# Assessing the Scientific Basis of the Food Safety Modernization Act Produce Safety Rule and Microbial Quality of Water Used to Grow Produce in Alabama

by

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Abstract

Small farms are a growing part of the fresh produce market. In Alabama, 93 percent of farms are defined as small and 2 percent of agricultural sales were from fresh produce in 2016. Only 1.3 percent of produce farms in Alabama are GAP certified. Alabama Agricultural Experiment Stations (AAES) conduct research and demonstrate technologies before they are adopted by commercial growers. For this reason, they were used to represent small growers. The 2016 Final Produce Safety Rule (PSR), currently scheduled to be implemented in 2018, will require growers to follow a new set of practices during growing, harvesting, packing, and holding of fresh produce. Among other criteria, the PSR establishes standards for water that directly contacts produce pre- and post-harvest. AAES can aid growers with PSR implementation. The objectives of this study were to (1) assess the scientific basis of the agricultural water provision of the PSR, (2) determine the average *E. coli* presence in agricultural water used to grow produce during a growing season at three locations across Alabama, and (3) determine which food safety practices a sample of AAES farm managers implement. Data from this study will determine the baseline level of food safety practice and knowledge of a sample of PSR-exempt growers, as well as the microbial quality of water used in produce production and harvest in relation to the PSR standards. All scientific studies cited in the Federal Registrar in support of the PSR were categorized and assessed for how well they matched the FDA's claims. It was found that overall, the FDA made a good faith effort, given the time and resources, to base the water provision of the PSR on "sound science." There are gaps in the literature, but the FDA

was required to publish the Final PSR within a timeframe before additional baseline funding could be established. Ground, county, and surface water were sampled from seven sample sites in three different geographical areas of the state over a four-month growing season. Under the PSR, growers must choose a water quality testing method that vary in cost, feasibility, and ease of use. Generic *E. coli* presence was enumerated with three methods equivalent to EPA Method 1603: EPA Method 1604 (MI), Hach Method 10029 (mColiBlue24), and EPA 1103.1 (mTEC). There was an average geometric mean (GM) of *E. coli* in the two surface water sources of 20 and 6 CFU/100 mL. There was no detectable *E. coli* in the ground and county water sources. The mTEC produced significantly different *E. coli* counts than the mColiBlue24. Next, a survey was sent to AAES farm managers across the state. From the 8 responses received, it was found that none had a food safety plan or had conducted an environmental microbial safety risk assessment; there were food safety risks such as wildlife, domestic animals, and use of untreated soil amendments close to the growing areas; only one farm had trained their workers in food safety practices; and one other farm tested their irrigation water. Although all water sources met the PSR criteria, the presence of food safety hazards indicates that risk assessments should be conducted. This study provides opportunities for AAES and growers to review implementation of the PSR and optimize training. Both parties would benefit from opportunities like on-farm safety demonstrations to fill the current gap in knowledge.

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|---|---|
| AAES | Alabama Agricultural Experiment Station |
| ACES | Alabama Cooperative Extension System |
| APLU | Association of Public and Land Grant Universities |
| AWP | Agricultural water provision |
| CDC | Center for Disease Control |
| CFU | Colony forming unit |
| CFR | Code of Federal Regulations |
| EPA | Environmental Protection Agency |
| FAO | Food and Agriculture Organization of the United Nations |
| FDA | Food and Drug Administration |
| FDCA | Food Drug and Cosmetic Act |
| FSMA | Food Safety Modernization Act |
| GAPs | Good agricultural practices |
| GM | Geometric mean |
| HHS | Health and Human Services |
| LGMA | Leafy Green Marketing Agreement |
| LOD | Limit of detection |
| MPN | Most probable number |
| NASS | National Agricultural Statistics Services |
| NIFA | National Institute of Food and Agriculture |

| | |
|---|---|
| NOP | National Organic Program |
| PFGE | Pulsed field gel electrophoresis |
| PFU | Plaque forming unit |
| PSR | Produce Safety Rule |
| QAR | Qualitative Assessment of Risk |
| SDWA | Safe Drinking Water Act |
| STV | Statistical threshold value |
| USDA | United States Department of Agriculture |
| WHO | World Health Organization |

## 1. Introduction

Fresh produce is an important source of nutrients, fiber, and vitamins in the human diet (Olaimat and Holley, 2012). Due to health promotions by organizations such as the United States Department of Agriculture (USDA), demand for fresh produce has steadily increased since the 1970s (You et al., 1998). At the same time, the number of foodborne outbreaks associated with fresh produce has been high compared to other food groups (Fischer et al., 2015). Foodborne outbreaks associated with fresh and processed produce in the United States account for an estimated $39 billion dollar annual economic loss (Scharff, 2010). Pathogenic microorganisms of potential concern in fresh produce include *Aeromonas hydrophila*, *Escherichia coli* , *Salmonella* spp., *Shigella* spp., *Vibrio cholera*, *Bacillus cereus*, *Clostridium botulinum*, and *Listeria monocytogenes* (Brackett, 1999).

Produce contamination can occur at many points along the production chain. The most common causes of foodborne outbreaks from produce are contaminated water and soil, inadequately composted manure and biological soil amendments, lack of worker health and hygiene, inadequate equipment and building sanitation, and the presence of nearby pathogen-harboring domestic animals or wildlife. Contamination can occur include during production (planting, growing, and irrigating), at the processing and packing stage, or during preparation (Uyttendaele et al., 2015). *E. coli* O157:H7 and other enteric pathogens are capable of survival in animal feces, water, soil, as well as on and in crops (Gagliardi and Karns, 2000; Kudva et al., 1998; Wang et al., 1996; Wang and Doyle, 1998). Pathogen contamination of produce is considered a serious public health risk because fruits and vegetables are often consumed raw or after minimal processing, therefore, any existing bacteria are not killed by cooking (Park et al., 2013).

The produce items most commonly associated with outbreaks include leafy vegetables such as spinach, lettuce and lettuce mixes, cucumbers, herbs, sprouted seeds, tomatoes, cantaloupes, mangoes, and peppers (Beuchat, 1996; Fischer et al., 2015; Sivapalasingam et al., 2003). The outbreak causing pathogen is usually zoonotic or human in origin. Common zoonotic organisms implicated in produce outbreaks are *Salmonella enterica* and verocytotoxin producing *E. coli* O157:H7 (Uyttendaele et al., 2015).

Produce contamination during production is most frequently caused by exposure to contaminated irrigation water or manure fertilized soil (Castro-Ibáñez et al., 2015; Franz and van Bruggen, 2008). Soil can become contaminated if the site was previously used as a grazing area for animals or with the application of animal waste as fertilizer or for waste disposal. Another important cause of produce contamination pre-harvest is the presence of domestic or wild animals. Water runoff from areas with accumulated animal feces can potentially contaminate produce (Uyttendaele et al., 2015). Produce can also become contaminated by workers with poor hygiene practices, improper sanitation in the field, improper equipment sanitation, and in the packing plant (Franz and van Bruggen, 2008).

The source of produce contamination that will be the main focus of this review is water that is used pre- or post-harvest (Uyttendaele et al., 2015). Pathogens such as viruses, protozoa, and bacteria can survive or grow in water, later causing human illnesses (Ferguson et al., 2003). For example, irrigation water has been demonstrated to harbor pathogens such as *E. coli* O157:H7 and *Salmonella* (Materon et al., 2007; Steele and Odumeru, 2004). In one case, pond water used to irrigate peppers was the cause of a 2005 multistate *Salmonella* Newport outbreak and a 2008 nation-wide *Salmonella* Saint Paul outbreak (Barton Behravesh et al., 2011; Greene et al., 2008).

Post-harvest, improperly sanitized wash, flume, or cooling water can transfer pathogens to produce (FDA, 2015a).

Due to the potential for produce to become contaminated by contact with tainted water, the FDA created agricultural water quality criteria as part of 2011's Food Safety Modernization Act (FSMA) Produce Safety Rule (FDA, 2015b). The FSMA Final Produce Safety Rule (PSR) of 2016 set forth regulations to ensure the safety of produce by focusing on causes of contamination during growing, harvesting, packing, and holding such as water, soil amendments, worker hygiene, and sanitation. Before this legislation, there was no regulatory oversight of produce safety at the production, packing, and holding level in the United States. These regulations established acceptable criteria for the presence of generic *E. coli* in any water that is used to irrigate or contact produce. They also established a water testing frequency that growers must use to create a rolling dataset of *E. coli* enumeration for ground water and surface water. Testing is not required for municipal water. Water that does not meet the required *E. coli* criteria must be treated, no longer used, or allowed die-off time between last application and harvest (FDA, 2015b).

The source of irrigation water and the irrigation method play an important role in the transmission of contamination to produce (Brackett, 1999; Leifert et al., 2008; Steele and Odumeru, 2004). The FDA identifies three source of the water in the PSR: municipal, ground and surface, which impacts its microbiological quality and potential for transmission of contamination to produce (Uyttendaele et al., 2015). According to the PSR, municipal water is water that was obtained from a public water system that meets microbial requirements defined in the EPA's Safe Drinking Water Act (SDWA), which requires that public water systems routinely take representative samples to test for total coliforms (EPA, 2009). Municipal water is the least likely to be contaminated because its quality is frequently monitored under the Act and disinfectants are

applied. Ground water is defined in the PSR as the supply of fresh water found beneath the earth's surface, usually in aquifers, which supply wells and springs. Ground water, generally accessed through wells, is usually of acceptable microbiological quality if contact with surface water due to runoff is avoided (Burch and Thomas, 1998). Under the SDWA, ground and surface water used for public water systems are tested for *E. coli* and coliforms at a routine frequency, with more samples in the case of a positive result. Surface water is any source of water open to the atmosphere including rivers, lakes, reservoirs, streams, impoundments, seas, and estuaries; and all springs, wells, or other collectors that are directly influenced by surface water. Because surface water is open to the environment, it may be contaminated by animals, agricultural or flood runoff, unintentional sewage system failures, or heavy rainfall. Therefore, microbial levels in surface water can be highly variable due to water runoff from sewage plants and agricultural operations, animal feces, precipitation, and other factors (Bihn and Reiners, 2010; Jones and Shortt, 2010; Steele and Odumeru, 2004).

The PSR *E. coli* water criteria received a substantial pushback from produce growers and other stakeholders concerning their practicality, complexity, and ease of implementation (Holcomb et al., 2013). For this reason, the FDA is proposing to extend the agricultural water regulation compliance dates and modify the requirements (FDA, 2017a). Currently, no studies have applied the PSR criteria to Alabama agricultural water. In an effort to contribute to the baseline understanding of agricultural water quality, a handful of studies have applied the PSR agricultural water criteria in Pennsylvania, Florida, and Georgia, with most finding that water met the criteria (Antaki et al., 2016; Draper et al., 2016; Hong et al., 2017; Topalcengiz et al., 2017).

Currently, agricultural water used pre- and post- harvest in Alabama is tested in accordance with Good Agricultural Practices (GAPs). These specify production practices and intervention

strategies that could be implemented on farms producing unprocessed or minimally processed fresh fruits and vegetables (FDA, 1998). According to the 2012 USDA Census of Agriculture, only 34 out of 2,655 total farms (1.3 percent) that grow produce in Alabama are GAP certified (USDA, 2017). The fresh produce industry in Alabama has a significant output of $161 million, and 1,121 new jobs on the state's economy. Fresh produce represents 2 percent of agricultural sales (Fields, 2017). A large concentration (93 percent) of these produce operations are small farms (USDA, 2012). Small farms and local product purveyors are an important part of the produce market in the United States and will face challenges implementing the PSR.

**Objectives**

The objective of this dissertation is to (1) assess the scientific basis of the agricultural water provision (AWP) of the PSR and determine barriers to its enforcement and implementation for the FDA and small growers, (2) determine the average *E. coli* presence in agricultural water used to grow produce during a growing season at three locations across Alabama, and (3) determine which food safety practices a sample of AAES farm managers implement. The objective will be addressed via eight research questions.

To represent small farms, the test sample is Alabama Agricultural Experiment Station (AAES) managers who, while PSR-exempt, provide an opportunity to test and exemplify food safety practices for commercial growers in the state. Therefore, AAES managers are used to represent small growers in this study.

The first research question asks if the AWP of the PSR is based on "sound science." The scientific basis of the PSR requirements have not previously been assessed. Assessing the scientific basis of the rule will verify if the requirements are properly substantiated by locating gaps in the literature and potential problems with implementation and enforcement for the FDA and small

growers. This information can then be conveyed to growers by AAES and Extension programs. The question will be answered by conducting a review of the FDA's scientific claims in the Final PSR and comparing them to the scientific literature.

The second research question of this study is to ask what the baseline quality of agricultural water used to irrigate produce in Alabama might be. Next, the third research question of this study is to determine if the agricultural water utilized to grow produce in three Alabama produce growing regions meets the PSR water quality standards. The fourth research question asks if it is feasible for small growers to implement the PSR water regulations. This will be determined by analyzing the scientific basis of the regulation and locating potential concerns for growers such as sampling costs and laboratory testing requirements. This will also be assessed by testing the practical aspects of the regulation such as *E. coli* enumeration methods and sample transportation times to simulate a small farmer carrying out the PSR water regulations.

The fifth research question of this study is to ask whether or not there are any food safety practices currently in place at AAES farms, and if so, what practices might be used. The question will be answered by administering a survey to AAES managers regarding on-farm food safety practice use. The sixth research question is to determine the knowledge of produce safety practices AAES managers possess before the implementation of the PSR. The seventh research question is to determine manager knowledge of the PSR. The eighth research question is to ask if the small farm PSR exemption is valid and appropriate. These questions will be answered with the survey responses and an analysis of the water quality results. The results of this study will identify areas for improvement and gaps in our collective knowledge in the land grant university experiment station-grower education system. They will also identify where and how AAES may ease the

transition to implementing the PSR for growers, ultimately supporting the produce industry in the state.

Chapter 2 provides an expanded literature review on the topics of foodborne outbreaks due to produce, routes of produce contamination, and the regulatory and land grant university response to these outbreaks. Chapter 3 examines the FSMA PSR broadly, how small farms are affected by the PSR, and the AWP specifically. Chapter 4 provides an overview of challenges with agricultural water sampling, water testing methods, and early assessments of agricultural water using the PSR standards. Chapter 5 describes the scientific basis of the AWP of the PSR. Finally, chapters 6 and 7 assess the microbial quality of produce irrigation water from a selection of PSR-exempt farms, as well as the food safety practices on AAES farms. Chapter 8 identifies possibilities for future research.

## 2. Literature Review: Foodborne Outbreaks Due to Produce

This chapter will provide an overview of the problem of foodborne outbreaks due to produce, routes of fresh produce contamination with a focus on agricultural water, the regulatory and land grant university response to these outbreaks, and how small farms are involved with these issues.

### 2.1. Foodborne Outbreaks Due to Produce

Fresh produce is an important source of nutrients, fiber, and vitamins in the human diet (Olaimat and Holley, 2012). The Dietary Guidelines for Americans promotes the daily intake of two and a half cups of vegetables and two cups of fruit per day for the prevention of chronic diseases such as heart disease, diabetes, cancer, and obesity (US Dept. Health and Human Services, 2015). As a result, produce consumption in the United States has increased (Callejón et al., 2015). Produce has also become more easily available and affordable globally (Garcia and Heredia, 2017).

All fruits and vegetables can be contaminated with bacterial pathogens (Brackett, 1999). From the 1970s to the 1990s, the incidence of foodborne outbreaks due to produce in the United States rose from 0.7 percent to 6 percent of all reported outbreaks (Sivapalasingam et al., 2004). In 1998, the Food and Drug Administration (FDA) published *The Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*, which outlined GAPs: production practices and intervention strategies that could be implemented on farms for use in the production of unprocessed or minimally processed fresh fruits and vegetables (FDA, 1998). They are meant to ensure the safe production, harvesting, and handling of fresh produce.

The number of foodborne outbreaks due to produce in the US has continued to rise. A March 2013 report from the U.S. Centers for Disease Control (CDC) stated that 46 percent of all

foodborne illnesses between 1998 and 2008 could be traced back to produce (Painter et al., 2013). In addition, 38 percent of hospitalizations from foodborne illnesses and 25 percent of deaths from foodborne illnesses could be traced back to produce (Painter et al., 2013). Fresh produce also accounted for an increasing proportion of foodborne outbreaks where the food was reported (an increase of 8 percent from 1998 to 2001 to 16 percent from 2010 to 2013) (Bennett et al., 2018).

The first explanation for this increase in fresh produce related foodborne outbreaks is an increase in consumption related to consumer health motivations (Olaimat and Holley, 2012). Other reasons include a trend towards consuming food prepared outside of the home such as at salad bars, and a higher consumption of intact and pre-sliced fruits and vegetables that are shipped from central locations and distributed over large geographical areas to many consumers. This last factor, in addition to increased global trade and importation of fresh produce, potentially increases consumer exposure to a wide variety of foodborne pathogens, the probability that outbreaks will be widespread or multistate, and the chances that an outbreak will be detected (Harris et al., 2003).

Another factor contributing to the increase in foodborne outbreaks due to produce is the increasing centralization of the industry. The bulk of produce production in North America has moved from a large number of farms towards a smaller number of centralized growing, packing, and shipping facilities in Mexico, California, and Florida (Sivapalasingam et al., 2004). California produces 70 percent of all leafy greens such as lettuce and spinach consumed in the United States (Agricultural Marketing Resource Center, 2017). This centralization has major implications for the spread of foodborne outbreaks due to produce from a single distribution center. For example, in 2006, there was an outbreak of *E. coli* O157:H7 linked to fresh, bagged baby spinach that spread to 26 states and Canada (CDC, 2006a). Contaminated spinach was linked to a single processing

plant and associated fields and ranches on the central California coast (Gelting, 2007). Most fresh produce receives minimal processing and is eaten raw (Olaimat and Holley, 2012).

A final factor contributing to the increase in foodborne outbreaks due to produce is the absence of a kill step during fresh produce production and consumption. This allows for pathogenic microorganisms that have contaminated fresh produce to survive until the consumer consumes the product (Olaimat and Holley, 2012).

### *2.1.1 Pathogens Associated with Fresh Produce*

Foodborne pathogens of particular concern in fresh produce include *Salmonella, E. coli* O157:H7, *Listeria monocytogenes,* Norovirus, *Bacillus cereus, Campylobacter jejuni, Shigella, Staphylococcus aureus, Yersinia enterocolitica, Cyclospora cayetanensis, Giardia lamblia,* Hepatitis A virus, *Clostridium botulinum*, *Vibrio cholera*, and *Cryptosporidium parvum* (Brackett, 1999; Garcia and Heredia, 2017). In the US from 1998-2013, Norovirus, *Salmonella enterica,* and Shiga toxin-producing *E. coli* accounted for the top three identified causes of foodborne outbreaks in produce (Bennett et al., 2018). *Salmonella*, *Listeria,* and *E. coli* O157:H7 are among the top causes of fresh produce outbreaks. All three of these pathogens have low infection doses, or the number of cells required to be ingested to cause illness. (Harris et al., 2003).

*Salmonella* has been isolated from, or caused an outbreak in, cantaloupes, cabbage, cauliflower, watermelons, tomatoes, lettuce, celery, sprouts, parsley, unpasteurized orange juice, and raw salad vegetables (Beuchat, 1996; Garcia-Villanova Ruiz et al., 1987; Gayler et al., 1955; Inami and Moler, 1999). Symptoms of *Salmonella* infection include abdominal pain, diarrhea, chills, fever, nausea, and vomiting (FDA, 2015a). *E. coli* O157:H7 has been isolated from and/or caused an outbreak in products such as alfalfa sprouts, apple juice, cabbage, celery, cilantro, and lettuce (Beuchat, 1996). An *E. coli* O157:H7 infection can result in bloody diarrhea, abdominal

pain, and can lead to hemolytic uremic syndrome and kidney failure (FDA, 2015a). *Listeria monocytogenes* has been isolated from or caused an outbreak in tomatoes, salad vegetables, radishes, potatoes, bean sprouts, cabbage, and cucumbers (Beuchat, 1996). A *Listeria monocytogenes* infection can cause gastroenteritis in healthy adults and may lead to spontaneous abortion or stillbirth in pregnant women (FDA, 2015a). In the young, immunocompromised individuals, and the elderly, *Listeria monocytogenes* manifests as septicemia, meningitis, or other infections of the central nervous system (Ferreira et al., 2014). To minimize the transmission of pathogens like *Listeria* to fresh produce, it is important to understand the routes in which pathogens gain access to produce.

### 2.2. Routes of Produce Contamination

The fresh produce production chain is complex and contains many access points where contamination can occur from human, animal, or environmental sources. This includes production, harvest, processing, storage, transportation, retail and consumer handling (WHO/FAO, 2008). Contamination of raw produce with pathogenic organisms can occur pre-harvest by contaminated soil, water, inadequately composted manure, dust, wild and domestic animals, and human handling (FDA, 2015a). Foodborne pathogens associated with fresh produce most often originate from enteric environments, or the intestinal tract and fecal material of humans or animals. An exception to this is *Listeria monocytogenes*, which is ubiquitous and can be isolated from soil, irrigation sources, decaying plant residue on equipment or bins, cull piles, packing sheds and food processing facilities (Harris et al., 2003).

In the post-harvest stage, human handling, harvesting equipment, transport containers, wild and domestic animals, dust, wash and rinse water, further-processing equipment, ice, transport vehicles, improper storage environment, improper packaging, cross contamination from produce,

water, equipment, and contact surfaces, and cooling water can contaminate fresh produce (FDA, 2015a).

There are current limitations in the scientific community's understanding of how foodborne pathogens are introduced onto produce (Garcia and Heredia, 2017). Each individual farm has a variety of unique interacting factors such as crop type, weather, human and animal relationships, and irrigation type that makes it difficult to develop a one-size-fits-all intervention system to identify and control the contamination (Brandl, 2006; Olaimat and Holley, 2012). There is also a gap in the scientific community's understanding of the interaction between human pathogens and growing produce (Delaquis et al., 2007). Due to the large variety of factors that can lead to fresh produce contamination, it is difficult to design well-controlled experiments that address risk factors for produce contamination that are broadly applicable (Harris et al., 2003).

A number of reports have shown that many outbreaks due to fresh produce are caused by contamination at the field level (Lynch et al., 2009). The relationship between plants and microbes can be influenced by the plant species and cultivar, the type of pathogen, the entry point of the pathogen into the growing environment, and various environmental features such as the phylloplane niche, which is a habitat for a variety of microbes (Mitra et al., 2009). The phylloplane niche is affected by the large variability in plant surface morphology, the plant species and cultivar, surface morphology, tissue composition, and metabolic activities (Beuchat, 2002; Brandl, 2006; Burnett et al., 2000; Takeuchi and Frank, 2000). Pathogenic bacteria may interact with these various ecological niches and bacteria that are naturally present, then adapt to the environment, and possibly penetrate the plant tissue (Brandl and Mandrell, 2002; Cooley et al., 2006; Critzer and Doyle, 2010). The most important factors influencing microbial survival on plants include temperature, pH, salinity, adsorption to particulate matter, solar radiation, soil

moisture level, and soil type (Gerba et al., 1975). The next section addresses the various sources of microbial produce contamination.

