.00033 when comparing responses "pre-" and "post-instruction."

Orange. The orange odorant was most familiar to the human subjects. The orange wordcloud for "pre-instruction" was consistent with formal common fruity, orange-like descriptors; the responses also included citrus, lemon, orange, sweet, and cleaning supplies. However, with the aid of the T&O Wheel, the list of descriptors in the wordcloud for the "post-instruction" odor study was more narrowed and constricted to formal orange, fruity, citrus, and lemon descriptors. The McNemar's Test comparing "pre-instruction" to "post-instruction" using

only T&O descriptors (Odor Comparison 1) showed a significant improvement with a p-value of 0.000175. The second McNemar's Test (Odor Comparison 2) that assessed "pre-instruction" using only T&O Wheel words to "post-instruction" using the Extended Descriptors list revealed a lower p-value of 0.0000002, indicating that adding descriptors (i.e., lemon, citrus) to the existing T&O Wheel list significantly improves consumer description of the orange odorant.

Dimethyltrisulfide (DMTS). Consistent with previous studies (Dravnieks 1992; Dietrich et al. 2014), DMTS was a difficult odorant for subjects to describe as shown in the wordclouds (Figure 2). This may be due to DMTS having a diverse list of formal descriptors that include: onion, garlic, sulfurous, septic, rotten-egg, swampy, decaying vegetation, and rubbery. In the "pre-instruction" study, consumers recorded numerous descriptors for DMTS; these descriptors ranged from musty and garlic to bad-smell, sewage, and rotten eggs. Other less common recorded descriptors included: sulfury, dirt, rust, sweat, metallic, swampy, and salty. The T&O Wheel did not help or hinder the human subjects in describing DMTS. As shown in the DMTS "post-instruction" wordcloud (Figure 2), the descriptors did not improve with the aid of the T&O Wheel. The recorded "post-instruction" descriptors varied as much as the "pre-instruction" descriptors, and included predominantly chemical, moldy, metallic, swampy, earthy, rancid, septic, musty, fishy, and cat-urine. The McNemar's Test using only T&O Wheel descriptors to compare "pre-" and "post- instruction" descriptors returned a p-value of 1. The Odor Comparison 2 McNemar's Test that included Extended Descriptors of "onion" and "garlic" in the "post-instruction" demonstrated an improvement with respect to the p-value, although it was not a significant difference (p-value = 0.211). However, the lower p-value in Odor Comparison 2 does suggest that adding "onion" and "garlic" to correct identification list could improve consumer description of DMTS. It is of interest to note that the descriptors "onion", "garlic" and

'cooked vegetables" were commonly used by untrained subjects in another study (Dietrich et al. 2014).

Human subject assessing value of T&O Wheel for describing odors. As shown in Table 2, 94.2% of the subjects answered that the T&O Wheel would be very helpful (31.4%) or helpful (62.8%) in identifying the *general category* of an odorant if they were to notice a change in their tap water's taste-and-odor. However, the subject's responses to actually describing the *specific odor* were more varied with only 14% stating it would be very helpful, 52% saying it would be helpful, 24% being neutral, and 4% and 6% stating it would be very not helpful and slightly not helpful, respectively. Regardless of the response in identifying a *specific odor*, all subjects rated either neutral (23%), helpful (59%), or very helpful (18%) in terms of improvements to their ability of identifying odors by using the T&O Wheel tool. No subjects rated the T&O Wheel as not helpful.

Table 2. Value of T&O Wheel for improving subjects' ability to describe/identify odorants

Questions	% of Subjects Responding				
	Not Very Helpful	**Slightly Not Helpful**	**Neutral**	**Helpful**	**Very Helpful**
How helpful would the T&O wheel be for **describing the *general category* of the odor?**	0	2	4	63	31
How helpful would **the T&O wheel be for describing the *specific* odor?**	4	6	24	52	14
How helpful was having a copy of the T&O Wheel in improving **your ability to identify odors?**	0	0	23	59	18

Consistent with the "pre-" and "post-instruction" wordcloud results (Figure 2), greater than two-thirds of the subjects found that the T&O Wheel was helpful in identifying the 2-MIB, geosmin, and DMTS odors (Table 3). Surprisingly, over two-third of subjects specified that the T&O Wheel helped them identify DMTS's odor even though the "pre-" and "post-instruction"

wordcloud results in Figure 2 revealed only a slight improvement. Only 56% of subjects believed that the T&O Wheel helped in identifying the orange odorant, which is expected as it was the odor that subjects had the most experience with prior to this study. Overall, 92% of subjects reported that they would prefer to have the T&O Wheel to assist them in identifying odors in their drinking water (Table 3).

Table 3. Value of T&O Wheel for identifying individual odorants

Questions	% of Subjects Responding	
Was the T&O Wheel helpful for identifying these odors?	YES	NO
2-Methylsioborneol	74	26
Geosmin	69	31
Orange	56	44
Dimethyltrisulfide	77	23
Would you prefer to have a copy of the T&O Wheel when attempting to identify aesthetic problems in drinking water?	92	8

DISCUSSION

This study focused on consumers' current lexicon on drinking water odors and evaluated if consumer access to the T&O Wheel tool would improve it. The results demonstrated that consumers report that using the T&O Wheel is helpful and the data reveal that access to it improved their descriptors for odors. The results of this study are consistent with previous research in the food and beverage industry. Research shows improvement in consumer feedback when structured tools containing product descriptors, such as checklists and wheels, are used to probe responses (Lawless 2013). Having a list of words can assist consumers and make it easier for them to describe important attributes.

Understanding descriptor differences among odorants. Consumers should have access to descriptive tools to help identify aesthetic issues in drinking water. The current T&O Wheel

with two inner levels was helpful in describing 2-MIB and geosmin. There were minor improvements for the familiar orange and hardly any benefits for DMTS.

The subjects were readily able to describe 2-MIB and geosmin odorants with the aid of the T&O Wheel. 2-MIB has formal descriptors of earthy-musty-moldy-woody-medicinal odors (Dravnieks 1992; Yu et al. 2014; Dietrich et al. 2014), and geosmin has similar odors to 2-MIB with earthy-musty-moldy-woody-grassy descriptors (APHA et al. 2012; Pepper et. al. 2014). There is consensus among the drinking water industry that these two odorants possess these odor properties and are commonly present in drinking water (Jüttner & Watson 2007; Piriou et al. 2009; APHA et al. 2012).

Citrus, oranges, and lemon odors are present in common beverages and can also be found in drinking water in contact with polyethylene pipes (Heim & Dietrich 2007). Orange was the most familiar odorant used in this study. The subject responses to orange were consistent with descriptors of fruity, orange-like odors. A study by Keller and Vosshall (2016) found that familiarity with an odorant has a strong positive correlation with the ability of the subjects to describe the smell. Subjects often described a familiar odorant using names of commercial products, which reveals that prior experience does play a key role in possessing the necessary lexicon for olfactory perception (Keller & Vosshalll 2016). This phenomenon is known as an odor memory, where humans encounter odors and can remember them over long periods of time; however, often times, humans can perfectly recognize the odor, but cannot quite pinpoint the descriptor and thus, associate it with a similar odor (i.e., lemon for orange) or use consumer product names as descriptors (i.e., “lemon head candy” or “sour patch kids” for orange) (Jonsson and Olsson 2003; Dietrich et al. 2014).

DMTS is a common aesthetic issue for drinking water (Yang et al. 2008; Zhang et al. 2013; Ma et al. 2015). It has diverse formal descriptors related to septic, sulfurous odors that many consumers would not normally associate with their drinking water (Dietrich et al. 2014). The T&O Wheel (Khiari et al. 2002; APHA et al. 2012) has a total of six formal descriptors alone: sulfurous, septic, rotten-egg, swampy, decaying vegetation, and rubbery. This broadness in descriptors can also be seen in the Odor Atlas (Dravnieks 1992), which had trained professionals describing odors. Keller and Vosshall (2016) found that nonspecific descriptors were applied to unfamiliar odorants, such as "chemical." As seen in the wordcloud for DMTS, "chemical" was predominantly used as a descriptor even with the aid of the T&O Wheel and the inconsistency among subjects' descriptors is clear. Furthermore, the Keller and Vosshall (2016) study found that with the presence of a sulfur group, subjects perceive the odorants as less pleasant, hence, the direct yet common "bad-smell", "disgusting", and "unpleasant" odor descriptors for DMTS.

Improving the Drinking Water T&O Wheel. Although the T&O Wheel has proven to be helpful, the lack of descriptors, such as citrus and lemon for the fragrant/vegetation/fruity/flowery *general category* or garlic and onion for the moldy/swampy/septic/sulfurous *general category*, can impact the effectiveness of the T&O Wheel as a tool to identify odors. Upon adding the Extended Descriptors for the orange (e.g., lemon, citrus) and DMTS odorants (e.g., garlic, onion), the p-values decreased substantially for correct descriptors (Figure 2). Thus, adding additional descriptors to the T&O Wheel could improve a consumer's ability to identify odors. However, as seen by the long list of formal descriptors and lack of consensus among sensory professionals (Dravniek 1992), DMTS still remains a complex odor to describe. It is important to recognize that in some cases, like with

DMTS, the T&O Wheel tool can show improvement in subjects' responses, but even with wide accessibility to an improved T&O Wheel, it will not always aide in resolving all aesthetic issues.

The "grassy" term is present on the T&O Wheel under the grassy/hay/straw/woody *general category*, however, it was not categorized under the earthy/musty/moldy *general category*. Since grassy is a common descriptor for geosmin (Pepper et al. 2014), this descriptive term should also be present under the earthy/musty/moldy *general category*. Other *general* and *specific odor* categories on the T&O Wheel should be explored and expanded accordingly.

Most importantly, the reference list of "formal" descriptors from the T&O Wheel and Odor Atlas did not always account for common terms used by the typical water consumer. For example, many subjects used "dirt-like" pre-instruction and then used "earthy" for 2-MIB post-instruction or "cleaning-agent-like" pre- then used "citrus" post-instruction for orange. Thus, the water industry's formal descriptors of odorants should include reasonable descriptors commonly used by consumers bridge the gap between consumers and the water professionals, i.e., dirt for earthy. Interestingly, the spice wheel, developed by professionals at the McCormick Spice Company, combines earthy/dirty as a formal descriptor (Lawless et al. 2012).

Making the Drinking Water T&O Wheel accessible to professionals and consumers. The T&O Wheel is an excellent tool that could be marketed, advertised, and published so that consumers can readily access it. Many drinking water providers maintain websites to which the T&O Wheel could be added. In the United States, (see WaterOne 2016 for an example) community drinking water providers are required to send an annual Water Quality Report to their consumers (Roy et al. 2015; Phetxumphou et al. 2016). These annual reports offer an excellent opportunity to communicate the T&O Wheel and its importance. The T&O Wheel provides a common lexicon that if widely used by both professionals and consumers, would aid in

identifying and discussing odor issues, which is the first step to determining its cause and then remediation (Dietrich et al. 2014).

Across the globe, consumers are interested in the aesthetic quality of their drinking water (Burlingame 2015; Burlingame & Mackey 2007; Devesa et al. 2004; Dietrich & Burlingame 2015; Doria 2010; Lin et al. 2012). Presently, drinking water quality is undergoing many changes due to drought, climate change, and source water/treatment changes such as water reuse (Agus et al. 2011; NRC 2012). Making the Drinking Water T&O Wheel readily accessible to consumers could improve its use. Potentially, it could improve water quality as consumers could provide more targeted feedback to their drinking water providers concerning taste-and-odor issues that may just indicate an aesthetic problem or may indicate a more serious health problem. A consistent lexicon for odors will aide in statistical analysis of drinking water odor problems (Gallagher & Dietrich 2014).

CONCLUSIONS

Consumers can serve as sentinels to their drinking water quality, as they are often the first ones to detect a difference in the taste-and-odors of their drinking water. However, they may be challenged in describing specific odorants without proper instruction in sensory analysis or without the aid of a descriptor tool, such as the T&O Wheel. Ninety-four percent of subjects reported that the Drinking Water T&O Wheel was helpful for describing common odorants (i.e., 2-MIB and geosmin) in drinking water. It is important to the note, however, that the T&O Wheel can be vague and limited for some odorants that do not have straightforward descriptors (i.e., DMTS). Nevertheless, results from this study indicated that 92% of the naïve subjects, representative of consumers, would prefer to have the T&O Wheel to assist them in describing and identifying odors in their drinking water and that using the T&O Wheel statistically improved their ability to describe an odor.

ACKNOWLEDGEMENTS

The authors appreciate the willingness and responses of the human subjects. Ms. Phetxumphou is supported by the Virginia Tech Water INTERface Interdisciplinary Graduate Education Program (IGEP). Ms. Raghuraman is an undergraduate researcher.

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CHAPTER 6: Tools for Better Communication of Water Quality to Consumers

This chapter has been published as: Katherine Phetxumphou, Siddhartha Roy, Brenda M. Davy, Paul Estabrooks, Wen You, and Andrea M. Dietrich. Tools for Better Communication of Water Quality to Consumers. *Tap Into Virginia*, 36-40, 2017. Accessed at http://www.vaawwa.org/magazine/30/

WHAT ARE CCRs?

Community water systems (CWS), or water utilities, serve over 300 million people in the US and are required to release annual Consumer Confidence Reports (CCRs), also known as water quality reports. The USEPA states that the purpose of CCRs is to "improve public health protection by providing educational material to allow consumers to make educated decisions regarding any potential health risk pertaining to the quality, treatment, and management of their drinking water supply." While all CWS write and distribute CCRs, many also acknowledge that they need to improve the ability of their CCR to communicate with their consumers.

CAN CONSUMERS READILY UNDERSTAND CCRs?

The simple answer is, NO. According to national surveys and the USEPA, consumers have concerns regarding their drinking water quality and these concerns are not addressed by CCRs. It is widely recognized that understandability is a problem for CCRs that could be addressed by writing the CCR at a level and in a manner more easily understood by the consumer. Furthermore, Virginia Tech researchers found that a nationally representative sample of CCRs were not effectively communicating drinking water information to the public. Virginia Tech

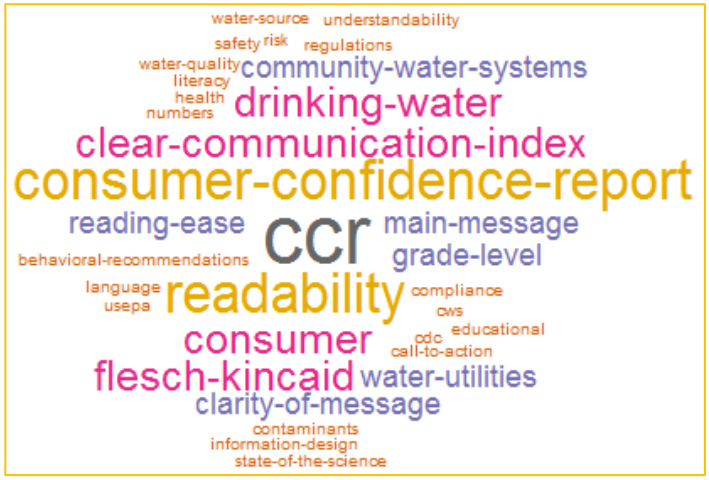

Figure 1. Word cloud of related to Consumer Confidence Reports

researchers who were not trained in environmental engineering or science were dumbfounded by the complicated table of maximum contaminant levels (MCLs) and language in the CCRs, such as “greensand filters”, “sodium hydroxide”, “curb-stop” and “cross-connections”. While these are everyday terms for water professionals, consumers are at a loss to interpret them. Thus, it is clear that the scientific jargon used and current CCR formats create difficulty for consumers to make informed decisions involving personal risks and their drinking water.

The lack of understandability is further confounded by the 11-14th grade reading level (e.g., high school graduate through some college) at which most CCRs are typically written. This reading level is equivalent to the Harvard Law Review, which is a document few consumers attempt to read. Even though the United States has a high literacy rate of 99%, the average reading level of typical consumers is only at the 7-8th grade level. To deliver an easily understood message to consumers, the National Institute of Health actually recommends documents be written at a 6-7th grade reading level. Water utilities must understand their respective constituents and target the NIH’s recommended reading level in writing their CCRs.

WHAT TOOLS ARE AVAILABLE TO IMPROVE CCRs?

Since CCRs are considered public health communications, their messages and information presented should be clearly understood by all consumers. Water utilities can improve consumer understandability and increase consumer readability of CCRs using communication tools that are readily available.

Tool #1: Clear Communication Index

The Centers for Disease Control and Prevention (CDC) published the Clear Communication Index (Index) in 2013 as an easily implemented tool for preparing effective and clear communication materials for the public. The Index focuses on important characteristics of

written documents that enhance and aid people's understanding of information. Through the use of 20 key questions (Table 1), the Index both can guide writers in preparing easy to read reports and also can be applied to assess the clarity of a written reports.

Table 1. Seven indices and related questions from the CDC's Clear Communication Index (CDC, 2014)

Indices	Questions
1) Main message/Call to action	• Does the material contain one main message? • Is the main message at the top, beginning, or front of the material? • Is the main message emphasized with visual cues? • Does the material contain at least one visual that conveys or supports the main message? • Does the material include one or more calls to action for the primary audience?
2) Language	• Do both the main message and the call to action use the active voice? • Does the material always use language the primary audience would use?
3) Information Design	• Does the material use bulleted or numbered lists? • Is the material organized in chunks with headings? • Is the most important information the primary audience needs summarized in the first paragraph or section?
4) State of the Science	• Does the material explain what authoritative sources, such as subject matter experts and agency spokespersons, know and don't know about the topic?
5) Behavioral Recommendations	• Does the material include one or more behavioral recommendations for the primary audience? • Does the material explain why the behavioral recommendation(s) is important? • Does the behavioral recommendation(s) include specific directions about how to perform the behavior?
6) Numbers	• Does the material always present numbers the primary audience uses? • Does the material always explain what the numbers mean? • Does the audience have to conduct mathematical calculations?

7) Risk	• Does the material explain the nature of the risk? • Does the material address both the risks and benefits of the recommended behaviors? • If the material uses numeric probability to describe risk, is the probability also explained with words or a visual?

Written reports are assessed to obtain a total score of 100, which is considered "perfect" and effectively communicating messages, scores ≥90% is considered "passing", and any scores <90% is not considered passing. The complete Index score sheet can be found on the CDC's website: http://www.cdc.gov/ccindex. Improving the clarity of message by CCRs can be achieved by targeting the seven indices:

1. *Main message/Call to action:*
    - The main message should be in the first section, top, beginning, or front of the CCR and emphasized with visual cues and graphics.
    - CCRs should highlight a single main message. Since most consumers are concerned about the safety and quality of their drinking water, a standard message should inform consumers if, "the drinking water is safe to drink according to all state and federal standards and regulations."
    - Any call to actions (e.g., phone numbers for contacting water utilities for more information) should be highlighted or boxed in to be easily identified by

2. *Language:*
    - Use active voice. Active voice sentences present direct messages and are less wordy and more to the point.
    - Use language that is common to lay-consumers. If scientific jargon is necessary, the terms must be defined and explained in lay-person language.
3. *Information Design:*
    - The main message must be easily identified by the consumer.
    - Use bulleted or numbered lists, with no more than 7 points per list and avoid subheadings.
    - Use simple color schemes, meaningful pictures, and sufficient pages to deliver messages.
4. *State of the Science:*
    - Use authoritative sources to provide information for water quality or explain the current science.
    - Acknowledge uncertainty in data and current science. The required contaminant table is an opportunity to increase the public's scientific literacy and educate them about water quality standards. For example, the public should be informed that

MCLs were developed though a scientific process designed to protect public health. Therefore, consumers should not be alarmed to know that regulations target specific contaminants like metals and microorganisms.

5. *Behavioral Recommendations:*
    - Use of standardized scripts required by the USEPA (e.g., text concerning immuno-compromised individuals) are unavoidable. Expand these messages to make them highly visible to individuals who are potentially sensitive to certain water contaminants.
6. *Numbers:*
    - Numerical data in the contaminant table should be in whole numbers since decimals or fractions are intimidating to many consumers. For example, the MCL for benzene is 0.005 mg/L, but reporting the MCL at 5 ppb in the table is more effective.
    - The consumers should never have to perform calculations.
7. *Risk:*
    - If there is a need to present uncertainty, probabilities should be in percentages or mathematically equal proportions (1/10 etc.).
    - Risk presented as numbers is not enough; it should follow with explanations about how the risk relates to the consumer. For example, if an incident occurs such as a spill or contaminant violation occurs, clarifications on the cause, the steps taken to remediate the issues, and potential health risks should be presented to the consumers.

Tool #2: Flesch-Kincaid Readability Tests

Readability means comprehension difficulty, and is often evaluated using the Flesch-Kincaid Readability Tests to determine "Reading Ease" and "Grade Level" for written reports. Applying these tests to a written report has the potential to increase reading comprehension by 60% if the report is delivered to the consumer at the appropriate reading level. The "Reading Ease" test scores documents ranging from 0 being very difficult to 100 meaning very easy and better comprehension (Table 2). The "Grade Level" readability test assigns an actual grade level with 6-7th grade level as the NIH's recommended reading level for health based information. The Flesch-Kincaid Readability Tests are readily available through Microsoft Word's "proofing" option.