### *2.2.1 Sources of Produce Contamination*

Sources besides water that can contaminate produce include domestic animals, worker health and hygiene, equipment and building sanitation, biological soil amendments, and damaged produce. One possible reason for the increase in foodborne outbreaks associated with fresh produce may be increased confinement of livestock production near areas of large produce growing operations (Suslow et al., 2003). Important sources of *E. coli* O157:H7 on the livestock farm are manure, water, and feed. *Salmonella*, *Campylobacter*, *E. coli* O157:H7, and *L. monocytogenes* are shed in the feces of infected animal hosts into the environment (Clough et al., 2003; Franz and van Bruggen, 2008). For this reason, produce grown near animal-rearing environments is subject to direct or indirect contamination from animals, run-off, bio-aerosols, and vectors associated with birds, rodents, and flies (Brandl, 2006; Gelting et al., 2011). Domestic animals can contaminate a growing area with fecal droppings. While the presence of domestic animals can be controlled, wild animals such as birds, frogs, rodents, and snakes can only be controlled to a limited extent, and can therefore be a further source of contamination (Harris et al., 2003).

Humans working with fresh produce are a potential direct vector of pathogenic microorganisms (Gil et al., 2015). Personal hygiene of farm workers is an important predictor of pathogen transfer to fresh produce on the growing fields, during harvesting, post-harvest handling, and distribution (Beuchat, 1996; Olaimat and Holley, 2012). The main reason for concern is the use of hands during the harvest and post-harvest stages and their potential to spread feces to the produce (Suslow et al., 2003). Infected workers are the primary source of viruses such as hepatitis

A and norovirus that can cause foodborne illness. Loading dock workers, truck drivers, workers involved with distribution, and retail workers can potentially spread contamination to produce (Brackett, 1999).

Another important cause of produce contamination is poor sanitary practices in environments where produce is handled. Produce itself is a potential source of contamination when it is brought into a plant. Once this contamination is introduced, it can spread by cross contamination to equipment and other produce items (Brackett, 1999). Any equipment, tools, and containers used to harvest, store, and transport produce from the field to the packinghouse must be properly sanitized. Packinghouse and processing equipment, drains, floors, walls, and ceilings can harbor pathogenic bacteria (Beuchat, 1996). Plant managers are recommended to choose a plant site in a location with good drainage and away from other manufacturing plants, landfills, excess foliage, standing water, and other areas that may harbor pests and contaminants (Marriot and Gravani, 2006). Produce can become contaminated on transport trucks when drivers "backhaul" a load of incompatible items such as raw meat or live animals before transporting produce (Brackett, 1999). Produce loading bins and containers, truck walls and floors must be sanitized to prevent contamination at the transportation level (FDA, 1998).

Soil is the natural environment for many human pathogens including *B. cereus*, *C. botulinum*, *C. perfringens*, and *L. monocytogenes* (Ivanek et al., 2006; Olaimat and Holley, 2012). A common practice used to dispose of animal waste and fertilize soil is to apply manure to fields (Brandl, 2006). Animal manure has fertilizing properties such as nitrogen, phosphorus, and potassium nutrients that aid in soil quality and fertility (Islam et al., 2004). However, manure that is not adequately treated or aged before application poses a risk of introducing pathogenic bacteria, viruses, and parasites to growing produce (Mawdsley et al., 1995). Studies have shown that farms

that applied manure to their land had a significantly higher proportion of *E. coli* positive produce samples than farms that did not use it (Mukherjee et al., 2007; Park et al., 2013).

The presence of animal waste on field surfaces increases the likelihood that surface runoff from heavy rain might wash contaminated manure onto other water environments such as ponds where pathogens can survive longer (Chalmers et al., 2000; Lim and Flint, 1989). Storing piles of manure near growing operations should be also be avoided because of potential contaminated run-off, aerosol spread, or vector transmission by rodents or insects (Brandl, 2006; James, 2006; Suslow, 2003).

If a produce item is physically compromised, this can promote contamination through each of the routes previously described. Sources of damage include physical or mechanical on-farm events such as high wind, hail, pests, and farm equipment. Damage promotes suitable nutritional and moisture conditions for microbial growth (Barker-Reid et al., 2009). Cut surfaces and wounds on produce are potential sites for biofilm formation, which then in part can reduce the effectiveness of sanitation washes (Aruscavage et al., 2006). *E. coli* O157:H7 and *Salmonella* in contact with produce leaves can move through leaf stomata and enter via damaged tissue (Gu et al., 2011; Itoh et al., 1998; Seo and Frank, 1999; Takeuchi and Frank, 2000). Produce that is damaged can be contaminated post-harvest by contaminated wash water, brushes, unhygienic workers, and contaminated equipment and surfaces.

Consumers expect year-round availability of produce and convenient pre-packed products that minimize preparation work (Warriner et al., 2009). Pre-packed items like pre-cut, sliced, or shredded items have had their protective epidermal barriers removed, which can facilitate the growth of microorganisms (Harris et al., 2003; Rodrigues and Fernandes, 2012). In addition,

abrasions or cuts can exude nutrients and facilitate the movement of microorganisms from the outer layer to the nutrient-rich inner layer of the produce (Takeuchi and Frank, 2000).

Efforts undertaken by producers at the farm-level to improve produce safety can be negated at the retail or consumer level. GAPs will not prevent post-harvest contamination by foodservice workers or consumers improperly handling the produce in their kitchens (Harris et al., 2003). At the retail and food-service level, the FDA Food Code specifies certain temperature controls for cut tomatoes and melons (FDA, 2017b). However, food handlers that are not trained in good hygiene practices can cause cross contamination while preparing fresh produce (Sagoo et al., 2003). At the consumer level, a survey found that 33 percent of consumers do not properly bag produce separately from other food items at the store, 20 percent washed their produce before storing it in the refrigerator, potentially promoting mold growth, 20 percent stored produce at room temperature, and 23 percent reported placing meat, fish, or poultry on the refrigerator shelf above other foods (Li-Cohen and Bruhn, 2002).

The focus of the remaining portion of this chapter will be a discussion of agricultural water contamination of fresh produce. To assess if agricultural water could be a source of contamination for produce, the microbiological quality of the water should be determined.

### *2.2.2 Water Quality Indicator Organisms*

Water quality is often assessed by determining the number of indicator organisms. Indicator organisms are microorganisms whose existence indicates probable presence of pathogenic microorganisms (Griffin et al., 2001). Testing for specific pathogenic microorganisms can be difficult because levels capable of causing human illness may be too low to be detectable by standard methods (Harwood et al., 2005). Testing for pathogens is also expensive, time consuming, and requires trained personnel and specific equipment (Harwood et al., 2005). Due to

these difficulties, water quality is most often assessed with indicator organisms (Harwood et al., 2005). Ideally, indicator organisms are non-pathogenic, occur regularly in pathogen-contaminated waters, do not multiply in water, occur primarily in the intestinal tract of humans and animals, are easy to isolate and enumerate, can be reliably detected at low concentrations, have similar survival time to pathogens, and are present in greater numbers than pathogens (Griffin et al., 2001). Figure 2.1 (US Geological Survey, 2016) shows the relationship between the indicator organisms used for water samples.

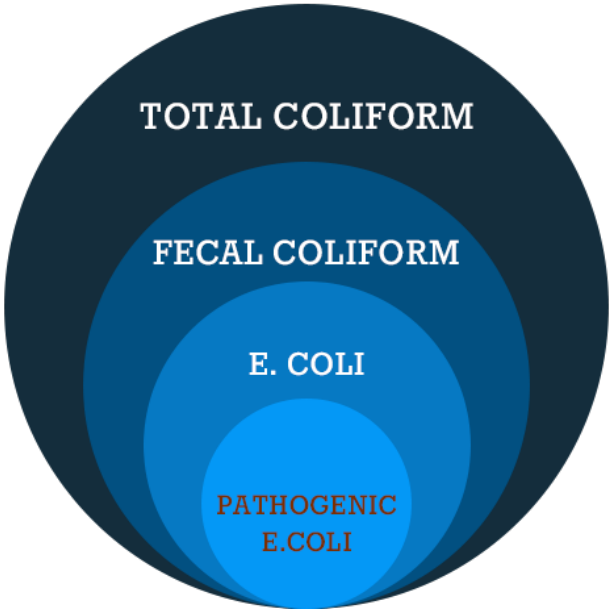


**Figure 2.1 The relationship between *E. coli* and fecal indicators.**

The coliform group are gram negative, non-spore forming, facultative anaerobes that ferment lactose to form acid and gas at 35-37 °C within 48 hours (American Public Health Association, 2005). They contain several genera of bacteria that belong to the family *Enterobacteriaceae* such as *Escherichia*, *Klebsiella*, and *Serratia* (Schraft and Watterworth, 2005). A drawback to the use of coliforms as fecal indicators is that the group contains many

harmless types of bacteria found in the soil that do not represent fecal contamination which results in false positive results (Griffin et al., 2001).

As shown in Figure 2.1, fecal coliforms are a subset of coliform bacteria which ferment lactose to form acid and gas between 44.5 and 45.5 °C (American Public Health Association, 2005). Fecal indicator organisms are found in the intestinal tracts of animals and humans and are shed in the feces (Griffin et al., 2001). Since most human pathogens are enteric pathogens that enter the body via the fecal-oral route, and pathogens are transmitted through human and animal feces, fecal coliforms may serve as a better indicator of pathogens than total coliforms (Griffin et al., 2001).

*E. coli* is a species of gram negative, rod-shaped, facultative anaerobic, non-sporeforming enteric bacteria within the fecal coliform group (Doyle and Beuchat, 2007). It is a predominant part of the human large-intestinal microflora (American Public Health Association, 2005). Because of this, it is the most widely accepted water fecal quality indicator (American Public Health Association, 2005). Some strains of *E. coli*, such as *E. coli* O157:H7, are pathogenic to humans, but the presence of *E. coli* does not guarantee the presence of pathogenic microorganisms (Odonkor and Ampofo, 2013).

If bacteria fecal indicator organisms are not used, coliphages are a good alternative. Coliphages are bacteriophages that infect coliform bacteria (Lin and Ganesh, 2013). They are good indicators for enteric viruses because they share properties such as composition, morphology, structure, size, and site of replication (Lin and Ganesh, 2013). They are also more resistant to environmental factors than fecal bacteria in water (Jurzik et al., 2010).

### *2.2.3 Agricultural Water Uses and Contamination*

Water can be used in produce production for irrigation, application of pesticides and fertilizers, cooling, and frost control. Post-harvest, water is used for produce rinsing, cooling, washing, waxing, and transport (FDA, 1998). The majority of produce grown in the United States is grown in semi-arid or arid regions where irrigation is necessary (Franz and van Bruggen, 2008). About 80 percent of land used to grow berries, orchards, and vegetables in the US is irrigated (USDA National Agricultural Statistics Service (NASS), 2012). Only 15 percent of land available for farming in Alabama is irrigated (Hollis, 2017). Pathogens such as viruses, protozoa, and bacteria can survive or grow in water, later causing human illnesses (Ferguson et al., 2003). Water can become contaminated by direct introduction of human or animal fecal contamination or in an indirect manner such as run-off from land to water (Hsu et al., 2011).

Irrigating the edible portion of produce with poor quality water can transfer bacteria such as *E. coli* and *Salmonella* from the water to produce (Bihn and Reiners, 2010; Guan et al., 2005; Takeuchi et al., 2001; Wachtel et al., 2002). Contamination is spread to produce via water either by uptake through the root system, direct deposit via irrigation, or indirectly by splash dispersal from the soil surface (Franz and van Bruggen, 2008). Studies have shown that irrigating fresh lettuce with water artificially contaminated with *E. coli* leads to adherence to the plant roots and contamination of the edible portions of the plants (Bernstein et al., 2007; Cooley et al., 2003; Wachtel et al., 2002). These studies also suggested that the edible portion of lettuce can become contaminated during growth by contaminated irrigation water which reaches the crop by traveling through the roots (Solomon et al., 2002b; Wachtel et al., 2002). In one study, soil used in lettuce production was irrigated with *E. coli* O157:H7-inoculated water. Three and five days after exposure to the inoculated water, two out of five plants tested on both occasions were positive for

*E. coli* O157:H7. *E. coli* O157:H7 was visualized throughout the plant tissue, including some internal areas inaccessible to post-harvest sanitizing agents (Solomon et al., 2002b). Contaminated irrigation water has recently been the cause of several *E. coli* outbreaks in California (lettuce), Arizona (lettuce), and in a *Salmonella* outbreak in Mexico (peppers) (Folster et al., 2011; Guan and Holley, 2003; Mody et al., 2011).

### *2.2.4 Sources of Agricultural Water*

Worldwide, irrigation water is sourced most commonly from surface water or ground water (Uyttendaele et al., 2015). In the United States in 2013, 55 percent of irrigated farm acres were irrigated with ground water, 10 percent with farm surface water, and 35 percent with off-farm water (USDA NASS, 2012). Availability and cost impact the source of irrigation water a farm uses (Benjamin et al., 2013). For example, municipal water is costlier than surface water, which is free. Municipal water in the United States is regulated by the Environmental Protection Agency (EPA) National Primary Drinking Water Regulations (EPA, 2009). In the state of Alabama, public water may contain up to 5 percent total coliform positive samples out of 40 or more per month. If more than 5 percent of samples are positive, the system must conduct an assessment in order to identify sanitary defects and issues with the distribution system coliform monitoring practices. Every total coliform positive sample must be further analyzed for *E. coli*. If two consecutive total coliform positive samples are obtained, and one is also positive for *E. coli*, the system obtains a maximum contaminant level violation (Alabama Department of Environmental Management Water Division, 2016; EPA, 2009).

Rainwater is usually of acceptable microbial quality but of lower quality than potable water. Its quality varies with how and where it is collected (Uyttendaele et al., 2015). Rainwater

collected from roofs can be contaminated with bird, animal, or insect droppings (Ahmed et al., 2012).

Ground water is less susceptible to contamination than surface water, but shallow, improperly constructed or older wells may be susceptible to contamination by surface water (FDA, 1998). Ground water is generally of acceptable microbial quality if contact with surface water is avoided. Flooding or storm related water runoff from areas where manure is present such as manure lagoons or sewage treatment facilities can contaminate ground water (Ibenyassine et al., 2007; Oron et al., 2001). Authors postulated that part of the cause of the 2006 California spinach outbreak was *E. coli* O157:H7-contaminated surface water that could have reached wells used for irrigation (Gelting et al., 2011). Additionally, some farmers pump well water into surface reservoirs for temporary storage before irrigation, which could lead to contamination of the well water (Benjamin et al., 2013).

Surface water is generally accepted as a greater risk to human health because it is susceptible to fecal contamination and runoff water from adjacent fields that properly constructed wells can prevent (Suslow et al., 2003). Various surveys of American surface water have detected the presence of *E. coli* O157:H7 (Armstrong et al., 1996; Olsen et al., 2002). A survey of surface waters in Georgia detected *Salmonella* in 79 percent of samples (Haley et al., 2009). Surface water is often contaminated from discharges of treated wastewater, feedlot runoff, storm water runoff, and livestock or wild animal feces intentionally or unintentionally present (Steele and Odumeru, 2004). It is also susceptible to runoff from storms, tides, and winds, which cause sediments to re-suspend in the water column, resulting in a high concentrations of fecal indicators (Ahn et al., 2005; Parker et al., 2010; Stumpf et al., 2010). In addition to susceptibility, higher concentrations

of bacteria are generally expected in surface water because of the structures overlying the water source (Benjamin et al., 2013).

Depending on factors such as temperature and UV radiation, *E. coli* and *Salmonella* can survive for days to months in surface and ground water (Bitton et al., 1983; Korhonen and Martikalnon, 1991; Santo Domingo et al., 2000). Low temperature in surface waters can increase the survival time of generic *E. coli* and *E. coli* O157:H7 (Barcina et al., 1986; Wang and Doyle, 1998). At 25 °C, *E. coli* O157:H7 survived in lake, reservoir, and municipal water for 28, 49, and 77 days, respectively (Wang and Doyle, 1998). *E. coli* O157:H7 survived for 21-56 days in lake water, and 71 days in reservoir water when both were held at 15 °C. When the temperature was reduced to 8 °C, *E. coli* survived for 91 days in lake, reservoir, and municipal water (Wang and Doyle, 1998). When cloud cover increased and solar radiation decreased, higher *E. coli* levels were detected in surface water (Whitman et al., 2004).

Pathogen survival in surface water is also impacted by nutrient levels, sediment concentrations and the type environment the water body is located in (Cooley et al., 2007; Czajkowska et al., 2005; Randall et al., 1999; Wang and Doyle, 1998). Water from different sources have different compositions of microflora, therefore two ponds inoculated with the same concentrations of *E. coli* may ultimately have different concentrations due to competition between species and predation (Kerr et al., 1999; Korhonen and Martikalnon, 1991).

Agricultural water can become contaminated by incorrectly managed animal or human waste (FDA, 1998). Contamination can occur at the water source with animal feces, due to a treatment failure, resulting in accidental entrance of raw sewage, or at a post-treatment occurrence (Chalmers et al., 2000). *E. coli* can enter a water supply through runoff from agricultural land onto the surface, through the soil, or via drainage systems (Chalmers et al., 2000). Irrigation water can

also be contaminated unintentionally with manure. Hilborn et al. (1999) speculated that a 1996 Connecticut and Illinois *E. coli* O157:H7 mesclun lettuce outbreak that caused illness in 53 individuals was due to the presence of cattle adjacent to the growing field, which led to contamination of the irrigation water. Studies have shown contamination of peppers, lettuce, and carrots with *E. coli* and *C. perfringens* by irrigating with wastewater from animal operations (Manshadi et al., 2001). The multistate San Benito County, California 2006 *E. coli* O157:H7 bagged spinach outbreak sickened 199 and killed three people. Investigators speculate that it was caused by either animal fecal contamination of the field or fecal contamination of the surface waterways, which then contaminated the irrigation wells (Jay et al., 2007).

### *2.2.5 Method of Application*

The type of irrigation system utilized impacts the microbial safety of fresh produce. Commonly used methods include gravity (flood) irrigation, spray, canal or furrow, and subsurface (drip) irrigation (Olaimat et al., 2017). Flood irrigation involves applying water over an entire field to infiltrate soil. Spray irrigation applies water in a form of spray throughout the air that reaches the soil and the plant in a sprinkle. Drip irrigation and subsurface irrigation are the application of water below the soil surface through emitters (Camp, 1998). Canal or furrow irrigation involves applying water between ridges, corrugations, or furrows (Uyttendaele et al., 2015). Of these listed forms of irrigation, flood and spray irrigation pose the greatest risk to the safety of fresh produce because contaminated water can be directly deposited on edible leaves of a crop (FDA, 1998). For example, root crops such as carrots, beets, and leafy vegetables are at a greater risk for contamination where the edible portion touches the soil (Guan and Holley, 2003). Drip, surface, and well maintained furrow irrigation can be of less risk compared to spray irrigation in spreading contamination because these methods can minimize the edible portion of crop that comes in

contact with contaminated water (Manzoor, 2007; Steele and Odumeru, 2004). Drip irrigation may also help to minimize cross contamination from the soil to plant surface when handled by workers (Song et al., 2006).

Contamination of produce through irrigation has been illustrated in several studies. A study comparing contamination levels of bell peppers, cantaloupe, and lettuce due to contaminated irrigation water applied using subsurface drip irrigation or furrow irrigation found significantly greater levels of coliphages on the lettuce that was furrow irrigated (0 and 1 log PFU/g), but not on cantaloupes and bell peppers (Song et al., 2006). Another study comparing the effects of spray and surface irrigation on *E. coli* O157:H7 internalization into lettuce found a significantly greater number of plants positive for the pathogen after spray irrigation (90.5 percent of plants) than surface irrigation (19 percent of plants). In addition, 20 days after spray irrigation with contaminated water, *E. coli* persisted on 9 out of 11 plants (Solomon et al., 2002a). In another study, *E. coli* O157:H7 that was dropped onto spinach simulating spray irrigation survived for 14 days (Mitra et al., 2009). These studies confirm that spray and furrow irrigation are more likely to contaminate produce than drip irrigation.

### *2.2.6 Timing of Application*

It has been found that the timing of the last crop irrigation pre-harvest impacts the postharvest microbial populations; the greater the time between the last irrigation application and harvest, the lower the effect of contaminated irrigation water on microbial levels of the harvested crop. In one study, lettuce outer head leaves that received “late” irrigation four days before harvest had 0.4 log CFU/g higher microbial counts than lettuce that received irrigation 16 days before harvest. An approximately 0.6 log CFU/g higher microbial load was found on outer lettuce leaves irrigated with spray irrigation compared to furrow irrigation 4 days before harvest Additionally,

the intermittent effect of rainfall increased microbial counts on lettuce heads by 1.5-3 log CFU/g (Fonseca, 2006). Similarly, a time period of more than five days after last irrigation was associated with a decreased risk of *E. coli* contamination of spinach (Park et al., 2013).

Rainfall increases concentrations of pathogens and indicator organisms in ponds, lakes, open wells, streams, and reservoirs due to a loosening and re-suspension of bacteria present in bottom sediments and an increase in surface runoff into waterways from surrounding lands (Pachepsky and Shelton, 2011). It was found that rainfall increased coliform counts in ponds up to 4.2 log MPN/100 mL (Topalcengiz et al., 2017). Alternatively, heavy rainfall can dilute surface waters and decrease indicator organism concentrations. For example, 400 CFU/mL *E. coli* was detected during dry months in a constructed wetland, and less than 100 CFU/mL was detected during a month with 70 mm of rainfall (McLain and Williams, 2008).

Intermittent flooding from a creek in Salinas Valley, California was found to be a contributing factor to an *E. coli* O157:H7 outbreak in 2002-2003. An investigation into these watersheds from 2004-2006 found a 6 percent incidence of *E. coli* O157:H7, and a positive correlation associated with increased rainfall and water flow (Cooley et al., 2007).

The time of day may also influence indicator organism concentrations. For instance, a later time of day (afternoon) was found to decrease *E. coli* concentrations in surface water anywhere from 0.2 to 2 log CFU/mL from the morning *E. coli* numbers (Meays et al., 2006; Traister and Anisfeld, 2006; Whitman et al., 2004). This is because solar radiation can inactivate or kill *E. coli* in surface waters (Burkhardt et al., 2000). One study determined that *E. coli* levels decreased 0.5 log CFU/mL from AM to PM hours on sunny days, and 0.2 log CFU/mL on cloudy days in Lake Michigan (Whitman et al., 2004).

The time of year also impacts irrigation water contamination levels. During the summer months, *E. coli* survived for shorter periods of time in lettuce fields, but the chances of introducing a pathogenic *E. coli* strain from surface water to the field was higher (Fonseca et al., 2011). Research has generally found higher incidence of *E. coli* in summer months (Cinotto, 2005; Holvoet et al., 2014; Kim et al., 2010; Shelton et al., 2011; Traister and Anisfeld, 2006). Seventy-four percent of *E. coli* O157:H7 outbreaks due to produce consumption in the United States from 1982-2002 were in the months of July through October (Al, 2005). These variations in pathogen prevalence in water by season appear to be influenced by the interactions between land use, water management, weather patterns, and the source and type of pathogen (Pachepsky et al., 2011). Specifically, land use affects site-specific concentrations of pathogens due to the proximity of contaminating elements like grazing animals and wildlife presence, water management practices like irrigation timing and type, and weather patterns like precipitation quantity and frequency (Pachepsky and Shelton, 2011).

### *2.2.7 Water Treatment*

If a grower is applying surface water to produce that will be consumed raw, one option is to treat the water. Treatment methods include filtration, chlorination, ozonation, UV light, electronic beam processing, and heat treatment, but not all of these are practical for large scale growing operations and may be cost prohibitive (EPA, 1999). Chlorine is a common disinfecting agent, but its effectiveness is reduced with increased concentrations of organic matter common in surface water (Suslow and Trevor, 1997).