Table 2. Flesch-Kincaid Reading Ease Scores and Grade Levels (Flesch, 1949)

Style	Flesch Reading Ease	Average words/sentence	Magazine Type	Grade Level
Very Easy	90 – 100	8 or less	Comics	4
Easy	80 – 90	11	Pulp fiction	5
Fairly Easy	70 – 80	14	Slick fiction	6
Standard	60 – 70	17	Digests	7 - 8 NIH Target
Fairly Difficult	50 – 60	21	Quality	Some high school
Difficult	30 – 50	25	Academic	High school/some college
Very Difficult	0 – 30	29 or more	Scientific	College

The reading ease scores and grade levels are based on defined equations with total words, total sentences, and total syllables as variables. Ways to improve readability scores and target appropriate grade level are the following:

- Use 14-17 words per sentence
- Eliminate unnecessary technical jargon
- Use active voice and simple vocabulary
- Implement pictures/videos (on-line versions)
- Incorporate bulleted or numbered lists
- Use whole numbers
- Explain standard violations in lay-consumer terms

HOW TO IMPROVE YOUR NEXT CCR?

- The main message of whether "the water is safe to drink or *not* according to all state and federal regulations" should be directly addressed and conveyed in the beginning or top of the document and emphasized with visual cues and pictures.
- Use short to-the-point sentences and simple words that target a 6-7th grade reading level.
- Health advisories, standard violations, and noticeable changes in water quality should be acknowledged and explained in detail and in common language for the lay-consumer.
- Apply the Clear Communication Index and Flesch-Kincaid Readability tools to improve communication of drinking water quality and safety to consumers.

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CHAPTER 7: Write Consumer Confidence Reports Customers Can Understand

This chapter has been published as: Phetxumphou, Katherine; Roy, Siddhartha; Davy, Brenda M.; Estabrooks, Paul; You, Wen; Dietrich, Andrea M. Write consumer confidence reports that customers can understand. *Op Flow, 43*(2): 24-26, 2017.
DOI: http://dx.doi.org/10.5991/OPF.2017.43.0010

PUBLIC NOTICE

Consumer Confidence Reports (CCRs) should be clearly understood by all consumers, but they often fall short of their goal. Using common communication tools, water utilities can improve their CCRs.

Water utilities are required to release annual Consumer Confidence Reports (CCRs), also known as water quality reports. According to the US Environmental Protection Agency (USEPA), CCRs are intended to "improve public health protection by providing educational material to allow consumers to make educated decisions regarding any potential health risk pertaining to the quality, treatment, and management of their drinking water supply." Although all water utilities write and distribute CCRs, many acknowledge they need to improve their CCRs as tools to communicate with their customers.

DO UTILITY CUSTOMERS UNDERSTAND CCRS?

The simple answer is no! According to national surveys and USEPA, consumers have concerns regarding their drinking water quality, but the concerns aren't addressed by CCRs. The understandability of CCRs is a widely recognized problem that utilities could address by writing CCRs at a level and in a manner that consumers can more easily understand.

Virginia Tech researchers found that a nationally representative sample of CCRs didn't effectively communicate drinking water information to the public. The research team consisted of environmental engineers and health researchers, but those who weren't trained in environmental engineering were dumbfounded by the complicated table of maximum contaminant levels (MCLs) and the language used in the CCRs, including terms such as "greensand filters," "sodium hydroxide," "curb stop," and "crossconnections." Although these are common terms for water professionals, consumers are at a loss to interpret them. The researchers found the scientific jargon and formats used in CCRs make it difficult for consumers to make informed decisions involving personal risks and their drinking water.

The lack of understandability is further confounded by the 11th- to 14thgrade reading level (i.e., high school graduate through some college) at which most CCRs are written. This reading level is equivalent to the *Harvard Law Review*, which is a publication few consumers attempt to read. Although the United States has a literacy rate of 99 percent, the average reading level of typical consumers is only at the 7th- to 8th-grade level. To deliver an easily understood message to consumers, the US National Institutes of Health (NIH) suggests documents should be written at a 6th- to 7^{th} grade reading level. Water utilities should strive to better understand their customers and use the NIH's recommended reading level when writing their CCRs.

IMPROVING CCRS

Because CCRs are considered public health communications, their messages should be clearly understood by all consumers. Water utilities can improve their CCRs' understandability, increasing the consumer readability, by using readily available communication tools.

Clear Communication Index. The US Centers for Disease Control and Prevention (CDC) published the Clear Communication Index in 2013 as an easily implemented tool for preparing effective and clear communication materials for the public. The index focuses on important characteristics of written documents that help people understand information. Using the 20 key questions listed in Table 1, the index can help writers prepare easy-to-read reports and can be applied to assess the clarity of written reports. Written reports are assessed to obtain a total score of 100, which is considered "perfect" in communicating messages; scores at or greater than 90 percent are considered "passing," and any scores under 90 percent aren't considered passing. The complete Index score sheet can be found on the CDC's website at www.cdc.gov/ccindex. Improving the message clarity of CCRs can be achieved by targeting the seven indices as follows:

1. Main Message/Call to Action. The main message should be in the first section, top, beginning, or front of the CCR and emphasized with visual cues and graphics. CCRs should highlight a single main message. Because most consumers are concerned about the safety and quality of their drinking water, a standard message should inform consumers if "the drinking water is safe to drink according to all state and federal standards and regulations." Any call to actions (e.g., phone numbers for contacting water utilities for more information) should be highlighted or boxed to be easily identified by consumers.

2. Language. Use active voice sentences, which present direct, less-wordy messages that are more to the point.Also, use language that is common to consumers. If scientific jargon is necessary, the terms must be defined and explained in layperson language.

3. Information Design. The main message must be easily identified by the consumer. Use bulleted or numbered lists, with no more than seven points per list and avoid subheadings. Also, use simple color schemes, meaningful pictures, and sufficient pages to deliver messages.

4. State of the Science. Use authoritative sources to provide information for water quality or explain the current science. Acknowledge uncertainty in data and current science. The required contaminant table is an opportunity to increase the public's scientific literacy and education about water quality standards. For example, the public should be informed that MCLs were developed through a scientific process designed to protect public health. Therefore, consumers shouldn't be alarmed that regulations target specific contaminants like metals and microorganisms.

5. Behavioral Recommendations. Use of USEPA-required standardized scripts (e.g., text concerning immunocompromised individuals) is unavoidable. Expand these messages to make them highly visible to individuals who are potentially sensitive to certain water contaminants.

6. Numbers. Numerical data in the contaminant table should be in whole numbers because decimals or fractions are intimidating to many consumers. For example, the MCL for benzene is 0.005 mg/L, but reporting the MCL at 5 ppb in the table is more effective. Also, consumers should never have to perform calculations.

7. Risk. If there's a need to present uncertainty, probabilities should be in percentages or mathematically equal proportions (1/10, etc.). Also, risk presented as numbers isn't enough; it should follow with explanations about how the risk relates to the consumer. For example, if an incident such as a spill or contaminant violation occurs, clarifications on the cause, the steps taken to remediate the issues, and potential health risks should be presented to consumers.

Table 1. Improving Written Reports
CDC's Clear Communication Index provides seven indices and related questions that can be applied to improve CCRs.

Indices Questions
1. Main Message/Call to Action
Does the material contain one main message?
Is the main message at the top, beginning, or front of the material?
Is the main message emphasized with visual cues?
Does the material contain at least one visual that conveys or supports the main message?
Does the material include one or more calls to action for the primary audience?
2. Language
Does the main message and the call to action use the active voice?
Does the material always use language the primary audience would use?
3. Information Design
Does the material use bulleted or numbered lists?
Is the material organized in chunks with headings?
Is the most important information summarized in the first paragraph or section?
4. State of the Science
Does the material explain what authoritative sources, such as subject matter experts and agency spokespersons, know and don't know about the topic?
5. Behavioral Recommendations
Does the material include one or more behavioral recommendations for the primary audience?

Does the material explain why the behavioral recommendation(s) is important?
Does the behavioral recommendation(s) include specific directions about how to perform the behavior?
6. Numbers
Does the material always present numbers the primary audience uses?
Does the material always explain what the numbers mean?
Does the audience have to conduct mathematical calculations?
7. Risk
Does the material explain the nature of the risk?
Does the material address the risks and benefits of the recommended behaviors?
If the material uses numeric probability to describe risk, is the probability also explained with words or a visual?

Table 2. Improving Readability
The Flesch-Kincaid "Reading Ease" test scores types of documents on a scale of 0 to 100 and grade levels.

Style	Flesch Reading Ease	Average words/sentence	Magazine Type	Grade Level
Very Easy	90 – 100	8 or less	Comics	4
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Difficult	30 – 50	25	Academic	High school/some college
Very Difficult	0 – 30	29 or more	Scientific	College

Flesch-Kincaid Readability Tests. Readability means comprehension difficulty, which is often evaluated using Flesch-Kincaid Readability Tests to determine "Reading Ease" and "Grade Level" for written reports. Applying these tests to a written report has the potential to increase reading comprehension by 60 percent if the report is delivered to the consumer at the appropriate reading level. As shown in Table 2, the "Reading Ease" test scores types of documents ranging from 0 (very difficult) to 100 (very easy, which equates to better comprehension). The "Grade Level" readability test assigns an actual grade level, with 6th to 7th grade level as NIH's recommended reading level for health-based information. The Flesch-Kincaid Readability Tests are readily available through Microsoft Word's "proofing" option. The reading ease scores and grade levels are based on defined equations with total words, total sentences, and total syllables as variables. Following are ways to improve readability scores and target appropriate grade level:

- Use 14–17 words per sentence.
- Eliminate unnecessary technical jargon.
- Use active voice and simple vocabulary.
- Implement pictures/videos (online versions).
- Incorporate bulleted or numbered lists.
- Use whole numbers.
- Explain standard violations in terms a layperson can understand.

HOW TO IMPROVE YOUR NEXT CCR

A few simple guidelines will go a long way toward improving your next CCR. For example, the main message of whether the water is safe to drink or not, according to all state and federal regulations, should be directly addressed and conveyed in the beginning or top of the document and emphasized with visual cues and pictures. Also, use short, to-the-point sentences and simple words that target a 6th- to 7th-grade reading level. Health advisories, standard violations, and noticeable changes in water quality should be acknowledged and explained in detail and in common language for consumers. Finally, apply the Clear Communication Index and Flesch-

Kincaid Readability tools to communicate drinking water quality and safety more effectively to consumers.

SECTION III: Food Safety

The works in this section are focus on beef safety, in particular Shiga-toxin producing *Escherichia coli* (STEC), which is a common foodborne pathogen found in beef products.

A quantitative microbial risk assessment (QRMA) was completed on beef that follows the farm-to-fork framework. It started at the peri-harvest stage, moved to processing, distribution, consumption, and then human risk assessment. Chapter 8 involves the processing plant stage that utilized a meta-analysis to determine intervention effectiveness measured as log reduction. Chapter 9 evaluated time and temperature during beef cooking to reduce STEC concentrations. Cooking is a main intervention strategy, but there are large data gaps in cooking time and temperature methods, thus, a meta-analysis was conducted on thermal death time values. Chapter 10 evaluated human risk and dose-response to support the STEC QMRA, where population susceptibility and matrix effects showed significant contribution to the dose-response.

As part of this grant, other researchers developed the 160isgood.com campaign to encourage food safety while cooking beef products. This campaign has contributed to beef safety through social media channels, conference booths, public education during tailgates, and the importance of proper thermometer usage.

CHAPTER 8: Meta-Analysis on the Effect of Interventions Used in Cattle Processing Plants to Reduce Escherichia coli Contamination

This chapter has been published as: Zhilyaev, S. Cadavez, V., Barron-Gonzales, U. Phetxumphou, K. and Gallagher, D.L. Meta-analysis on the effect of interventions used in cattle processing plants to reduce *Escherichia coli* contamination. *Food Research International*, 93: 16-25, 2017. DOI: https://doi.org/10.1016/j.foodres.2017.01.005

ABSTRACT

Cattle coming from feedlots to slaughter often harbor pathogenic *E. coli* that can contaminate final meat products. As a result, reducing pathogenic contamination during processing is a main priority. Unfortunately, food safety specialists face challenges when trying to determine optimal intervention strategies from published literature. Plant intervention literature results and methods vary significantly, making it difficult to implement interventions with any degree of certainty in their effectiveness. To create a more robust understanding of plant intervention effectiveness, a formal systematic literature review and meta-analysis was conducted on popular intervention methods. Effect size or intervention effectiveness was measured as raw log reduction, and modeled using study characteristics, such as intervention type, temperature of application, initial microbial concentration, etc. Least-squares means were calculated for intervention effectiveness separately on hide and on carcass surfaces. Heterogeneity between studies (I^2) was assessed and factors influencing intervention effectiveness were identified. Least-squares mean reductions (log CFU/cm^2) on carcass surfaces (n=249) were 1.44 [95% CI: 0.73 – 2.15] for acetic acid, 2.07 [1.48 – 2.65] for lactic acid, 3.09 [2.46 – 3.73] for steam vacuum, and 1.90 [1.33 – 2.47] for water wash. On hide surfaces (n=47), least-squares mean reductions were 2.21 [1.36 – 3.05] for acetic acid, 3.02 [2.16 – 3.88] for lactic acid, 3.66 [2.60 – 4.72] for sodium hydroxide, and 0.08 [-0.94 – 1.11] for water wash. Meta-regressions showed that initial microbial concentrations and timing of extra water washes were the most important predictors of intervention effectiveness. Unexplained variation remained high in carcass, hide, and lactic acid meta-regressions, suggesting that other significant moderators are yet to be identified. The results will allow plant managers and risk assessors to evaluate plant interventions, variation, and factors more effectively.

Keywords: Beef, Cattle, *E. Coli*, Intervention, Meta-analysis, O157

1. Introduction

Shiga-toxin producing *Escherichia coli* (STEC) has been recognized as a serious source of illness since it was first identified in 1982 (CDC, 2015). Young children, the elderly, and immunocompromised individuals are especially susceptible to illness and death from STEC infections (CDC, 2015). An estimated 176,000 U.S. foodborne STEC infections occur annually, with approximately 63,000 due to *E. coli* O157:H7 and 113,000 from non-O157 STEC (Batz, Hoffmann, & Morris, 2012; Scallan, Hoekstra, Angulo, Tauxe, Widdowson, Roy, Jones, & Griffin, 2011). STEC is estimated to cause 1% of food borne illnesses in England and 3% in Scotland. O157 is the predominant STEC organism in both the U.S. and the U.K. Continental Europe generally has a lower outbreak rate than the U.S or U.K., but they are caused by a broader range of STEC organisms (Vanaja, Jandhyala, Mallick, Leong, & Balasubramanian, 2013). In the U.S., thirty-nine percent of O157 infections and 30% of non-O157 STEC infections are linked to beef sources (Painter, Hoekstra, Ayers, Tauxe, Braden, Angulo, & Griffin, 2013).

Consequently, reducing STEC concentration and prevalence in beef is a high priority (Sofos, 2008). Through the implementation of plant hazard analysis critical control point (HACCP) principles, sanitary conditions at cattle processing plants have improved (Ropkins & Beck, 2000; Sofos, 2008). The risk and impact of product contamination has significantly decreased through plant interventions (Antic, Blagojevic, Ducic, Nastasijevic, Mitrovic, & Buncic, 2010; Arthur, Bosilevac, Nou, Shackelford, Wheeler, Kent, Jaroni, Pauling, Allen, & Koohmaraie, 2004; Sheridan, 1998). However, current plant intervention literature provides conflicting results. Some authors, for instance, report very high reductions, such as 5.05 log CFU/cm^2 for a water wash spray, while others recorded increases in bacterial counts from water washes on cattle surfaces (Scanga, Buschow, Kauk, Burk, Koohmaraie, De La Zerda, Motlagh,

Samadpour, & Koohmaraie, 2011; Yoder, Henning, Mills, Doores, Ostiguy, & Cutter, 2010). These discrepancies among reported intervention effectiveness are found throughout the literature and make it difficult to determine optimal decontamination strategies. It is likely that variations in experimental design (i.e., temperature, surface type, indicator organism, etc.) contribute to these discrepancies.

A systematic literature review coupled with meta-analysis is one method used to address differences between experimental methods and results within a body of literature (O'Connor, Sargeant, & Wang, 2014; Sargeant, Rajic, Read, & Ohlsson, 2006). Reported results, as intervention effectiveness, can be aggregated to provide weighted averages, or summary effects, among similar trials. Summary effects draw from a larger pool of information and therefore, create a more robust estimate of an intervention's effectiveness. When heterogeneity between trials is high, other tools, such as meta-regressions, can be used to explain the differences in intervention effectiveness (O'Connor et al., 2014; Prado-Silva, Cadavez, Gonzales-Barron, Rezende, & Sant'Ana, 2015). Systematic reviews and meta-analysis are powerful tools that are currently being used in food safety to measure intervention effectiveness with reduced bias and increased transparency (Bucher, Fazil, Rajić, Farrar, Willis, & McEwen, 2012; Greig, Waddell, Wilhelm, Wilkins, Bucher, Parker, & Rajić, 2012; Sargeant et al., 2006). A recent report on abattoir-level plant intervention studies supported current industry practices as effective methods for the reduction of STEC (Greig et al., 2012). However, the report was only limited to abattoir-level studies and did not appear to account for substitution practices in the recorded data. Substitution practices refer to the replacement of a non-detection or zero count (i.e., either a true zero or a value below the limit of detection) by some fraction of the detection limit to calculate

descriptive statistics. These substitution methods are an issue because they often lead to biased and inaccurate summary statistics.

This meta-analysis research had two objectives: (i) to determine the effectiveness of various plant interventions to mitigate Shiga-toxin producing *E. coli* using all published intervention data since 1990; and (ii) to apply meta-regressions to determine significant moderators, or covariates, (e.g., temperature of rinse, pressure of application, etc.) that could explain the variability observed across studies. It is expected that this research will help plant operators determine which combination of interventions and intervention parameters are optimal for the reduction of STEC.

2. Methods

2.1. Intervention Selection and Search Design

The 2011 Food Safety Inspection Service report was used to compile the list of potential plant interventions (Alvares, Lim, & Green, 2008). Only primary interventions that were (a) continuously applied throughout the year and (b) applied at 5% or more of plants surveyed were included as potential candidates for this meta-analysis. This method was chosen because a meta-analysis on each intervention should include several studies, but the number of studies for uncommon interventions was expected to be low (Borenstein, Hedges, Higgins, & Rothstein, 2009). Nine interventions that met the above criteria were: rinsing with water, lactic acid, acetic acid, sodium hydroxide, peroxyacetic acid, steam vacuum, citric acid, hypochlorite, and acidified sodium chlorite.

In June 2015, a published systematic literature review process (Knobloch, Yoon, & Vogt, 2011; Moher, Liberati, Tetzlaff, & Altman, 2009) was followed in order to effectively search for preliminary interventions and identify potential explanatory variables that could influence the

effectiveness of interventions. A full search of databases including Google Scholar, PubMed, Agricola, CAB, and Food Science and Technology Abstracts was completed in August of 2015. Journal articles within the previous 25 years were used. The general format of the searches was: *intervention type AND (beef OR carcass OR subprimal OR hide) AND ("Escherichia coli" OR O157 OR "non-O157" OR coliform OR "E. coli").*

When these terms were too broad, restrictive terms against other products (e.g., poultry, produce, etc.) were added. A full list of search terms are available in Table 1, and a diagram of the systematic review procedure (Knobloch et al., 2011; Moher et al., 2009) is available in Figure 1. All search results were screened for relevance, except for Google Scholar where only the first 40 results were screened. All the papers that passed the first round of screening were collected for further evaluation.

2.2. Screening and Eligibility Criteria

The screening criteria followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) method (Knobloch et al., 2011; Moher et al., 2009). Primary screening was purposefully broad; titles and abstracts from the initial searches were checked for any possible relevance to plant interventions. Papers were more rigorously screened in the second round by two independent reviewers. Differences between reviewers' findings, that could not be reconciled initially, were brought to a third reviewer for a final decision. Articles in the second round were required to have information to obtain a mean difference in log CFU/cm^2 of bacteria and a variance of the difference. If not explicitly provided, mean differences could be extracted from papers reporting concentrations on a before and after group. Visual information, such as graphs, representing bacterial reduction were not considered due to reduced precision.

Studies that applied interventions by dipping samples in solutions were also excluded. Only primary studies reporting the author's original work were included. Furthermore, the treated specimens must have originated from cattle. The tracked organism must have been one of the STEC-7 (O157, O26, O45, O103, O111, O121, and O145), generic *E. coli*, or coliforms. The STEC-7 were chosen because they are the pathogenic organism of interest, while generic *E. coli* and coliforms are often used as surrogates (Ingham, Algino, Ingham, & Schell, 2010). Trials that reported log reduction statistics from substituted values were not included if 20% or more of the data was substituted. This occurred when experimenters recorded initial or final concentrations below a detection limit. Values below the detection limit were often substituted for some fraction of the detection limit and incorporated into the analysis of the original papers. The procedure for identification and screening is summarized in Table 1.

Table 1. Procedure for Identification and Screening During Systematic Review

(Knobloch et al., 2011; Moher et al., 2009)

Step	Procedure
Identification	Plant intervention studies were searched in Google Scholar, PubMed, Agricola, CAB, and Food Safety and Technology Abstracts databases using the following terms: *("sodium hydroxide" OR "lactic acid" OR "citric acid" OR "acidified sodium chlorite" OR ASC OR hypochlorite OR "sodium hypochlorite" OR bleach OR chlorine OR "steam vacuum" OR "peroxyacetic acid" OR "water wash" OR "hot water" OR "water washes" OR "water rinse" OR "water spray" OR "spray washing" OR "spray wash" OR "water rinsing" OR "water washing") AND ("Escherichia coli" OR O157 OR "non-O157" OR coliform OR "E. coli") AND (beef OR carcass OR hide OR subprimal).* Restrictive terms applying only to the abstracts were: *(poultry OR chicken OR cilantro OR lettuce OR dairy OR milk OR biofilm OR brine OR broiler OR pigs OR pork).*
Primary Screening	Initial screening included scanning abstracts and figures to check if studies were relevant. In other words, the studies must have included at least one of the nine interventions (water wash, lactic acid, acetic acid, sodium hydroxide, peroxyacetic acid, steam vacuum, citric acid, hypochlorite, or acidified sodium chlorite), on cattle surfaces, and one of the organisms of interest (STEC-7 [O157, O26, O45, O103, O111, O121, and O145], generic *E. coli*, or coliforms).
Secondary Screening	Articles must have contained the following information to pass secondary screening: Reported intervention effectiveness in log CFU/area and standard error (SE) of the intervention effectiveness or information to calculate effectiveness and SE; published after 1990; sampled within 2 hours of intervention application; and measured reductions must have been from previously uncleaned surfaces (i.e., before any other treatment). Experiments were excluded if the above criteria were not met, if the data was only presented visually, if the interventions were applied by dipping/soaking samples instead of spraying, or if more than 20% of values were being substituted.

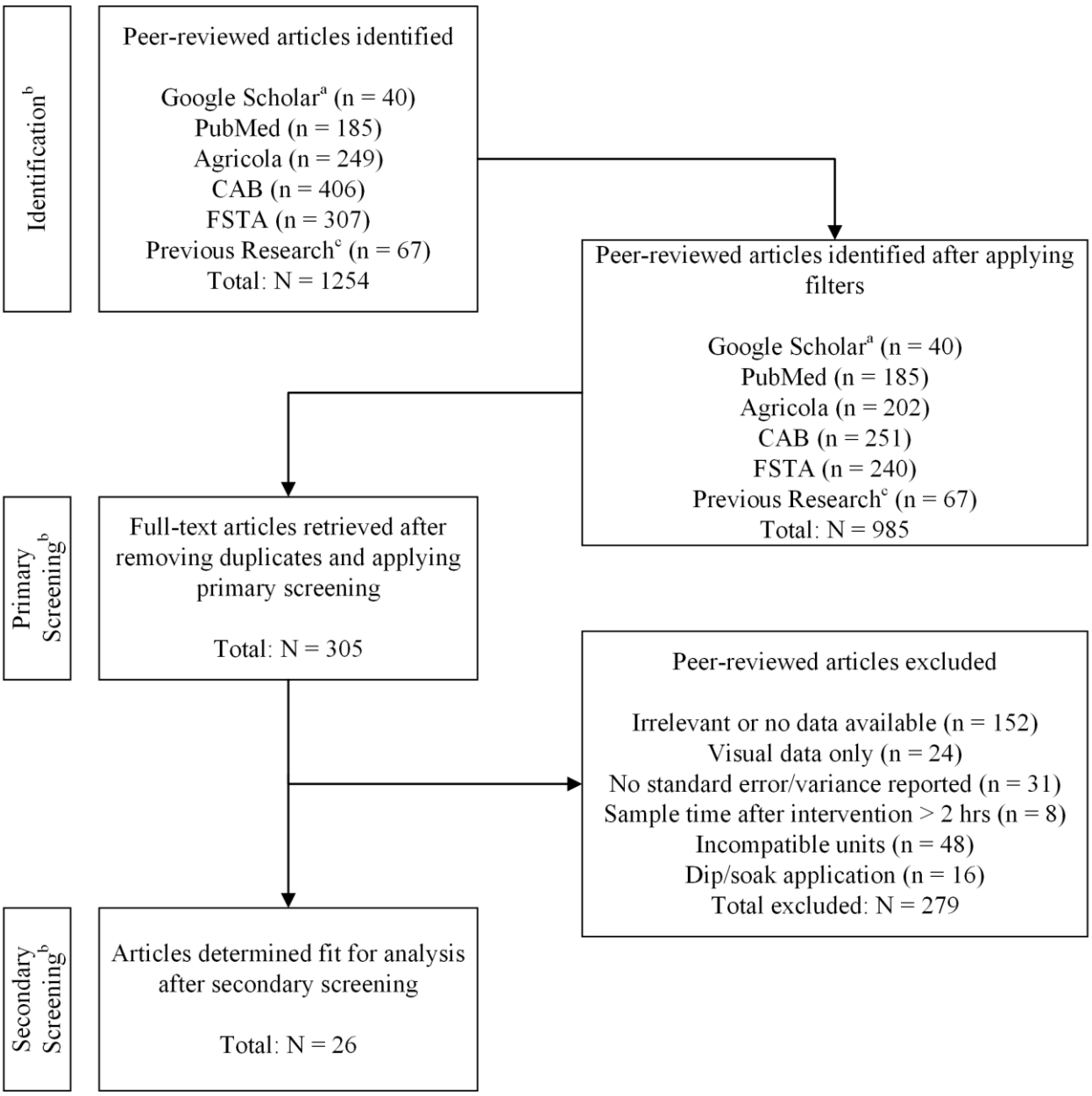

Figure 1. Results from the Systematic Review: Identification and Screening

[a] Only the first 40 results of Google Scholar were checked.
[b] Table 3 defines procedures for identification and screening.
[c] Some articles were already gathered from previous research on plant interventions.

Based on initial screening, insufficient data were available for peroxyacetic acid, citric acid, hypochlorite, and acidified sodium chlorite. Data were considered insufficient when there were

less than three eligible studies on a particular intervention. These interventions were not considered further.

2.3. Assessing Critical Variables and Data Extraction

After the preliminary search, several factors were selected as possible explanatory variables. In an effort to explain the differences in intervention effectiveness between trials, the following study characteristics were extracted from the papers: temperature of application, intervention type, duration of application, sampling method (viz. excision or sponge), pressure of application, microbial concentrations on samples before intervention, concentration of antimicrobial, microorganism being tracked (viz. coliforms, *E. coli* O157, non-O157 STEC, or generic *E. coli*), surface type (viz. hide or carcass), type of contamination (viz. natural or inoculated), and rinsing with water (viz. no rinse, rinse before/after main treatment). For further details on study characteristics, see Table 2. These study characteristics were used as moderating variables in the meta-regressions. The final list of journal articles used in this meta-analysis is available in Supplemental Information, Appendix A. For clarification, in this analysis, a "study", "article", or "paper" refers to a single, unique, peer-reviewed publication. "Trials" are separate experiments within a publication. "Carcass" trials are those experiments that were conducted on the dehided carcass surface while "hide" trials were conducted on the hair and epidermis sections of the cattle.

Table 2. Covariates Used in the Meta-Regressions

Variable	Definition	Values Taken[a]	Baseline used in meta-regressions[b]
Organism Type	Microbial organisms used to track reductions	Coliforms, *E. coli* O157, non-O157 STEC, or generic *E. coli*	Coliforms
Sample Method	Sample collection techniques used for enumeration	Excision or sponge	Excision
Duration	Intervention application time	2.5 to 150 seconds	0 seconds
Inoculation Type	Origin of the microbe being tracked	Naturally contaminated or artificially inoculated	Natural contamination
Surface Type	Sample surface	Hide or carcass	Carcass
Extra Water Wash	Water wash(es) in addition to main treatment[c]	No wash, wash after, wash before, or both before and after	No extra wash
Temperature	Maximum temperature of intervention applied on samples	15 to 95°C	0°C
Pressure	Maximum pressure of the intervention applied	23 to 8274 kPa	0 kPa
Initial Microbial Concentration (IMC)	Levels of contamination before sanitization	-0.32 to 8 log CFU/cm^2	0 log CFU/cm^2
Intervention Type	Method or antimicrobial used to remove contamination	Water wash, acetic acid, lactic acid, sodium hydroxide, steam vacuum	Water wash
Antimicrobial Concentration (AMC)	Concentration of antimicrobial	1.6 to 10% antimicrobial	0% antimicrobial

[a] Values taken represents the range of categorical and continuous variables observed across all trials.

[b] The baseline refers to the one option within a variable that is set as the default in the meta-regression. For example, meta-regression results including inoculation type as a variable will only explicitly show the effect of inoculated samples, as the effect of naturally contaminated samples, is accounted for in the intercept term. Regressions use a baseline of 0 for continuous variables as a default to calculate intercepts. However, for predictive purposes, it is not recommended to use values outside those observed for continuous moderators.

[c] The water wash applied at a higher temperature was considered the main intervention for sequential water wash treatments.

2.4. Data Description

Table 3 is provided to give some understanding of the data structure used in the meta-regressions. A key characteristic of the data is the skewed information among covariates and intervention types. For example, the most information collected on interventions was for lactic acid, with 135 trials, and water wash, with 99 trials. On the other hand, the total trials for acetic

acid, steam vacuum, and sodium hydroxide were 21, 18, and 11, respectively. The same issue exists within covariate data; there are a total of 241 trials on carcass surfaces while only 43 trials on hides. A total of 251 inoculated trials were collected to 33 naturally contaminated ones. The organism type, temperature, and initial microbial concentration variables were generally more uniformly distributed; while the extra wash, inoculation type, surface type, sample method, pressure, antimicrobial concentration, and duration variables had frequent issues with skewed representation. Additionally, there were issues with missing data; specifically, the pressure, initial microbial concentration, and duration of application were not always reported for every trial used in the analysis. An alternative version of the data structure based on surface type is provided in Supplemental Information Table SI-1.

The non-uniformity and sparseness of the data in certain covariates and interventions impede the analysis of all the trials under one meta-regression. As a result, several meta-regressions were split by surface type, intervention type, and available covariate data. Details of each model and methods are given in the *Meta-Regression* section.

Table 3. Data Structure for Meta-Regressions by Covariate and Intervention

Intervention (# of trials)	Organisms type	Count	Extra water wash	Count	Inoculation type	Count	Surface type	Count	Sample method	Count		Continuous Variables[a]				
												Temp	Pres	IMC	AMC	Duration
Acetic acid (21)	Coliforms	7	After	6	Inoculated	15	Hide	12	Excision	6	Min	23	203	3.4	2	5.6
	E. coli	8	Before	5							Max	55	207	6.6	10	15
	O157	6	B & A	4	Natural contamination	6	Carcass	9	Sponge	15	Mean	47.6	206.5	4.93	6.2	7.2
	Non-O157	0	No wash	6							Count	21	21	11	21	21
											% Reported	100	100	52	100	100
Lactic acid (135)	Coliforms	9	After	6	Inoculated	127	Hide	12	Excision	123	Min	15	69	1.23	2	7
	E. coli	45	Before	17							Max	55	850	8	10	60
	O157	49	B & A	0	Natural contamination	8	Carcass	123	Sponge	12	Mean	37.2	273.5	5.76	3.8	14.4
	Non-O157	32	No wash	112							Count	135	127	125	135	33
											% Reported	100	94	93	100	24
Sodium hydroxide (11)	Coliforms	4	After	7	Inoculated	4	Hide	11	Excision	3	Min	10	203	3.7	1.6	7
	E. coli	3	Before	1							Max	60	8274	5.2	3	30
	O157	4	B & A	0	Natural contamination	7	Carcass	0	Sponge	8	Mean	24	1214	4.63	2.8	11.7
	Non-O157	0	No wash	3							Count	11	8	4	11	11
											% Reported	100	73	36	100	100
Steam vacuum (18)	Coliforms	11	After	6	Inoculated	12	Hide	0	Excision	15	Min	82	23	-0.32	-	6
	E. coli	5	Before	0							Max	95	103	5.3	-	6
	O157	2	B & A	0	Natural contamination	6	Carcass	18	Sponge	3	Mean	90.9	40.5	3.93	-	6
	Non-O157	0	No wash	12							Count	18	15	18	-	6
											% Reported	100	83	100	-	33
Water wash (99)	Coliforms	39	After	8	Inoculated	93	Hide	8	Excision	82	Min	15	138	1.9	-	2.5
	E. coli	37	Before	9							Max	95	2760	8	-	150
	O157	23	B & A	2	Natural contamination	6	Carcass	91	Sponge	17	Mean	47.4	717	5.02	-	30.5
	Non-O157	0	No wash	80							Count	99	79	93	-	87
											% Reported	100	80	94	-	88

[a] Temp = temperature in ^{o}C; Pres = pressure in kPa; IMC = initial microbial concentration in log CFU/cm^2; AMC = antimicrobial concentration in percent; Duration in seconds

2.5. Summary Effects

In anticipation of high heterogeneity between trials, a random effects model was used to produce summary effects for the interventions that were listed earlier (Borenstein et al., 2009). High or statistically significant heterogeneity occurs when intervention results differ more than expected by random error alone (Higgins & Green, 2006). Statistically significant heterogeneity is often the product of differences in experimental design or application that influences trial results, causing results to vary significantly (Higgins & Green, 2006). Summary effects are the weighted averages of trial results. In the random-effects model, the weights for each trial is the inverse of the sum of trial variance (w_i) and between-trial variance (τ^2) (Borenstein et al., 2009):

$$Weight_i = \frac{1}{w_i+\tau^2}$$

The "meta" package v4.3-0 in R v3.2.2 (R Development Core Team, 2015) was used to obtain single summary effects, forest plots, funnel plots, and heterogeneity (I^2) measurements for each intervention. I^2, which is calculated on the Q statistic and by degrees of freedom, allows for heterogeneity to be compared on a relative scale by measuring the amount of true heterogeneity over the total observed variation (Borenstein et al., 2009; Gelman, 2015; R Development Core Team, 2015). The Q statistic is based on a chi-squared test; it has low power as a test for heterogeneity when the number of trials are low and too much power when trial size is high (Higgins & Green, 2006). As a result, the I^2 is usually regarded as a better gauge of heterogeneity as it measures the percentage of variability that is due to heterogeneity rather than sampling error (Borenstein et al., 2009; Higgins & Green, 2006). Generally, I^2 between 0-25% is considered low, 25-75% is moderate, and 75-100% is high (Higgins & Green, 2006). Meta-regressions were run when moderate to high heterogeneity was found in the summary effects.

Microbial concentrations in log CFU/cm^2 along with their respective standard deviations were used as inputs into the meta-analysis. The mean difference was used when an author failed to report before and after values. Trials within a single paper were entered as independent entries into the meta-analysis. This procedure likely underestimated the variance of the effects and overestimated the precision of the model (Borenstein et al., 2009). This issue was more thoroughly addressed in the meta-regressions by nesting the effect of trials within a paper as additional random effects to account for the correlation among trials (Borenstein et al., 2009; Pinheiro & Bates, 2000).

2.6. Meta-Regressions

Meta-regressions are similar to simple linear regressions except they use the variance of each trial to allocate more weight to trials with smaller variances. This procedure is similar in effect to the random-effects model summary effect, except that meta-regressions can incorporate continuous and categorical variables as moderators. Results from a meta-regression can be used to assess the impact of a unit increase in explanatory variable on the effect size, which is intervention effectiveness (Higgins & Green, 2006). Following recommendations, meta-regressions were only carried out on interventions that had more than one study and over ten total trials (Borenstein et al., 2009; Higgins & Green, 2006). Meta-regressions, performed with the "metafor" package v1.9-7 and "nlme" package v3.1-124, could further reduce heterogeneity by incorporating the continuous and categorical variables into the meta-analysis (Pinheiro, Bates, DebRoy, & Sarkar, 2016; Viechtbauer, 2010). However, papers did not always report all of the variables of interest. As mentioned previously, surface type, temperature, inoculation type, antimicrobial concentrations, extra wash type, and organism type were always reported, while duration, pressure, and initial microbial concentrations were not always stated. Data were split

into 7 categories before regression analysis. The first two subsets were separated by surface type, with trials conducted on hide surfaces analyzed separately from those on carcass surfaces. The other five subsets were arranged by intervention type, with trials on water wash, lactic acid, acetic acid, steam vacuum, and sodium hydroxide analyzed separately.

For each of the seven subsets, two meta-regressions were conducted in order to utilize and obtain as much information as possible. The first meta-regression approach only included the variables of interest that were always reported in the literature as moderators; it utilized all the trials within a data set, thus, it is referred to as the "full-trial" meta-regression. An analysis of 20 trials, for instance, with each reporting a temperature, but with incomplete reporting of initial concentrations, would be run with temperature as a covariate, but not initial concentration. Given adequate information, a second meta-regression was performed that also contained the less frequently reported variables (e.g., initial microbial concentration, duration of application, or pressure) to observe their impact on log reductions. This second model is referred to as the "full-variable" meta-regression because it encompassed more variables, but often had fewer trials. Covariates were added to the full-variable meta-regression when they were reported in less than 100% of trials, but more than 75%. Seventy-five percent was chosen as a limit below which too many trials were being lost. As a minimum, two trials per study were required to calculate the random effects terms. Therefore, studies with only one trial were excluded from mixed model meta-regressions.

2.7. Meta-regression Models

Non-significant predictors were removed from the models so that their correlation with other covariates did not adversely affect the standard error estimates of other predictors (O'brien, 2007). A simple backward selection process was used to determine which variables to eliminate

from each model and the results were confirmed by a forward selection process (Chatterjee & Hadi, 2006). All significance tests to determine covariate significance used an alpha of 0.10. Table 4 shows all of the covariates that remained significant after backward selection for each data subset. The full-trial hide meta-regression is explicitly reproduced below as an example:

$$LR_{ijmnqr} = \beta_0 + \beta_{1i} + \beta_{2j} + \beta_{4im} + \beta_{5n} + \varepsilon_{ijmnqr}$$

Where LR_{ijmnqr} is the expected log reduction in log CFU/cm^2; β_0 is the mixed effects intercept term equal to $\dot{B} + \nu_q + \eta_{qr}$ for paper q and trial r; and $\dot{B}$ is the fixed effect intercept. The random effects terms, ν_q and η_{qr}, were added to separate the between-trial variation from the between-paper variation. This allowed the correlated between-trial results to be entered as separate entries and provided more information on the relationship between covariates and log reduction (Pinheiro & Bates, 2000).

β_{1i} is the effect of intervention i (i = lactic acid, sodium hydroxide, water wash, or acetic acid), β_{2j} is the effect of inoculation type j (j = lab inoculation or natural contamination), β_{4im} is the nested effect of an extra wash m (m = no extra water wash, water wash before intervention, water wash after intervention) within intervention i, and β_{5n} is the effect of sample method n (n = excision or sponge). The nested effect for water wash allows the effect to be calculated within each intervention, e.g., the effect of water wash within the lactic acid data is calculated separately from the effect of water wash within the sodium hydroxide data. The random effect terms for paper (ν_q), trial (n_{qr}), and residual error (ε) were expected to follow normal distributions with mean of zero and variance of s_p^2, s_t^2, and s^2, respectively. These definitions for ν_q, η_{qr}, and ε are the same for all meta-regressions.

Table 4. Final Meta-Regression Model Structure From Backward Variable Selection[a]

Equation Number	Data Group[b]	Model Type[c]	Meta-regressions after backward selection procedure for each data sub-group
1	*Hide*	*FT*	**Log Reduction = $\beta_0 + \beta_{1i} + \beta_{2j} + \beta_{4im} + \beta_{5n}$**
NA	*Hide*[d]	FV	
2	*Carcass*	*FT*	**Log Reduction = $\beta_0 + \beta_{1i} + \beta_{2j} + \beta_{3k} + \beta_{4m} + \beta_7$*IMC + β_8*T**
NA	*Carcass*[d]	*FV*	
4	*WW*	*FT*	**Log Reduction = $\beta_0 + \beta_{2j} + \beta_{4m} + \beta_{5n} + \beta_{6p} + \beta_8$*T**
5	*WW*	*FV*	**Log Reduction = $\beta_0 + \beta_{5n} + \beta_7$*IMC + β_8*T + β_9*t**
6	*LA*	*FT*	**Log Reduction = $\beta_0 + \beta_{3k} + \beta_{4m}$**
7	*LA*	*FV*	**Log Reduction = $\beta_0 + \beta_{4m} + \beta_{6p} + \beta_7$*IMC**
8	*AA*	*FT*	**Log Reduction = $\beta_0 + \beta_{3k}$**
NA	*AA*[d]	*FV*	
9	*SH*	*FT*	**Log Reduction = $\dot{B}^e + \beta_{2j} + \beta_{4m} + \beta_{11}$ *AMC**
NA	*SH*[d]	*FV*	
10	*SV*	*FT*	**Log Reduction = $\beta_0 + \beta_{4m} + \beta_7$*IMC**
NA	*SV*[f]	*FV*	

[a] β_0 is the mixed effects intercept term equal to $\dot{B} + \nu_q + \eta_{qr}$ for paper q and trial r, β_{1i} is the effect of intervention i (i = lactic acid, sodium hydroxide, water wash, steam vacuum, or acetic acid), β_{2j} is the effect of inoculation type j (j = lab inoculation or natural contamination), β_{3k} is the effect of organism k (k = generic *E. coli*, *E. coli* O157, non-O157 STEC, or coliforms), β_{4m} is the effect of an extra wash m (m = no extra water wash, water wash before, water wash after, or both before and after), β_{4im} is the nested effect of an extra wash m (m = no extra water wash, water wash before, water wash after) within intervention i, β_{5n} is the effect of sampling method n (n = excision or sponge), β_{6p} is the estimated effect of surface p (p = carcass or hide), β_7 is the increase in log reduction observed with each log increase in IMC, β_8 is the unit increase in effectiveness given a Celsius increase in T for temperature, β_9 is the estimated effect of a unit increase in t for time (s), β_{10} is the effect of an incremental increase in P for pressure (kPa), and β_{11} is the unit increase in effectiveness given a percent concentration increase in antimicrobial.