### *2.2.8 Postharvest Applications of Agricultural Water*

It may be difficult to eliminate or counteract microbial contamination that occurred before harvest (Materon, 2003). Post-harvest, water utilized to wash produce must be sanitary to prevent

an introduction of contaminants and to prevent cross contamination from one produce item to another. If water is reused and recycled, it has the potential to encourage cross contamination (Luo et al., 2011). Therefore, produce operations that utilize water to wash, cool, and transport produce must ensure that an effective dose of a sanitizing agent is applied to the water (Suslow, Trevor, 2001). Produce is commonly washed post-harvest with additions such as chlorine, chlorine dioxide, ozone, hydrogen peroxide, and peroxyacetic acid (FDA, 1998). The efficacy of chlorine-based antimicrobials is based on the amount of free chlorine (Suslow, 2000). Factors that impact free chlorine concentrations in wash water include the load level of organic matter, or turbidity, pH, temperature, initial dose, and contact time (Luo et al., 2011; Suslow and Trevor, 2001).

Washing produce is a good practice to reduce the total microbial load on its surface, but natural plant contours, openings, cracks, uneven surfaces, and wounds make it difficult for the disinfecting agent to reach all microorganisms present on a plant surface (Suslow and Trevor, 2001). Therefore, in these areas of the plant surface, microbes are largely unaffected by permitted doses of post-harvest treatments. Studies have visualized microbes on *E. coli* O157:H7 inoculated cut lettuce leaves with confocal scanning microscopy within stomatas and cut edges, and attached to trichomes. A three-dimensional reconstruction of the interior portions of the lettuce leaves revealed that *E. coli* cells were 20 to 100 micrometers below the surface in stomatas and cut edges (Seo and Frank, 1999).

When lettuce leaves were inoculated with *E. coli* O157:H7 at high and low doses and sprayed with either water or 200 ppm chlorine, both were equally effective at reducing *E. coli* populations when the inoculum level was low, but chlorine was more effective when the inoculum level was high (Beuchat, 1999). The recommended chlorine concentration of 200 ppm can result in a 2.5 log CFU/g reduction of *E. coli* O157:H7, which was equivalent in effectiveness to a

deionized water spray of 1 or 5 minutes (Beuchat, 1999). A 10 percent organic load of lettuce slurry reduced the effectiveness of 30 ppm chlorine and 10 and 20 ppm peroxyacetic acid on *E. coli* O157:H7 to the same effectiveness as water (Zhang et al., 2009). Some studies have shown that chlorine is limited in its disinfecting power (Brackett, 1994; Solomon et al., 2002a), however, it is the most widely used sanitizing agent to prevent cross contamination during washing (Olaimat and Holley, 2012). For example, Seo and Frank (1999) showed that viable *E. coli* O157:H7 cells were present on cut lettuce stomata and edges exposed to a 20 ppm chlorine solution for 5 minutes. In contrast to the obvious antimicrobial activity, some studies have shown that the presence of chlorine can enhance the uptake of water into the plant, and therefore increase incidence of plant disease (Bartz, 1988; Boshoff et al., 1995). Other sanitation methods including chlorine dioxide, bacteriophage cocktails, and alkaline produce wash showed promising results better than the 2.5 log CFU/g reduction chlorine showed in other studies (Han et al., 2000; Harris et al., 2001; Leverentz et al., 2001).

The temperature of wash and cooling water that contacts produce post-harvest is an important potential cause of microbial infiltration. When produce is warmer than the environment, a negative temperature differential is created, which can draw contaminants present in improperly sanitized wash water through the item's pores (Bartz and Showalter, 1981; Zhuang et al., 1995). This occurs due to a contraction of gases in the produce item, and the resulting hydrostatic pressure pulling surface contaminants into the fruit or vegetable (Penteado et al., 2004). The area around the stem is most susceptible to pathogen entry (Bartz and Showalter, 1981; Buchanan et al., 1999; Eblen et al., 2004; Penteado et al., 2004). When warm tomatoes were immersed for 10 minutes or longer in cool water suspensions contaminated with *S. marcesens*, *P. marginalis*, or *P. aeruginosa*, they were infiltrated with water and these bacteria (Bartz and Showalter, 1981). Similarly, tomato

core tissues took up a significantly higher number of *S.* Montevideo when the temperature differential between the water and the tomatoes was 15 °C, than when the difference was 0 or 12 °C (Zhuang et al., 1995). Similar results have been obtained with mangoes (Penteado et al., 2004), oranges (Eblen et al., 2004), and apples (Buchanan et al., 1999; Burnett et al., 2000). The nature of the pathogen might not affect internalization frequency, as *Salmonella* and *E. coli* O157:H7 were found to internalize pathogens into oranges at similar frequencies (Eblen et al., 2004). Spoilage bacteria internalization into tomatoes has been demonstrated at a temperature differential as low as 8 °C (Bartz and Showalter, 1981). Although a temperature differential appears to increase bacterial infiltration, it does not necessarily need to be present to allow bacterial infiltration, as has been demonstrated with almonds (Danyluk et al., 2007), lettuce (Takeuchi and Frank, 2000), and parsley (Duffy et al., 2005). The land grant university system in the United States is well-positioned to respond to the increase in foodborne outbreaks due to produce with research, education, and outreach.

### 2.3. Land Grant University Responses to Produce Outbreaks

The Morrill Act of 1862 created land grant universities and instructed them to educate the public on the subject of agriculture (Martin, 2001). The Hatch Act of 1887 charged land grant universities with conducting research and experiments in the public interest at agricultural experiment stations (Martin, 2001). Agricultural experiment stations conduct scientific research that advance a state's agricultural industry. The scientists employed at the stations research biological, economic, and social problems of food and agriculture in the state. The Association of Public and Land Grant Universities (APLU) has a goal to provide leadership in ensuring a safe, secure, and abundant food supply. One of the areas of focus is "developing effective methods to prevent, detect, monitor, control, trace the origin of, and respond to potential food

safety hazards, throughout production, processing, distribution, and service of food crops and animals grown under all production systems" (APLU, 2010, p. 40). They state that more research is required into the nature and causes of foodborne contamination and mediation measures, and that universities must conduct food safety research to generate the knowledge to develop science based-food safety policy (APLU, 2010). The APLU has a goal to "develop more effective preventative controls by developing and testing technologies that assess critical points, mitigate risks in the food chain, and allow the timely detection of potential foodborne illness outbreaks, particularly in fresh produce" (APLU, 2017). One of the missions of Alabama Experiment Stations is "reducing foodborne illnesses in humans and monitoring food products from production to consumption, thereby guaranteeing the safety and accessibility of our nation's food supply" (AAES, 2018).

The Smith-Lever Act of 1914 established cooperative extension systems as a funding partnership with the USDA and land grant universities (Martin, 2001). Extension agents are mandated to provide educational opportunities and research-based information to citizens regarding agriculture (Tobin et al., 2013). Conveying information through educational programs about on up-to-date farm food safety practices is a major mission of extension agents (Tobin et al., 2013). Ideally, extension agents act as a conduit for information flow among land grant institutions, experiment stations, and farmers. These agents can then assess stakeholder needs through their close interactions with farmers and relay this feedback to land grant university researchers (Dunning et al., 2012). Experiment stations often demonstrate techniques before small farmers in the state invest in them, as well as help develop strategies for commercial growers. This research related to developments in agricultural science can then be conveyed to small farmers in the state by extension agents (Kahan, 2013).

The mission of the Morrill Act in 1862 was to promote sound and prosperous agricultural and rural life with the creation of land grant universities (Zimdahl, 2003). According to Harl (2003), several decades after the passage of the Hatch Act of 1887, the three land grant university functions: education, research, and extension, have shifted in priority towards research. Critique of traditional agricultural research that is supported by the Hatch Act is that it tends to focus on site-specific, short term problems (Budiansky, 1984). Budiansky claims that this type of work has ignored basic research and instead concentrated on narrowly focused applied research applicable only to small groups of farmers, especially large-scale operations.

In contrast, Harl (2003) argues that the predominance of research is a result of each university's push to be the "best" in the country. In more recent years, university rankings have been measured in terms of research and publication output with a premium on theoretical rather than applied research. Lockeretz (1995) believes that there is an inherent conflict of goals in the research mission statements of land grant universities. Research can be a service to farmers or a traditional academic activity, but not both. The audience for university research is either scientific peers or farmers and agribusiness companies that will adopt and utilize what is learned. The drive for researchers to obtain limited public funding sometimes leads to acceptance of funding from private firms with agendas separate from the university. Pollack (2001) suggests that agricultural research is increasingly being controlled by private companies, not land grant universities. The land grant university mission of extension may be losing priority at some institutions, but others have initiated new produce safety related education and outreach programs.

### *2.3.1 Produce Safety Extension Efforts*

In terms of produce safety, Cooperative Extension Systems offer food safety courses on the topics of GAPs and the PSR. The Extension system is supported by federal, state, and local governments, and can allocate funds to different priority areas, such as food safety education programs, at their discretion (Tobin et al., 2013; Wang, 2014). Food safety educators might only have one opportunity to educate small scale growers about best food safety practices on the farm face-to-face due to limited availability of trainers (Laury-Shaw et al., 2015). GAP and PSR workshops in the US tend to last one work day.

The safety of fresh produce is important to the economies of several large states. There has been increased university effort to research, educate, and perform outreach on the topic of on-farm food safety practices in some of these states. In 2017, the top vegetable and melons producers in the country were California, Arizona, Florida, Washington, and Idaho. The top fruit and nut producers were California, Washington, Florida, Oregon, and Michigan (USDA ERS, 2018). California fresh produce contributed approximately 27 billion dollars to the state economy in 2016 (California Department of Food and Agriculture, 2017). Fresh produce accounted for 55 percent of cash income in 2016 in California (California Department of Food and Agriculture, 2017).

For example, the University of California-Davis created the Western Center for Food Safety, a joint FDA cooperative that conducts research on pre- and post-harvest produce safety issues. They also provide technical educational assistance to the farming community in the form of outreach materials, food safety workshops, and educational programs (Western Center for Food Safety, 2018). In 2007, the University of California-Davis created the Center for Produce Safety which integrates academic, industry, and government research to enable produce safety. It

has funded numerous research projects and showcases the work for industry, academic, and government leaders at an annual symposium (The Center for Produce Safety, 2018). Another joint organization, the Produce Safety Alliance, is a project between Cornell University, the USDA, and the FDA. Its mission is to provide training and education to the produce industry and associated groups. The main curricula consists of GAPs and the PSR, as well as outreach focused on produce growers, packers, and cooperatives with an emphasis on small and very small sized farms (Produce Safety Alliance, 2018). The newly established (2015) Cornell Institute for Food Safety, a separate initiative, partners experiment stations with extension agents to teach on-farm food safety practices (Institute for Food Safety, 2018). In the Southeastern US, the Southern Center at the University of Florida was created to build a collaborative infrastructure to support PSR training, education, extension, outreach, and technical assistance for small and medium size growers (Southern Center, 2018). Auburn University is not a part of the Southern Center. There has been no research on the effectiveness of these types of PSR-focused outreach programs. The relatively small size of the Alabama produce industry explains the lack of produce safety-specific education and outreach efforts compared to the other states mentioned.

### *2.3.2 Alabama Agriculture Industry*

Agriculture represents a large portion of the state of Alabama's economy. Of $4.7 billion worth of agricultural related sales in 2010, the sector was dominated by poultry and egg production (66 percent of sales), cattle (8 percent of sales), and greenhouse, nursery, and floriculture (5 percent of sales), while vegetables and fruit represented 1.4 percent of total sales (Fields et al., 2013). Nevertheless, specialty crops like fresh produce are still a large area for potential growth to the state's economy (Fields, 2017). Fresh produce contributed approximately 160 million dollars in sales to Alabama's economy in 2016 (Fields, 2017). The USDA definition

of a small family farm is a farm that has a gross sales of less than $350,000 (USDA, 2018). Approximately 93 percent of Alabama fruit and vegetable farmers in 2012 would fall under the small family farm definition (USDA, 2012). Small farms often receive education about farming practices from the Cooperative Extension System.

In the state of Alabama, GAP training is provided by members of the Cooperative Extension system. As of September 2018, Alabama has 34 (1.3 percent) GAP certified farms out of 2,655 total farms that grow produce (according to the 2012 USDA Census of Agriculture) (USDA, 2017).

### 2.4. Policy and Regulatory Responses to Produce Outbreaks

The authority of the FDA to legally regulate on-farm activities has been an issue since its creation in 1938 (Burrows, 2007). Therefore, before the implementation of the Produce Safety Rule, the FDA was limited to promoting "guidance" in the form of codified Good Agricultural Practices (GAPs), which are voluntary produce safety standards. GAPs were not created until the US Department of Health and Human Services (HHS), USDA, and EPA sent a letter to the president in 1997 identifying fresh produce as an area of public health microbial concern (EPA et al., 1997). GAPs are designed to ensure that fruits and vegetables are produced, packed, handled, and stored as safely as possible. They are not required by law, but the government and private sectors are more commonly encouraging produce growers to implement them (Tobin et al., 2013). Members of the private sector such as supermarkets have been more frequently implementing their own food safety policies requiring growers to provide evidence of compliance with GAPs (Palma et al., 2010; Ribera et al., 2012). Some supermarkets may require growers to create a food safety plan for their operation, while some may insist that growers verify their on-farm food safety practices through a third party certification (Tobin et al., 2013).

GAPs address pre-harvest and post-harvest activities related to safe harvesting, production, and handling of produce, food quality, food security, and environmental effects due to agriculture (Hobbs, 2013; Tobin et al., 2013). The FDA's guide to GAPs identifies hazards associated with fresh produce, the scientific basis of the concerns, and good agricultural or management practices that can be applied to reduce the risk of contamination of fresh produce (FDA, 1998).

Despite the existence of federal GAP guidelines and increased grower awareness of their role, foodborne illnesses due to produce continued to occur in the 2000s (Paggi et al., 2013). A series of notable produce outbreaks led to heightened consumer, government, and industry concern in the US about fresh produce safety. For instance, in 2006, a case of spinach contaminated with *E. coli* O157:H7 caused 205 illnesses, 103 hospitalizations, and 3 deaths in 26 US states (CDC, 2006b). In the same year, three other high-profile outbreaks were caused by *Salmonella*-contaminated tomatoes and *E. coli* O157:H7-contaminated lettuce (Doyle and Erickson, 2008). Consequently, negative media attention and consumer pressure led to increasing calls for a more effective food safety system (Fisher, 2015; Nucci et al., 2009). This acted as a catalyst to develop industry-based food safety standards (Paggi et al., 2013).

The California leafy greens industry responded to this growing pressure by developing a voluntary set of field-level food safety standards in 2007 known as the Leafy Greens Marketing Agreement (LGMA) (Calvin et al., 2017). The core of LGMA is specific good agricultural practices (GAPs), water quality standards, food safety practices such as controlling runoff from animal production operations, and mandatory third party audits to ensure compliance (CLGMA, 2018). Similarly, the California Cantaloupe Board developed a mandatory commodity-specific

food safety program following the cantaloupe *Listeria* outbreak that killed 36 Americans (CDC, 2012; Paggi et al., 2013).


**Figure 2.2 Timeline of produce safety regulations.**

Drawing on this LGMA, the US Congress passed an amendment to the 1938 US Food, Drug, and Cosmetic Act (FDCA), known as the Food Safety Modernization Act (FSMA) (2011). Figure 2.2 shows the order in which these regulations were passed. FDCA charged the U.S. Food and Drug Administration (FDA) with overseeing produce safety and focused almost exclusively on post-harvest inspection, particularly processing and packing plants. FSMA, however, required the FDA to establish science-based minimum standards for the production and harvest of raw fruits and vegetables, and to develop a set of regulations based on known safety risks. Thus, the amendment was the first major change to FDCA in 70 years (Strauss, 2011). FSMA is the topic of Chapter 3, which will address the PSR, small farm compliance requirements, and the PSR AWP. It is also the focus of Chapter 5, which will detail the scientific basis of the AWP of the PSR and implementation challenges for small farmers and the FDA.

# 3. Literature Review: The FSMA Produce Safety Rule

This chapter will discuss the Food Safety Modernization Act (FSMA) Produce Safety Rule, the Produce Safety Rule (PSR) agricultural water requirements, and requirements for small farms.

## 3.1. The FSMA Produce Safety Rule

The Food Safety Modernization Act (FSMA) is an amendment to the Federal Food, Drug, and Cosmetic Act (FDCA) that enables the Food and Drug Administration (FDA) to better protect public health (FDA, 2011a). It was created to shift the nation's food safety system from responding to food safety problems to preventing them. It was signed by Barack Obama into law in January of 2011. The FSMA includes the Produce Safety Rule, which requires the FDA to establish science-based minimum standards for the safe production and harvesting of raw fruits and vegetables, and to adopt a final regulation based on known safety risks, which are defined by a history of repeated and confirmed foodborne illness outbreaks of at least two people from a common source (CDC, 2000; FDA, 2015b).

As detailed in Chapter 5, during the crafting regulations, the produce safety standards received high levels of input from scientists, industry stakeholders and consumers during the comment periods (FDA, 2015b, Gradl and Worosz, 2017). The standards identified procedures, processes, and practices that will prevent the introduction of known or foreseeable hazards into produce, and provide reasonable assurances that it is not adulterated under section 402 of the FDCA (FDA, 2015b). Before the introduction of the PSR, there was no mandatory regulatory oversight of produce safety in the United States.

There are seven provisions throughout the Code of Federal Regulations (CFR) concerning the Produce Safety Rule (FDA, 2015b):

- **Worker training and health and hygiene.** Establishes qualification and training requirements for all personnel and their supervisors who handle produce or food-contact surfaces.
- **Agricultural water.** Defines water used in agriculture and requires that it must be safe and of adequate sanitary quality for its intended use.
- **Biological soil amendments**. Establishes requirements for determining the status of an amendment of animal origin as treated or untreated, and for their handling, conveying, and storage.
- **Domesticated and wild animals**. Requires measures to assess relevant areas during growing if there is a reasonable probability that grazing animals, working animals, or animal intrusion will contaminate produce.
- **Growing, harvesting, packing, and holding activities**. Will minimize the risk of serious adverse health consequences or death from the use of, or exposure to, covered produce.
- **Equipment, tools, and buildings.** Establishes requirements for equipment, tools, instruments, controls, and buildings that contact produce.
- **Sprouts.** Establishes measures that must be taken related to seeds or beans for sprouting, and for their growing, harvesting, packing, and holding.

The Produce Safety Rule became effective January 26, 2016. The bulk of the PSR applies to "covered produce," which is the harvestable portion of edible crops (FDA, 2015b). Items exempt from the definition of covered produce include crops that are "rarely consumed raw" such as potatoes and beans, and crops that receive adequate commercial processing such as canned tomatoes to reduce the presence of pathogens.

### *3.1.1 Food Safety and Farm Size under FSMA*

A farm that sells more than $25,000 worth of produce in the previous three years is considered subject to the PSR. These farms are divided into categories. A “very small” business, is any farm that sells less than $250,000 worth of produce in the past 3 years, whereas a “small” business is one that sells less than $500,000 but more than $250,000 worth of produce. Farms not defined as very small or small businesses are given 2 years past the effective date of the Rule (January, 2016) to comply with the Rule (January, 2018), excluding the sprout and agricultural water standards. Very small businesses are given 4 years past the effective date, and small businesses are allowed 3 years to comply. Very small businesses, small businesses, and all other businesses have different compliance dates for the water regulations (January 2022, 2021, and 2020, respectively).

The Tester-Hagan amendment is an amendment to FSMA, which provides an exemption from the produce safety standards “if a farm can demonstrate a previous three-year average gross income of less than $500,000, and over 50 percent of sales were to consumers, restaurants, or retailers within a certain geographic region” (Schieber, 2013). This region is defined as a food establishment in the same state or within 275 miles of the farm. The amendment was criticized by both food safety proponents and the agriculture industry, who observed that food safety issues can arise on farms of any size (Beyranevand, 2013). One compromise that led to the adoption of the Tester-Hagan Amendment was the addition of the "exemption withdrawal" provisions in the Act. The Tester Amendment provided the means of withdrawing an exemption from a farm under two circumstances: when it is directly linked to an active outbreak investigation; and when the FDA deems that a foodborne outbreak could arise from the farm due to conditions that affect the

safety of the produce grown, harvested, packed, or held. With the exemption withdrawal in place, all produce is potentially subject to the requirements of the PSR.

### *3.1.2 Food Safety and Small Farms*

Data concerning the safety of produce from small farms is very limited. As mentioned in Chapter 2 and directly above, there is no single federal definition of "small farm." In 2008, farms with gross annual sales less than $50,000 accounted for 81 percent of sales at farmers' market and roadside stands (Low and Vogel, 2011). It has been found that growers who direct market their product (e.g., farmers market, roadside sales) are significantly less likely to be GAP certified than growers who market wholesale (Marine et al., 2016a). Produce sold directly to consumers has caused foodborne outbreaks. For instance, *Campylobacter* contaminated peas sickened 18 people in Alaska in 2008 (Gardner et al., 2011), and *E. coli* O157:H7 contaminated strawberries sold at roadside and at farmers' markets sickened 16 and caused one death in Oregon in 2011 (Food Safety News, 2011). In a sampling of herbs at thirteen farmers' markets in Los Angeles and Seattle, one parsley sample was positive for *Salmonella*, and 24 percent of the samples were positive for generic *E. coli* (Levy et al., 2015). In a sampling of produce from farmers' markets in West Virginia and Kentucky, 18.6 percent of spinach, 11 percent of tomatoes, 18.5 percent of peppers, and 56.3 percent of cantaloupes were positive for *Salmonella enterica* subsp. *enterica*. Approximately four percent of all samples were positive for *Listeria*, with half of these samples positive for *Listeria monocytogenes* (Li et al., 2017).

The knowledge that small growers possess of produce safety practices must be gauged because many preventative efforts can be made throughout the supply chain. A select number of surveys of small produce grower GAP knowledge have been conducted. A survey of 160 small-scale Kentucky growers found that 90 percent were familiar with GAPs, 55 percent used GAP

protocols for soil amendments, and 47 percent used water quality GAP protocols. Yet, 55 percent to 72 percent of survey participants were unable to identify causes of potential microbial contamination such as soil, ice, cooling, and refrigeration (Vincent et al., 2015). A survey of 226 growers in Georgia, South Carolina, and Virginia indicated that 34 percent of farmers used raw manure or a mixture of compost and raw manure. Only 66 percent of farms had easily accessible hand washing or bathroom facilities, 51 percent indicated that domestic animals had access to production areas, 27 percent used untested water to irrigate produce, and 16 percent used untested water to wash produce. Only 43 percent of farmers indicated that they sanitize surfaces that come in contact with produce and 33 percent sanitize transport containers between uses (Harrison et al., 2013a).

## 3.2. Produce Safety Rule Agricultural Water Regulations

### *3.2.1 Microbial Water Quality Criteria*

The AWP of the Produce Safety Rule establishes two sets of criteria for microbial water quality, both based on generic *E. coli,* which is an indicator of fecal contamination. This criteria is found at 21 CFR sections 112.44 (a) and (b). At the beginning of each growing season, "as appropriate, but at least once annually," the grower must inspect all of his or her water systems to identify conditions that are reasonably likely to introduce known or foreseeable hazards into or onto produce or food contact surfaces in light of the grower's practices and conditions. Under this inspection, growers must consider the nature of each water source (e.g., well, surface), the extent of his or her control over the source, the degree of protection of each source, the use of nearby land, and the likelihood of introducing hazards to the agricultural water by another user of the water before it reaches the farm. In addition, growers must maintain all water systems and sources on a regular basis to ensure that they are not a source of contamination to produce, food contact

surfaces, areas used for growing, harvesting, or holding activities, or water. Such maintenance includes regularly inspecting the sources to identify hazardous conditions, correcting deficiencies such as broken equipment, and keeping the source free of debris, trash, animals, and other sources of contamination.