[b] Data subset by trial design, Carcass = Regression on intervention data for carcass surface only, Hide = Regression on intervention data for hide surface only, WW = Water Wash, AA = Acetic Acid, SH = Sodium Hydroxide, SV = Steam Vacuum.

[c] FT = Full-Trial, FV = Full-Variable.

[d] Meta-regression was conducted but converged to the same model as in the Full-Trial

[e] The sodium hydroxide model did not have trial or paper as random effects.

[f] There was not enough data to test the Full-Variable regression. Quality information on time and temperatures were missing.

Sodium hydroxide had the smallest pool of data, thus, the mixed effects model could not be used while meeting the minimum trial criteria. Therefore, the random effects terms, ν_q and η_{qr}, were dropped to incorporate the two studies with only one trial each so the total number of trials could pass the 10 trial minimum.

3. Results

3.1. Summary Effects

Sodium hydroxide had the highest estimated log reduction, with a summary effect at 3.17 log CFU/cm^2, while steam vacuum had an estimated log reduction of 3.08 log CFU/cm^2. The estimated impact of acetic acid, lactic acid, and water wash were similar at approximately 2 log CFU/cm^2. Moderate to high I^2 was observed in all data sets (Table 5). Additionally, all summary effects were for overall intervention effectiveness and those presented were not sub-grouped by factor (e.g., effectiveness of lactic acid on hide vs. on carcass surfaces). Examples of intervention forest plots of extract data from the different intervention studies can be found in Supplemental Information, Appendix C, Figures SI-1 through SI-5.

Table 5. Meta-Analysis Summary Effects Results

Intervention	Summary Effects[a]	Between-Trial Variation (τ^2)	Heterogeneity (I^2)	Trials	Papers
Sodium Hydroxide	3.17	0.906	89.7	11	4
Steam Vacuum	3.08	3.233	99.3	19	6
Acetic Acid	2.10	0.717	95.9	23	5
Lactic Acid	2.01	0.171	99.4	139	14
Water Wash	1.81	0.254	98.5	105	20

[a] Measured in log CFU/cm^2 reduction

3.2. Meta-Regressions

The results for the full-trial and the full-variable meta regressions are given in Tables 6 and 7 respectively. The full-trial meta-regression maximized the amount of trials being used, but limited the covariates considered in the backward selection process.

Table 6. Full-Trial Meta-Regression Results (mean and standard error)[a]

Covariates	Carcass (Trials = 249, papers = 22)	Hide (Trials = 47, papers =4)	Acetic acid (Trials = 21, papers = 3)	Lactic acid (Trials = 135, papers = 10)	Water wash (Trials = 99, papers = 14)	Sodium hydroxide (Trials = 11, papers = 4)
Fixed Effects						
Intercept	-0.79 (0.43)[c]	-1.63 (0.49)[b]	2.28 (0.60)[b]	2.21 (0.55)[b]	3.17 (0.82)[b]	1.06 (0.54)[b]
Acetic acid	-0.46 (0.30)	3.22 (0.43)[b]	NT	NT	NT	NT
Lactic acid	0.16 (0.18)	3.94 (0.45)[b]	NT	NT	NT	NT
Sodium hydroxide	NT	3.91 (0.45)[b]	NT	NT	NT	NT
Steam vacuum	1.19 (0.23)[b]	NT	NT	NT	NT	NT
Inoculation	0.92 (0.45)[c]	0.82 (0.31)[b]	NS	NS	-1.66 (0.74)[b]	0.55 (0.24)[b]
Generic *E. coli*	0.19 (0.14)	NT	0.72 (0.35)[c]	0.06 (0.34)	NS	NS
E. coli O157	-0.55 (0.15)[b]	NT	-0.86 (0.46)[c]	-0.70 (0.34)[b]	NS	NS
Non-O157 STEC	-0.58 (0.15)[b]	NT	NT	-0.72 (0.34)[b]	NS	NS
Wash after	1.02 (0.203)[b]	NT	NS	-0.5 (0.72)	0.74 (0.35)[b]	-1.06 (0.27)[b]
Wash before	0.80 (0.206)[b]	NT	NS	1.47 (0.74)[c]	0.43 (0.28)	0.69 (0.67)
Wash before & after	1.01 (0.301)[b]	NT	NT	NT	-1.22 (0.67)[c]	NT
IMC	0.31 (0.043)[b]	NT	NT	NT	NT	NT
AMC	NS	NS	NS	NS	NT	0.93 (0.18)[b]
Temperature	0.003 (0.001)[c]	NS	NS	NS	0.014 (0.003)[b]	NS
Sponge	NS	1.65 (0.43)[b]	NS	NS	-1.21 (0.43)[b]	NS
Hide	NT	NT	NS	NS	-2.53 (0.63)[b]	NT
Wash after AA	NT	-0.92 (0.43)[b]	NT	NT	NT	NT
Wash after LA	NT	-1.41 (0.45)[b]	NT	NT	NT	NT
Wash after SH	NT	-1.48 (0.46)[b]	NT	NT	NT	NT
Wash after WW	NT	1.11 (0.37)[b]	NT	NT	NT	NT
Wash before AA	NT	-0.94 (0.72)	NT	NT	NT	NT

Wash before LA	NT	-0.16 (0.73)	NT	NT	NT	NT
Wash before SH	NT	1.92 (0.96)[c]	NT	NT	NT	NT
Wash before WW	NT	0.33 (0.92)	NT	NT	NT	NT
Random Effects[d]						
S_p^2	0.285	0.024	0.86	1.603	0.23	
S_t^2	0.026	0.113	0.455	0.021	0.355	
S^2	11.049	1.669	<0.001	4.04	<0.001	<0.001

[a] NT = not tested because of insufficient data, NS = not statistically significant at α=0.10.
[b] Significant at α=0.05
[c] Significant at α=0.10
[d] Estimated variance for paper (s_p^2), trial (s_t^2) and residual error (s^2)

Table 7. Full-Variable Meta-Regression Results (mean and standard error)[a]

Covariates	Lactic acid (Trials = 125, papers = 9)	Water wash (Trials = 81, papers = 12)	Steam vacuum (Trials = 18, papers = 5)
Fixed effects			
Intercept	-0.27 (0.29)	-0.50 (0.45)	0.23 (0.36)
Wash after	NT	NS	1.43 (0.34)[b]
Wash before	2.00 (0.18)[b]	NS	NT
IMC	0.36 (0.05)[b]	0.27 (0.07)[b]	0.57 (0.09)[b]
Sponge	NS	-1.44 (0.50)[b]	NS
Temperature	NS	0.02 (0.003)[b]	NT
Hide	2.24 (0.59)[b]	NT	NT
Duration	NT	0.013 (0.003)[b]	NT
Random Effects			
S_p^2	<0.001	0.49	0.021
S_t^2	0.154	0.27	0.322
S^2	2.822	<0.001	0.569

[a] NT = not tested because of insufficient data, NS = not statistically significant at α=0.10.
[b] Significant at α=0.05

For the full-trial regressions on carcass, steam vacuum had the highest estimated impact at 1.19 log CFU/cm^2 higher reduction than water wash. Increases in temperature resulted in higher reductions by 0.003 log CFU/cm^2 per ^{o}C, which translates to approximately 0.3 log CFU/cm^2 at 95^{o}C. Washing with water before, after, or both before and after the main treatment added to microbial reduction by 0.80, 1.02, and 1.01 log CFU/cm^2, respectively. The data limitations of the extra wash data within the carcass trials did not allow for a nested analysis of extra wash within intervention type. Higher initial concentrations were predicted to increase reported log reductions at a slope of 0.31 log CFU/cm^2 per log CFU/cm^2 increased starting concentration. Samples that were inoculated were predicted to have higher levels of reported reduction, by 0.92 log CFU/cm^2, than those that were naturally contaminated.

The full-trial meta-regression on hide sample results estimated that acetic acid, lactic acid, and sodium hydroxide were over 3 log CFU/cm^2 more effective than water wash alone

(Table 6). Inoculated samples were associated with higher reductions than their naturally contaminated counterparts by 0.82 log CFU/cm^2. Adding an extra water wash after the application of an acid or base was predicted to substantially decrease effectiveness. The use of water after the application of acetic acid, lactic acid, or sodium hydroxide reduced the effectiveness by 0.92, 1.41, and 1.48 log CFU/cm^2, respectively. So, for example, a lactic acid application followed by a water wash had a combined effectiveness of only 2.53 log CFU/cm^2 (the lactic acid effectiveness of 3.94 log CFU/cm^2 together with the negative water wash after effectiveness of -1.41 log CFU/cm^2).

For the full-trial meta-regression on acetic acid, organism type was the only variable that remained significant after testing, with generic *E. coli* associated with increased reductions by 0.72 log CFU/cm^2 when compared to trials using coliforms (Table 6). *E. coli* O157, on the other hand, was estimated to decrease reduction by 0.86 log CFU/cm^2 when compared to coliforms. For example, the estimated 2.28 log reduction for coliforms with acetic acid would yield a 3.00 log reduction for generic *E. coli*, but only a 1.42 log reduction for O157.

Lactic acid data was available to produce both the full-trial meta-regression and full-variable meta-regression (Table 6 and Table 7). Having an extra wash before was estimated to increase effectiveness by 1.47 and 2.00 log CFU/cm^2 in the full-trial and full-variable meta-regressions, respectively. Organism type was significant in the full-trial meta-regression, with the pathogenic strains of *E. coli* associated with increased resistance to intervention, but not in the full-variable. Hide samples increased reductions by 2.24 log CFU/cm^2 when compared to carcass samples. Initial microbial concentration, in the full-variable meta-regression, was highly significant with a slope of 0.36 log CFU/cm^2 per increased log CFU/cm^2 starting concentration.

Water wash also produced both full-trial and full-variable meta-regressions. The full-trial water meta-regression showed inoculation type, extra wash, sample method, surface type, and temperature as significant predictors (Table 6). Temperature increased effectiveness by 0.014 log CFU/cm^2 per ^{o}C. Initial microbial concentration and duration were both estimated to have substantial impacts on final log reductions with slopes of 0.27 log CFU/cm^2 per increased log CFU/cm^2 and 0.013 log CFU/cm^2 per second, respectively. Sampling with a sponge was predicted to decrease reported reductions by approximately 1.21 and 1.44 log CFU/cm^2 in the full-trial and full-variable water wash regressions, respectively.

For the full-trial sodium hydroxide meta-regression, the number of trials available for analysis fell below the 10 trial minimum so only the residual error term remained to account for variance in the full-trial regression for sodium hydroxide (Table 6). The results showed that antimicrobial concentration, extra water wash, and inoculation were all statistically significant predictors. The resulting residual term was less than 0.001 (log CFU/cm^2)^2 as the three covariates described all of the between-trial variability. Concentration was estimated to have the most significant impact at a slope of 0.93 log CFU/cm^2 per percent NaOH. Following previous trends, the water rinse after the sodium hydroxide was linked to decreased effectiveness by about 1 log CFU/cm^2. Inoculated samples were associated with higher reported reduction.

The full-variable steam vacuum meta-regression found that extra water wash and initial microbial concentration variables were the only covariates found to be statistically significant. Each unit increase in initial concentration was estimated to increase reductions by 0.57 log CFU/cm^2, while adding an extra wash after would further increase reductions by 1.43 log CFU/cm^2 (Table 7). Variability between trials and residual variation remained high.

3.3. Least-Means Squares

The results of the surface models for intervention effectiveness are summarized as least-squares means (Figure 2 and Figure 3). Based on the hide model (Table 6), sodium hydroxide was estimated to be the strongest sanitizer followed by lactic acid, acetic acid, and water in the hide trials at reductions of 3.66, 3.02, 2.21, and 0.08 log CFU/cm^2, respectively (Figure 2). In the full-trial carcass meta-regression, steam vacuum had the greatest reductions followed by lactic acid, water wash, and acetic acid at reductions of 3.09, 2.07, 1.90, and 1.44 log CFU/cm^2, respectively (Figure 3). The full-trial carcass regression, however, estimates that the application of water, lactic acid, and acetic acid on carcasses are all statistically the same.

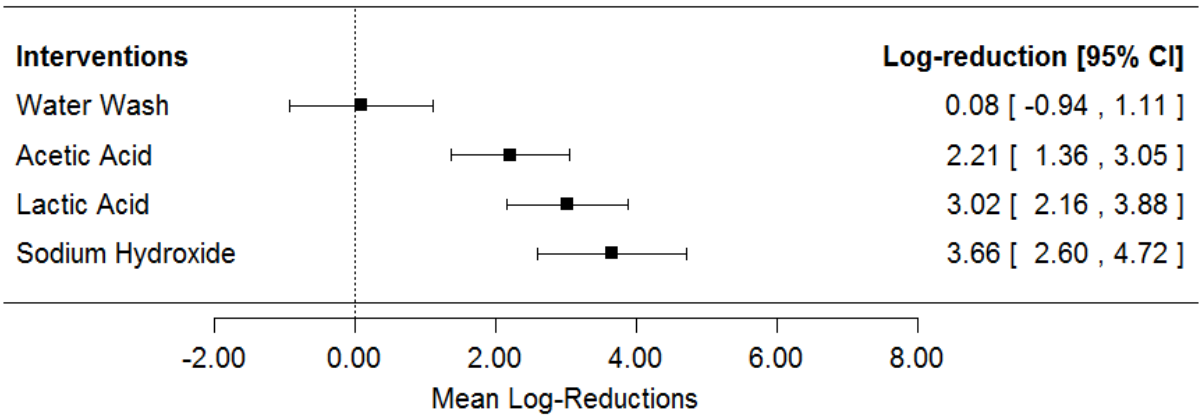

Figure 2. Least-Squares Means for Full-Trial Hide Model

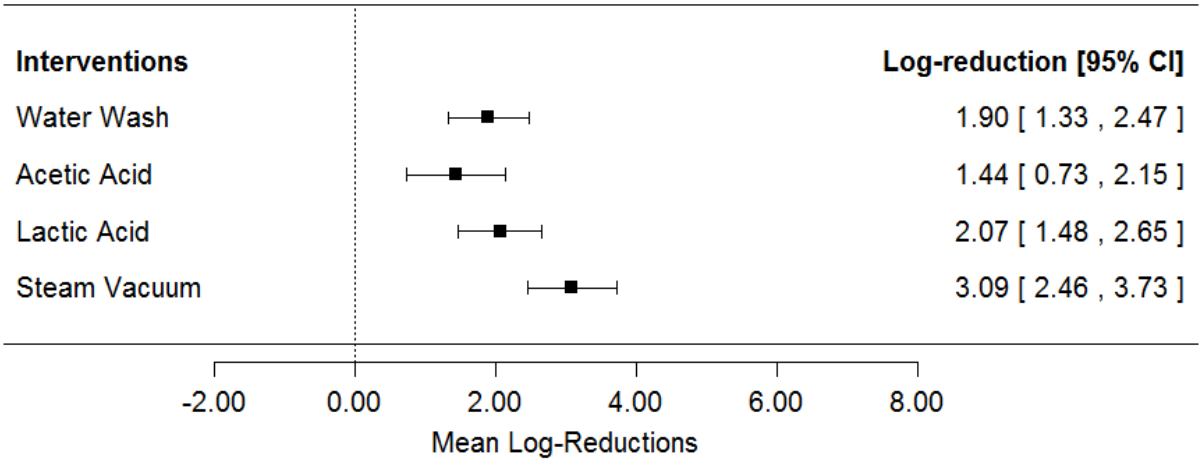

Figure 3. Least-Squares Means for Full-Trial Carcass Model

4. Discussion

4.1. Summary Effects

Caution should be taken when interpreting the summary effect results presented in Table 5 because of the high unexplained heterogeneity. The range of reported reductions vary substantially between trials, more than would be expected by sampling error alone (Borenstein et al., 2009; Higgins & Green, 2006). Therefore, the use of meta-regressions is appropriate to explain the variability between trials.

4.2. Meta-regressions: Explanatory Variables

Although heterogeneity remained high in some of the meta-regression models, the results clearly support that moderators are statistically significant predictors of intervention effectiveness. Initial microbial concentration and extra water wash were the most consistent predictors of log reduction, both in terms of being frequently statistically significant and

estimated impact in effectiveness. Antimicrobial concentration, sample method, inoculation type, organism type, duration of application, temperature, and surface type were also observed as important explanatory variables, but their impact was not as distinct and/or significant. Also, it is important to reiterate that all meta-regressions used baselines of 0 for any continuous variables as a default to calculate intercepts. However, for predictive purposes, it is not recommended to use values outside those observed for continuous covariates (e.g., setting temperature equal to 0^{o}C in a regression is not recommended for predictions).

Initial microbial concentration was arguably the most critical predictor of intervention effectiveness. Initial microbial concentrations were highly statistically significant ($p < 0.001$) predictors of intervention effectiveness in every meta-regression that tested initial concentration as a variable (Tables 6 and 7). These results show that interventions become less effective as concentrations decrease, even when excluding papers with major detection limit issues. Direct comparisons of similar trials with different initial concentrations in the literature show the same effect (Delmore, Sofos, Schmidt, & Smith, 1998; Youssef, Yang, Badoni, & Gill, 2012). As the top layers of contamination are washed away or disinfected, microorganisms may continue to survive in the microenvironments within the hide or subcutaneous tissue. This attribute of reduced effectiveness at low concentrations has critical implications for pathogenic contamination in plants. Specifically, food safety specialists are at risk of overestimating the effectiveness of interventions if they are using studies with moderate to high initial concentrations to estimate intervention effectiveness at plants. This can have negative effects on HACCP plans and plant sanitary conditions. For example, food safety managers deciding between implementing two or more sanitizers that were tested at different initial concentrations

may choose the less effective intervention if the impact of initial microbial concentration is not considered.

The significance of initial concentration as a critical predictor also has implications on the use of intervention data more broadly. Some epidemiologists argue that analysis of intervention effectiveness should be based on naturally contaminated studies in the field rather than the inoculated lab studies (Greig et al., 2012). The general sentiment is that the different environments produce systematically different results and therefore, it is often misleading to extrapolate findings from inoculated labs samples to the real world (Greig et al., 2012). The physiological state of cells also varies between inoculated samples versus naturally occurring samples so that results of inoculation studies cannot be easily extrapolated to naturally contaminated samples. However, if these differences can be adequately explained by the covariates of the experimental design, such as initial concentration, then it allows for a more effective use of a larger body of information in the realms of risk assessment and risk management.

While water washes before the main intervention often increased reductions, water rinses after antimicrobial treatments were linked to decreased reductions in the hide, sodium hydroxide, and acetic acid meta-regressions (Tables 6 and 7). This effect is likely the result of the water rinse removing or diluting the antimicrobial, leading to decreased application times, concentrations, and ultimately reductions (Carlson, Geornaras, Yoon, Scanga, Sofos, Smith, & Belk, 2008; Sapers, Miller, Jantschke, & Mattrazzo, 2000). For this reason, the same negative effect is not seen on the water wash and steam vacuum trials, where washing after improves reductions.

Following trends seen in previous research, temperature was statistically significant in the carcass and water wash regressions (Tables 6 and 7) (Anderson & Marshall, 1989; Fouladkhah, Geornaras, Yang, Belk, Nightingale, Woerner, Smith, & Sofos, 2012; Gorman, Sofos, Morgan, Schmidt, & Smith, 1995; Yoder et al., 2010). Temperature, however, was not significant in the other meta-regressions although this may be due to the limitations of the data available.

The antimicrobial concentration was largely seen as a non-significant factor in the meta-regressions. The sodium hydroxide data, composed of 11 trials, was the only set of information that showed a statistically significant effect from increased antimicrobial concentration (Table 6). Both the lactic acid and acetic acid data sets had sufficient ranges of applied concentrations, 2 to 10%, but failed to show them as statistically significant predictors (Tables 6 and 7). Although increases in antimicrobial concentrations are expected to increase reductions, previous research has shown that the effectiveness is not always significantly different (Anderson & Marshall, 1989; Heller, Scanga, Sofos, & Belk, 2007). It is also possible that the presence of an extra wash has an interaction with the antimicrobial concentration, making it difficult to assess the impact of both individually. As mentioned, residual water left on before or added after may change the actual antimicrobial concentration on the sample surface by diluting the sanitizer, leading to concentrations on the surface that are substantially different than those expected by the concentration reported in the original mixture (Carlson et al., 2008; Sapers et al., 2000).

The duration of application was the least reported covariate, and therefore, its impact is difficult to compare to the other covariates. The water wash trials, however, did have sufficient information on application times and estimated the reduction to be 0.013 log CFU/cm^2 per second (Table 7). Food safety specialists may be able to increase reductions by exploring increased application times.

Washing with water was shown to be less effective on hide than carcass samples, while washing with lactic acid was more effective on hide samples than carcass (Tables 6 and 7). This may be due to the means by which each intervention reduces bacteria. It is possible that the hair on hide samples makes removal by physical means more difficult, but antimicrobial effects of acids may actually benefit in the environments of hair samples. While no research directly comparing the same intervention on hide and carcass samples were available, comparison of clipped versus unclipped hides shows significant differences (Baird, Lucia, Acuff, Harris, & Savell, 2006). Reinforcing the idea that the surface type plays a major role, these results suggest plant operators may gain increased reductions if cattle are sprayed with acidic interventions before dehiding rather than solely after dehiding.

The inoculation variable was frequently significant, showing that there are statistical differences between reductions reported on artificially inoculated samples and naturally contaminated ones. Inoculated organisms were more easily removed from hide and carcass surfaces compared to naturally contaminated organisms. Specifically, researchers should be wary of overestimating the effectiveness of a particular intervention on naturally contaminated specimens if inoculated samples are used as a reference.

Information from the meta-regressions on organism type indicated that pathogenic strains of *E. coli* were less vulnerable to intervention than indicator organisms (Tables 6). Specifically, the carcass, acetic acid, and full-trial lactic acid meta-regressions suggest *E. coli* O157 and non-O157 STEC may be more resistant to intervention that generic *E. coli* or coliforms. As previous research has suggested, both non-O157 and O157 STEC were predicted to behave similarly (Fouladkhah et al., 2012). While some studies do show O157 being less responsive to certain interventions, the opposite effect, or negligible differences, have also been recorded (Castillo,

Lucia, Goodson, Savell, & Acuff, 1998a, 1998b; Ingham et al., 2010; Yoder, Henning, Mills, Doores, Ostiguy, & Cutter, 2012). Nevertheless, the ability of O157 to survive in low pH environments has been established in the literature (Byrne, O'Kiely, Bolton, Sheridan, McDowell, & Blair, 2002; Feng, 1995). Therefore, the current meta-regression results coupled with the findings in the literature should encourage food safety specialists to be wary of overestimating pathogenic reductions when using indicator organisms to track effectiveness for acidic interventions. In addition, other than for water wash, there were too few studies to evaluate the overall effect of different interventions applied sequentially, e.g., an acetic acid wash followed by a steam vacuum. Further research is needed in this area.

4.3. Random Effects

The moderators used in the meta-regressions explained some variation among trial results, reducing unexplained heterogeneity particularly for water wash and sodium hydroxide meta regressions. However, the variation attributed to residual error and between-papers remained moderate to high for some of the meta-regressions (Tables 6 and 7).

While variation between trials, studies, and due to residual error is expected, the estimates are likely artificially high for two reasons. First, without a sufficient number of trials, the models cannot accommodate all of the covariates that could explain heterogeneity and variation. The results have shown that many of the covariates tested are highly statistically significant. The data, however, are limited with many covariates highly correlated. For instance, sponge sampling was often done on naturally contaminated samples, which only measured coliform or generic *E. coli* levels, and never STEC concentrations. Testing all possible covariates becomes impossible and the result is unexplained heterogeneity.

Additionally, although incorporating trials with high levels of substitution was avoided to a large extent, many experiments did not report whether they used substitution methods. It is possible that fabricated results were incorporated into the meta-analysis if authors failed to mention the use of substitution. If true, the variation within-trials would decrease and the between-trial variation would increase, resulting in higher levels of heterogeneity.

4.4. Real World Applications

Several recommendations can be made following the meta-analysis results. First, on hide surfaces, water washes should be avoided, as they are largely ineffective and may contribute to diluting any antimicrobial applied. Instead, sodium hydroxide, or possibly lactic acid, should be used for hide decontamination (Figure 2). Although they were not statistically different in the least-squares means, lactic acid was predicted to be less effective on STEC in the full-trial lactic acid regression. Sodium hydroxide may be a more appropriate solution to STEC contamination, however the sodium hydroxide data were scarce and therefore its estimated effect should be used with caution.

For decontamination after dehiding, steam vacuum presented the largest reductions (Figure 3). Lactic acid is also an effective sanitizer on carcass surfaces and is recommended if steam vacuum is not available. While water washes are viable interventions on carcass surfaces, they should be implemented before any antimicrobial. Finally, further microbial reductions can be gained by increasing the application temperature of any water washes.

5. Conclusion

The meta-regressions revealed that initial microbial concentration, extra water washes, intervention type, surface type, inoculation type, temperature, duration, antimicrobial concentration, organism type, and sample method all had impacts on the intervention

effectiveness. The most compelling evidence was for initial microbial concentration and extra water washes as the constant and most impactful predictive variables across interventions. The steam vacuum was the most effective intervention on the carcass and sodium hydroxide was the most effective on the hide. High heterogeneity remained, but this may be due to data limitations and substitution issues. Overall, the models and covariates helped explained differences across study results, and these findings can be used by the industry and risk assessors to improve safety and sanitary conditions.

6. ACKNOWLEDGEMENTS

This material is based upon work that was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2012-68003-30155 (STEC CAP grant) and by Virginia Tech's "Water INTERface" Interdisciplinary Graduate Education Program.

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CHAPTER 9: Systematic Literature Review of Thermal and Cooking Time Inactivation of STEC O157:H7 Contamination in Beef Products

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ABSTRACT

There are no formal systematic literature review completed on published thermal death time calculation values (D- and z-values) in the beef industry. Linear regression on published D-values calculated a z-value at 5.48°C, which can then be used to determine D-values at any cooking temperature for beef products. By weighting each reported D-value at 60°C, the reported summary effect D-value was 2.70 min (95% CI: 2.47-2.93 at 60°C). Summary effect D-values can be determined at other cooking temperatures systematically using published D-values or through the calculated z-value reported in this study. Based on fat:lean percentages in beef products, normalized D-values at a reference 60°C temperature showed no statistical differences. In order to protect public health from foodborne illnesses due to STEC in beef, the results of this review can ensure proper handling and cooking temperatures for beef products.

1. INTRODUCTION

The STEC O157:H7 strain has been a known foodborne pathogen since 1982 (Luchansky, Porto-Fett, Shoyer, Phillips, Chen, Eblen, Cook, Mohr, Esteban, & Bauer, 2013), and there are an estimated 176,000 foodborne STEC infections per year in the U.S (Lund, 2015; Scallan, Hoekstra, Angulo, Tauxe, Widdowson, Roy, Jones, & Griffin, 2011). O157 has been regulated as an adulterant in ground beef and nonintact (beef with tenderization processing) beef since 1993 and 1999, respectively (Luchansky et al., 2013), but several studies found that 40-70% of O157 foodborne illnesses are attributed to beef and lamb products (Lund, 2015). O157 regulations in beef products should be reevaluated as 20% of the US population is more susceptible to foodborne infections than the general public (Lund & O'Brien, 2011). A study in *Foodnet* (CDC, 2016) found that the incidence rate of STEC infections in 2014 were much higher for children under the age of 5 and the elderly over 60 years versus healthy adults. It is not uncommon for persons of any age to become infected, but young children, the elderly, and immunocompromised individuals are the ones most susceptible to develop illnesses (CDC, 2016).

The U.S. Department of Agriculture (1998) estimates that 42% of beef consumed in the US is ground, 20% are steaks, and the remaining portion are processed or in cuts for stews and other beef dishes (Davis and Lin, 2005). The cut of beef plays a significant role in STEC contamination and resulting foodborne infections after cooking and consuming by the consumer. STEC foodborne illnesses are more commonly attributed to undercooked ground beef (Kassenborg, Hedberg, Hoekstra, Evans, Chin, Marcus, Vugia, Smith, Ahuja, Slutsker, & Griffin, 2004; Slutsker, Ries, Maloney, Wells, Greene, & Griffin, 1998; Taylor, Holt, Mahon, Ayers, Norton, & Gould, 2012) as there is a greater risk of pathogens spreading through the grinding process (Røssvoll, Sørheim, Heir, Møretrø, Olsen, & Langsrud, 2014). However,

contamination in other cuts, such as steaks and roasts, should not be ignored as pathogens are still present on the surface of the meats (Chancey, Brooks, Martin, Echeverry, Jackson, Thompson, & Brashears, 2013).

Furthermore, consumers should consider if their beef product is intact (not tenderized) vs. nonintact (blade or needle tenderized) as epidemiologic data do show that several O157 outbreaks are linked to undercooked nonintact beef consumptions (Porto-Fett, Shoyer, Thippareddi, & Luchansky, 2013). Beef can be mechanically tenderized with blade penetration or application of pressure to improve palatability and tenderness (Chancey et al., 2013). In a 2003 survey by National Cattlemen's Beef Association, mechanical tenderization is used by 95% of beef producers (Chancey et al., 2013; Luchansky, Phebus, Thippareddi, & Call, 2008); this process increases translocation of STEC on contaminated meat surfaces to inside of beef cuts (Chancey et al., 2013). STEC may not be initially found inside deep tissue of intact meat, however, any puncturing with a blade, fork, knife, injection needle or through any precooking tenderization process, can contaminate the interior of meat (Adler, Geornaras, Belk, Smith, & Sofos, 2012; Luchansky et al., 2008; Luchansky, Porto-Fett, Shoyer, Phebus, Thippareddi, & Call, 2009; Wiegand, Ingham, & Ingham, 2012). Even though most commercially purchased steaks are mechanically tenderized, there is a misconception among consumers and food service personnel that nonintact meat cuts are not contaminated as they appear intact (Adler et al., 2012; Sofos, 2008). Subsequently, nonintact, mechanically tenderized meat products are often cooked to rare or medium-rare temperatures, increasing the survival rate of internally transferred pathogens like *E. coli* (Adler et al., 2012; Sofos, 2008).

Despite the USDA recommended internal temperature of 71.1°C for cooking ground beef (Wiegand et al., 2012), many consumers prefer their beef hamburgers undercooked or with some

pink (Røssvoll et al., 2014). The regulations are even more lax for intact beef, where guidance for internal cooking temperature-hold time combinations are 54.4°C for 112 min and ≥70.0 °C for instantaneous preparation (Wiegand et al., 2012). Studies found that 25-53% consumers opt for beef, particularly steaks, to be cooked at an endpoint internal temperature of rare to medium rare (54.4-60°C) (Adler et al., 2012; Cox, Thompson, Cunial, Winter, & Gordon, 1997; Franken, 2005; Schmidt, Keene, & Lorenzen, 2002; U.S. Dept. of Agriculture Food Safety and Inspection Service, 2002). The fallacy that cooking kills all pathogenic bacteria is alarming as consumers often consume beef cooked to suboptimal temperatures for microbial destruction (Chancey et al., 2013).

Food health specialists and researchers encourage consumers to use thermometers as the only sure and effective method to determine pathogen reductions to safe levels when cooking beef products (Luchansky et al., 2013). However, over 80% of consumers reported not using a thermometer when cooking ground beef (American Meat Institute, 2010; Luchansky et al., 2013). Consumers and foodservice providers often use color as an indicator for beef doneness during cooking. Hunt et al.(1995) demonstrated that the "cooked beef color test" alone is unreliable; many beef patties may have the brown cooked color, but their internal temperatures can be as low as 55°C, which is far from the minimum 71.1°C. Franken et al. (2005) evaluated consumer perception of doneness of cooked beef: for steaks, 81% stated that they use color, 35% indicated cooking time, and 12% used thermometers; for roasts, 57% relied on cooking time, 36% used color, and 26% used thermometers. Yet, less than half of those who reported using thermometers for cooking steaks or roasts knew the minimal internal temperature to reach (Franken, 2005).

Cooking time and temperature are important factors in reducing STEC concentrations in beef products. To model the effectiveness of these factors on inactivation of O157 under different conditions, such as food cooking, researchers measure thermal death time calculations (thermal inactivation) in terms of Decimal reduction (D, equivalent to $\log_{10}$ removal) and z-values (Stringer, George, & Peck, 2000). The D-value reflects the heat resistance of a microorganism; in a given medium and given temperature, it is the time required for one log microbial reduction (Stringer et al., 2000). The z-value measures the temperature dependence of the microbial inactivation; it is the equivalent degree change for one log reduction in D-value (Stringer et al., 2000). The holding time at a particular temperature is set at time zero after come-up internal temperature is reached. These cooking temperature/time parameters are important as the D-value is the time calculated for one log microbial reduction after holding and cooking at a set temperature. An example cooking profile used to determine time zero for D-value calculations at a holding temperature of 71.1°C is shown in Figure 1, where come-up time is 8 min (time = 0 minute, starting time for holding) for a come-up temperature of 71.1°C (holding-temperature).

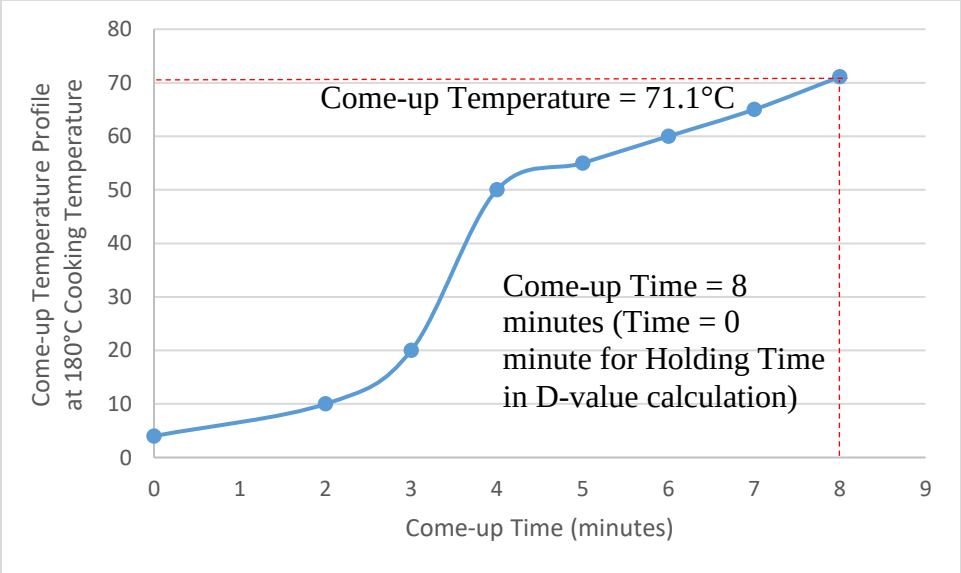

Figure 1. Example cooking come-up time and temperature for beef product refrigerated at 4°C then cooked at 180°C to internal temperature of 71.1°C.

Comparable tables do exist to show the thermal inactivation of STEC when cooking foods at different temperatures and time (Stringer et al., 2000), but the data are quite disjointed. Completed tables with D- and z-values for different cooking conditions and food matrixes would be beneficial to food service providers and the beef industry since most thermal inactivation studies and data for STEC O157 is very fragmented, with varying beef cuts, cooking times, temperatures, and methods to account for. Furthermore, with STEC contamination in beef being a major culprit in foodborne illnesses, there is a need to combine all known STEC thermal inactivation data and summarize microbial reductions in terms of cooking time and temperature dependency. Such information will lead to improved microbial concentration policy and regulation in the beef industry, as well as reducing potential STEC foodborne infection and improving public health.

The objectives of this review are to:

1. Conduct a systematic literature review for STEC O157:H7 thermal death time values (D- and z- values) in beef industry
2. Complete meta-analysis on published D- and z-values

2. METHODS

2.1 Literature Search and Screening

A systematic literature review process (Knobloch, Yoon, & Vogt, 2011; Moher, Liberati, Tetzlaff, & Altman, 2009) was followed to search for thermal inactivation studies involving O157 in beef published after 1982. Targeted databases included: Google Scholar, PubMed, Agricola, CAB, and Food Science and Technology Abstracts. The general terms format for each database search was:

(inactivation OR "thermal inactivation" OR time OR temperature OR thermal OR "die off" OR cooking) AND (beef OR hamburger OR steak OR roast OR meat) AND ("Escherichia coli" OR O157 OR EHEC OR "E. coli" OR STEC OR coliforms)

Abstracts and citation information was downloaded into the citation management software, EndNote. Once all abstracts were collected, a screening criteria following the Preferred Items for Systematic Reviews and Meta-analyses (PRISMA) similar to Figure 1 was used (Knobloch et al., 2011; Moher et al., 2009). Primary screening was broad, where titles and abstracts were checked for any significance to inactivation of O157 in beef products and duplicates were removed. The second round of screening was more rigorous; the papers must have had D- and z-values present or data that allowed for D- and z-value calculations. Such information included, but was not limited to cooking time, cooking temperature, and microbial concentrations and reductions. Visual data, such as graphs and tables, was the main source of data extracted from the articles.

Tracked organisms were STEC O157 and non-O157: O26, O45, O103, O111, O121, and O145, generic *E. coli*, and coliforms. These STEC serogroups were chosen because O157 is the pathogenic organism of interest and non-O157 organisms often co-exist with the former, while generic *E. coli* and coliforms are known surrogates (Ingham, Algino, Ingham, & Schell, 2010).

2.2 Data Extraction

The ultimate goal of this review was to evaluate cooking impact on STEC inactivation based on time and temperature. In addition to imperative cooking temperature and holding time, initial microbial concentration and reduction, and any D- and z-values data, other parameters

also play into the effectiveness of thermal microbial reductions. Table 1 summarizes data criteria extracted from each article that met the full analysis criteria after secondary screening.

Table 1. Summary of potential extracted items from each study

Type	Examples
Food group/beef cut	Hamburger, roast, prime rib, sausages, steak, meatballs, veal
Beef type	Beef, veal
Process type	Intact, non-intact, ground
Cooking temperature	°C
Cooking time	minutes
Cooking method	Grilling, baking, pan sear, pan broiled, double pan broiled
Cooking appliance	Conventional oven, Convection oven, toaster oven, George Foreman, slow cooker, oil skillet, gas grill, charcoal grill, deep fryer
Organism	*E. coli*, O157, non-O157 (O26, O45, O103, O111, O121, and O145), coliforms
Source of contaminant	Naturally contaminated, inoculated
Inoculated location	Surface, certain depth, geometric center
Initial STEC conc.	log CFU/g
State of product before cooking	Fresh, frozen (storage time), thawed
Thickness of cut	cm
Weight	grams
Coatings	Breading, wrapped (i.e., bacon), naked
Mean recovered	log CFU/g
± SD recovered	log CFU/g

2.3 Meta-Analysis

Systematic literature reviews are rigorous searches for peer review articles to extract data, and meta-analysis is the process of combining that data to estimate summary effects and explore factors that may affect the subject matter of interest. These are powerful tools used across many disciplines that combines data from individual studies to create a balanced, overarching picture of the issue at hand (Adolphus, 2014; Lean, Rabiee, Duffield, & Dohoo, 2009). Two independent researchers were involved in the selection of literature used in this paper. Statistical analyses that utilize first order kinetics and linear regressions were used to model D-value relationship with

different cooking temperatures and holding times. The D-values at different reference temperatures were depicted in forest plots to determine summary effect D-values at respective holding temperatures. R version 3.1.2 (R Core Team, 2014) was the statistical software used.

3. RESULTS AND DISCUSSION (TO DATE)

Systematic Literature Review

In this review, the systematic literature review process followed a standard protocol that examines STEC thermal inactivation data in an explicit and transparent manner. Much of this data was fragmented without a cohesive understanding of D- and z-values. These parameters are important to recognize in order to improve regulation and policy of beef cuts, processing steps, and cooking times and temperatures for retail/food service and consumer preparation (Stringer et al., 2000). There is a need for mean thermal death calculation values as studies report contradicting or inconsistent results. At 55°C and similar fat percentages, Byrne et al. (2002)'s reported D-value was 14.8 min, but for Juneja et al. (2003), the reported D-value was 21.0 min.

After an exhaustive literature search as shown in Figure 2, 123 papers were included for secondary screening and from that, 59 papers were excluded due to not being associated with beef, *E. coli*, and cooking temperature/time, or not enough data for thermal death time calculations. Twenty-seven papers had published D- and z-values, and 37 papers contained cooking time and temperature data.