The first water quality criteria requires that "no generic *E. coli* is detected in agricultural water in which it is reasonably likely that potentially dangerous microbes would be transferred to produce through direct or indirect contact" (FDA, 2015b). Examples include hand washing water used pre- and post-harvest, water used on food-contact surfaces, water that comes into direct contact with produce, including water used to make ice, applied to produce pre- and post- harvest for washing and cooling activities, and to irrigate sprouts. If generic *E. coli* is detected, the use of this water must be immediately discontinued and corrective actions taken before re-use for any of the previously listed purposes. The rule prohibits use of untreated surface water for any of the above purposes (FDA, 2015b).

The second water quality criteria is for agricultural water that is directly applied to growing produce other than sprouts. The criteria, based on the Environmental Protection Agency (EPA) standards for recreational water, include two values: the geometric mean (GM) and the statistical threshold value (STV). These criteria do not specifically consider *E. coli* data from agricultural water, but instead, they were created for recreational water testing. The geometric mean represents the average amount of generic *E. coli* in a water source. The STV is the amount of variability in the water *E. coli* results. It can be more simply described as the level at which 90 percent of the samples are below the required value. The required GM of samples is 126 or less colony forming units (CFU) of generic *E. coli* per 100 mL of water, and the required STV of samples is 410 CFU or less of generic *E. coli* in 100 mL of water. If water does not meet this criteria, corrective actions

must be taken as soon as practical or within one year. Farmers with agricultural water that does not initially meet the microbial criteria have options such as treating the water (21 CFR 112.43) or allowing die-off time before the water is suitable to use on their crops (FDA, 2015b).

Untreated surface water that directly contacts growing produce is considered the most vulnerable to outside pathogenic influences (Whitman et al., 2011). The FDA requires farms to conduct an initial survey with a minimum of 20 samples, collected as close as is practical to harvest, and represent the grower's use of the water over a two to four year time span. The initial survey findings are used to calculate the GM and STV and to determine if the water meets the required microbial quality criteria. After the initial survey, an annual survey of a minimum of five samples per year is required to update the calculations of GMs and STVs. In total, the five new samples, plus the previous most recent 15 samples, create a rolling data collection of 20 samples for use in confirming that that the water meets the microbial quality criteria by recalculating the GM and STV (FDA, 2015b). Untreated ground water that is directly applied to growing produce has less strict sampling frequency requirements than that of untreated surface water. A minimum of four samples must be collected over a harvest season or one year initially, then one sample per year must be collected, creating a rolling dataset of four samples (FDA, 2015b). Untreated ground water used for purposes where no detectable generic *E. coli* is allowed must be sampled, initially, four times during a growing season or year. If these tests show no detectable *E. coli*, the test can then be repeated once annually. If the test is positive for *E. coli* presence, the four sample scheme must resume during the next growing season or year (FDA, 2017c). Again, if these tests show no detectable *E. coli*, the test can then be repeated once annually.

There are no requirements in FSMA to test agricultural water from public water systems that are defined under the Safe Drinking Water Act regulations (section 112.46(a)), the microbial

quality criteria of section 112.44(a), or water treated according to section 112.43 (i.e., water treated with EPA-approved antimicrobial pesticides or other methods to make water safe and of adequate sanitary quality for its intended use). The FDA explained their scientific basis, and underlying statistical analysis, for testing untreated surface water in a reference memo that accompanied the PSR supplemental notice (Bowers, 2015). In this memo, the FDA evaluated sample size requirements for estimating the microbial water quality profile of untreated surface water sources with a focus on the use of a rolling approach to calculating the microbial water quality profile. If a grower has scientific data that an alternative method would provide the same level of public health protection use of said method is permitted (FDA, 2015b). Regardless of testing method, sufficient records must be kept for all aspects of water inspection, testing, or treatment by the grower.

### *3.2.2 Water that Fails to Meet Agricultural Water Criteria*

According to section 112.45, if generic *E. coli* is detected in water at levels above the allowable microbial criteria, the water must immediately be discontinued. Before re-use, growers must (1) re-inspect the entire agricultural water system to identify conditions that may introduce hazards, make the needed changes to correct the problem, and determine if the measures were effective by sampling the water and ensuring the water meets the microbial criteria of 112.44, or (2) treat the water in accordance with section 112.43.

If water does not meet the FDA's microbial criteria for growing produce, it must be discontinued as soon as practical, and no later than the following year (FDA, 2017c). However, there are four possible approaches in which to apply corrective actions and continue use of the water source. As mentioned in the microbial water quality criteria section, the first option to meet the criteria is to allow time for microbial die-off on the field during production between last

irrigation and harvest using a die-off rate of 0.5 log per day for no longer than four days (see previous discussion in Chapter 2 on factors such as timing of application that affect agricultural water as a route of produce contamination). An alternate die-off rate and maximum time interval on the field during production can be utilized if scientifically valid. A second potential corrective action is to allow time for microbes to die off between harvest and end of storage, or to be removed during commercial activities such as washing and sanitizing. A third option is for the grower to *re-inspect* the entire agricultural water system to identify conditions that may introduce hazards, make the needed changes to correct the problem, and determine if the measures were effective by sampling the water. The fourth option is to treat the water according to section 112.43; growers may use a method such as a pesticide device (e.g., an instrument that is intended to mitigate a pest or any form including bacteria (EPA, 2013)) such as UV lights and ozonators, or an antimicrobial pesticide (e.g., a substance intended to disinfect, sanitize, or reduce the growth of microbial organisms) such as chlorine.

### *3.2.3 Opposition*

The FDA received feedback from many stakeholders regarding the practicality of the water testing regulations. As of December 2018, the FDA proposed to extend the agricultural water compliance dates by another two years. The FDA states in its proposed extension of the compliance date that additional time would allow them to consider additional approaches to address issues concerning the water testing requirements such as sampling frequency and location, as well as additional options for reducing the costs and improving the flexibility of the testing requirements (FDA, 2017a). In light of this opposition, Chapter 4 provides an overview of challenges with agricultural water sampling, water testing methods, and early assessments of agricultural water using the PSR standards.

# 4. Literature Review: Agricultural Water Sampling Methodology and Quality

In the context of the AWP of the PSR, this section will address challenges associated with sampling and testing water for pathogens, the various water quality testing methods and associated issues, and early assessments of agricultural water using the PSR criteria.

## 4.1. Challenges Associated with Sampling and Testing Water for Pathogens

The PSR establishes criteria for testing agricultural water that contacts produce pre- and post-harvest. There are challenges associated with testing any biological sample for pathogen presence. Factors that influence microbiological testing results in the case of agricultural water include sampling (the sampling procedure, the location of the water sample (i.e., field, packing house), the quantity and volume of samples, frequency of sample collection), transportation (sample temperature maintenance during transport to the laboratory, time transporting sample to laboratory), testing method (volume of sample tested and dilutions utilized, the method of pathogen detection), and potential cross contamination during sampling, transport, or testing (Fresh Produce Safety Center, 2015).

As previously discussed in Chapter 2.3, the generally accepted method to determine the microbiological quality of a water body is to use certain bacteria as indicators of potential pathogenic bacteria (Hamilton et al., 2005). One of these indicator bacteria is total coliforms. The "total coliform" group bacteria are generally not of fecal origin, more often originating from vegetation and soil (Edberg et al., 2000). The detection of fecal coliforms indicates a higher safety risk than total coliform detection, and that recent fecal contamination might have occurred (NMCL, 2017). *E. coli* is a major subgroup of the fecal coliform group, and its presence indicates fecal contamination by warm blooded animals (US Geological Survey, 2016). However, there is a frequent discrepancy between indicator bacteria and pathogen concentrations due to the fact that

traditional fecal indicator bacteria occur in nature (Pillai and McElhany, 2011). The fecal coliform and total coliform tests, can detect bacteria of non-fecal origin such as *Klebsiella*, as it is part of the total coliform group (Barrell et al., 2000; Edberg et al., 2000). *Klebsiella* is associated with vegetation, agricultural products, wood pulp, and paper mill effluent. Therefore, false positive results could be obtained that overestimate actual fecal contamination levels in the water. In addition, the fecal coliform test must be performed under exact temperature standards and often results in incorrect and difficult to interpret false negative and positive results (Edberg et al., 2000). Many fecal indicator organisms are ubiquitous in the water environment (Edberg et al., 2000). For these reasons, generic *E. coli*, which is the only fecal coliform bacteria of true fecal origin, has been shown to be the coliform most consistently associated with fecal contamination (Diez-Gonzalez, 2011; Gerba, 2009).

*E. coli* is specific in human and animal feces where it is present in high amounts ($10^9$ CFU/g). It also does not appreciatively multiply in the environment outside the host (Edberg et al., 2000). *E. coli* is the accepted fecal indicator by the environmental science community because it is almost exclusively associated with a fecal source and its detection methods are rapid, accurate, specific, inexpensive, and sensitive (Diez-Gonzalez, 2011; Leclerc et al., 2001; Tallon et al., 2005). *E. coli* was demonstrated to be a useful indicator of *Salmonella enterica* serovar Typhimurium originating from manure applied to vegetables (Natvig et al., 2002). It also has been shown to be an indicator for the presence of *Salmonella* species and *E. coli* O157:H7 (Ceuppens et al., 2014; Ogden et al., 2001; Park et al., 2013). However, in a California produce irrigation water study, generic *E. coli* presence was not correlated with *Salmonella* or *E. coli* O157:H7 presence from the same water sources (Benjamin et al., 2013). Therefore, the validity of generic *E. coli* as an indicator of pathogen presence should be assessed for each specific situation (Ceuppens et al., 2014).

There are some problems with using *E. coli* as an agricultural water contamination indicator. *E. coli* is ubiquitous in agricultural water: water masses move past their original sampling site, therefore indicator concentrations at a single site can vary, *E. coli* can take various pathways in agricultural water systems, and stream sediments have been shown to harbor generic *E. coli* (National Research Council, 2004b; Whitman et al., 2011). For these reasons, when the objective is to detect the presence of a pathogen in low concentrations such as *E. coli* O157:H7 instead of the more common generic *E. coli*, some studies have used the "Moore swab" methodology, which is the collection of bacteria on a cotton swab in a flowing water body over one or more days (Benjamin et al., 2013; Cooley et al., 2007). However, the problem with this method is that the bacteria cannot be quantified because of the unknown volume of water that is associated with the cotton material (Benjamin et al., 2013). An alternative to this method is "grab sampling," which involves collecting a small amount of water at a particular time and location. This method can quantify bacteria present per a volume of water. However, grab sampling might result in false negative results if the *E. coli* is present in low levels or is not equally distributed in the water source (Benjamin et al., 2013).

Microbial results obtained from surface water vary spatially and temporally (Nnane et al., 2011). Therefore, it is important that a sampling frequency is established that detects these changes. Environmental factors that can cause variance in the concentrations of microbial indicators should be considered when creating a sampling frequency plan (Won et al., 2013). These factors include non-point source pollution (e.g., run-off that can not be traceable back to a single source) and extreme precipitation events leading to runoff (Curriero et al., 2001; Gould, 1990; Jamieson et al., 2002; Kistemann et al., 2002). Scientific justification for irrigation water sampling frequency is lacking. Sampling infrequently does not allow growers to assess the true level of risk

in a water body, but sampling too frequently creates an economic burden for growers (Won et al., 2013). It was also found that a single water sample did not reflect the microbiological quality of water over an irrigation period (Won et al., 2013).

### 4.2. Water Testing Methods

The FSMA Produce Safety Rule permits various methods to test water samples for the presence of generic *E. coli*. The method stated in the text of the PSR (§ 112.46 and 112.151) is EPA Method 1603: *E. coli* in Water by Membrane Filtration Using Modified membrane-Thermotolerant *E. coli* Agar (Modified mTEC). Method 1603 is a membrane filtration method using the selective and differential media modified mTEC. The modified mTEC is incubated at 35 °C for 2 ± 0.5 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5 °C for 22 ± 2 hours. Red or magenta colonies are counted after this period. Another method besides EPA's 1603 can be utilized if it is "at least equivalent to the method of analysis in 112.151(a) in accuracy, precision, and sensitivity (FDA, 2015b).

FDA permits other equivalent methods such as (a) EPA Method 1103.1: *E. coli* in Water by Membrane Filtration Using membrane-Thermotolerant *E. coli* Agar (mTEC), (b) EPA Method 1604: Total Coliforms and *E. coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium), and (c) Hach Method 10029 for Coliforms – Total and *E. coli* (mColiBlue24 medium) (FDA, 2017f). Similar to EPA Method 1603, mTEC media and filter are incubated at 35 °C for 2 ± 0.5 hours, and then at 44.5 °C for 22 ± 2 hours. Unlike EPA Method 1603, however, following incubation, the filter is transferred to a filter pad saturated with urea substrate. After 15 minutes, yellow, yellow-green, or yellow-brown colonies are counted. The EPA Method 1604 membrane filtration method involves placing the filter on MI agar followed by a single incubation step at 35 °C for 24 hours. The colonies are inspected for the presence of blue

color from the breakdown of Indoxyl-β-D-glucuronide by the *E. coli* enzyme β-glucuronidase. The Hach 10029 Method uses 2 mL aliquots of mColiBlue24. After membrane filtration, the plates and filters are incubated at 35 °C for 24 h. After incubation, blue colonies are indicative of *E. coli*, which hydrolyze the enzyme substrate BCIG (5-bromo-4-chloro-3-indolyl-beta-D-glucuronide) to an insoluble salt.

Most of the EPA 1603 equivalent methods use the membrane filtration method, which is subject to competition from high levels of background microflora on the growth media from the water sample. Background microflora can be reduced by diluting the sample, however dilutions to reduce the background microflora may also reduce the concentration of the target organism to below detectable limits (Mattelet, 2006). Another hindrance in the membrane filtration method is sediment. Sediment present in water samples can get trapped in the membranes, providing an extra source of nutrients and potentially altering the results (Mattelet, 2006). A pitfall of conventional *E. coli* enumeration methods is that they use an end product of a metabolic pathway such as lactose fermentation to signal the presence or absence of a particular organism (Alonso et al., 1998; Sartory and Watkins, 1998). However, if bacteria have been subject to thermal stress, short incubation times, or an inhibitory culture medium, they may fail to create the end product of lactose fermentation, and will not be counted even if they are present and viable (Edberg et al., 2000; Sartory and Watkins, 1998).

Some of the FDA approved *E. coli* detection equivalent methods, such as Hach 10029, EPA 1603, and EPA 1604 use chromogenic media, which present some challenges depending on the water source. The chromogenic method for *E. coli* detection targets the enzyme activity of β-glucuronidase (Sartory and Watkins, 1998). Studies comparing various *E. coli* detection methods have found that chromogenic media often overestimated the number of bacteria present in ground

water and drinking water (Landre et al., 1998; Pitkänen et al., 2007). One PSR approved method using mColiBlue24 was found to have a failure rate of 23 percent in detecting *E. coli* and coliforms in ground water (Olstadt et al., 2007), particularly when the water was characterized with a low pH, high numbers of heterotrophic bacteria, and high alkalinity. Authors speculate that stress experienced by the target organism affects the expression of the target enzyme on the chromogenic media (Clark et al., 1991; Sartory and Howard, 1992; Sartory and Watkins, 1998; Schets et al., 1993). These stresses include oxidative stress from chlorine and other disinfectants, environmental stress, and competitive inhibition by non-target bacteria on overloaded membrane filters.

Standard methods for the quantification of generic *E. coli* in water samples dictate that the sample must be processed within 6 hours of collection, and subsequently analyzed in the laboratory in under two hours (American Public Health Association, 2005). Water samples also must be maintained below 10 °C. In a study assessing the effect of sample holding time on *E. coli* concentrations at 0, 8, 24, 30, and 48 hours after sample collection, variable results were found. The majority of samples held at 10 °C did not exhibit a significant change in *E. coli* counts at 8, 24, and 30 hours after sample collection. However, there were samples held at 10 °C analyzed using both the Colilert and the membrane filtration methods in all trials that showed significant decreases in *E. coli* densities as early as 8 hours after sample collection (Pope et al., 2003). Regardless, authors have concluded that most samples can be retained at this temperature for more than 8 hours and up to 24 hours after sample collection without significantly impacting *E. coli*, total coliform, or fecal coliform results (McCarthy et al., 2008; Pope et al., 2003; Selvakumar et al., 2004). However, other studies have concluded that holding water samples at 4-5 °C beyond 24 hours generates significantly different *E. coli* and total coliform results compared to zero hours (McDaniels et al., 1985; Selvakumar et al., 2004; Toranzos et al., 2007). It has also been found

that the temperature the samples are stored at highly impacts the survival of *E. coli* in water samples (McDaniels and Bordner, 1983; Pope et al., 2003; Toranzos et al., 2007). Approximately 60 percent of samples held at 20 and 35 °C had significantly lower *E. coli* densities from 8 to 48 hours after sample collection (Pope et al., 2003). It was also found that water samples sometimes froze during cold storage, which can cause lysis of the bacterial cells (Pope et al., 2003).

### 4.3. Early Assessments of Water Quality using Produce Safety Rule Standards

Several studies have applied the Produce Safety Rule water quality criteria to regional areas of the United States. Results of these studies as well as a select few other water quality assessments are presented in Table 4.1. One study sampled three Southern Georgia farms during three growing seasons. Generic *E. coli* was detected in both farm ponds and irrigation distribution systems, but the concentrations met the water quality standards. Low concentrations of *Salmonella* were detected in the irrigation systems (Antaki et al., 2016). Ten irrigation ponds were sampled in Southern Georgia and Northern Florida. All ponds and 28 percent of samples were positive for *Salmonella,* and levels correlated with increased rainfall and temperature. All ponds met the current PSR standards for generic *E. coli* presence, and it was found that levels of *E. coli* were a significant predictor for the probability of *Salmonella* occurrence (Luo et al., 2015). *E. coli* concentrations in stream water in southeastern Pennsylvania were assessed using watershed-scale water quality modeling. It was found that a change in sampling location, rain levels, and time of year impacted *E. coli* concentrations. Fifteen to seventy percent of samples exceeded the regulatory threshold (Hong et al., 2017). All ponds sampled in a Central Florida study met the produce safety rule standards for *E. Coli,* but the *invA* gene, a virulence gene from *Salmonella,* was detected in all of the six ponds sampled (Topalcengiz et al., 2017) .

**Table 4.1 Survey of the quantity of generic *E. coli* present in surface, ground, and municipal water from recent studies.**

| Study | Water type | Water source | Detection Method | Arithmetic mean concentration generic *E. coli* log CFU/ml | Region |
|---|---|---|---|---|---|
| **Draper et al. (2016)** | Surface water used for irrigating produce | Ponds, creeks, rivers, streams, lakes, open wells at same depth of water intake source | *E. coli*/Coliform Petrifilm | 0.905 | Pennsylvania, US |
| **McLain and Williams (2008)** | Constructed wetland receiving treated municipal effluent | Wetland water column | mColiBlue24 | 2.92 | Arizona, US |
| | | Wetland outlet | | 2.76 | |
| **Topalcengiz et al. (2017)** | Surface water fed with well water used for irrigating produce | Ponds | QuantiTray 2000/Colilert | 1.25 $\log_{10}$ MPN/100 mL (geometric mean) | Central Florida, US |

| Study | Water Type | Water Source | Detection Method | Arithmetic mean concentration generic *E. coli* log CFU/ml | Region |
|---|---|---|---|---|---|
| **Antaki et al. (2016)** | Surface water and wells used to irrigate produce | Ponds (1 m below surface) | ChromeEC agar | 2.08 | Southern Georgia, US |
| | | Pond-surface | | 0.88 | |
| | | Irrigation output (sprinkler heads, end of drip line) | | 1.72 | |
| | | Wells | | 0 | |
| **Luo et al. (2015)** | Surface water used to irrigate produce | Ponds (surface and 50 cm below surface) | Quantitray | 0.8 $\log_{10}$ MPN/100 mL (geometric mean) | Suwanee watershed, SE US |

| Study | Water Type | Water Source | Detection Method | Arithmetic mean concentration generic *E. coli* log CFU/ml | Region |
|---|---|---|---|---|---|
| **Hong et al. (2017)** | Surface water located in an agricultural watershed | Stream water in a watershed | Not specified | 2.06 $\log_{10}$ CFU/ 100 mL (geometric mean) | SE Pennsylvania, US |
| **McEgan et al. (2013)** | Surface water used for agriculture | Lakes, ponds, rivers, canals | Colisure | 1.7 $\log_{10}$ MPN/100 mL | Central Florida, US |
| **Holvoet et al. (2014)** | Well water used for lettuce irrigation | Well water and sprinkler taps | Rapid *E. coli* 2 chromogenic media | 1.5 $\log_{10}$ CFU/mL | Belgium |
| **Benjamin et al. (2013)** | Surface water used for produce irrigation | Ponds, reservoir water, streams, rivers, creeks, ditches | Quantitray 2000/Colilert | 2.78 $\log_{10}$ MPN/100 mL | California central coast, US |
| | | Well water | | 0 MPN/100 mL | |

One study evaluated the Produce Rule's agricultural water quality criteria utilizing data from Topalcengiz et al. (2017) mentioned in Table 4.1 It was found that 20 samples over a 2 to 4 year period was not sufficient to accurately characterize water quality at a surface water source because of high variability in *E. coli* levels. The observed standard deviations of the *E. coli* data were up to three times higher than a theoretical pond that would comply with the Rule. A pond in compliance would have a standard deviation of 0.92 $\log_e$ units (Havelaar et al., 2017). Similarly, surface water used for irrigation in Pennsylvania had standard deviations of *E. coli* of 2.19 and 2.76 log units in 2 consecutive years (Draper et al., 2016). Therefore, to obtain the same precision of the geometric mean required by the Rule, 180 samples would be required compared to 20. The authors believe that the current Produce Safety Rule microbiological water criteria are statistically limited, and high *E. coli* counts will only lead to action after a few years of data collection (Havelaar et al., 2017). More agricultural water quality data is needed from various production regions across the United States to better create agricultural water *E. coli* criteria to serve all growers (Havelaar et al., 2017). Chapter 5 will examine the agricultural water requirements in more depth, particularly in the context of the scientific basis of the PSR. Chapters 6 and 7 will assess the microbial quality of produce irrigation water from a selection of Alabama PSR-exempt farms, as well as the food safety practices on AAES farms.

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## 5. Assessing the Scientific Basis of the Agricultural Water Provision of the FSMA Produce Safety Rule

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### 5.1. Abstract

The Food Safety Modernization Act of 2011 ("FSMA") requires the U.S. Food and Drug Administration ("FDA") to establish science-based minimum standards for the safe production and harvesting of raw produce. This paper examines the scientific basis of the Produce Safety Rule's agricultural water provision, highlighting several criteria: microbial indicators for fecal contamination and decay rates; water source testing; wash water temperature; and water treatment. Analysis finds that FDA made a good faith effort in rulemaking. Implementing the rule, however, is complex for both producers and regulators, requiring additional research to fill gaps in the scientific literature and gaps in knowledge about application of the standards.

### 5.2. Introduction

FSMA (FDA, 2011b), a 2011 amendment to the Federal Food, Drug, and Cosmetic Act (FDCA), is intended to enable FDA to better protect public health (FDA, 2011c). It requires FDA to establish science-based minimum standards for the safe production and harvesting of raw fruits and vegetables, and to adopt a final regulation based on known safety risks. This final rule identifies the procedures, processes, and practices designed to meet at least two goals: 1) to prevent the introduction of known or foreseeable hazard into produce; and 2) to provide a reasonable assurance that produce is not adulterated in accordance with 21 U.S.C. § 342.

The purpose of this paper is to examine the scientific basis of the regulations that implement FSMA, particularly the agricultural water provision of the final rule on Produce Safety, known hereafter as the Final PSR. We focus on water as it is a provision of the Final PSR in which

FDA used the largest number of scientific studies in rule development (FDA, 2015c). The agricultural water provision of the Final PSR is hereafter known as AWP. Also examined are the challenges to interpretation and implementation of these standards at the farm level, and the limitations for FDA in both rulemaking and in providing science-based guidance for compliance.

Previous studies examined the development and implementation of FSMA broadly (Drew and Clydesdale, 2015; Oldfield, 2015); whether FDA has the capacity to fully enforce subsequent regulations (Eads and Zwagerman, 2011; Fortin, 2011; Strauss, 2011) and meet the regulatory training requirements; the economic impacts on small-scale production and international trade (Hassanein, 2011; Pouliot, 2014; Roland, 2015); and the cost of implementation as it relates to operator size (Hassanein, 2011; Pouliot, 2014). Accordingly, we will evaluate the AWP of the Final PSR to examine whether it is based on "sound science" and risk assessment, the foundation of FSMA. This is typically understood to be work that originates from "organized investigations and observations conducted by qualified personnel using documented methods and leading to verifiable results and conclusions" (Society of Environmental Toxicology and Chemistry, 1999). Drawing on this understanding, FDA defines "sound science" as an approach that is based on scientific information, data, and results that are published in peer-reviewed journals, textbooks, or other proprietary research (FDA, 2015c). The justification for using proprietary research is in the Current Good Manufacturing Practices requirements for dietary ingredients and dietary supplements (FDA, 2003).

FDA's risk assessment includes four components (FDA, 2015d):

(1) Hazard Identification: a summary of the biological agents capable of causing adverse health effects that may be present in produce.

(2) Hazard Characterization: a qualitative description of the nature, severity and duration of the negative effects of microbiological hazards that may result from ingestion of contaminated produce (Joint FAO/WHO Codex Alimentarius Commission et al., 2003).

(3) Exposure Assessment (Food and Agriculture Organization and World Health Organization, 2008): an accounting of the likelihood of on-farm contamination from water, soil amendments, animals, workers, equipment and buildings, including estimates of the likelihood and frequency that contamination remains at the point of consumption.

(4) Risk Characterization: an integration of information from hazard identification, hazard characterization, and exposure assessment to qualitatively estimate the negative effects likely to occur in the population.

In the section that follows, a brief background on the rulemaking process contextualizes the regulatory landscape of the Final PSR. Next, is a summary of the AWP including the associated comments and scientific justification for the respective standard. We show that the AWP of the Final PSR is generally supported by the scientific literature. Yet, also acknowledged are weaknesses and gaps in the literature that support the provision, and the subsequent reservations that arise for both producers and regulators enacting the AWP of the Final PSR. We conclude by laying out three issues that have policy implications: 1) the gaps in water safety research; 2) the hidden compliance costs; and 3) the capacity to provide adequate guidance.

### 5.3. Background of FSMA rule-making

On January 16, 2013, FDA published its proposed rule titled Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (21 CFR § 11, 16, and 112). At the same time, FDA published a draft of the Qualitative Assessment of Risk (QAR) (FDA, 2013a) that informs the proposed science-based standards. The draft QAR received approximately

1,300 public comments on five topics outlined by FDA: the risk levels of certain commodities; the belief that larger farm size correlates to higher produce contamination risk; the need for incorporating risk management and assessment into the QAR; the lack of quantitative, science-based, risk assessment for microbial contamination; and the limitations of using CDC and FDA epidemiological records for assessing biological hazards in produce (FDA, 2015e).

Five experts in medical microbiology and pre-to-post-harvest food safety from industry and academia conducted peer reviews of the draft QAR (FDA, 2015e). While the reviews were generally positive, one comment noted that gaps in the data limited the ability to understand the process of microbial contamination. Therefore, a degree of scientific inference in qualitative risk rankings was required. Reviewers also provided comments to improve the document including the selection of scientific references to support the QAR.

The final QAR was published on November 13, 2015, noting five key findings (Joint FAO/WHO Codex Alimentarius Commission et al., 2003):

(1) produce can be contaminated, and the vast majority of related illnesses are associated with biological hazards;

(2) the known routes of contamination from growing, harvesting, and on-farm postharvest activities are associated with seed, water, soil amendments, animals, worker health and hygiene, and buildings and equipment;

(3) all produce has the potential to become contaminated through one or more routes;

(4) commodity specific growing, harvesting, and on-farm postharvest conditions and practices may influence the potential routes and likelihood of contamination and possibly lead to illness; and

(5) postharvest handling practices such as washing, peeling, and cooking before consumption may impact the likelihood of contamination, and thus, may increase (i.e., cross-contaminate) or decrease (i.e., remove a possible contaminate) the possibility of consumer exposure.

The initial comment period for the proposed Produce Safety Rule was extended three times from the initial publication date in January to August in response to complaints that the original 30-days was insufficient. On September 29, 2014, FDA proposed new provisions and amendments, which were open for public comments until December 15, 2014 (FDA, 2014). This later set of comments are addressed in this paper as they immediately preceded the Final PSR that was published on November 27, 2015 (FDA, 2015f). In total, approximately 15,000 comments[1] were made by consumers, legal firms, producers and cooperatives, trade and public health organizations, advocacy and consumer groups, and governmental organizations during the final comment period. These comments addressed four broad issues.

First, several comments were specific to the *science* supporting the proposed Produce Safety Rule including the use of a commodity-specific versus an integrated approach for developing the risk profiles and the inadequacy of empirical support (e.g., lack of comparative risk studies of different supply chain types). Second, the comments addressed *interpretation* of the proposed rule including the lack of guidance for both aquaponics and produce safety generally. Stakeholders also requested clarification of both the terminology and the equivalency of alternative methods of pathogen control including the associated analytical requirements.

[1]Comments on the Final Produce Safety Rule were found by accessing the Final PSR, then clicking on "public comments." The number of comments on any particular topic is an estimate because FDA does not provide the number that were received on that issue. Comments were collected both by tracking the topics mentioned in the Final PSR and searching the comment database for particular word combinations.

A third set of comments were directed toward *implementation,* including how producers ought to reconcile the proposed PSR with existing industry guidelines and certification programs, handle non-biological hazards, and manage the seemingly excessive recordkeeping requirements. This group also included questions about the use of market incentives in lieu of regulation; the inclusion of prescriptive and/or inflexible quantitative metric requirements; the eligibility criteria for variances; as well as inquiries about why farm specific food safety plans were not required. A last set of comments focused on *equity* of the proposed PSR including concerns about FDA singling out on-farm produce contamination rather than the occurrence of contamination along the supply chain after produce leaves the farm; its fair application to both foreign and domestic farms; and the suspected burden for small-scale farms.

In addition, there were numerous concerned stakeholders of intersecting groups—those who have small-scale operations, those who are involved in short supply chains (e.g., direct and/or local sales), and those who use sustainable agricultural practices (e.g., organic, natural). Together, these stakeholders opposed the Final PSR.[2] Ultimately, their concerns were generally satisfied by the Tester-Hagan Amendment to FSMA (§ 112.5). Tester-Hagan exempted farms "that could demonstrate a previous three-year average gross income of less than $500,000 and over 50 percent of sales were to [consumers, restaurants, or retailers within a certain geographic region], or the average value of produce sold over the previous 3 years was less than $25,000" (Schieber, 2013). Both food safety proponents and the agricultural industry criticized the amendment, stating that food safety issues could arise on farms of any size (Beyranevand, 2013). The compromise that led to the Tester-Hagan adoption was the addition of the "exemption withdrawal," which permits FDA to require small farms not meeting the minimum safety standards, or found to be linked to an

[2]See comment from Carolina Farm Stewardship Association on the Proposed Rule for Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption 1 (Nov. 14, 2013) and comment from Slow Food (Oct. 31, 2013), docket FDA-2011-N-0921.

outbreak, to comply with the Final PSR.[3] With the exemption withdrawal in place, all produce is potentially subject to the requirements of the Final PSR.

The Final PSR is specific to domestic and imported "covered produce," which is the harvestable part of edible crops (Roland, 2015). "Covered produce" exemptions include those items that are rarely consumed raw and/or those that receive adequate commercial processing to reduce the presence of pathogens (e.g., *Salmonella, Listeria, E. coli*). For simplicity's sake, we drop the word "covered." Foreign farms that export to the United States are subject to the Final PSR. These facilities are required to implement a food safety program to verify that their produce meets the standards (Drew and Clydesdale, 2015).[4]

The seven provisions of the Final PSR establish a set of science-based minimum standards for the safe growing, harvesting, packing, and holding of produce on farms. The agricultural water provision (AWP) is summarized in Section IV. Also detailed are the studies and references[5] FDA used to justify their responses to stakeholder comments on the proposed Produce Safety Rule.[6]

### 5.4. Provision: Agricultural Water

The AWP defines what is meant by water used in agriculture and requires that it must be safe and of adequate sanitary quality for its intended use. The AWP addresses three substantive issues. First, it establishes inspection and maintenance requirements related to water use, sources, and distribution systems associated with growing, harvesting, packing, and holding produce.

[3]See 21 U.S.C. § 350g(l)(3)(A) (providing that if the Secretary is investigating a "foodborne illness outbreak that is directly linked to a qualified facility" under the Act, and if the qualified facility is exempt from the hazard-analysis and risk-based preventive controls required under the Act, the Secretary may withdraw the facility's exemption after making certain safety-based determinations); 21 U.S.C. § 350i(f)(3)(A) (providing a similar provision for farms linked to outbreaks).

[4]Foreign governments may request a variance from one or more of the standards in the Produce Rule if they can demonstrate that they are able to provide the same level of public health protection as the requirements. Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption, 80 Fed. Reg. at 74,354.

[5]We define "references" as published books or other documents that compile various sources of information that are generally regarded as scientific.

[6]All documents in the References section of the Final PSR were analyzed to determine if they fit the definition of "references" for the purpose of this paper. These peer-reviewed studies and books were then compiled in a spreadsheet and categorized by study purpose, study findings, FDA's claims made in the Final PSR, and study outcomes related to FDA's claims.

Second, the water provision creates testing, treatment, and monitoring requirements including a set of specific standards designed to establish and to maintain microbial safety. Third, the AWP sets recordkeeping requirements for six criteria: inspection, including the water testing and treatment results; scientific data collection and other information to support microbial die-off and removal rates; time intervals to allow for microbial die-off; microbial treatments applied; alternative testing and treatment approaches; and corrective actions that are taken including any alternative approaches that may have been used (FDA, 2015g, p. 5).

The provisions are reviewed in light of stakeholder comments. These comments covered five key themes: (A) the microbial indicator for fecal contamination; (B) the testing of water sources; (C) the microbial decay rate; (D) the wash water temperature; and (E) the treatment of contaminated water. Addressed below are each of the comment themes and FDA responses. Often, FDA responses consisted of a statement indicating that the Final PSR is based on appropriate scientific sources, a reference to additional sources such as supplemental notices, and/or revisions to the Final PSR. In some cases, FDA responses were simply an agreement or disagreement with the stakeholder comment.

### *5.4.1 Microbial Indicator*

Stakeholders questioned FDA's proposed requirement (§ 112.44) to use generic *Escherichia coli*, a broad category of "*E. coli*" bacteria, as the indicator of microbial water quality because its detection does not necessarily indicate the presence of certain bacterial and non-bacterial pathogens. FDA agreed, but also stated that failure to detect an indicator does not guarantee pathogens are absent. Support for this statement came from a literature review showing a frequent discrepancy between indicator bacteria and pathogen concentrations due to the ubiquitousness of traditional fecal bacteria (Pillai and McElhany, 2011). A study highlighting this

point found that generic *E. coli* from watershed samples, taken in a produce farming region of California, was a poor indicator of *E. coli* O157. This lack of detection was due to the fact that individual sample sites did not contain a significant number of *E. coli* O157 cells to correlate with the presence of generic *E. coli* (Cooley et al., 2007).[7]

FDA explained that in addition to generic *E. coli*, several indicators have been used to predict the presence of fecal pollution including total coliforms, fecal coliforms, enterococci, and coliphages. However, there have been varying degrees of success in the effectiveness of these other indicators. While FDA did not discuss enterococci and coliphages, FDA stated that neither total nor fecal coliforms serve as a reliable indicator of fecal contamination because many pathogenic sub-species are ubiquitous (Leclerc et al., 2001; McLellan et al., 2001; Tallon et al., 2005). Two literature reviews supported this determination. Both reviews showed that many total coliforms[8] and fecal coliforms[9] are capable of growth in both the broader biophysical environment and in drinking water distribution systems.

Furthermore, many sub-species like the coliform *Klebsiella* are naturally present (Leclerc et al., 2001; Tallon et al., 2005). In a study of beach water,[10] 82 percent of bacterial isolates from water samples were confirmed to be *E. coli* and 16 percent were identified as other fecal coliforms. These findings demonstrate that *E. coli* is a better indicator of fecal pollution than fecal coliforms. Additionally, biochemical testing produced a wide range of pulsed-field gel electrophoresis (PFGE)[11] patterns similar to the patterns found in *E. coli* isolates from known host sources (Gerba,

[7]Approximately 1000 EcO157 isolates obtained from cultures of more than 100 individual samples were typed using Multi-Locus Variable-number-tandem-repeat Analysis (MLVA) to assist in identifying the potential fate and transport of the pathogen.

[8]Defined as all facultative anaerobic, gram-negative, non-spore-forming, oxidase-negative, rod-shaped bacteria that ferment lactose to acid and gas within 48 h at 35 ◦C or members of Enterobacteriaceae which are β-galactosidase positive. American public health association; standard methods for the examination of water abd wastewater, (Lenore S. Cleserl et al. eds., 20th ed. 1998.

[9]Defined as coliforms that produce gas in EC broth at 44.5±0.2 ◦C within 24±2 h (APHA, 1998).

[10]In this study, bacterial strains were isolated from beach water samples using the EPA method for *E. coli* enumeration and analyzed by pulsed-field gel electrophoresis (PFGE). (McLellan et al., 2001)

[11]Pulsed field gel electrophoresis; (McLellan et al. 2001).

2009; McLellan et al., 2001). *Klebsiella, Citrobacter,* and *Enterobacter* share characteristics with fecal coliforms such that they may falsely increase fecal indicator levels due to environmental replication. Moreover, coliform bacteria may originate from soil and vegetation, as well as other aquatic environments unrelated to fecal pollution (Gerba, 2009; Tallon et al., 2005). In sum, FDA explained that generic *E. coli* is a member of both the coliform and fecal coliform groups, and it has been shown to be the coliform most consistently associated with fecal contamination (Diez-Gonzalez, 2011; Gerba, 2009; Tallon et al., 2005).

Two key studies formed the basis of FDA response to questions about the use of generic *E. coli* as an indicator of fecal contamination, both of which show that the presence of indicators does not always guarantee the presence of pathogens. One study involved in-mill water and external effluent treatment systems of seven typical Canadian pulp and paper mills (Gauthier and Archibald, 2001). With the exception of an operation that disinfected their input water, the mills sampled did not have sewage input, yet all samples supported the growth of numerous coliforms and the fecal indicator organism *E. coli*. Following disinfection, viable enteric bacteria were detected in all of the mills tested. The study investigators claimed that pulp-paper water systems were similar to produce water systems in that both should contain coliforms and indicator organisms while having no fecal input (Gauthier and Archibald, 2001). The second study evaluated Bifidobacterium spp. as a potential fecal contamination indicator in a Puerto Rican rainforest watershed (Carrillo et al., 1985). Investigators found that all viable bacteria counts were related to the nutrient levels, regardless of the contaminants present at the sample site. The implication is that coliforms can become “normal” flora in tropical environments. Most importantly, these findings suggest that coliforms are a poor indicator of fecal contamination (Carrillo et al., 1985).

### *5.4.2 Ground and Surface Water Testing*

The Final PSR (§ 112.42) requires producers to test the entire agricultural water system under their control. In particular, §§ 112.44 & 112.46 direct growers to meet the requirements of a geometric mean of 126 or less colony forming units (CFU) of generic *E. coli* per 100 mL of water, and a statistical threshold of samples at 410 CFU or less of generic *E. coli* in 100 mL of water. Some stakeholders questioned the feasibility and the frequency of water sampling for crops with different growing seasons, while others requested the freedom to design their own *E. coli* water-sampling program.

In response to these comments, FDA addressed several sampling challenges—frequency, location, persistence, and transport—that influence feasibility. The reviews cited support for the notion that *E. coli* detection methods are rapid, accurate, specific, and sensitive (Diez-Gonzalez, 2011; Leclerc et al., 2001; Tallon et al., 2005). FDA defended its claim with both a reference document and a study showing that sampling frequency and location relative to the source of contamination can affect the performance of generic *E. coli* as a fecal indicator (Howell et al., 1995; National Research Council, 2004a). The first investigation found that detection of generic *E. coli* can be difficult because water masses move past their original sampling site, thus indicator concentrations at a single point may vary (National Research Council, 2004a). The second, which focused on Kentucky watersheds, determined that monthly sampling can be used to accurately assess the extent and variability of fecal contamination (Howell et al., 1995).[12]

FDA noted two additional points associated with water testing: *E. coli* may take different paths in different watersheds and *E. coli* may escape detection when they settle into sediments,

[12]Water quality variation due to flow dynamics impact how frequently samples should be taken. Sampling location is also important because "fecal contamination in agricultural water reflects complex interactions affecting the survival, infiltration, and movement of . . . organisms in soil, water, and sediment." (Howell et al., 1995)

sediments that then act as reservoirs (Garzio-Hadzick et al., 2010; Pachepsky and Shelton, 2011; Pillai and McElhany, 2011; Whitman et al., 2011). Depending on the sampling location, bacteria in freshwater sediments may not be detected (Whitman et al., 2011). One reason for the lack of detection is stream re-suspension. If sediment-borne organisms are not distributed throughout the entire water column, samples taken in any one place may not represent the full column. In addition, sampling an entire water column is highly impractical (Pachepsky and Shelton, 2011). Another study tested the survivability of *E. coli* in a manure contaminated streambed to determine if it was affected by the organic carbon content.[13] It was found that *E. coli* survived in the sediments much longer than the overlaying water (Garzio-Hadzick et al., 2010).

Comments regarding the frequency for testing untreated surface water were quite critical stating that it is overly complicated, burdensome, lacks scientific justification, is a statistical construct, does not take into consideration site-specific variables of surface waters, and is not sufficiently flexible. Further, some argued that the time and location of sampling are more important than the number of samples. FDA explained their position and underlying statistical analysis in a self-authored reference document that accompanied the supplemental notice (Bowers, 2015). The document focused on the use of a rolling approach for calculating the microbial water quality profile. This "rolling approach" requires producers to collect initial samples (4 for groundwater, 20 for surface water), and then sample annually, as necessary, to create a dataset that retains the same number of total samples (1 for groundwater, 5 for surface water) on a yearly basis (FDA, 2015h, p. 46).

Several comments supported a greater testing frequency for untreated surface water as compared to untreated groundwater sources used for the same purposes, while others suggested

[13]Indigenous *E. coli* populations in stream sediments ranged from $10^1$ to $10^3$ MPN/g, while approximately $10^3$ manure-borne *E. coli* MPN/g were added by inoculation.

that there is no difference between the two. FDA responded by stating that groundwater is less likely to contain microorganisms due to the soil's natural filtering mechanism (Gerba, 2009). FDA recognized that groundwater can still be contaminated if wells are inadequately constructed, poorly maintained, improperly located (e.g., near extensive livestock production, near fields where raw manure is applied), and/or draw water from a contaminated aquifer (Close et al., 2008; Gelting et al., 2011; Gelting and Baloch, 2012; Howell et al., 1995; Leifert et al., 2008). For example, agricultural runoff from pastures often contains fecal bacteria. Subsurface transport of bacteria to shallow springs and wells is a concern in areas where groundwater is utilized as drinking water (Howell et al., 1995; Leifert et al., 2008). A study in New Zealand, which has an agro-ecological environment similar to that found on the U.S. western and southern borders, evaluated the leaching of *E. coli* and *Campylobacter* from intensive dairying and border-strip irrigation. Groundwater samples were collected over a three-year period, mostly during the irrigation season, with 75 percent found to contain *E. coli* (Close et al., 2008).[14]

Another study examined the environmental factors related to the 2006 spinach outbreak. Analysis of available data suggested that both the depth of the groundwater[15] and the surface and groundwater interactions may pose a risk to ready-to-eat crops such as spinach. Other potential sources of contamination include surface runoff, well construction, and direct or indirect application of irrigation water to crops (Gelting and Baloch, 2012). In the spinach case, CDC and California Emergency Response Team investigators found that the outbreak was most likely due to the use of contaminated irrigation water. The source of this contamination was thought to have

[14]*Campylobacter* was identified in twelve percent of samples.

[15]Winter rain is stored in reservoirs and then released during the dry summer season to recharge aquifers used for irrigation. Analysis of the farm watershed system indicated that pathogens in surface water could have reached wells on the farm and contaminated irrigation water. During the growing season, groundwater levels dropped below the level of the San Benito river, allowing surface water to interact with groundwater on the farm.

been surface water recharge of the groundwater that was used for irrigation (Gelting and Baloch, 2012). Nevertheless, FDA concluded that surface water sources are inherently subject to a greater potential for contamination than well-maintained groundwater sources. Therefore, when both irrigation water source types are used under § 112.44(b), fewer groundwater tests are required compared to surface water.

### *5.4.3 Microbial Decay Rate*

If agricultural water does not meet the requirements of § 112.44, producers may apply a time interval between last irrigation and harvest using a microbial die-off rate of 0.5 log per day to meet the standards. One comment requested flexibility to the standard in § 112.45(b)(1)(i) so as to make possible the application of a 0.5 log per day die-off rate on an hourly rather than a daily basis. FDA stated that there is little evidence to support a modification. As the literature indicates, decay constants have been found to vary within the 24 hour cycle, depending on climate and other conditions such as plant age, water application rate, time of inoculation, and presence of other natural flora (Fonseca et al., 2011; Moyne et al., 2011; Petterson et al., 2001; Wood et al., 2010). FDA cited four studies of leafy greens to support their position on variable decay rates. First, it was found that the consumption risk was high in cases associated with secondary-treated wastewater irrigation of lettuce (Petterson et al., 2001).[16,17] Second, the survival rates of two *E. coli* strains, following leaf and soil irrigation of spinach, decreased over time (Petterson et al., 2001).[18,19] Third, *E. coli* survived longer after sprinkler irrigation than subsurface drip or surface

[16]If the viral rate of decay on the lettuce crop follows a first-order rate expression, then the decay coefficient is the slope of the linear regression line on a log scale versus time.

[17]Viral decay constants varied up to five logs on single days since last irrigation.

[18]Various *E. coli* strains were applied at rates of $10^4$ to $10^7$ cfu/100ml to the spinach plants (secondary-growth). Culturable *E. coli* were recovered from plants up to six days post-inoculation.

[19]*E. coli* decay constants varied 2. 5-3 logs within a 24 hour period at one, two, and three days post inoculation.

furrow irrigation (Fonseca et al., 2011).[20,21,22] Finally, drip and furrow irrigation had no effect on the persistence of *E. coli* O157:H7 on lettuce.

### *5.4.4 Wash Water Temperature*

Section 112.48(c) of the Final PSR requires that producers maintain and monitor water used during harvesting, packing, and holding at temperatures appropriate for the commodity and operation. Some stakeholders believe that produce wash water should be warmer than the produce, while others question this assertion. FDA stated that when there is a certain degree of wash water temperature difference compared to the produce, it may influence the processes leading to infiltration of microorganisms. The QAR notes that this type of infiltration has been demonstrated in apples (Buchanan et al., 1999), oranges (Merker, 1999), tomatoes (Bartz, 1988; Bartz and Showalter, 1981), and mangoes (Penteado et al., 2004; Sivapalasingam et al., 2003). The first cited study assessed the extent and location of *E. coli* O157:H7 contamination in four room temperature and refrigerated apple varieties subjected to immersion in cold *E. coli* inoculated peptone water.[23] When immersed in cold water, warm fruit was more likely to take up the pathogen than cold fruit (Buchanan et al., 1999). In a similar study, tomatoes were immersed in various suspensions with differing bacteria[24] and a negative suspension/fruit temperature differential. In general, weight increases were correlated with bacterial infiltration (Bartz and Showalter, 1981).[25]

[20]Overhead sprinkler, subsurface drip, and surface furrow irrigation methods were tested.