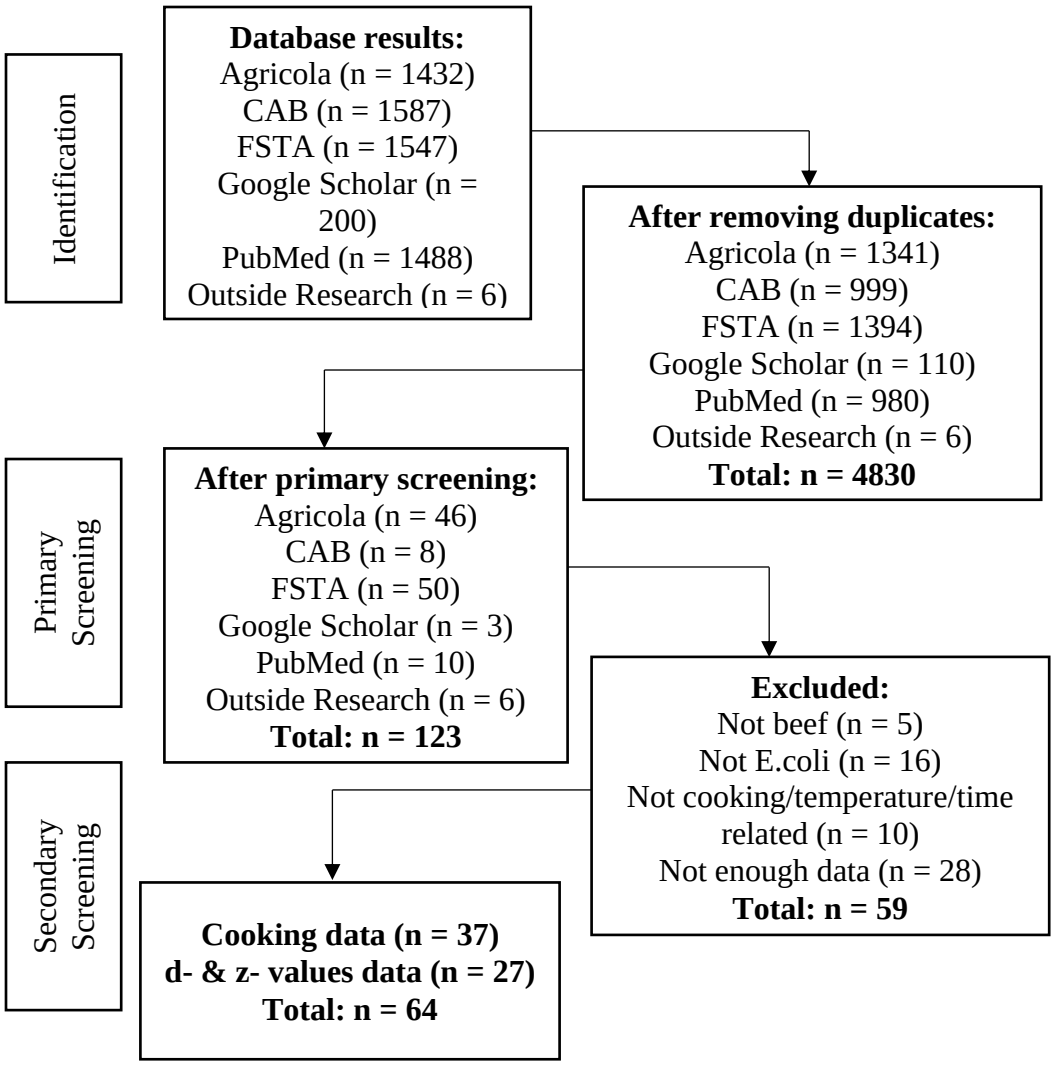

Figure 2. Identification and Screening of Systematic Literature Review Results

Linear Regression of Published D-values

Published decimal reduction values were directly extracted from the 27 final papers (with multiple trials within each paper) without normalizing for a cooking temperature reference point (Figure 3). The published D-values are log-normal and follow a linear relationship for multiple holding temperatures ranging from 50 to 71.1°C. The linear plot of log D-values versus temperature provides the inverse of the slope of the line for z-value = 5.48°C, which is the temperature required for a one-log reduction in the D-value. This calculated z-value can be used to determine D-values at any cooking temperature. D-values are lower for higher holding

cooking temperatures, which is expected, as microbial destruction increases at higher temperatures (Figure 3).

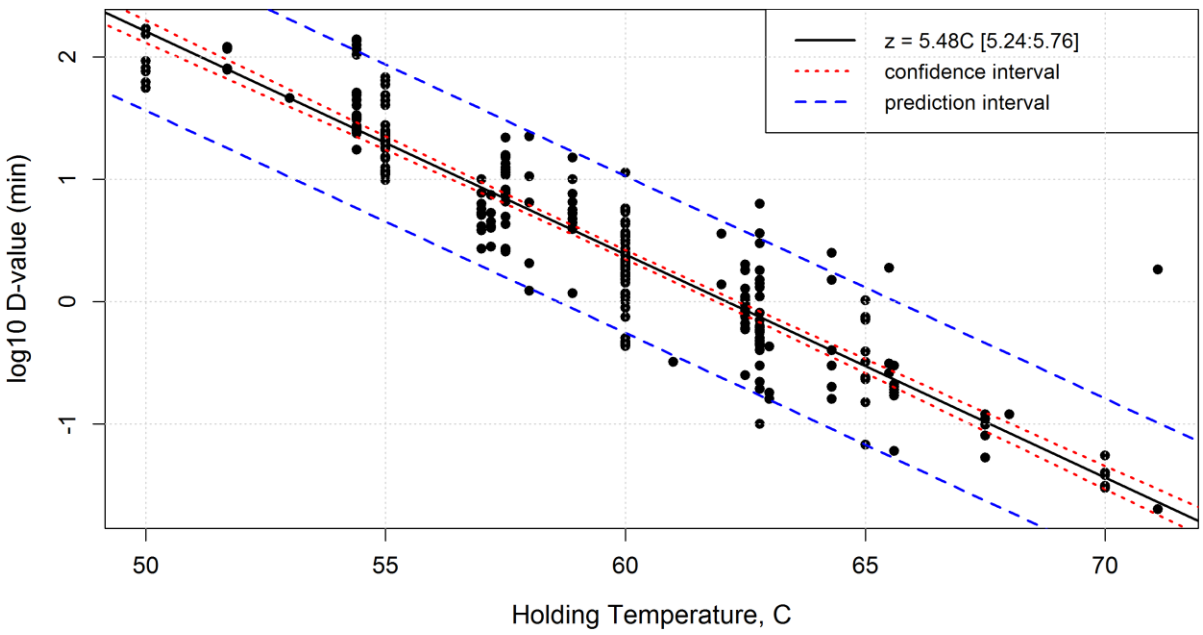

Figure 3. Published D-values at respective holding temperatures (n = 27)

The USDA recommends that the minimum cooking temperatures for ground beef and steak are when they reach a core temperature of 71.1°C (held for 0.25 min) and at a core temperature of 62.8°C (held for 0.25 min), respectively (Wiegand et al., 2012). Franken et al. (2005) found that most consumers prefer their hamburgers at medium temperatures and Adler et al. (2012) suggest that almost half of consumers prefer their steaks at rare to medium rare temperatures. An example cooking time and temperature is that steak doneness to rare to medium rare preferences should be removed from the grill at 54.4-57.2°C and 60°C, respectively, to reach 54.44-60°C and 62.78°C for final cooked temperatures (HEB, 2018).

Figure 3 shows that the minimum holding time necessary for a one-log reduction of O157 cooked to the medium rare temperature is greater than 2.10 min. For steaks cooked to medium to

well-done temperatures, they should be removed once internal temperatures are 68.33°C and 73.89°C, respectively, to reach 71.1°C and 76.68°C for final cooked temperatures (HEB, 2018). Figure 2 indicates that the holding time needed for one-log reduction of O157 for steaks cooked to medium preferences is 0.10 min, which is a 21-fold lower holding time compared to cooking to only 60°C.

Summary Effect D-value

Forests plots can also be depicted at different holding temperatures for the published D-values. Figure 4 is an example of a forest plot at reference temperature of 60°C; these values were not normalized using given z-values from their respective papers or from the calculated z-value in Figure 2. As shown in Figure 4, the published D-values a 60°C is very fragmented with values ranging from as low as 1.0 minutes to more than 11 minutes, which if modelled by microbial destruction properties, the D-values should theoretically all be the same. Thus, a summary effect D-value at 60°C is 2.70 min (95% CI: 2.47-2.93).

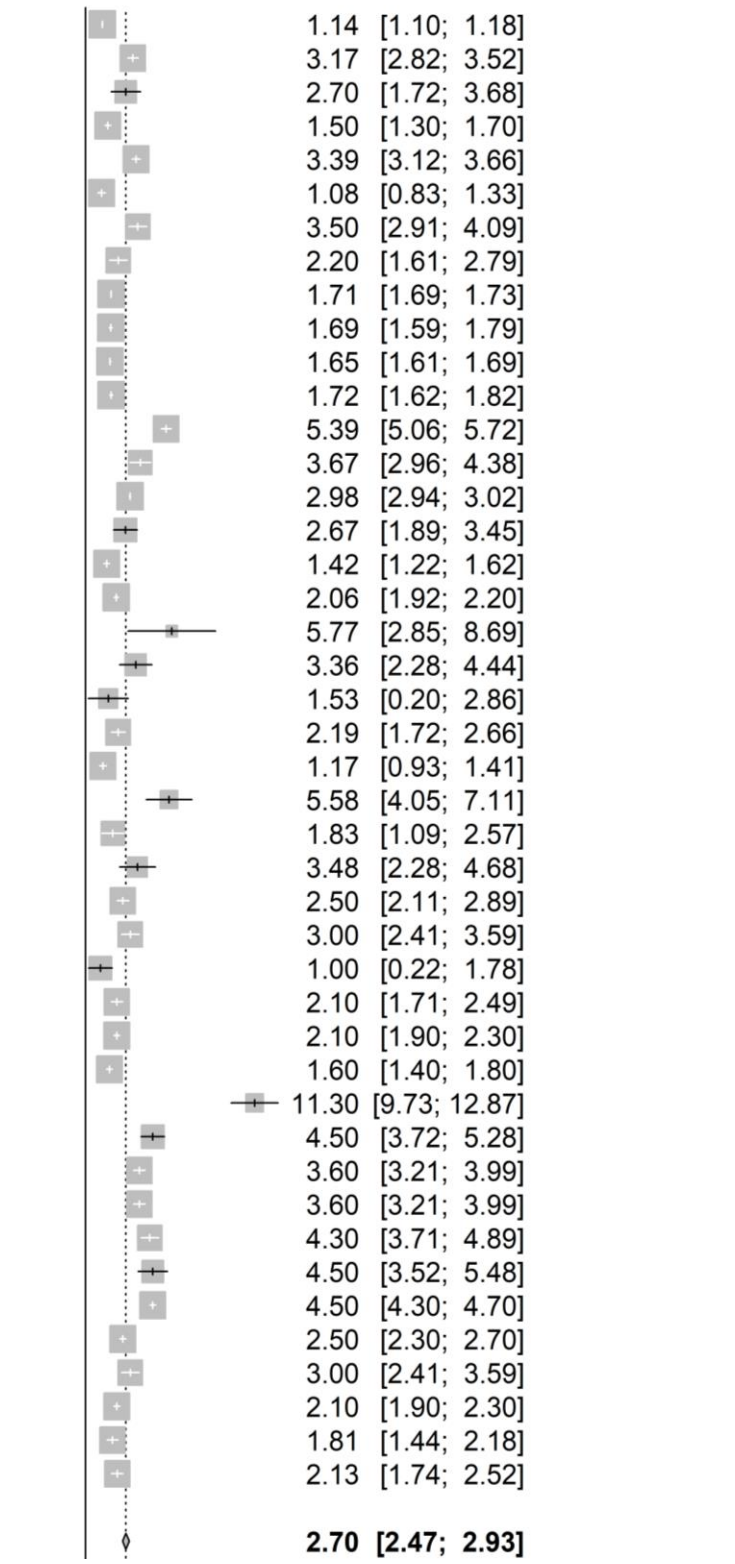

Figure 4. Forest plot of published D-values at 60°C (not temperature normalized)

Normalized D-values and Fat% Effects

There was a total of 172 data points that had published z-values within the 27 papers. With given z-values, D-values at reference temperature 60°C were calculated as shown in Figure 4. In order to be considered "lean", ground beef must have a lean point of 92% lean to 8% fat or higher. Only 34 studies/data points reported fat percentages, where 13 of those had an 8% fat level or lower and 21 had greater than 8% fat (Figure 5). It is known that fat percentages increase required cooking time as the fatty properties inhibits the inactivation of O157 due to the transfer of heat being delayed. The median D-value for fat percentages over 8% was 0.026 min compared to 0.005 min for the fat percentages below 8%; however, formal analysis showed no statistical difference among these data points

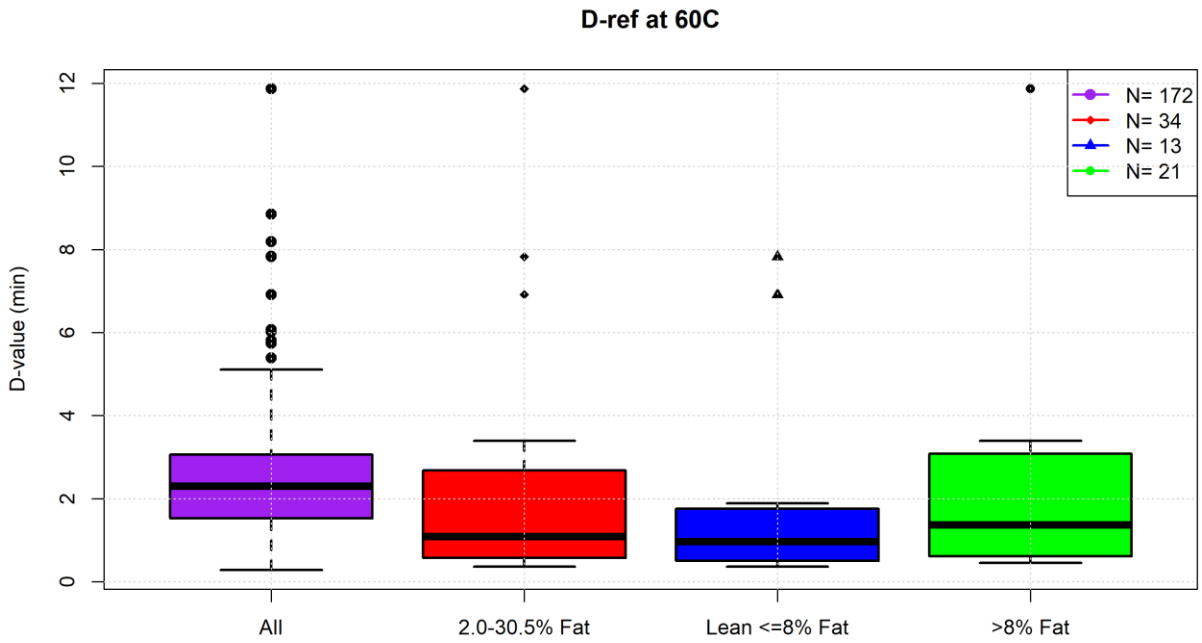

Figure 5. Box plot of normalized D-values at reference temperature 60°C and categorized by fat percentages

Taylor et al. (2012) survey study found that, on average, ground beef is consumed 1.7 times per week and steaks are consumed 1-3 times per month by those who eat beef (McCarty,

2013). Educating the public about potential health effects from consuming undercooked ground beef and tenderized steaks should be a priority. The use of a thermometer is necessary. With increased public awareness, there will be improved public health.

4. **CONCLUSIONS (Tentative)**

There are hundreds of STEC thermal inactivation studies in the literature yet no formal systematic literature review has been completed. This review serves as a stepping stone to ensure proper handling and cooking temperatures for beef products to protect the public from foodborne illnesses. A linear regression on published D-values calculated a z-value at 5.48°C, which can then be used to determine D-values at any cooking temperature. Furthermore, weighting each reported D-value at 60°C reported a summary effect D-value of 2.70 min (95% CI: 2.47-2.93 at 60°C). Summary effect D-values can be determined at other cooking temperatures. Using normalized D-values, no statistical differences were reported for D-values based on beef product fat:lean percentages. Beef industry policies and regulations should consider the results from this review and utilize D- and z-value table as a Hazard Analysis and Critical Control Points tool.

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CHAPTER 10: Population Susceptibility Effects on Dose-Response Modeling of STEC O157:H7 Outbreak Data

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ABSTRACT

The Shiga toxin-producing *E. coli* (STEC) O157:H7 strain is frequently associated with foodborne outbreaks. Young children, the elderly, and immunocompromised individuals are most susceptible to develop illnesses, yet it is not uncommon for persons of any age to become infected. To support quantitative risk assessment of this pathogen, a meta-analytical dose-response model was developed to summarize the relationship between numbers of ingested STEC O157 cells and the probability of someone falling ill as a result. After an exhaustive literature search, 12 STEC observations from eight outbreak studies were fitted to a logistic regression dose-response model. Variables such as population type (healthy adult and susceptible), matrix (environment, produce, and meat), dose, number of exposed persons, and number of ill cases were extracted from outbreak studies. The logistic regression model with random effects placed on matrix was fitted to assess the effect of population susceptibility, matrix, and dose. The model demonstrated that population susceptibility ($p<0.001$) had significant impact on probability of illness. The median effective dose (ED_{50}) for the susceptible population was 3.61 $\log_{10}$ CFU compared to ED_{50} of 4.19 $\log_{10}$ CFU for the healthy adult population. The matrix analysis indicated that for equivalent doses, meat exposures were more risky than produce exposures, which were more risky than environmental exposures. Understanding the dose-response relationship for this pathogen will promote increased food safety and as a result, can reduce the number of foodborne illnesses.

KEYWORDS: beef, dose-response, foodborne outbreaks, matrix effects, STEC, *E. coli*, O157, meta-analysis, susceptibility

INTRODUCTION

Shiga-toxin producing *E. coli* (STEC) is commonly associated with foodborne outbreaks and as a result, is highly studied during foodborne outbreak investigations; the STEC O157:H7 strain was identified as a pathogen in 1982 (CDC, 2015a). Non-O157 serogroups are not as fully studied, but they can still cause severe illnesses nonetheless (CDC, 2015a). There are an estimated 176,000 foodborne STEC infections per year in the U.S, where approximately 36% of these infections are due to O157, and the remainder are caused by non-O157 (CDC, 2015a; Scallan et al., 2011). In a study by Scallan et al. (2011), both O157 and non-O157 serogroups are reported as major contributors to foodborne outbreaks as they are ranked among common pathogens, such as Norovirus and Campylobacter, that cause illnesses.

In the early 1990s, there was a foodborne outbreak at a major fast food chain, where over 600 people became ill from consuming beef hamburgers infected by O157 (Frame, 2013). This outbreak was the momentum behind the USDA-FSIS declaring O157 as an adulterant in ground beef in 1994 (Frame, 2013). However, it was not until over a decade later in 2005, that O157 was regulated in beef trimmings and other non-intact beef products (Frame, 2013). Additionally, it was not until 2012 that non-O157 serotypes, including O26, O45, O103, O111, O121, and O145, were declared adulterants in beef products (Frame, 2013).

Studies estimate that 40-70% of O157 foodborne illnesses are attributed to beef and lamb products, and that 10-30% of O157 foodborne illnesses are caused by contaminated produce (Tam et al., 2014; Lund, 2015). Both O157 and non-O157 from cattle are major contributors to foodborne illnesses in the U.S, but unfortunately, the literature lacks models for hazard characterization of STEC post-processing in the beef industry (USDA-FSIS, 2001b, 2012). Hazard characterization is important as 20% of the U.S. population is more susceptible to foodborne diseases than the general public (Lund & O'Brien, 2011). It is not uncommon for

persons of any age to become infected, however, young children, the elderly, and immunocompromised individuals are the ones most susceptible to develop foodborne illnesses (CDC, 2015a; Lund & O'Brien, 2011; Nwachuku & Gerba, 2004). A study in *Foodnet* (CDC, 2016) found that the incidence rate of STEC infections in 2014 were much higher for children under the age of 5 and the elderly over 60 years versus healthy adults. By understanding the hazard characterization of this pathogen in beef products, which entails exposure assessment and particularly, the dose-response relationship, risk characterization and assessment of STEC can be utilized to improve public health and safety (Nwachuku & Gerba 2004).

When humans exposed to STEC become ill, the dose-response is defined as "the relationship between the dose received and resulting health effects" (George, Divya, & Suriyanarayanan, 2013). Evaluating a dose-response involves mathematically modeling the dose and the probability of the exposed population becoming ill by using both available outbreak and experimental data to fit the model. Dose-response models can aid in the quantitative microbial risk assessment (QMRA) of the O157 strain by predicting the resulting human health impact from exposure to this pathogen (George et al., 2013). Once a dose-response relationship for O157 is estimated, risk assessors and public health officials can suggest regulations on how to improve public health and decrease the number of people getting infected.

ED_{50} is the dose in which there is a 50% probability a person will become infected if consuming a contaminated product at that dose (Bailer & Piegorsch, 1997). A total of eight historical O157 dose-response articles from 1996-2008 with reported median effective doses (ED_{50}) associated with different matrices are shown in Table 1. At this point, more recent studies were not found. Unfortunately, there is little consistency among the studies with median effective doses ranging from as low as 2.3 log CFU to as high at 6.5 log CFU. Furthermore, no

studies have completed a meta-analytical evaluation of population susceptibility and effects of matrix on dose-response.

Table 1. Historical STEC O157 Median Effective Dose

Year	Paper	Host	Pathogenic Used Organisms in Model	Model	ED_{50} (log_{10} CFU)
1996	Crockett et al.	Humans	*Shigella*	Beta-Poisson	3.05
1998	Cassin et al.	Humans	*Shigella*	Beta-Binomial	3.49
2000	Powell et al.	Humans	EPEC, *Shigella*	Beta-Poisson	5.29
2000	Haas et al.	Rabbits	O157	Beta-Poisson	5.77
2001	Strachan et al.	Humans	O157	Beta-Poisson	5.73
2004	Ebel et al./USDA-FSIS, 2001	Humans	O157, *Shigella*, EPEC	Beta-Poisson	6.53
2004	Teunis et al.	Humans	O157	Beta-Poisson	6.07
2008	Teunis et al.	Humans	O157 (meta-analysis)	Beta-Poisson	2.33

Further complicating the dose-response modeling issue is that current data estimating minimum effective dose, which is the lowest threshold dose that a person will become ill, is either inconsistent or there is a lack of data. For O157, the range is as low as 10 CFU to as high as 700 CFU, and most non-O157 serogroups have no data available (USDA-FSIS, 2012). For this reason, the meta-analysis dose-response model in this paper focuses only on the O157 serogroup.

The overall goal of this review is to conduct a meta-analysis that accounts for population susceptibility and matrix to evaluate the relationship between the dose of ingested O157 and the probability of someone falling ill as a result. The initial task is to complete a systematic literature search through major journal databases for STEC, particularly the O157 serogroup, concerning foodborne and environmental-related outbreaks and historical dose-response models.

The objectives of this research:

1) To support quantitative risk assessment of STEC O157 by developing a meta-analytical dose-response model
2) To better understand population susceptibility and matrix effects on the dose-response relationship of STEC O157

METHODS

2.1 Systematic Literature Search

This Review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, 2009). Database searches included: Web of Science, AGRICOLA: Proquest, Cabdirect, and PubMed. Search terms used were:

("dose response" OR "foodborne pathogen" OR "foodborne illness" OR "minimum effective dose" OR "median effective dose" OR dose) AND/OR (beef OR hamburger OR steak OR roast OR meat) AND/OR (susceptible OR children OR elderly OR immunocompromised) AND ("Escherichia coli" OR O157 OR EHEC OR "E. coli" OR STEC OR coliforms)

Where available, filters were applied, i.e. 1) Publication type: Books and Documents, Peer Reviewed Journal Article, Case Study, Meta-Analysis, Review, Systematic Reviews, Reports, Data Study, Abstracts; 2) Date: 1982 to recent; and 3) Language: English. Additional references were added from "related citations" and "cited by" functions in the databases and duplicates were removed. The literature search was concluded in December 2017.

A total of 76,580 references were initially retrieved from the databases, but after filters/limits use, duplicate removal, and related citations additions, 376 were imported into the reference management software EndNote X7 for Windows (Figure 1) for the abstracts and titles

screening stage. Eighty-three external articles from previous STEC work were also included for full review. The final count eligible for full screening after abstract and title screening was 303. These references were eligible for the review if it discussed STEC dose-response models or contained any STEC outbreaks. After the final screening and eligible selection stages, 14 articles were used in the historical dose-response model discussion and eight were used for STEC foodborne outbreaks. The final articles selected were reviewed by a second researcher.

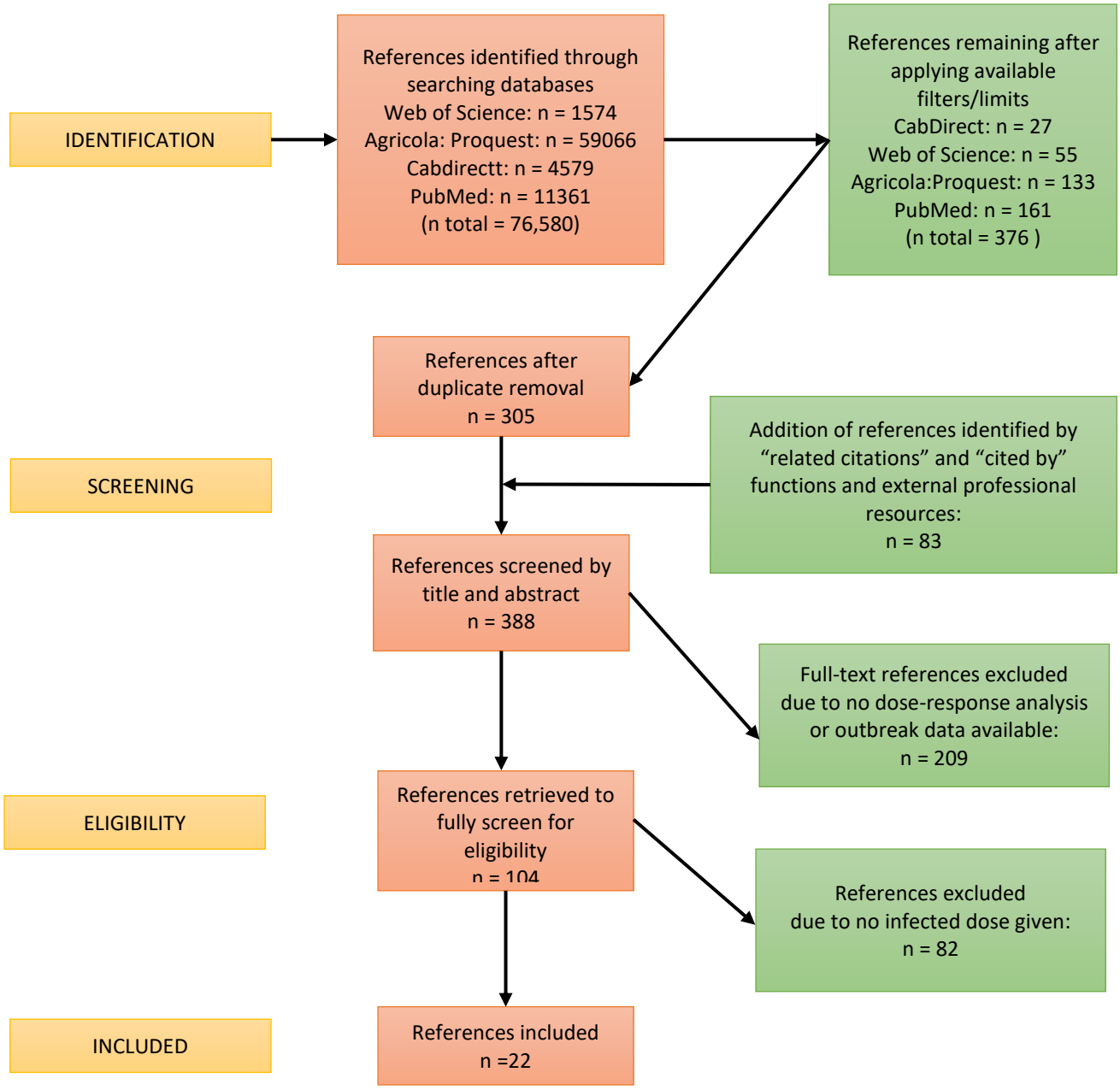

Figure 1. Map of Systematic Literature Search Process

2.2 Data Extraction

Reported O157 foodborne outbreak data were collected. Variables such as population type (healthy adult, children, and elderly), matrix (categorized as environment, produce, and meat), mean dose, exposed population, and number of ill cases were extracted from outbreak

studies as shown in Table 2. If outbreak studies did not contain data to evaluate all items in the "Parameters" column of Table 2, it was not included in the meta-analysis.

Table 2. Summary of extracted items required from each study

Parameters	**Examples**
Organism	*E. coli*, O157, non-O157 (O26, O45, O103, O111, O121, and O145), coliforms
Dose-response model	Logistic regression, beta-Poisson, beta-binomial, Weibull-gamma, exponential, Gompertz
Matrix	Meat, produce, environment
Location	City, State, Country
Population	Children <5 yr, adult, elderly >65 yr
Dose	log CFU
# of exposed persons	Numeric count
# of ill	Numeric count

2.3 Dose-response Modeling

The dose-response relationship of outbreak data was modeled using the linearized form of the logistic regression. The linearized logistic regression was selected because it is able to model the dichotomous data, e.g., the subject was sick or not sick, while also accounting for fixed and random effect covariates to assess the impact of dose, susceptibility, and matrix. Commonly used in epidemiology for these reasons (Chatterjee and Hadi, 2006), the logit transformed logistic regression is expressed below in Equation 1:

$$\ln \frac{P_{ill}}{1-P_{ill}} = \beta_0 + \beta_1 * Susceptibility + \beta_2 * Dose + \eta_{f1} + \beta_2 * \text{Dose} * \eta_{f2} + \varepsilon_{random}$$

(Equation 1)

P_{ill} is the probability of illness; β_0 is the intercept; β_1 is the effect of being in the susceptible populations; *susceptibility* is an indicator variable which is either 1 for the susceptible population and 0 otherwise; β_2 is the effect of dose; *dose* is the amount of reported pathogen ingested in log CFU; η_{f1} represents the random effect of matrix on the intercept, modeled as $N \sim (0, \sigma_{f1})$, where σ_{f1} is estimated from the model given that the outbreaks were associated with produce, meat, or

through the environment; η_{f2} represents the random effect of matrix on the dose slope, modeled as N ~ (0, σ_{f2}), where σ_{f2} is estimated from the model given that the outbreaks were associated with produce, meat, or through the environment; and ε_{random} is the residual error term.

Matrix as a random effect allowed the model to account for the variability introduced by contaminated source. Since each matrix was expected to impact the probability of illness, it was modeled to impact both the intercept and slope. From a conceptual framework, $\beta_1 * Susceptibility$ assesses the difference in response between the susceptible and non-susceptible populations, $\beta_2 * Dose$ assess the increased odds of becoming infected with increased dose, and $\eta_{f1} + \beta_2 * Dose * \eta_{f2}$ captures the effect of different matrix on the dose-response model.

2.4 Statistical Analysis

The "lme4" package v1.1-10 in R v3.1.2 (R Core Team, 2014) was the statistical software used to model dose-response of the outbreak data. A logistic regression model with random effects placed on matrix (environment, produce, and meat) was fitted to assess the effect of population susceptibility, matrix, and dose using the *glmer* function.

RESULTS AND DISCUSSION

3.1 Outbreak Data

After an exhaustive literature search, 12 STEC O157 observations from 8 outbreak studies were recorded in Table 3 with details below:

Outbreak #1: MMWR 1995: In 1995, there was an *E. coli* O157 outbreak associated with ingestion of contaminated water at a lake swimming beach in Illinois. Twelve cases of illnesses were identified among 2350 exposed persons. The estimated dose was 75 CFU (Strachan et al. 2005). No conclusive detail was given for the ages of infected persons.

Outbreak #2: Strachan et al. 2001: In 2000, twenty people out of 228 attendees at the New Deer agricultural camp ground in the UK were infected with *E. coli* O157. The age range was 8 to 20 years old. The estimated ingested dose was between 4 to 24 organisms. The source was through contaminated soil/mud from a field recently grazed by sheep.

Outbreak #3: Swerdlow et al. 1992: Between December 1989 and January 1990, residents in Cabool, Missouri were exposed to water contaminated with *E. coli* O157. Eighty-eight out of 1465 adults, 12 out of 164 children under 5 years old, and 38 out of 461 elderly older than 64 years became ill as a result. The estimate dose was 10-100 cells/L of water. With an illness onset at day 7 and an estimated water consumption rate of 3.7, 1.3, and 3.3 L/day for adults, children, and the elderly (Sebastian et al. 2011), respectively, the estimated consumed dose was 86, 59, and 54 organisms.

Outbreak #4: Nauta et al. (Shinagawa et al. 1997): In 1996, *E. coli* O157 contamination of pumpkin salad and seafood sauce during preparation caused illness in 322 among 828 exposed children and 11 out of 43 exposed adults after a school lunch period in Morioka, Japan. Based on average food portions, the estimated consumed dose for children was 31 CFU and 35 CFU for adults. The exact source for *E. coli* O157 was never identified.

Outbreak #5: Uchimura et al. 1997: In 1997, *E. coli* O157 contaminated melon were exposed to 71 people at a daycare center in Kashiwa, Japan. With an estimation of 1 caregiver per 6-7 children (http://factsanddetails.com/japan/cat23/sub150/entry-2797.html), 9 adults and 62 children were assumed. An estimated 4 out of 9 adults and 28 out of 62 children became ill.

Outbreak 6: Keene et al. 1997: In 1995, 10 out of 11 people in Oregon developed gastroenteritis after consuming homemade venison jerky contaminated with *E. coli* O157. The estimated dose was 10,000 CFU when the average person consumed 200 g of the jerky. No

specific age range was given except one of the exposed persons was an infant who became ill through person-to-person contact, thus, this subject was not included in the total number of 10 persons becoming ill.

Outbreak #7: Tilden et al. 1996: In 1994, 17 cases of *E. coli* O157 infections out of 2778 exposed people to pre-sliced, dry, fermented salami were reported in California/Washington. Tilden et al. (1996) assumed 50 g portions that yielded an estimated 2778 exposed persons. The concentration of *E. coli* O157 was 0.3-0.4 CFU/g of salami. Four of the infected case subjects consumed 6-113 g of salami, equating to an estimated *E. coli* O157 consumption of 2-45 CFU, with the average dose at 23 CFU.

Outbreak #8: National Academy of Sciences 2002: From November 1992 to February 1993, an estimated 5634 hamburger patties from a major fast food restaurant in Washington were undercooked and contaminated with *E. coli* O157. There were 398 primary cases (Bell et al 1994) of illness as a result. Seventy-six of the remaining patties' *E. coli* O157 concentrations after undercooking ranged from <0.3 to 15 CFU/g. With each patty weighing 45g, the number of organisms per patty was <13.5 to 675, but the median was 67.5 CFU/patty. Powell's model (2000) estimated that the median was 23 CFU. USDA-FSIS (2002) estimated 183 CFU.

Table 3. Historic STEC O157 Outbreak Data

Outbreak #	Organism	Matrix	Susceptibility	Dose (CFU)	# Ill	# Exposed
1	O157	environment	healthy adults	75	12	2350
2	O157	environment	healthy adults	14	20	228
3	O157	environment	healthy adults	1166	88	1465
3	O157	environment	children	410	12	164
3	O157	environment	elderly	1040	38	461
4	O157	produce	children	31	322	828
4	O157	produce	healthy adults	35	11	43
5	O157	produce	children	1100	28	62
5	O157	produce	healthy adults	1100	4	9

6	O157	meat	healthy adults	10000	11	12
7	O157	meat	healthy adults	23	17	2778
8	O157	meat	healthy adults	23	398	5634

As with existing dose-response models, a completed record of foodborne outbreak data is limited in our model. Thus, it is important for risk assessors to meticulously track future foodborne outbreaks in order to refine dose-response models and determine representative median effective doses among different population groups. As empirical data shows, children, the elderly, and immunocompromised individuals are more susceptible than healthy adults (CDC 2016).

3.2 Fitted Model Results

The results of the fitted logit transformed regression model from Equation 1 is shown in Table 4. The fixed effects parameters include β_0, β_1, and β_2. The random effects parameters based on food matrix is accounted for as $\eta_{f1} + \beta_2 * Dose * \eta_{f2}$.

Table 4. Logit transformed fitted dose-response model results

Parameter	**Definition**	**Fitted Model Results**
β_0	Intercept	-4.2121
β_1	Effect of being in the susceptible populations	0.5861
Susceptibility	Indicator variable	1 for susceptible population; 0 for healthy adults
β_2	Effect of dose	1.0045
Dose	Amount of reported pathogen ingested in log CFU	Food Matrix Modeled as Random Effect: Healthy adults: ED_{50} = 4.20 $\log_{10}$ CFU Susceptible: ED_{50} = 3.61 $\log_{10}$ CFU Environment: Healthy adults: ED_{50} = 6.17 $\log_{10}$ CFU Susceptible: ED_{50} = 5.54 $\log_{10}$ CFU Meat: Healthy adults: ED_{50} = 3.02 $\log_{10}$ CFU Susceptible: ED_{50} = 2.69 $\log_{10}$ CFU

		Produce: Healthy adults: ED_{50} = 5.84 $\log_{10}$ CFU Susceptible: ED_{50} = 3.41 $\log_{10}$ CFU
η_{f1}	Random effect of matrix on the intercept	Environment = -1.540848 Meat = -1.170707 Produce = 2.807104
η_{f2}	Random effect of matrix on the dose slope	Environment = -0.07192501 Meat = 0.77645994 Produce = -0.76406932

3.3 Dose Response: Susceptible vs. Healthy Adults

Age plays a major role in susceptibility (Lund & O'Brien, 2011). In this study, the susceptible population is defined at children under five (due to limitations from outbreak studies defining children as under 5 years old) and the elderly over 65 years old (due to limitations from outbreak studies listing elderly as 65+ years old). The second population subgroup was healthy adults. Figure 2 depicts the logistic dose-response model of O157 outbreak studies from Table 3. The points are relative to each outbreak and only included to convey the number of exposed persons (n_{max} = 5634) by increasing size, with the black points as healthy adults and the red points as the susceptible population. Similarly, the black line is the dose-response for healthy adults and the red line is the dose-response for the susceptible population. The dose-response for the susceptible population is shifted to the left compared to the healthy adult population. As expected, this logistic regression model depicts a statistically different and lower dose-response (more sensitive) for the susceptible population versus the healthy adult population ($p < 0.001$). The dose-response model demonstrates that population susceptibility had significant impact on probability of illness. The median effective doses from this study's meta-analysis are within the range of historical dose-response model data (Table 1). However, it is important to note that there is almost a one-half log CFU lower dose-response for the susceptible population (ED_{50} =

3.61 $\log_{10}$ CFU) compared to the effective dose for healthy adults (ED_{50} = 4.20 $\log_{10}$ CFU). The susceptible population's ED_{50} is 3.8 times lower than the ED_{50} for healthy adults.

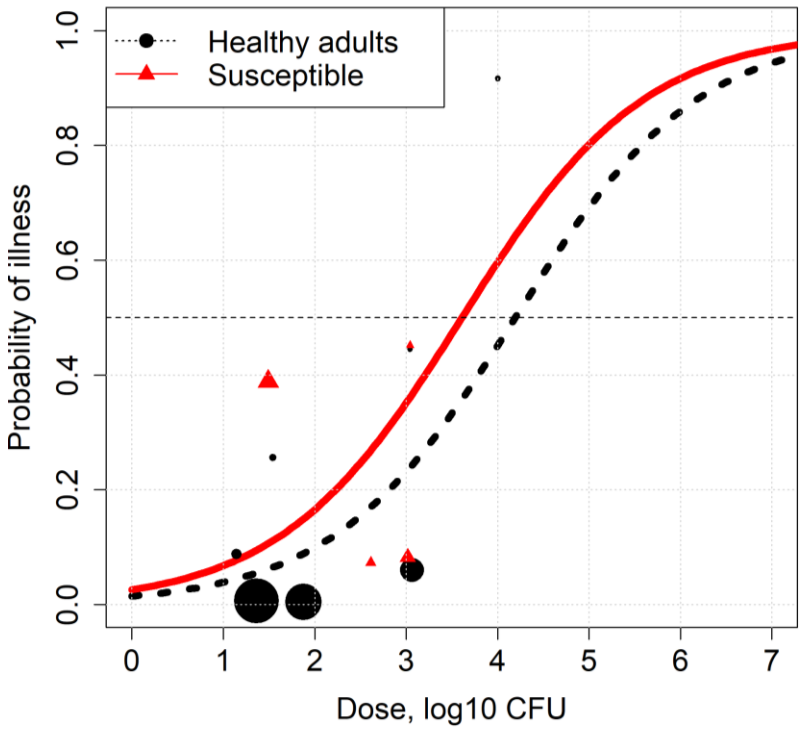

Figure 2. Logistic regression dose-response model fitted to O157 outbreaks data from Table 3

It is expected that young children are highly susceptible because their immunological, neurological, and digestive systems are still developing (Nwachuku & Gerba, 2004). A study published in *Foodnet* (CDC, 2016) reveals that children under the age of 5 have the highest incidence rate of STEC infections as shown in Figure 3. The data reveals an incidence rate ratio for children age <5 to adults age 30-39 as 5:1 (CDC, 2016). Furthermore, the immune system diminishes with age, resulting in increased susceptibility to foodborne illnesses in the elderly (McBride et al., 2012; Smith, 1998; Aw et al., 2007; Aspinall et al., 2010; Lund & O'Brien, 2011).

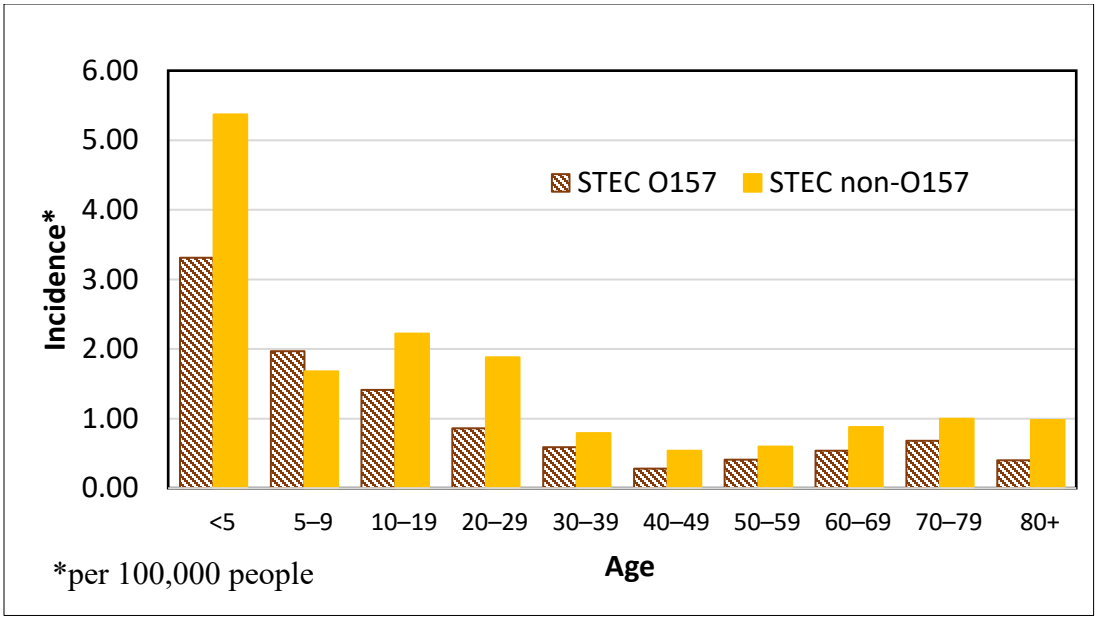

Figure 3. Incidence rate of STEC infections by age group; data adapted from (CDC, 2016)

Other studies have considered age as a factor in their dose-response model but reported different values and criteria. McBride et al. (2012) utilized a comparative risk assessment model to evaluate population susceptibility overall to common foodborne pathogens on the basis of age only. They reported that the highest relative risk is associated with young children with a relative risk of 1, where the relative risk is 0.7 for the general population, and 0.1 for predominantly adults (McBride et al. 2012). Consistent among CDC studies, Ebel et al. (2004) found that the probability of illness for children ages 0-5 years is 2.5 times higher than for the healthy adult population for exposure to O157. Lammerding (1999) also found that the probability of illness for STEC producing *E. Coli* was 6.4×10^{-4} per serving for adults and slightly lower at 4.6×10^{-4} for a child <5 years old. Additionally, the susceptibility of children is not contained and reported only within the United States. For reported O157 infections in England, children in the 1-4 years old age group have consistently been the highest reported group (Palmer & Parry, 2002).