[21]In two trials, *E. coli* strains were injected into the water stream of the different irrigation systems to determine survival in the field. Results showed that product samples were positive for *E. coli* for up to seven days when using sprinkler irrigation, while only one product sample was found positive for *E. coli* when using other irrigation methods.

[22]It found decay constants in a twenty-four hour period were different for the inner and outer portion of lettuce when irrigated with the sprinkler.

[23]Containing approximately $3x10^7$ CFU/ml *E. Coli* O157:H7.

[24]*S marcesens, Erwinia carotovora, Pseudomonas marginalis,* or *P. aeruginosa*.

[25]The marker bacterium was isolated from fruit subject to negative pressure differential, with more isolated from fruits that gained more water weight.

In December 1999, there was a nationwide increase in *Salmonella* serotype Newport infections due to tainted mangoes from a single Brazilian farm. In this case, hot water was used as a treatment for fruit flies and it was identified as the point of contamination (Sivapalasingam et al., 2003).[26] Immature and ripened mangos were positive for *Salmonella* internalization at a frequency of 80 percent and 87 percent, respectively (Penteado et al., 2004). This study supports FDA's position that infiltration of hot water containing pathogens could play a role in a produce outbreak.

Other studies demonstrated that pathogen infiltration can occur without a temperature differential (Merker, 1999; Xia et al., 2012). For example, a different study of tomatoes found factors such as the variety and the time delay between stem removal and water immersion to have a significant impact on the frequency and population of internalized *Salmonella* spp.[27] However, the temperature differential had no significant effect on the incidence of *S.* enterica internalization (Xia et al., 2012). Another study examined orange and grapefruit water-immersed dye uptake finding some evidence of low levels of uptake in grapefruit when there was no temperature differential. Yet, the authors also suggested that infiltration of water and dye can occur into an intact fruit when the temperature differential between fruit and water favors uptake (Merker, 1999). Regardless of the conflicting studies, the final version of § 112.48(c) requires water temperature to be determined by the producer and to be considered appropriate for the commodity and operation. The time and depth of submersion and the method's adequacy in minimizing the potential for infiltration of pathogenic microorganisms must also be considered.

[26]A simulation was conducted to evaluate this treatment and to assess whether this process promotes internalization of *Salmonella* into mangos. Dye internalization potential was determined: untreated domestically grown immature and ripened Tommy Atkins variety mangos were immersed in water at 47 degrees C for 90 min and then immersed in 21 degrees C water containing blue dye for 10 min. and the experiment was repeated using 21 degrees C water containing $10^7$ CFU/ml *Salmonella* Enteritidis expressing constitutive green fluorescent protein. Fruit was then stored at 10, 20, or 30 degrees C for up to one week.

[27]Different tomato varieties, temperature differentials between tomato and bacteria suspension (-5.6, 0, and 5.6 °C), and post-stem removal times were evaluated for their effects on *S. enterica* internalization. Mature green tomatoes at 32.2 °C were immersed in water containing approximately $10^6$ CFU/ml *S*. enterica. The incidence and density of internalized cells were determined by culture enrichment and most-probable-number methods, respectively.

### *5.4.5 Water Treatment*

When agricultural water does not meet the microbial quality criterion (§ 112.44(a)), the Final PSR requires one of two approaches that a covered farm must take (§ 112.45(a)): using a different source of water; or re-inspecting the water system, making necessary changes to bring the water system into compliance, and testing to confirm that the changes were effective. Several stakeholder comments express concern about the potential for adverse environmental impacts from implementing the water treatment provisions in § 112.43, namely the application of antimicrobial pesticides to ground water and the chemical treatment of irrigation water.

FDA responded that failures in treatment systems are largely attributed to suboptimal particle removal and treatment malfunction (Reynolds et al., 2008; Westrell et al., 2003). A cited review and study of municipal water treatment supported FDA's position, indicating that if properly applied, current protocols are effective at eliminating pathogens from water. However, inadequate, interrupted, and intermittent treatments repeatedly have been associated with waterborne disease outbreaks (Reynolds et al., 2008).[28] Water treatment failure may also occur from equipment malfunction and microbial pollution of reservoirs and local networks (Westrell et al., 2003).

Several comments requested additional instruction and examples regarding how to comply with the AWP. Citing three articles (Ritenour et al., 2014; Suslow, Trevor, 2001, 1997), FDA provided an example of an effective orange post-harvest sanitation program using a chlorine-based wash,[29] but also explained that the water temperature and pH modifications, and/or allowing time for microbial die-off between last irrigation and harvest, could also be used successfully. Also

[28]Contamination levels are affected by the number of pathogens in the source water, the age of the distribution system, and the quality of the delivered water, as well as climatic events.

[29]The antimicrobial activity of chlorine compounds depends on the amount of hypochlorous acid present in the water after the treatment is applied.

noted were parameters (e.g., water temperature, amount of antimicrobial substances used) that require continuous monitoring and adjustment as hypochlorite activity is reduced by organic material (e.g., soil, plant debris), and it is ineffective if the pH values are outside its normal range (i.e., pH 6.0-7.5) (Ritenour et al., 2014; Suslow, Trevor, 2001, 1997). In addition, as one commenter stated, there are several non-chemical treatments for agricultural water—mechanical (e.g., filtration) and physical (e.g., pesticide devices)—under examination. FDA acknowledged these possible alternatives indicating that there may, in fact, be other technologies that are effective, including several pesticide devices (e.g., filter units, ultraviolet light units, ozonator units), the use of reverse osmosis, and solar radiation (Shannon et al., 2008). In response, a revision to § 112.43(a) included some additional acceptable means of treating agricultural water to meet the relevant microbial quality criteria in § 112.44.

**5.5. Challenges to Implementation**

An endless number of access points between production and consumption complicates the prevention of microbial contamination along the produce supply chain. FDA considers the Final PSR to be a grand "food safety plan," designed to identify and mitigate said complication system-wide (FDA, 2015f, p. 74). Focusing strictly on the water provision, the AWP addresses two key points—irrigation during growing and washing at harvest—where contamination is likely to occur at the farm level. Analysis of the standards within the provision finds that they are generally supported by scientific studies published in the peer-reviewed literature, and secondarily via microbial reference materials. Yet, there are notable questions about the science and gaps in the literature. Most problematic for both growers, and regulators charged with application of the Final PSR, are those areas in which uncertainty exists. In the proceeding sections, the science behind the AWP and associated uncertainty will be discussed.

### *5.5.1 Science*

The use of "sound science" is the foundation of FSMA and its associated risk assessment (FDA, 2015f, p. 358). Valid science used in rulemaking is publicly available, peer-reviewed work published in scientific journals or textbooks. FDA also includes proprietary research that is not open to review, evaluation, or use by supply chain actors, which are critical aspects in the "practice" of science. The context in which these proprietary studies are used is unclear. In addition, there is no information about FDA oversight of proprietary studies (FDA, 2013b) or the ways in which FDA may have assessed these works for their scientific merit. For example, an FDA authored study examined the average variability among surface water sources. This project determined the sample size requirements for estimating microbial water quality profiles (Bowers, 2015). Since the FDA document was not peer-reviewed, and cannot be easily obtained,[30] its applicability for a scientist or a grower is unclear.

The practice of science also requires accurate application and representation of existing work. Yet, there were cases in which FDA used studies that did not necessarily support its assertion. For instance, the use of generic *E. coli* as a microbial standard is based on the EPA drinking water criteria, which might not translate to irrigation water.[31] In addition, several cited studies did not look at generic *E. coli* at all, but instead examined other pathogens, namely *Salmonella*. In the case of microbial die-off criteria (i.e., 0.5 log die off rate per day), a source FDA cited did not completely support the regulation, because the source failed to assess die-off rates over a 24-hour period (Moyne et al., 2011). Detailed in Section IV was the case of wash water

[30]Efforts were made to search both the FDA database and Google for documents with this title. Academic search engines, including Westlaw, were also utilized without success. The only known way to access this document without a FOIA request is with the docket number FDA-2011-N-0921-18658. However, this docket number is not provided in the Reference section of the Final PSR. Simply searching for the title of the document on regulations.gov does not yield the correct document.

[31]Letter from Judith McGeary, Executive Director, Farm & Ranch Freedom Alliance, et al., to FDA (Dec. 15, 2014) http://farmandranchfreedom.org/wp-content/uploads/2014/12/FSMA-organizational-comments-PRODUCE-RULE-2014-Submitted.pdf. (Farm & Ranch Freedom Alliance, 2014)

temperature. FDA's concern was "pathogen uptake" during rinsing, but the cited study focused on dye uptake (Merker, 1999), and it did not use water temperature as a variable. Another FDA claim recommended chlorine as a water treatment, yet the cited literature indicated that chlorine may enhance microbial infiltration (Bartz and Showalter, 1981).

Second, there are gaps in the literature. Some commenters suggested that further research is warranted to fill the lacuna such as microbial die-off or removal rates associated with washing, harvest, and storage;[32] and determining water quality standards with greater precision should be required. FDA denied these stakeholder comments, citing the QAR. As noted in Section III, however, a QAR reviewer indicated that the incomplete microbial die-off data limited his or her ability to understand fully the processes involved in microbial contamination (FDA, 2015e). In addition, directly comparable studies appear to be lacking. For example, during the rulemaking, FDA drew on several studies conducted in agro-ecological regions and in production environments that may not be directly comparable to the dominant or relevant growing regions and/or commodity systems from which the US primarily sources its produce (Carrillo et al., 1985; Close et al., 2008; Cooley et al., 2007; Howell et al., 1995).[33] Another gap in the current body of research is that which addresses the question of scale. Organizations such as the Produce Marketing Association and the Food Marketing Institute argue that exempting farms based on revenue (see Section III) is not a scientific or risk-based approach as contamination may occur on any farm with unsafe practices (Wiseman, 2015).[34] A study of small-scale organic farms in Maryland illustrated

[32]"As noted in the supplemental notice, at this time, FDA is not establishing specific microbial die-off rate(s) between harvest and end of storage, or specific microbial removal rate(s) during postharvest activities such as commercial washing, because they do not have sufficient information to support the derivation of appropriate, broadly applicable, microbial die-off or reduction rate(s) for these purposes." Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption, 80 Fed. Reg. 74,354, 74,444 (Nov. 27, 2015).

[33]Studies were conducted in New Zealand, Puerto Rico, Kentucky, and California, for example. It is important to note, however, that FSMA applies to imported produce, some of which may be grown and processed in tropical regions in which these studies took place.

[34]A key argument supporting the exemption is that an outbreak from a small business would have a minimal and relatively contained impact in contrast to an outbreak from a large-scale operation that would have large domestic implications.

this point when investigators found indicator organisms in their produce and water samples (Pan et al., 2015; Xu et al., 2015).[35]

### *5.5.2 Uncertainty*

While FDA did not avoid any particular topic, the very large number and wide range of comments during the rulemaking process suggest a high level of uncertainty about the standards, many of which are tied to Final PSR *implementation*. Most of the comments related to the AWP fell into three categories: expertise, ambiguity, and data. When FDA responded to questions about an approach in the Final PSR, uncertainty appeared to be the underlying issue.

First, implementation of the AWP requires scientific expertise. For example, it is up to the producer to evaluate how often water sources ought to be tested and treated beyond the initial requirements, and to create a management system that ensures safe produce. Water testing itself requires a working knowledge of *E. coli* detection methods, aseptic sampling procedures, appropriate sample timing and location, pH and antimicrobial testing, and the ability to utilize geometric means and statistical threshold values for evaluating *E. coli* presence. Further complicating the matter, the AWP is in flux as FDA is currently reviewing ways to simplify the microbial agricultural water standards, recognizing that they "may be too complex to understand, translate, and implement" (FDA, 2017d).

Expertise related to "on-the-ground" application of the standards is also required as stakeholders requested guidance on a number of issues. These issues included data sharing; inspection timing and sampling program design; reconciling regulatory or certification compliance (e.g., NOP certification, EPA pesticide use registration, state water treatment regulations); hazard identification (e.g., conditions likely to introduce known or foreseeable problem when water

[35]In a study of local foods, vegetables at California farmers markets were found to be positive for several strains of *Salmonella* and fecal coliforms.

cannot be treated); and potential hazards of water decontamination (e.g., treatment causing pollutant discharge). In some cases, FDA referred the reader to other FDA or EPA authored documents (EPA, 2015; FDA, 2015i). Thus, while FDA responded to these questions, and often provided an assurance that the methods mentioned would comply with the AWP, it did not always provide science-based evidence in their response.[36]

Second, implementation requires *interpretation* of vague information. When the Final PSR was published, FDA did not have a clear picture of the on-farm application of the standards, and thus, had not developed guidance documents. Consequently, there were 650 comments requesting clarification of the central concept of the provision, the definition and identification of "agricultural water" (e.g., inclusion of pooled water in produce fields, identifying discrete sources, harvest related uses including packing and holding). Another 500 comments requested clarification of the sample timing standard. The Final PSR also included 326 instances in which producers were simply instructed to use measures that are "reasonably necessary" to prevent contamination. Currently, FDA permits growers to determine, with little guidance, water temperature, adequate procedures to prevent contamination both pre- and post-treatment, and alternative measures.[37] And, while FDA provided additional clarification for some standards (e.g., the EPA recreational water criteria, indicators of quality, sanitation assurance, criteria for specified purposes, alternative quality criteria), it did so without scientific justification.

[36]For instance, one commenter expressed concern that treating water with antimicrobials would be considered a point source discharge of a pollutant and would make producers liable under the Clean Water Act. FDA referred the reader to the EIS (Final Environmental Impact Statement (EIS) for the Proposed Rule: Standards for Growing, Harvesting, Packing, and Holding of Produce for Human Consumption) for a detailed discussion of the potential environmental effects of the agricultural water provision.

[37]The current draft guidance published only applies to sprouts, which have more specific regulations. U.S. Food and Drug Administration., Draft Guidance for Industry: Compliance with and Recommendations for Implementation of the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption for Sprout Operations (2017). https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm510578.htm (last visited April 1, 2017).

A third point is that implementation requires access to generally accepted scientific data. There are several circumstances in which the AWP allows producers to use an "alternative" approach (e.g., microbial criteria, testing frequency, die-off rates). In these cases, an alternative is permitted when it is supported by adequate scientific data that illustrates effectiveness in providing the same level of public health protection as the method stated in the AWP. Yet, access to, and development of, an alternative will be difficult, particularly for small-scale and remote producers. As noted above, there are gaps in the literature. Of the literature that is available, a majority is published in publicly availably peer-reviewed journals, but retrieval requires physical access to a research library and/or a credential to gain access remotely. Additionally, these studies are written for an academic audience, making them generally inaccessible to others without discipline specific expertise.

### 5.6. Conclusion

While some instances in the AWP were found to be weakly supported, analysis of stakeholder comments suggest that FDA did make a good faith effort to address thousands of comments, to provide assurances where possible, and to offer science-based rule modifications. Overall, the comments and responses illustrate that the application of FSMA is problematic for both growers and regulators. Our research found three policy implications that are specific to the science used to bolster the AWP. First, there are key gaps in the water safety research as it relates to on-farm production, and these gaps may cause ambiguity for both producers and regulators. Second, there are costs associated with implementing the science-based standards that may not be readily discernable to decision-makers. Third, there are concerns about the capacity of regulators to provide producers with adequate application guidance, and a lack of sufficient guidance, may put growers at an increased risk of non-compliance (Boys, 2013; Johnson and Endres, 2012).

As FDA acknowledged, further research on produce pathogen growth on farms is needed to support the Final PSR (FDA, 2015d). Also lacking is work on determining the origin, survival, and distribution of pathogens in an agro-ecological environment (i.e., pathogens from wild animals and livestock, from soil contamination, from post-harvest operations). This work ought to include, but not be limited to, questions about how pathogens transfer to, and reproduce on, produce; questions about the appropriate on-farm methods to reduce contamination; and questions about the number and prevalence of pathogenic cells remaining in contaminated produce at consumption (FDA, 2015d). Stakeholder questions about rule implementation were understandably difficult for FDA as the agency began with a deficit—FDA has not traditionally been involved in production agriculture—and many of the comments came from producers throughout the country, many of whom access and use agricultural water differently. These questions brought up a number of topics that require further investigation: ascertaining the effects of farm size and region on produce safety, determining how to describe the hurdles faced by growers of all sizes, and understanding how the relationship between production practices and crop type may influence contamination.

To supplement the science available, FDA will need access to investigators with expertise in production agriculture. Traditionally, scientific questions about agricultural production, water, and food safety have been addressed via competitive funding opportunities associated with the USDA (e.g., National Institute of Food and Agriculture ("NIFA") Foundational Program grants). This funding is most commonly awarded to investigators at research universities (e.g., land grant institutions and agricultural experiment stations), regional laboratories (e.g., National Food Safety and Toxicology centers), and government agencies (i.e., USDA Agricultural Research Service). Going forward, FDA might create new opportunities through the National Science Foundation (NSF), work within the existing USDA funding structure, and/or collaborate directly with state

Agricultural Experiment Stations, state Cooperative Extension Services, and state Departments of Agriculture. Additionally, FDA ought to develop a means for making publicly available any proprietary research used in rulemaking and implementation standards.

Not addressed, above (Section IV), were comments rooted in concerns of *equity*. The dominant concern among these comments was whether the financial cost of implementation is fair for small-scale farms, as it has been addressed elsewhere (Drew and Clydesdale, 2015; Eads and Zwagerman, 2011; Fortin, 2011; Hassanein, 2011; Oldfield, 2015; Pouliot, 2014; Roland, 2015; Strauss, 2011). Others also note that regulations may increase the vulnerability of small farms should an outbreak occur because contamination may be more easily traced-back (Johnson and Endres, 2012); retailers, buyers, and institutional food providers have begun to require small farms to carry liability insurance (Boys, 2013); and tort law has been used to hold producers liable when found negligent (Johnson and Endres, 2012). Nevertheless, with respect to retaining the notion of a science-based Final PSR, there is a dearth of studies examining small-scale farms *before* implementation. Such studies are necessary to determine whether the regulations have an impact on an entity's economic viability and public health more broadly.

Often overlooked in assessing food safety rules is that there is a "cost" in the "science" of the individual standards. For instance, implementation of the AWP will require water samples to be tested at certified labs that will undoubtedly charge user fees (Roland, 2015). These labs are required to meet a range of scientific and technical standards for analysis that may not be feasible for producers to meet on-farm, except the very largest corporate entities (FDA, 2017e). Moreover, some costs are less visible; there will be a costly, and high, learning curve for the vast majority of producers because only very large-scale operations are likely to have dedicated food safety

personnel. There will be costs in learning the details of the Final PSR and in understanding at least some of the underlying science including the associated jargon, techniques, and data.

Capacity in rule implementation is a third issue. While some growers may already meet GAP and other buyer standards that have similar criteria to the agricultural water provision, this is not the case for most (Hatanaka et al., 2005).[38] Growers of all sizes will need sufficient investment in education to mitigate a range of limitations in knowledge and gaps in the accessible literature. One option is to seek out third-party training, which can exceed $400 per course/per individual (Produce Safety Alliance, 2017), a cost and commitment that will push some to pay for outside "experts."[39] Growers unable to "buy" scientific expertise will have few places to turn for guidance as state Departments of Agriculture lack funding and state Cooperative Extension programs are already overburdened (Al-Kaisi et al., 2015).

FDA is currently working on guidance documents intended to provide "user friendly" suggestions on how to meet some of the Final PSR requirements. Two points considered "high priority" are documents detailing the definition of "agricultural water" and sample timing (Morris, 2016). Yet, the financial resources for assisting growers via research funding and direct assistance is unclear. FDA has begun to collaborate with NIFA to provide funding for food safety technical assistance, training, and education, and its 2017 objectives include increasing states' capacity for rule implementation. However, the expected budget across all key agencies and organizations is woefully lacking.[40] Potentially more problematic, at the time of this writing, is that President Trump has called for an 18 percent cut to FDA's budget (Whitworth, 2017a). In addition, the new

[38]In a study of GAP certified farms in Vermont, it was found that they tended to be larger in acreage than non-certified farms. (Marine et al., 2016a)

[39]Similar examples include integrated pest management (IPM) scouts and crop consultants, as well as the use of soil testing laboratories.

[40]The National Association of State Departments of Agriculture (NASDA), for instance, has stated that it is unable to assist without significant federal funding. Barbara Glenn, NASDA Letter to Senate Appropriators on the FY 17 President's Budget and FSMA Implementation (Mar. 1, 2016). http://www.nasda.org/Policy/filings/Letters/40009/41263.aspx

Trump Administration has introduced a bill that is intended to strip regulations and hinder regulatory rulemaking, which food safety groups refer to as the “Filthy Food Act” and interpret as an intent to cut science from the regulatory process (Whitworth, 2017b).

## 5.7. References


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## 6. Introduction: Agricultural water sampling methodology and quality in Alabama

One of the most controversial and important provisions of the Produce Safety Rule is the agricultural water provision (AWP). Pathogens can be transferred from contaminated water to the harvestable portion of produce via irrigation, aerial crop sprays, wash water, and other routes (Harris et al., 2012). The AWP of the PSR establishes allowable levels of generic *E. coli* in water that directly or indirectly contacts produce during growing (pre-harvest) and post-harvest activities. Factors that influence water microbiological testing results include sampling (the sampling procedure, the location of the water sample (i.e., field, packing house), the quantity and volume of samples, frequency of sample collection), transportation (sample temperature maintenance during transport to the laboratory, time transporting sample to laboratory), testing method (volume of sample tested and dilutions utilized, the method of pathogen detection), and potential cross contamination during sampling, transport, or testing (Fresh Produce Safety Center, 2015).

The PSR establishes generic *E. coli* as the preferred indicator organism for water quality. Generic *E. coli* is the only fecal coliform bacteria of true fecal origin and has been shown to be the coliform most consistently associated with fecal contamination (Diez-Gonzalez, 2011; Gerba, 2009). It also does not appreciatively multiply in the environment outside the host (Edberg et al., 2000). However, there are some problems with using *E. coli* as an agricultural water contamination indicator. *E. coli* is ubiquitous in agricultural water: water masses move past their original sampling site, therefore indicator concentrations at a single site can vary, *E. coli* can take various pathways in agricultural water systems, and stream sediments have been shown to harbor generic *E. coli* (National Research Council, 2004b; Whitman et al., 2011).

Microbial results obtained from surface water vary spatially and temporally (Nnane et al., 2011). Therefore, it is important that a sampling frequency is established that detects these changes. Environmental factors that can cause variance in the concentrations of microbial indicators should be considered when creating a sampling frequency plan (Won et al., 2013). These factors include non-point source pollution (e.g., run-off that can not be traceable back to a single source) and extreme precipitation events leading to runoff (Curriero et al., 2001; Gould, 1990; Jamieson et al., 2002; Kistemann et al., 2002).