In combination with the immune systems of the elderly deteriorating, this population subgroup is also affected by chronic ailments further increasing their susceptibility to foodborne

pathogens (CAST, 1994; Lund & O'Brien, 2011; Smith, 1998; Aw *et al*., 2007; Aspinall *et al*., 2010). The elderly is the most likely subgroup to die after an O157 infection (Lund & O'Brien 2011; Gould et al., 2009). In fact, the case fatality in nursing homes for bacterial gastroenteritis outbreaks is 10 times higher than the general population; the case fatality rate is 11.8 for O157 outbreaks versus 0.2 for the general population (Gerba et al., 1996). Even though only one outbreak data for immunocompromised individuals was reported in Table 3, it is important not to ignore this population subgroup in microbial risk assessment of foodborne pathogens. This subgroup has an increased risk for foodborne infections because their immune systems are inadequate to fight off illnesses. As Gerba et al. (1996) stated, "Enteric pathogens are among the many agents that take advantage of their impaired or destroyed immune system to set up persistent and generalized infections in the immunocompromised host." As a result, this vulnerability leads to a lower dose of foodborne pathogens needed to cause infections and increase severity of illness (Lund & O'Brien, 2011).

Population susceptibility, with respect to host age and health status, must be recognized when evaluating virulent pathogens. Evaluations of infectious doses that are reported in clinical trials mostly used healthy adult volunteers, not accounting for preeminent risk factors faced by children, the elderly, and immunocompromised individuals (McBride et al., 2012; USEPA 2000b; Nwachuku & Gerba 2004; Wade 2008). The awareness of increased risks for susceptible populations must always translate into the work of risk assessors and public health officials. Studies on listeriosis, which is the disease caused by consuming *Listeria Monocytogenes* contaminated products, have made superior efforts in recognizing the susceptibility of different subpopulations by reporting ranges over 1000-fold difference between the healthy adult population versus immunocompromised groups (McBride et al. 2012; Marchetti, 1996). As

stressed by Lund & O'Brien (2009), "Suppliers of food, including water and beverages, to hospitals, nursing homes, elderly-care homes, schools, and day-care centers for children aged nine or less, and to vulnerable people in the community should have in place a food safety management system based on Hazard Analysis Critical Control Point principles." Furthermore, the Food and Drug Administration does have guidelines for food suppliers serving highly susceptible populations (FDA. 2009), but these guidelines should be clear and continually updated with newly available research.

3.4 Dose Response: Matrix Effects

The dose-response models represented in Figure 4 demonstrate that food matrix as a fixed effect does have an impact on probability of illness. The median effective doses for the susceptible population are 5.54, 3.41, and 2.69 $\log_{10}$ CFU, and the median effective doses for healthy adults are 6.17, 5.84, and 3.02, respectively, for environmental, produce, and meat matrices. Probability of illness is higher for consumption of the same dose in meat products over produce, which are both higher than the probability of illness for environmental exposures; however, it is important to recognize the limitation of available outbreak data. The environmental exposures included soil contamination and ingestion of contaminated water during recreational uses, where the risk of becoming ill is lower than direct consumption through foods.

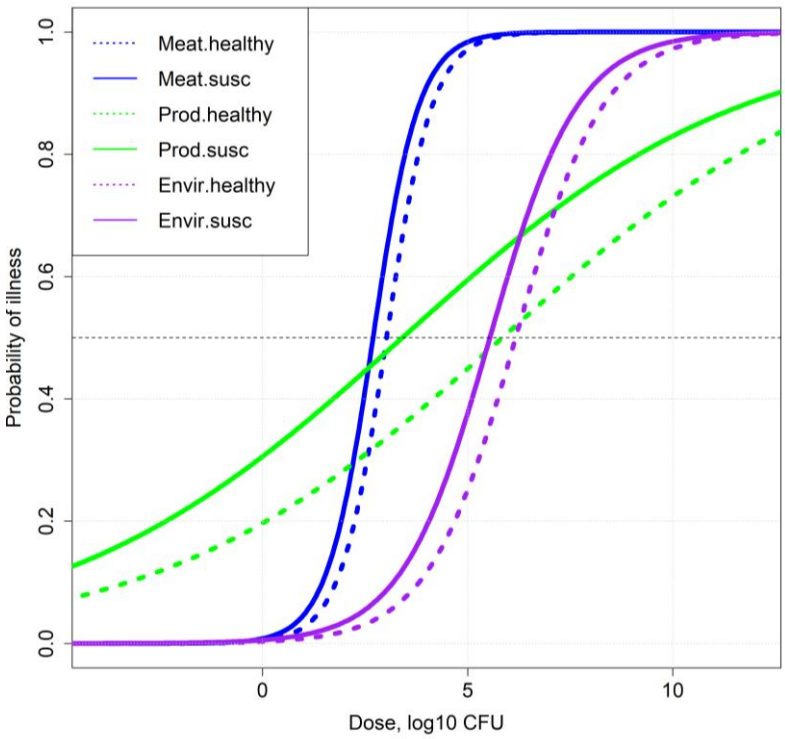

Figure 4. Logistic regression dose-response models due to different matrices fitted to O157 outbreaks from Table 3

Furthermore, produce contamination are regulated differently than meat products, as produce are often consumed raw, but washed prior to consumption (Tam et al., 2014; Lund, 2015). Buchanan et al. (2000) found that entrapment of bacterial cells within fat droplets serve as a microbial protection layer from stomach acid. Additionally, the heat of transfer is inhibited in fatty products, such as ground beef, in comparison to produce, which consist of mostly water, allowing for microbes to survive. The beef industry has done excellent work imposing intervention strategies to minimize STEC contamination, but undercooked hamburger and non-intact meat by consumer can increase the risk for illnesses (USDA-FSIS, 2002). Thus, it is imperative that consumers are properly educated in consumption and cooking of raw meats products (Hochstein et al., 2018).

3.5 Comparative Dose-response with Previous Models

The logistic regression model is commonly used for analysis of chemical toxicity, where it is assumed that the host has a tolerance distribution for a contaminant (Haas et al. 2014). When sub-populations are exposed to a certain level of contaminant, it is expected that all members within that sub-population will experience adverse effects if their tolerance is lower or equal to the exposed level (Haas et al. 2014). Bollaerts et al.'s (2008) study evaluated the dose-response of Salmonellosis from foodborne outbreak data and employed the logistic regression method. Similar to the dose-response model in this study, Bollaerts (2008) modeled population susceptibility as fixed effects and the differences in matrix as random effects. The logistic regression model does not follow mechanistic assumptions as used for exponential or beta-Poisson dose response models (Haas et al. 2014). These mechanistic assumptions include: 1) the host consuming one or more organisms that cause illness, and 2) the organism decaying or is inhibited from multiplying to cause illness in host; and 3) only a fraction of consumed organisms migrating to a position that initiates infection (Haas et al. 2014).

Haas (1983) first applied the beta-Poisson model to estimate microbial dose-response; however, the model was originally discussed by Furumoto and Mickey (1967a, b) (Crockett et al. 1996). The beta-Poisson model has been widely used to model microbial data because microbe infectivity varies and exposed hosts differ by degree of susceptibility (Crockett et al. 1996). The parameters are usually determined by the maximum likelihood method (Crockett et al. 1996), and the Poisson-distribution represents the number of organisms present in a given serving of food and the beta-distribution represents the variability of illness susceptibility among the population. The logistic regression model, on the other hand, is also designed for testing the effects of covariates. It accounts for susceptibility as a dichotomous variable, but also considers

random effects associated with matrix and allows for weighting of the data. The logistic regression models for both the susceptible and healthy adult populations from this study are conservative when compared among other historical dose-response models in Figure 5.

Most of the historical models in Table 1 are developed from data that include human clinical trials, epidemiological studies from food poisoning outbreaks, animal clinical trials, in-vitro studies using cell lines, and biomarkers or expert opinion. These methods are not uncommon as volunteer human clinical trials to develop a dose-response for O157 is limited due the virulent properties of O157 (Duffy, 2003). As a result, surrogate organisms, like *Shigella dysenteriae* are often used (Crockett et al., 1996). The susceptible model is comparable to the Cassin et al.'s (1998) beta-binomial healthy adult dose-response model from ground beef hamburger outbreaks (the dose-response model was more conservative for children and the elderly), but Cassin et al.'s model does not account for random effects due to other matrices or consider all outbreaks in this study. Additionally, Cassin et al.'s model parameterized its assumptions with a virulence similar to *Shigella.*

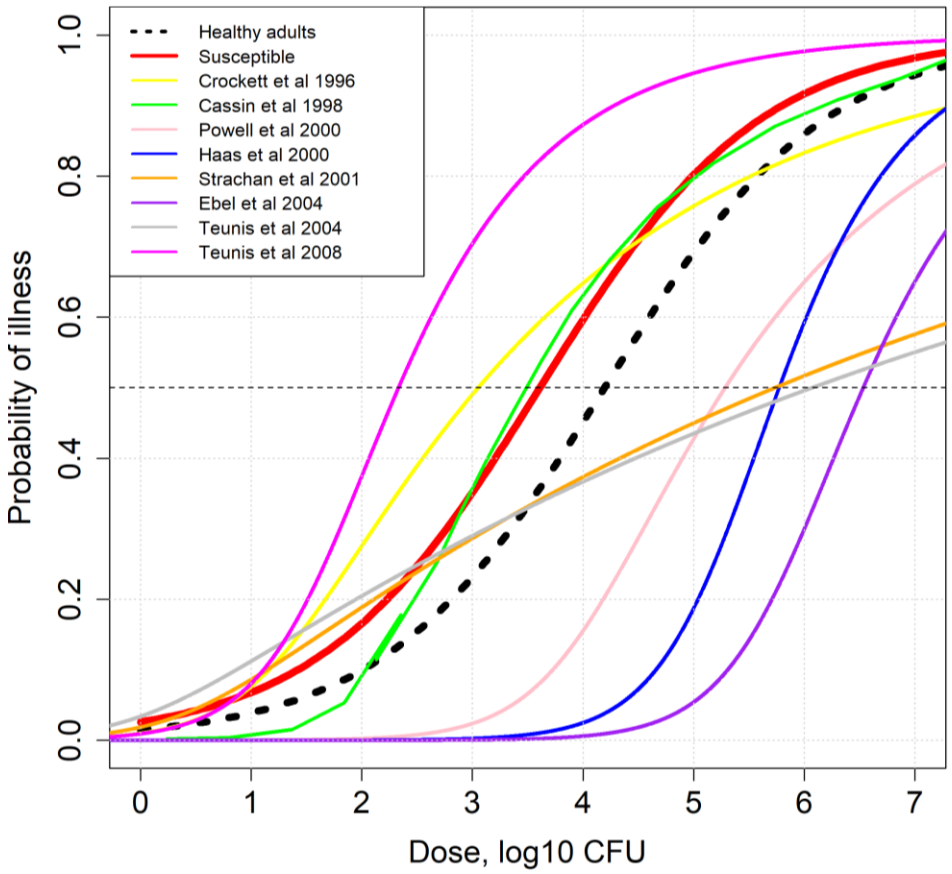

Figure 5. Logistic regression dose-response model fitted to outbreak data from Table 3 plotted against historical dose-response models

Crockett et al. (1996) used a *Shigella* feed study on humans to replicate the O157 dose-response relationship, and modeled the data using a beta-Poisson approach. The study by Haas et al. (2000) used injected O157 in 3-month old rabbit's data and a beta-Poisson dose-response relationship. Powell et al. (2000) also utilized a beta-Poisson model based on effective doses for humans infected with the surrogate organisms, *S. dysenteriae* and EPEC. EPEC is assumed to be less pathogenic than O157, thus its response was modeled as the lower bound of the O157 dose-response (Powell, Ebel, Schlosser, Walderhaug, & Kause, 2000). *S. dysenteriae* was the upper - response by assuming that it was less pathogenic than *Shigella.* The beta-Poisson model by Powell et al. (2000) and USDA-FSIS (2001) estimated ED_{50} of O157 by using the number of

illnesses due to O157 in ground beef annually, contaminated servings of ground beef, and dose-response upper bounds modeled by S.dysenteriae and EPEC data. Other beta-Poisson dose-response models including Stratchan et al. (2005), Teunis et al. (2004), and Teunis et al. (2008) models were developed using quantitative data from documented human outbreaks, but did not include all outbreaks used in this study nor did they evaluate population susceptibility.

The major take away from this study is that important factors affecting dose-response are not considered in previous studies. There are variability responses among matrix, and the random effects parameter built into the dose-response model in this study accounts for this variation. The most compelling result is that population susceptibility is a major factor affecting dose-response. Even though illnesses resulting from ingesting STEC can affect all people, those who are immunocompromised, children, and the elderly are the most susceptible. Results from this study reveal that the susceptible population's ED_{50} is 3.8 times lower than the ED_{50} for healthy adults.

The hazard characterization stage of a risk assessment involves a well-defined dose-response relationship. Unfortunately, the limitation in this study is the lack of existing dose-response models for STEC in the literature that accounts for all available STEC outbreak data. Thus, there remains the stress for future outbreaks to be well-documented with a thorough epidemiology study documenting the number of exposed and number of ill persons, along with a complete population demographic breakdown, and a thorough systematic tracking of the source and estimated dose of contamination. Thus, it is imperative that this model is revisited if additional outbreak data information becomes available.

CONCLUSIONS

The logistic regression model in this study demonstrated that population susceptibility impacts probability of illness by revealing a 3.8 times lower dose-response for the susceptible population. Future use of this study is widely applicable due to the need for developing better dose-response models and parameters to simulate real world interactions of STEC O157 consumption and resulting foodborne illnesses. Although data is limited, preliminary analysis of matrix effect reveals that exposure matrix can have an impact on dose-response with environmental exposure, produce, and meat, respectively, imposing increased probability of illness at the same consumption dose. Risk assessors, public health officials, and the food industry need a model that accurately predicts the median effective dose from consuming O157 in foods as there is a threat to public health and huge economic loss potential. The discussed dose-response models can lead to more accurate risk estimates and better food handling practices, especially in the beef industry; however, it is acknowledged that additional outbreak data will be needed to obtain a larger picture of how this organism attacks humans when a minimum effective dose is consumed. Better understanding this dose-response relationship will promote increased food safety and reduce the number of foodborne illnesses, especially among susceptible populations.

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CHAPTER 11: Summary

This dissertation developed interdisciplinary analytical and quantitative methods to assess the risk from contamination in water and food. Tools for communicating risk to the public were also developed. This interdisciplinary research occurred at the intersection of regulations, modeling, dose-response, contaminant inactivation, and industry/policy controls. The overarching goal was to improve public health.

Processing, distribution, and human consumption of food and water share similarities, as shown in Figure 1. Successful application of the risk assessment framework for food and water safety will improve individual consumer and the public health.

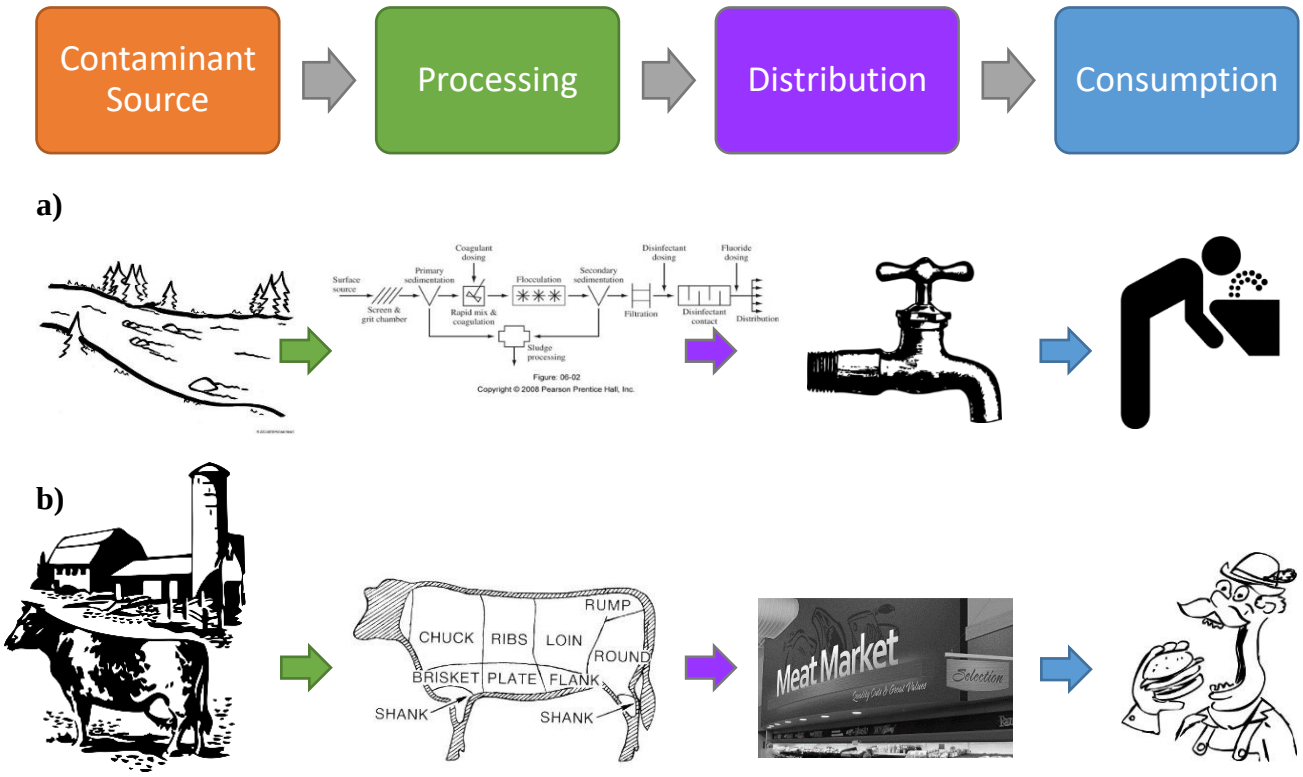

Figure 1. Processing flow patterns: a) drinking water industry; and b) beef industry

This interdisciplinary research demonstrated that collaborations among engineers, scientists, and regulators are essential to produce the data needed for hazard identification, exposure assessment, and dose-response and then integrate these components into a risk characterization. The hazard can be chemical or microbiological contaminants; the exposure can be to children or adults; the dose response can be a health effect or an aesthetic effect; the risk characterization can be regulations or industry controls. The ultimate goal of the risk assessment is to protect the public health. Engineers, scientists, and regulators can be do this through interventions that control the contaminants at their source, during processing, or during distribution. Communicating risks and risk control strategies to the public will integrate consumers into the risk assessment framework and provide them an active role in protecting their individual health and also the public health.

The crude MCHM chemical spill highlighted the importance of fate and transport and biological properties of different components in a mixture, whether major or minor. Additionally, the route of exposure (ingestion, inhalation) must be considered, as well as the differences in population exposed (adults, children). Lessons learned from the MCHM spill stress the need for better overall monitoring and testing of all chemical components and media involved, as well as improved communication between the public and the water utilities.

Public perception is important when it comes to water safety as consumers judge the quality of their drinking water through aesthetics. Human intuition is to avoid products that are unpleasant or do not look “right” (Renn, 1991; Jardine et al., 1999; Dietrich, 2006; Dietrich et al., 2014). Consumers are in fact concerned about the safety of their drinking water, and research suggest that clear communication of water quality information is needed (Dietrich et al., 2014). The Drinking Water Taste and Odor Wheel and Consumer Confidence Reports are useful public

communication tools. With minor improvements, they can be very useful in communicating with consumers.

Pathogenic STEC organisms in food represent another potential health risk for consumers. Like drinking water, where a variety of different disinfectants can be used at the treatment plant to reduce pathogen concentrations, both beef processing facilities and consumers have interventions available to reduce STEC concentrations. The plant intervention meta-analysis research and the cooking time-temperature study helped identify appropriate and achievable bacterial reductions. The plant intervention analysis helped distinguish which interventions actually work well from those that are less effective or those where little data is available. The work also identified common pitfalls, e.g. lab inoculation and data substitution practices, which both the industry and regulators need to be aware of for effective risk management. The dose-response paper better elucidate the risks to different populations, adults and children, and identified potential differences in the dose-response relationship based on the food/water media consumed.

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