The FSMA Produce Safety Rule permits various methods to test water samples for the presence of generic *E. coli*. FDA permits equivalent methods to EPA Method 1603 such as EPA Method 1103.1, EPA Method 1604, and Hach Method 10029 (FDA, 2017f). Most of the EPA 1603 equivalent methods use the membrane filtration method, which is subject to competition from high levels of background microflora on the growth media from the water sample (Mattelet, 2006). Some of the FDA approved *E. coli* detection equivalent methods, such as Hach 10029, EPA 1603, and EPA 1604 use chromogenic media, which present some challenges depending on the water source. Studies comparing various *E. coli* detection methods have found that chromogenic media often overestimated the number of bacteria present in ground water and drinking water (Landre et al., 1998; Pitkänen et al., 2007). There are many variables that can influence agricultural water microbiological results in addition to challenges associated with enumerating water samples for *E. coli*. Chapter 7 will assess the microbial quality of produce irrigation water from a selection of Alabama farms using three *E. coli* enumeration methods, as well as the food safety practices on AAES farms.

# 7. Assessing the food safety practices and agricultural water used to grow produce of farms in Alabama

## 7.1. Abstract

The Food Safety Modernization Act Produce Safety Rule (PSR), enacted in 2018, will require produce growers to follow standards for agricultural water used during production, harvest, and post-harvest activities. Growers that sell >$25,000 of produce annually will be required to test their agricultural water for the presence of generic *E. coli*. The majority of growers in Alabama are small and very small in scale. To represent these growers, Alabama Agricultural Experiment Stations (AAES) was used as a proxy. AAES conduct research and demonstrate technologies that may be adopted by commercial growers. Little is known about how the PSR will impact Alabama small growers or the baseline microbial quality of AL water that may be used in produce production. The objectives of this study were, therefore (1) to determine the average *E. coli* presence in agricultural water used to grow produce from three regions across Alabama, (2) to compare three *E. coli* enumeration methods, and (3) to determine the food safety practices PSR-exempt AAES managers have implemented. Data from this study will help determine if following the current water requirements will be feasible for small scale growers.

Ground, county, and surface water were sampled from seven sites in three different geographical areas of the state over a four month growing season. A survey of produce safety practices was sent to AAES managers, including the farms where water samples were obtained (n=10). *E. coli* was enumerated with three methods equivalent to EPA Method 1603 as specified by the PSR: EPA Method 1604 (MI), Hach Method 10029 (mColiBlue24), and EPA 1103.1 (mTEC). Over the study period, the average geometric mean (GM) of *E. coli* in the two surface

water sources sampled were 20 and 6 CFU/100 mL. There was no detectable *E. coli* in the ground and county water tested. The mTEC was found to produce significantly different *E. coli* counts than the mColiBlue24. Complying with the water standards will be difficult for farms located far from an accredited laboratory. Survey responses (n=8, 80%) indicated that while all had access to food safety training, only half were trained in programs such as GAPs or the PSR. Only one farm trained their workers in food safety practices, and one tested their irrigation water. Although all water sources tested met the PSR criteria, the acknowledged presence of food safety hazards suggests that risk assessments are warranted. AAES managers and commercial growers would benefit from on-farm produce safety demonstrations to fill the current gaps in knowledge.
Keywords: produce safety, agricultural water, Produce Safety Rule, FSMA

## 7.2. Introduction

Fresh produce has increasingly been implicated as the cause of numerous foodborne outbreaks globally (Callejón et al., 2015). In 2015, fresh produce was linked to 41 percent of reported foodborne illnesses in the United States (CDC, 2017). These high profile produce outbreaks, particularly the 2006 *Escherichia coli* O157:H7 outbreak caused by contaminated California spinach, resulted in calls from the public, industry, and government to regulate the production of fresh produce (Calvin et al., 2017). The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR), published in 2015, sets science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits and vegetables for human consumption (FDA, 2015). It also establishes record-keeping requirements for various food safety related activities. The PSR regulates agricultural water, biological soil amendments, worker training, health and hygiene, domesticated and wild animals, equipment, tools, buildings, and sprouts. The

rule applies to any farm that has sold more than $25,000 on average annually over three preceding years. Very small businesses are defined in the PSR as generating an annual income between $25,000 and $250,000 from produce in the previous three years. Small businesses are those that generated an income between $250,000 and $500,000, and large businesses are those that generated an income greater than $500,000. Large, small, and very small businesses must comply with the PSR by 2018, 2019, and 2020, respectively. Farms that sold less than an average of $25,000 worth of produce are eligible for an exemption from the PSR. Farms are also exempt if sales to end users, such as restaurants or retailers, within 275 miles of the establishment exceeded all other sales.

### *7.2.1 Agricultural Water Provision*

One of the most controversial and important provisions of the PSR is the agricultural water provision (AWP). Pathogens can be transferred from contaminated water to the harvestable portion of produce via irrigation, aerial crop sprays, wash water, and other routes (Harris et al., 2012). The AWP of the PSR establishes allowable levels of generic *E. coli* in water that directly or indirectly contacts produce during growing (pre-harvest) and post-harvest activities. The scientific basis of the AWP was assessed in a previous publication (Gradl and Worosz, 2017). Responding to the Final PSR, stakeholders indicated that the testing requirements may be too complex to understand, translate, and implement. Due to the acknowledged complexities related to this Rule, the current compliance dates for the water provision have been extended to 2022, 2023, and 2024 for large businesses, small businesses, and very small businesses, respectively. This extension is intended to address questions about rule implementation and flexibility and decrease their regulatory burden (FDA, 2017a).

#### *7.2.1.1 Pre-Harvest Water Use*

Agricultural water that is directly applied pre-harvest has specific requirements in the PSR (21 CFR section 112.44). Either surface, ground, or municipal water can be used for this purpose. Irrigation water must contain a geometric mean (GM) of *E. coli* of no more than 126 CFU/100 mL water and a statistical threshold value (STV) of 410 or less CFU/100 mL water. For surface water, a rolling sample plan consisting of 20 initial samples during a growing season, and 5 samples following that, must be used for these calculations. Ground water must be sampled four times initially, then once a year to create a rolling dataset of four samples. Municipal water does not require testing because it has been treated to meet certain federal and state microbial requirements (i.e., less than 5 percent total coliform positive samples and no consecutive *E. coli* positive samples). For the purpose of enumerating *E. coli* in water samples in accordance with the PSR, the FDA considers EPA Method 1103.1, Hach Method 10029, the Quantitray 2000 system, and EPA Method 1604 equivalent to EPA 1603 in sensitivity, accuracy, and precision (FDA, 2017f), and therefore all are suitable for this purpose.

#### *7.2.1.2 Post-Harvest Water Use*

No detectable *E. coli* is allowed in water used post-harvest (e.g., hand or produce washing). Ground water and municipal water are permitted to be used post-harvest, while surface water is not. This is because surface water is likely to contain pathogens or indicator organisms. Ground water used post-harvest is required to be sampled four times initially, then once a year to create a rolling dataset of four samples. Testing of municipal water that is intended for post-harvest use is not required.

### *7.2.2 Agricultural Water Assessments*

Several recent studies have tested agricultural water used on farms and compared the results to the PSR requirements in an effort to establish a baseline. There have been differences in generic *E. coli* counts from agricultural water from different regions, but no explanatory model for interregional differences in irrigation water quality has been proposed to date (Pachepsky et al., 2011). *E. coli* levels in surface water ponds used to irrigate produce in Pennsylvania were found to be approximately 90 CFU/100 mL on average (Draper et al., 2016), which is lower than the current PSR threshold. Similar studies assessing generic *E. coli* presence found a GM of 6 CFU/100 mL in Florida agricultural ponds, a GM of 3.7 CFU/100 mL and 6.26 MPN/100 mL in Georgia ponds, and a GM of 144 CFU/100 mL in a Pennsylvania watershed (Antaki et al., 2016; Hong et al., 2017; Luo et al., 2015; Topalcengiz et al., 2017). All of these water sources met the PSR threshold except the latter. A study of California produce irrigation water found a mean of 210 CFU *E. coli*/100 mL in irrigation water in general, and means of 1,000, 2,500, and 230 CFU/100 mL in streams and rivers, standing water, and ponds, respectively (Benjamin et al., 2013). The mixed results of previous studies (e.g., Benjamin et al., 2013; Draper et al., 2016; Luo et al., 2015) is likely due to the fact that surface water *E. coli* can vary by geographical area, sampling time, sampling frequency, sediment presence, and precipitation and runoff events (Pachepsky and Shelton, 2011). Surveys of ground water found no detectable *E. coli* in Georgia and California wells (Antaki et al., 2016; Benjamin et al., 2013). The results of these studies illustrates the large variation of *E. coli* levels in agricultural water sources.

A major facet of the water rule, which may require adjustment, is sampling frequency. Havelaar et al. (2017) found that using data from 20 samples was not sufficient to describe the

bacterial quality of an irrigation water source. Analyzing an additional 5 samples per year to update the 20 sample dataset resulted in uncertain results and delays in detecting changes in water quality (Havelaar et al., 2017). This is due the nature of the calculations and the large shift in *E. coli* levels that is required over multiple sampling occurrences to create a change in the average GM (Havelaar et al., 2017). More data are needed to assess the variability of *E. coli* levels in agricultural waters across the country, establish a baseline, and help determine the minimum number of samples required to describe water qualiy (Havelaar et al., 2017).

### *7.2.3 Produce Farm Scale and Food Safety Practices*

Small farms and local product purveyors are an important part of the produce market in the United States. These farms often use direct-to-consumer marketing channels such as farmers markets or through Community Supported Agriculture programs (Low et al., 2015; Martinez et al., 2010). While some small growers may already meet GAP and other buyer standards that have criteria similar to the PSR, most do not fall into this category (Hatanaka et al., 2005). There is a limited body of literature assessing the produce safety practices of small farmers during production, harvest, storage, transportation, and point of sale (Astill et al., 2018). Table 7.1 displays estimates of selected food safety practices from previous produce grower surveys. Rather than gross income, farm sizes were generally classified by acreage in these studies (small=<100 acres, medium=101-499 acres, large=>500 acres).

Some of this literature has analyzed the topic of agricultural water use during production. A New England study assessing GAPs found that 18 percent of small farmers tested ground water that was used to irrigate produce for fecal indicators (Cohen et al., 2005). A survey of small- and medium-sized New York growers found that 19 percent of growers tested their surface irrigation water for fecal contamination indicators (Bihn et al., 2013). Seventy-six

percent of small growers surveyed in Maryland and Delaware did not test their irrigation water yearly for fecal contamination indicators (Marine et al., 2016b). A survey of small and medium growers in South Carolina, Georgia, and Virginia (Harrison et al., 2013b) found that 27 percent use untested irrigation water.

To ensure the microbial quality of water that contacts produce post-harvest, growers can test and treat the water. However, several studies have shown that varying numbers of farmers do not test the microbial quality of their post-harvest water. Harrison et al. (2013b) found that post-harvest, 16 percent of growers use untested wash water. A national study of produce growers found that 31 percent of PSR exempt farms tested their irrigation and/or wash water, while 79 percent of medium and large farms tested their water (Adalja and Lichtenberg, 2018). Twenty-five, sixteen, and sixty-six percent of growers of various sizes nationwide, in New York, and Minnesota, respectively, treated their wash water with disinfectants (Astill et al., 2018; Hultberg et al., 2012; Rangarajan et al., 2002).

Worker training is essential to produce safety at the production, harvest, transport, storage, and point of sale stages. Various surveys of produce growers of all sizes found that anywhere from 25 - 100 percent of these establishments trained employees in health and hygiene practices (Adalja and Lichtenberg, 2018; Astill et al., 2018; Cohen et al., 2005; Harrison et al., 2013b; Hultberg et al., 2012; Lichtenberg and Tselepidakis Page, 2016; Marine et al., 2016b). At the harvest stage, 50 percent of small and medium growers (n=226) in South Carolina, Georgia, and Virginia harvested produce with bare hands (Harrison et al., 2013b).

Sanitizing food contact surfaces is also important throughout the produce production chain. Surveys found that 57 - 90 percent of growers of all sizes sanitized surfaces and equipment (Adalja and Lichtenberg, 2018; Astill et al., 2018; Cohen et al., 2005; Harrison et al.,

2013b; Hultberg et al., 2012; Lichtenberg and Tselepidakis Page, 2016; Marine et al., 2016b). The availability of accessible toilet and handwashing facilities near the growing area contributes to the safety of the produce during production and harvest. Sixty-six to ninety-five percent of growers of all sizes had accessible facilities (Adalja and Lichtenberg, 2018; Astill et al., 2018; Cohen et al., 2005; Harrison et al., 2013b; Hultberg et al., 2012). Many of these farms would be considered PSR-exempt based on income or sales locations. However, exempt farms may decide to adopt PSR practices for their own risk management purposes or to meet the demands of buyers (Astill et al., 2018).

Table 7.1. Survey data on estimated numbers of produce growers engaging in selected food safety practices.

| Study | Study region | Sample size | Water testing | Use of raw manure | Use of treated soil amendments | Wash water treatment | Worker health and hygiene training | Accessible wash/toilet facilities | Regularly sanitize surfaces/ equipment |
|---|---|---|---|---|---|---|---|---|---|
| Rangarajan et al. (2002)[a] | New York | N=213 | 15% | 17% | 16% | 16% | NA[1] | NA | NA |
| Cohen et al. (2005)[c] | New England | N=297 | 20% | NA | NA | NA | 50% | 93% | 63% |
| Hultberg et al. (2012)[c] | Minnesota | N=246 | NA | NA | NA | 66% | 87% | 95% | 87% |
| Harrison et al. (2013b)[b] | Georgia, Virginia, South Carolina | N=226 | 40% | 15% | 76% | NA | 41% | 66% | 57% |
| Bihn et al. (2013)[b] | New York | N=84 | 27% | 17% | NA | NA | NA | NA | NA |

| Study | Study Region | Sample size | Water testing | Use of raw manure | Use of treated soil amendments | Wash water treatment | Worker health and hygiene training | Accessible wash/toilet facilities | Regularly sanitize surfaces/ equipment |
|---|---|---|---|---|---|---|---|---|---|
| Marine et al. (2016b)[c] | Mid-Atlantic | N=313 | 32% | NA | NA | NA | 43% | NA | 90% |
| Lichtenberg and Tselepidakis Page (2016)[a] | Mid-Atlantic | N=47 | 36% | 53% | NA | NA | 100% | NA | 71% |
| Adalja and Lichtenberg (2018)[a] | Nationwide | N=394 | 31% | 66% | 74% | NA | 61% | 84% | 61% |
| Astill et al. (2018)[a] | Nationwide | N=4,618 | 39%* | 6% | 7% | 24.5% | 25%* | 77%* | 72%* |
| **This study[a]** | Alabama | N=8 | 16% | 0% | 37.5% | 0% | 50% | 100% | 60% |

[a]Small, medium, and large growers (small=<100 acres, medium=101-499 acres, large=>500 acres), [b]Small and medium growers (small=<100 acres, medium=101-499 acres), [c]Small growers (small=<100 acres)

*Not covered by PSR and exempt growers, N=1,625

[1]NA indicates variable was not measured.

### *7.2.4 Alabama Farm Size and Water Quality*

Alabama has a large concentration of small produce farms. About 93 percent of all farms in the state are small, as defined by the USDA (USDA, 2012). The fresh produce industry in Alabama has an estimated value of $161 million, contributing 1,121 jobs to the state's economy (Fields, 2017). Only 1.3 percent of produce farms in Alabama are GAP certified (USDA, 2017).

The quality of Alabama's water in previous surveys has varied. GMs of *E. coli* ranged from 181 - 462 CFU/100 mL at a mixed-use watershed that is on Alabama's list of impaired waterbodies due to high fecal coliform concentrations (Wijesinghe et al., 2009). Influent river water destined for an Alabama water treatment plant ranged from 4 - 62 MPN *E. coli*/100 mL. Well water samples collected from the same area before and after disinfection yielded no detectable *E. coli* (Okeke et al., 2011).

### *7.2.5 Experiment Station Food Safety Research*

Experiment station managers are in a unique position to educate and train growers about food safety practices and PSR implementation. Food safety research is a driving mission at land grant university experiment stations (APLU, 2010); and in 2016, 14 million dollars were awarded by the USDA National Institute of Food and Agriculture to fund food safety research at these facilities (AFRI, 2017). However, this work does not necessarily translate to the best, most current food safety practices being implemented at the experiment stations. There is no known research assessing the food safety knowledge or practices of experiment station managers. The newly established (2015) Cornell Institute for Food Safety partners experiment stations with extension agents to teach on-farm food safety practices (Institute for Food Safety, 2018). However, assessment of the prevalence or effectiveness of these on-farm teaching programs is also lacking.

### *7.2.6 Objectives*

Another gap in the literature is an evaluation of Alabama's agricultural water quality in relation to the upcoming PSR standards and produce safety landscape. The objectives of this study were (1) to determine the average *E. coli* presence in agricultural water used to grow produce from three regions across Alabama, (2) to compare three *E. coli* enumeration methods, (3) to determine whether or not PSR-exempt Alabama Agricultural Experiment Station (AAES) managers have adopted any food safety practices that align with the PSR, and (4) to determine how AAES farms might best aid small and very small farms in the state. This work aims to identify not only the current food safety practices and knowledge of AAES produce growers, but also critical gaps in their practice and knowledge, as well as the potential points (e.g. strategies, information) where AAES may be able to assist growers. It will also identify the microbial quality of water used in produce production and harvest. Ultimately, the goal is to help identify a broader range of challenges for small growers and contextualize the implications of the small farm PSR exemption.

## 7.3. Materials and Methods

Agricultural water from three different geographical areas of the state was sampled and enumerated for *E. coli* during a growing season. A survey of produce safety practices was sent to AAES managers including the farms where water was sampled. These data are synthesized in section 7.5.

### *7.3.1 Water Sampling Locations*

The experimental design was a repeated measures arrangement conducted over spring and summer 2018 in triplicate. Water samples from sources used to irrigate produce were collected from three Alabama counties. The three locations (identified as A, B, and C) were

chosen to represent the primary produce growing areas in Alabama. The three locations encompassed seven individual water sampling sites. Location A had one surface (a pond), one ground, and one county (municipal) water site. Location B had a ground and a surface water site (i.e., spring), and Location C had a ground water source with two sampling sites. Two of the three counties sampled were located are the top vegetable and fruit producers in the state. Sampling took place at four farms. Three farms were AAES. Location B included a local independent farm in addition to AAES. Locations A, B, and C were a 0.5 hour, 1.5 hour, and 3.5 hour drive from the laboratory. Since the majority of the produce grown at these locations is sold directly, they are PSR-exempt.

### *7.3.2 Water Sampling Process*

Water from the pond was sampled with a sterile bottle near the irrigation water intake source at approximately a one-foot depth. All other water samples were collected at the source from a tap. Samples were collected monthly over the course of a growing season, from March to July, 2018 (n=12). In each case, 1 L water samples were collected in triplicate in sterile plastic bottles. Samples were transported to the laboratory in a cooler with ice. Microbial analysis began less than 6 hours after collection in accordance with the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 2005).

### *7.3.3 Generic E. coli Enumeration*

From each 1 L bottle of water, serial dilutions were performed in phosphate-buffered saline. A volume of 90 - 100 mL was filtered via the membrane filtration method through a 47 mm diameter, 0.45 µm pore size filter (Pall Laboratory, Port Washington, NY, USA) and plated onto three different media types to enumerate generic *E. coli*. The methods used (see Table 7.2)

were: EPA 1103.1, EPA 1604, and Hach method 10029. Results from the three replicates were averaged for each enumeration method and month.

Table 7.2. Description of generic *E. coli* enumeration methods

| Method | Media | Definition | Media type | Procedure | Cost/plate |
|---|---|---|---|---|---|
| **EPA 1103.1** | mTEC (Hardy Diagnostics, Santa Maria, CA, USA) | Membrane-thermotolerant *E. coli* agar | Selective, differential | Incubate at 35 °C for 2 h, then incubate at 44.5 °C for 22-24 h in water bath. Transfer filter to urea-phenol substrate | $0.09 |
| **EPA 1604** | MI (BD Difco, Franklin Lakes, NJ, USA) | Membrane filter medium for total coliforms and *E. coli* | Chromogenic, fluorogenic, selective, differential | 24 h incubation at 35 °C | $0.35 |
| **Hach 10029** | mColiBlue 24 (Hach, Loveland, CO, USA) | Hach mColiBlue 24 for total coliforms and *E. coli* Membrane Filtration | Chromogenic, selective, differential | 24 h incubation 35 °C | $1.16 |

Each sample was plated in duplicate on 9 x 50 mm petri dishes and incubated according to the respective procedure (Table 7.2). The presumptive generic *E. coli* colonies were enumerated and the averages of the results are reported in Table 7.4. The limit of detection (LOD) was 1 CFU/100 mL. A plate was considered below the LOD when the average of the two enumerations were below 1 CFU. ND (none detected) indicates that no colonies were counted on a plate. *E. coli* was used as a positive control, and *Pseudomonas aeruginosa* (Hach, 2017) was used as a negative control on each trial sampling day with all three types of media.

### *7.3.4 Produce Grower Survey*

An electronic survey was sent to all (n=15) AAES managers and associate managers with Qualtrics software (Seattle, Washington, US).[41] Approximately 10 of these experiment stations grow produce such as tomatoes, squash, watermelon, peaches, and apples. Three AAES locations corresponded to the water samples; locations A, B, and C. The survey (Table 7.6) consisted of 45 questions covering 5 key topics including information about the farm size, crops grown, biological soil amendment use, food safety practices, agricultural water use, and demographics. The questions were adapted from Bihn et al. (2013). The survey was administered in June 2018 with 2 reminders sent in July. The questions were predominately closed-ended with a yes or no response option. Data were collected between June 25 and August 2, 2018. The survey results are compared to pre- and post-harvest on-farm food safety practices of other recent produce grower surveys in Table 7.1.

### *7.3.5 Analysis*

The geometric means and statistical threshold values for the *E. coli* surface water data were calculated with the UC Davis Surface Water MWQP Calculator. The Shapiro-Wilk test was

[41] Survey was approved by Auburn University Institutional Review Board (#18-246 EX 1806).

performed to assess the normality of the surface water data ($P<0.05$). To assess the *E. coli* enumeration methods, a mixed effect model was created with method and location as the main effects and month and replication as the repeated measures. The least-square means *post hoc* test with the Tukey adjustment was run to determine significant differences between groups ($P<0.05$).

Survey data results were compiled and mean response results for each question were computed. R software was used for statistical analysis (Vienna, Austria). For the water microbial data, 0.01 was added to the results that were zero in order to allow log transformations on all numbers, and the data was log transformed. The Shapiro-Wilk test was performed to assess the normality of the data ($P<0.05$). The Kruskal-Wallis test was performed with the Nemenyi *post hoc* test ($P<0.05$) when the data was found not to be normally distributed.

## 7.4. Results and Discussion

We first discuss the microbial quality of the water sources sampled, then the questionnaire results, and finally compare the questionnaire results to the corresponding microbial quality of the farms surveyed.

### *7.4.1 Agricultural Water Quality*

The prevalence of generic *E. coli* in the surface, ground, and county water are shown in Table 7.3. *E. coli* was present in all of the surface water samples across the collection period (n=24) and one ground water sample (n=48) during one month. *E. coli* was not present in the municipal water samples across the collection period. The average GMs of *E. coli* in the surface water at locations A and B were 6 and 20 CFU/100 mL. No detectable *E. coli* was found in the ground or municipal water. The *E. coli* counts in surface water samples were significantly higher than those of the county and ground water ($P<0.01$). This was expected, as surface water is

generally found to be of lower microbial quality than groundwater (Steele and Odumeru, 2004) due to a range of environmental factors such as presence of human or animal fecal material, and other biophysical factors such as weather, season, and water chemistry (Pachepsky et al., 2011; Udenika Wijesinghe et al., 2009).

Table 7.3. Prevalence of generic *E. coli* in seven surface, ground, and county agricultural water sampling locations in Alabama over four sampling periods during a produce growing season.[1]

| Water type | Location | Month 1 | Month 2 | Month 3 | Month 4 |
|---|---|---|---|---|---|
| **Surface** | Location A | + | + | + | + |
| | Location B | + | + | + | + |
| **Ground** | Location A | + | - | - | - |
| | Location B | - | - | - | - |
| | Location C | - | - | - | <LOD[2] |
| | Location C | - | - | - | <LOD |
| **County** | Location A | - | - | - | - |

[1]From 100 mL water sample

[2]LOD=limit of detection (1 CFU/100 mL)

The county water taken from Location A contained no detectable generic *E. coli*, which is expected as US municipal water is chlorinated to eliminate potentially harmful microorganisms. The results from surface and ground waters (GM of 20 and 6 CFU/100 mL, STV of 282 and 32 CFU/100 mL at Locations A and B in surface water and no detectable *E. coli* in ground water) are comparable to the results of Antaki et al. (2016), who assessed ponds and wells used to irrigate produce during three growing seasons in Georgia, a state neighboring Alabama. Also, no

detectable generic *E. coli* was found in ground water, and low levels of *E. coli* were found in two sources of surface water (GM of 3.68 CFU/100 mL and STV of 30.3 CFU/100 mL), the latter of which met the PSR criteria. These results are also within the upper acceptable threshold of the PSR (i.e., 126 CFU/100 mL GM and 410 CFU/100 mL STV).

Studies evaluating the quality of agricultural water in different US regions have also found varying levels of generic *E. coli*, although most have also been within the acceptable limits of the PSR. *E. coli* data from irrigation water has high spatiotemporal variability (Partyka et al., 2018; Rafi et al., 2018). For instance, studies of surface water in Florida and Pennsylvania sampled over 3-4 years found GMs of *E. coli* in various ponds ranging from 1 - 142 CFU/MPN/100 mL, with STVs ranging from 4-513 CFU/MPN/100 mL (Hong et al., 2017; Topalcengiz et al., 2017). This could be due to different sites being sampled and time of year. Our results fit within this range.

Table 7.4 shows the numbers of generic *E. coli* in the surface water samples evaluated by the three EPA Method 1603 equivalent methods as noted in the PSR. While FDA considers the methods equivalent, the results showed that at both locations, EPA 1103.1 (mTEC) produced significantly higher and lower results than Hach (mColiBlue24) at locations A and B, respectively ($P<0.05$). Grant (1997) found that mColiBlue24 performed significantly better than mTEC in agreement, sensitivity, and specificity in detecting *E. coli* in natural and spiked water samples, with mColiBlue24 recovering significantly more *E. coli* than mTEC. Similarly, Hamilton et al. (2005) found a significant overall difference with mColiBlue24 recovering more *E. coli* than mTEC from natural water samples.

Two studies found no significant difference in *E. coli* recovery rates from natural and spiked water samples and recreational water between MI medium and mTEC (Brenner et al.,

1993; Francy and Darner, 2000). Our results were similar, as the mTEC *E. coli* counts were not found to be significantly different from the EPA 1604 (MI) counts. Differences found in the performance of the media could be due to the fact that mColiBlue24 and MI target $\beta$-glucuronidase, an enzyme specific to *E. coli*, and they are incubated at 35-37 °C, which is the optimal growth temperature for *E. coli* (Leclerc et al., 2001). This optimal temperature allows for improved recovery of stressed organisms. In addition, the culture based procedure using mTEC involves a 45 °C incubation step that could be lethal to stressed *E. coli* cells (Hamilton et al., 2005).

Table 7.4. Descriptive statistics for generic *E. coli* populations in surface water determined by three equivalent methods to EPA 1603.[1]

**Microbial Count (CFU/100 mL) mean[2] ± SD**

| **Location** | **Month** | **EPA 1103.1 (mTEC)** | **EPA 1604 (MI)** | **Hach 10029 (mColiBlue24)** |
|---|---|---|---|---|
| **Location A** | 1 | 8 ± 9 | 2 ± 1 | 2 ± 1 |
| | 2 | 54 ± 61 | 11 ± 2 | 30 ± 38 |
| | 3 | 205 ± 205 | 16 ± 4 | 8 ± 4 |
| | 4 | 460 ± 365 | 15 ± 1 | 11 ± 5 |
| | **Average** | 182 ± 270[c] | 11 ± 6[abc] | 13 ± 21[ab] |
| **Location B** | 1 | <LOD | 7 ± 4 | 21 ± 13 |
| | 2 | 2 ± 1 | 3 ± 1 | 3 ± 2 |
| | 3 | 11 ± 12 | 2 ± 1 | 6 ± 2 |
| | 4 | 13 ± 4 | 15 ± 10 | 9 ± 5 |
| | **Average** | 6 ± 8[a] | 7 ± 7[ab] | 12 ± 11[bc] |

[abc]Denotes significant differences across the two "Average" rows (i.e., c is significantly different from a, b is significantly different from c, a and ab are not significantly different, abc and c are not significantly different, b and bc are not significantly different)

[1]PSR requirements are GM <126 CFU/100 mL

[2]Average of three replicates

n=12

### *7.4.2 Produce Grower Survey*

In addition to determining the average *E. coli* presence in agricultural water from three Alabama regions and comparing three *E. coli* enumeration methods, the other two objectives of

this study were to determine whether or not PSR-exempt AAES managers have adopted any food safety practices that align with the PSR, and to determine how AAES farms might best aid other small and very small farms. Eight AAES manager responses were received (response rate = 80 percent). Table 7.5 shows the survey responder farm size and operation information. The survey questions are shown in Table 7.6 with responses to the closed-ended questions. Half of the respondents had been employed at the position for 0-3 years (n=4), all respondents were male (n=6), and all respondents had a four-year degree (n=4).

Table 7.5. Descriptive summary of farm size and operations of survey responders.

| | |
|---|---|
| **Land Use** | Row crops (41.67 percent, n=6) |
| | Animal production (33.67 percent, n=3) |
| | Fruit and vegetable production (20 percent, n=7) |
| | Forest or undeveloped land (18.8 percent, n=6) |
| | Other uses (ornamentals, nuts) (34.67 percent, n=3) |

Table 7.6. Survey questions posed to Alabama Agricultural Experiment Station managers and related responses.

| | **Response (Y=yes, n=# responses)** |
|---|---|
| **Biological Soil Amendments** | |
| Do you use biological soil amendments? | Y=37.5 percent, (n=8) |
| Do you use untreated biological soil amendments like raw manure? | Y=0 percent, (n=3) |
| **Food Safety Practices** | |
| Do you have a written food safety plan for your farm? | Y=0, (n=7) |
| Have you ever had a third-party audit of your farm to verify food safety practices? | Y=0, (n=1) |

| | |
|---|---|
| Do you have domestic animals (cows, dogs, cats, pigs, chickens, turkeys, etc.) in the area of your farm used for growing crops? | Y=50 percent, (n=8) |
| Do you have access to farm safety/sanitation training? | Y=100 percent, (n=8) |
| Are you trained in farm food safety practices such as those listed in the Food Safety Modernization Act Produce Safety Rule regulations, or GAPs? | Y=50 percent, (n=8) |
| Do you use additional farm workers in your operation? | Y=37.5 percent, (n=8) |
| If yes, have your workers received food safety training that covers the Food Safety Modernization Act Produce Safety Rule or GAPs? | Y=33 percent, (n=3) |
| Do you have permanent or mobile toilet facilities? | Y=100 percent, (n=8) |
| Do you have permanent or mobile hand washing facilities? | Y=100 percent, (n=8) |
| Do you and/or your farmworkers harvest produce while wearing gloves? | Y=37.5 percent, (n=8) |
| **Agricultural Water** | |
| Do you use supplemental irrigation? | Y=100 percent, (n=6) |
| Has an environmental impact survey been done of the area surrounding your irrigation water source to determine potential contamination factors? | Y=0 percent, (n=6) |
| Do you wash your produce before it leaves the farm? | Y=33 percent, (n=6) |
| Have you ever tested your irrigation or wash water source(s)? | Y=50 percent, (n=6) |
| Are you currently testing your irrigation or wash water source(s)? | Y=33 percent, (n=3) |

High microbial risk crops, or crops with a greater history of associated foodborne outbreaks, grown by the survey respondents included leafy greens (n=4), tomatoes (n=4), herbs (n=1) and melons (n=4). Other crops grown included berries (n=3), pome fruits (n=1), stone fruits (n=2), onions and garlic (n=1), potatoes (n=3), other vegetables such as squash, eggplant, sweetcorn, and peppers (n=5), as well as other miscellaneous fruit crops such as grapes, citrus, and nuts (n=4). Of the managers surveyed, the farm crop acreages were <100 acres (n=3), 101-499 acres (n=2), and 501-1,000 acres (n=3).

#### *7.4.2.1 Training*

All respondents (n=8) indicated they had access to farm food safety training. Of the responders, 50 percent (n=4) were trained in food safety management programs such as GAPs or the FSMA PSR. This is comparable to about 30 percent of PSR exempt growers in a nationwide survey, and 54 percent of small growers who had received this training in New England (Astill et al., 2018; Pivarnik et al., 2018). In our survey, three out of eight respondents reported that they use additional farm workers at their operations. Of those respondents, one trained their workers in food safety practices such as GAPs and indicated that workers received training every 2 years. In contrast, more than a third of PSR-exempt growers (35 percent, n=1,625), nationwide train their workers in food safety practices (Astill et al., 2018).

#### *7.4.2.2 Food Safety Plan*

No respondents had a written food safety plan for their farm nor had they ever had a food safety audit to verify practices on their farms (n=7, n=1). Similarly, one fourth of PSR-exempt growers (26 percent, n=1,625) in a national survey, 17 percent of small growers country-wide and 17% of small New York growers had food safety plans (Adalja and Lichtenberg, 2018; Astill et al., 2018; Bihn et al., 2013). Previous studies found that small growers surveyed in New

England (16 percent) (Pivarnik et al., 2018) and New York (13 percent) (Bihn et al., 2013), as well as nationwide (7 percent of PSR exempt growers) (Astill et al., 2018) received few on-farm inspection audits.

#### *7.4.2.3 Biological Soil Amendments*

Only three respondents reported that they use biological soil amendments (n=8), none of which reported that they use untreated biological soil amendments such as raw manure. Seven to seventy-six percent of New York, Georgia, Virginia, and South Carolina growers of various sizes use treated biological soil amendments (Astill et al., 2018; Harrison et al., 2013b; Rangarajan et al., 2002; Lichtenberg and Tselepidakis Page, 2016). Between six and sixty-six percent of New York, Georgia, Virginia, South Carolina, and nationwide growers of various sizes reported use of untreated raw manure as soil amendments (Astill et al., 2018; Bihn et al., 2013; Harrison et al., 2013b; Rangarajan et al., 2002; Lichtenberg and Tselepidakis Page, 2016).

#### *7.4.2.4 Environmental Assessment*

Of the six who responded to the survey, each indicated that an environmental impact survey of the area surrounding the irrigation water source had not been conducted. In contrast, twenty-three percent of small New York growers in a recent study had conducted an environmental impact survey (Bihn et al., 2013). Known factors present within 1 mile of the AAES manager's operations (n=7) included: confined animal operations (n=1), wildlife (n=5), use of manure as a soil amendment (n=2), sewage treatment plant (n=1), and a river, stream, or other body of water running through the property (n=2). Half of the respondents indicated that they had domestic animals in the area of the farm used to grow crops (n=8), similar to 52 percent of small Georgia, Virginia, and South Carolina growers (Bihn et al., 2013; Harrison et al., 2013b).

#### *7.4.2.5 Sanitation*

All respondents indicated they had permanent toilet and handwashing facilities (n=8). One respondent had both permanent and mobile handwashing facilities. These toilet and handwashing facilities were either a 0 - 5 minute walk from the harvesting field (62.5 percent, n=5), or 5 or more minute walk (37.5 percent, n=3). These results are in line with other grower surveys, where 66 - 95 percent of respondents had these facilities (Adalja and Lichtenberg, 2018; Becot et al., 2012; Cohen et al., 2005; Harrison et al., 2013b; Hultberg et al., 2012). Three out of eight respondents (37.5 percent) indicated that workers harvest produce wearing gloves. This is similar to 28 percent and 50 percent in other surveys of small growers (Harrison et al., 2013b; Lichtenberg and Tselepidakis Page, 2016).

Of the five who responded, one participant indicated that produce contact surfaces such as bins, cutting boards, equipment, and tables are sanitized before every use. This is comparable to other grower surveys, which found that 68 - 70 percent of respondents sanitized contact surfaces and equipment regularly (Adalja and Lichtenberg, 2018; Hultberg et al., 2012).

#### *7.4.2.6 Agricultural water*

All respondents indicated that they use supplemental irrigation (n=6). Of these respondents, all use drip and overhead irrigation, while only one uses microjet irrigation. In a normal growing season, respondents (n=6) irrigate: 3-5 times (n=2, 33 percent), more than 5 times (n=2, 33 percent), and at least weekly (n=2, 33 percent). The six respondents use well water (n=3, 50 percent), municipal water (n=4, 66 percent), and surface water (n=3, 50 percent) to irrigate produce. Two respondents wash their produce using municipal water.

Half of the respondents (n=3, 50 percent) indicated that they had tested their irrigation or wash water, but only one of these three respondents is currently testing for *E. coli* and fecal

coliforms. This value is comparable to other surveys of produce growers (ranging in farm size from small to large) which obtained results ranging from 15 - 51 percent of respondents (Adalja and Lichtenberg, 2018; Becot et al., 2012; Cohen et al., 2005; Harrison et al., 2013b; Rangarajan et al., 2002). Twenty to 41 percent of small growers surveyed in previous studies tested their water (Adalja and Lichtenberg, 2018; Cohen et al., 2005; Marine et al., 2016b). No survey respondents in our study treated agricultural or wash water with chemicals such as chlorine or ozone. A survey of New York small, medium, and large growers found that 16 percent of respondents added chemicals such as these to wash water (Rangarajan et al., 2002).

### 7.5. Experiment Station Microbial Water Quality and Produce Safety Practices

This section synthesizes through case study the AAES water quality and farm manager survey results. It also identifies through case study gaps in food safety practices and opportunities for educating the managers. AAES locations are well-situated to work with Alabama's Cooperative Extension program and small growers to bridge the divide between classroom food safety education and on-farm education. At location A, there is no food safety plan and no environmental impact survey has been conducted. Wildlife is present near the growing area. Drip and overhead irrigation are used weekly during a growing season. Surface, ground, and municipal water are used to irrigate produce. The water sources have not been tested. Although this farm's surface water fell within the PSR agricultural water criteria, using ground or county water to irrigate produce would be a less risky option, since *E. coli* is expected to be present in lower numbers than in surface water.

Location B consisted of a ground water source that contained no detectable *E. coli* over the growing season. No food safety plan is present, and environmental risk assessment has not been conducted. Risks within 1 mile of the land include common wildlife sighting, and other

domestic animals. The manager is trained in GAPs, but workers have not received this training. Surfaces such as bins and cutting boards are sanitized once a year. Drip and overhead irrigation is used daily to irrigate produce with well water. Produce is not washed. The well water source has been tested "once" for *E. coli* and fecal coliforms. The relative risk of produce contamination at Location B is low due its use of well water and drip irrigation. Nevertheless, the manager would benefit from creating a food safety plan, conducting an environmental risk assessment, training his workers, and sanitizing food contact surfaces more frequently. The manager at Location B had a close relationship with the nearby local farmer. This type of relationship could be used to engage the grower in discussion of the PSR and the requirements of agricultural water use.

Location C had no detectable *E. coli* from the two ground water sites sampled over the growing season. There is no written food safety plan and an environmental impact survey has not been conducted. There is common wildlife presence within 1 mile of the growing fields, as well as domestic animals in the area. Supplemental irrigation with well water is used via drip, overhead, and microjet "more than 5 times" during a growing season. This location does not wash produce. The manager has previously tested the irrigation water. Given that only well water is used to irrigate produce at this location, the risk level of produce contamination is relatively low. All AAES locations could send representatives to PSR trainings, begin testing their water sources, conducting environmental impact surveys, and creating a food safety plan according to the PSR requirements. AAES sites have the facilities to develop and conduct on-farm regulation-based food safety demonstrations. These demonstrations may fill an important gap in knowledge about the challenges and best strategies for implementing the PSR for small-scale farmers.

### *7.5.1 Small Farm Agricultural Water Provision Implementation Feasibility*

The PSR AWP establishes microbial testing requirements for agricultural water that contacts produce pre- and post-harvest. This will create new challenges for small growers and laboratories. In accordance with standard methods, agricultural water samples must be transported to the laboratory within 6 hours of sample collection. Location C was a 3.5 hour drive away from the Auburn University laboratory. Therefore, in a state like Alabama, where the nearest accredited laboratory might be several hours away, the trip to transport the cooled water sample is likely to require a full work day. Smaller farms might not have the resources to meet this requirement

The *E. coli* enumeration methods considered equivalent to EPA Method 1603 vary in cost and ease of procedure. The FDA-approved *E. coli* enumeration methods range in cost per plate (Table 7.2), with mTEC being the most affordable, followed by MI, modified mTEC, and then mColiBlue24. Of these methods, those that use mColiBlue24 media require the fewest steps, followed by the MI, then the mTEC media-based techniques, which require an additional incubation step in a water bath. The mTEC media also requires a post-incubation urease test. Another of the approved methods, the Quantitray-2000, requires a substantial monetary investment in equipment that might be prohibitory to a small laboratory, however it does not require media preparation, and therefore saves time.

Currently, the PSR will require water samples to be tested for *E. coli* at certified labs which will undoubtedly charge user fees (Roland, 2015). These laboratories have trained personnel that must adhere to standardized methods. It is unlikely that small producers will be able to establish certified water testing laboratories on-farm (FDA, 2017e). Perhaps only the very largest corporate entities can manage the cost of building and maintaining a certified laboratory

with trained personnel. Moreover, analysis of the water samples must begin immediately upon receipt, which may be a struggle for some small laboratories because a minimum number of trained personnel must be present to conduct the procedure, and supplies such as media must be prepared in advance. For example, the laboratory must have the appropriate amount of media in stock the week of the analysis.

### 7.6. Conclusion

The passage of the FSMA Food Safety Rule gave the FDA authority to regulate the growing, harvesting, packing, and holding of fresh produce. Overall, all water sources tested would pass the PSR criteria for agricultural water. There was no detectable *E. coli* present in the ground or municipal water at the farms tested, which suggests the lower testing frequency required for ground water and lack of testing required for municipal water may be acceptable. The GMs of *E. coli* present in the surface water sources tested were relatively low, but high STVs indicate high variability in *E. coli* results that are likely impacted by factors such as sampling time of day, precipitation, runoff events, and water chemistry. These overall low numbers indicate that based on this small sample size tested during a single growing season, the qualified exemption from the PSR for very small growers was possibly valid for the 2018 growing season. However, future research with more frequent sampling and more geographical areas in the state and over a longer time period is necessary. In addition, since surface water conditions are always changing due to weather patterns and issues like wildlife presence, frequent samples at various sample points must be taken to help determine Alabama's agricultural water quality.

Since the PSR allows growers to choose their preferred *E. coli* enumeration method, growers will have questions about their differences and associated costs and practicalities. The

fact that the equivalent EPA Method 1103.1 and Hach 10029 generated significantly different *E. coli* results indicates that consideration is needed for which *E. coli* testing method might be utilized in a specific context. Given that *E. coli* yields were significantly different between methods, analysts should consider the type of media and method used when interpreting the results (Hamilton et al., 2005). As stated previously, the FDA approved *E. coli* enumeration methods vary widely in cost, time required, and ease of procedure. If only presence or absence data is needed, as for postharvest water, one of these presence/absence methods should be considered. It is important to pick an accurate and reliable enumeration method as it ultimately determines whether a water sample passes or fails the water criteria.

Large corporate farms may have the resources to build and staff an on-site certified laboratory to conduct water testing, but other farms will need to have their samples analyzed by a third party. This may not be practical for some operations with limited income and expertise, or farms located far from a certified laboratory.

There will be challenges for growers in implementing the Final PSR and in understanding at least some of the underlying science including the associated language, techniques, and data. Given the AAES mission to research food safety, more food safety techniques and policies should be implemented and tested at these sites to further aid growers at the farm level in Alabama. Our study found that AAES managers currently have gaps in knowledge regarding on-farm food safety practices. The survey results indicated that overall, AAES managers may require more food safety and PSR information and training themselves. Small growers rely heavily on Cooperative Extension food safety programs to convey the PSR. Some AAES locations currently have the infrastructure in place with demonstration farms to convey

information about growing fresh produce. These farms could also be used as on-site demonstration areas for on-farm food safety techniques like water sampling and hazard analysis.

If AAES managers were to voluntarily implement the PSR provisions, they would need to create a record keeping system, regularly test their agricultural water, train their workers on health and hygiene topics, and enforce more barriers to wildlife and other external food safety risks. Development of PSR demonstrations on AAES farms may fill an important gap in knowledge about the challenges and best strategies for implementing food safety regulations and testing choices, particularly for small-scale farmers.

There is still time ahead of the upcoming PSR compliance dates for more small growers to attend trainings led by Cooperative Extension. Since Cooperative Extension conveys information to small growers about research activities conducted at Experiment Stations, more food safety investment at the Stations would make it possible for AAES to research and test practical and cost-effective food safety practices. To take advantage of the Experiment Station-Cooperative Extension link, AAES managers should create produce food safety plans with an outreach component to reach small growers in the state.

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## 8. Future Study and Suggestions

One of the objectives of this work was to determine the baseline quality of agricultural water in Alabama. Due to financial and time limitations with this study, data was only collected for one growing season. The PSR recommends that surface water samples are collected for two to four years to establish a baseline water quality level. In order to establish a stronger baseline of Alabama's agricultural water, future work should sample the surface and ground water sources in Chilton, Shorter, and Baldwin counties for two to four more years. These results will represent a closer value to the true GM and STV of *E. coli* in these water sources. The baseline would also be strengthened by sampling the water as close as possible to harvest time and sampling from the same physical locations on each sampling occasion. Future work could also determine the variability of Alabama's agricultural water quality due to precipitation changes, presence of animals, and other environmental factors over time.

Another way to improve the calculation of Alabama's baseline water quality in the future would be to add more locations across the state to sample agricultural water from. Ideally, these locations would represent Alabama farms, which are mostly small in size. Further study could also collect separate samples from various types of irrigation systems and compare the results to the microbiological quality of the produce being irrigated. In addition, future work could compare the trend of Alabama's water quality data to similar data from neighboring states such as Georgia and Tennessee. Once the AWP is implemented for small farms, water samples could be again collected to compare the results to previously obtained *E. coli* results.

Using AAES locations as a proxy for small farms, another objective of this work was to determine the water quality and food safety practices at AAES locations. Future work could determine the similarities and differences between Alabama small farms and AAES locations in

terms of water use, food safety practices, and size. In addition, future studies should collaborate with Auburn University Cooperative Extension to help identify the farms in Alabama that will be impacted by the PSR. These farms could be surveyed in a similar manner in which AAES managers were surveyed to determine what food safety practices are currently in place and areas for improvement and education. These surveys to both AAES managers and small growers could be repeated once the PSR is fully implemented to identify positive changes in results.

Future research should continue to investigate if the methods designated by the FDA as equivalent to EPA 1603 are truly interchangeable. The results obtained from the EPA 1103.1 method in particular might need to be re-evaluated and re-tested with new water samples. Overall, the results from this baseline water quality survey would be strengthened with more sampling locations, more frequent sampling, and a multi-year plan of action. The results from the AAES manager survey should promote more PSR training that will ultimately lead to conversations between AAES manager and small grower about the PSR. Further work could evaluate if these types of programs have a positive impact on produce safety.