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Agricultural Water Use in U.S. Fresh Produce Growing Operations – Part II: Assessing Contamination Risks from Microbiological Hazards

ABSTRACT

In this second part of a series on agricultural water use in fresh produce growing operations, we explore the elements of the U.S. Food and Drug Administration's requirements for inspection, monitoring, and maintenance of agricultural water systems and for a written agricultural water assessment as promulgated in subpart E of the Produce Safety Rule. Although inspecting and monitoring agricultural water systems has typically been a routine activity for produce growers, the requirement to write and perform an agricultural water system assessment is new and shifts growers' food safety efforts away from water quality testing to identifying sources of microbial hazards and evaluating the likelihood that those hazards will contaminate the water supply. To this end, we reviewed scientific studies that provide information and insights relevant to these activities to assist growers in their efforts to comply with the rule's requirements and better manage the microbial quality of their agricultural water systems. Studies exploring the microbial quality and management of numerous types of water systems (e.g., drinking, agricultural, recreational, watershed, etc.) provide practical, science-based information that growers can apply to inspection and monitoring activities and when assessing the likelihood that human pathogens in the production environment will contaminate their agricultural water.

INTRODUCTION

In the confines and scope of the *Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption* (Produce Safety Rule), the U.S. Food and Drug Administration (FDA) defines agricultural water as water that "is intended to, or is likely to, contact covered produce or food contact surfaces, including water used in growing activities (including irrigation water applied using direct water application methods, water used for preparing foliar crop sprays, and water used for growing sprouts) and in harvesting, packing, and holding activities (including water used for washing or cooling harvested produce and water used for preventing dehydration of covered produce)" *(177)*. Section § 112.42 in subpart E of the Produce Safety Rule

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(PSR) requires that growers inspect, monitor, and maintain all agricultural water systems *(178)*. Inspection must occur at least once a year, preferably at the beginning of the growing season. In addition to inspection and maintenance of all agricultural water systems, for agricultural water that is applied directly to crops prior to harvest, section § 112.43 requires growers to conduct an agricultural water assessment at least annually *(178)*. All these activities must be documented in writing. As described in Part I of this series, during produce crop cultivation and growing operations, water is used in soil preparation, germination, transplanting, ground and foliage chemigation and fertigation, irrigation, foliar sprays, crop microenvironment modification, and dust abatement *(57, 100, 165)*. Not all these water uses would be considered "agricultural water" by the FDA's definition because, with some of these applications, the water does not directly contact the crop or a food-contact surface *(100, 178)*. However, all these uses have the potential to introduce human pathogens into the production environment and, therefore, pose a risk of direct or indirect crop contamination *(57, 100, 165)*.

Inspecting and monitoring activities are not new to produce growers. Many commodity-specific food safety guidelines (e.g., California avocados, the fresh tomato supply chain, Arizona and California leafy greens, and California cantaloupes) require inspection and monitoring of agricultural water systems *(27, 28, 54, 195)*. Select commodity-specific guidelines also require an agricultural water assessment (sometimes referred to as a "sanitary survey") *(28, 195)*. In their 1998, *Guidance for industry: Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables (175)*, the FDA recommends that growers monitor microbiological hazards and consider practices such as well maintenance, adjacent land use, manure storage and use, etc. to help ensure adequate water quality.

Despite this legacy of guidance, many produce growers are still challenged with managing microbiological hazards that could potentially contaminate their agricultural water systems. A review of publications surveying farmer's food safety knowledge over a 10-year span from 2010 through 2019, showed that handling of agricultural water was one of two topics least understood by growers *(34)*. Pre- and post-training assessments given to growers before (n = 2494) and after (n = 2460) each PSR training, showed that training modules addressing agricultural water had lower post-training assessment scores compared to other modules indicating, as noted by the study authors, "the amount of knowledge gained did not necessarily correspond with a sufficient understanding of the material" *(146)*.

For much of the regulated community attending PSR trainings before and after release of the final subpart E, multiple areas of subjective and impractical provisions, though well-intended, have served as barriers to comprehension of expectations and understanding of the means and options to achieve compliance. While arguably supported by principles of produce safety, these open-ended enforceable requirements (i.e., "must") and quasi-enforceable recommendations (i.e., "should") are not self-evident by data-bounded metrics. Based on their prior experiences, the concern of produce growers and company owners, as communicated in interactions with the authors, is that agency inspections and investigations will be conducted with variable interpretations of agricultural water provisions, despite efforts at internal training and harmonization.

Although numerous engagements between the FDA and produce industry have been held to increase the understanding of the requirements outlined in subpart E, many growers still have questions about implementing the requirements and/or lack confidence that their produce-growing operations are meeting or will be able to meet the requirements *(146, 172, 173, 174, 176, 184)*. As stated in Part I *(100)*, the primary objective of this series on agricultural water use in produce growing operations is to provide growers with pertinent information from the scientific literature that will assist them in making science-based decisions when inspecting, monitoring, maintaining, and assessing their agricultural water system as required under subpart E of the PSR. Considerable research relevant to preventing contamination of agricultural water systems has been published but remains relatively inaccessible to industry as individual studies scattered throughout the body of published literature. For this purpose, we examine the elements of subpart E of the PSR and review relevant published studies that: 1) provide insights and practical applications to consider when inspecting, monitoring, maintaining, and assessing agricultural water systems; and 2) examine risk factors, as specified in subpart E, and others related to the contamination of agricultural water.

Inspecting and maintaining agricultural water systems

As outlined in section § 112.42 of the PSR's revised subpart E, inspection, monitoring, and maintenance are core features of the FDA's agricultural water requirements *(178)*. As a core feature of other industry guidance for good agricultural practices (GAPs), these PSR requirements are familiar elements to produce growers and the industry at-large *(28, 54, 175, 195)*. From their inception, most produce growing operations have been inspecting, monitoring, and maintaining their agricultural water systems as a routine part of their activities. However, the purpose of these activities may primarily have been to ensure the system is working properly and not necessarily to identify potential microbial hazards that may contaminate the water. In the context of the PSR, the purpose of inspecting agricultural water systems is to collect and maintain information regarding how conditions and activities in produce fields and on land adjacent to and nearby produce fields may affect the water's microbial quality from a human food safety perspective

(178). Monitoring is a routine activity that follows up on the inspection findings and looks for significant changes in identified hazards or the emergence of an unanticipated new hazard(s) that may have appeared since the initial inspection. In the PSR, "monitor" is broadly defined as conducting "a planned sequence of observations or measurements to assess whether a process, point, or procedure is under control" *(177)*. Maintaining an agricultural water system is keeping it in a state or condition that excludes or reduces the likelihood of contamination.

System inspection and maintenance

Visual observations are foundational to inspecting and monitoring agricultural water systems for potential sources of microbiological hazards that could contaminate agricultural water. The various GAP programs used in produce growing operations typically require a visual inspection of agricultural water systems before use at the beginning of the season with follow-up inspections conducted throughout the growing and harvesting season *(28, 54, 195)*. In subpart E, the FDA defines "agricultural water system" as "a source of agricultural water, the water distribution system, any building or structure that is part of the water distribution system (such as a well house, pump station, or shed), and any equipment used for application of agricultural water to covered produce during growing, harvesting, packing, or holding activities" *(178)*. The agency requires agricultural water systems to be inspected at least annually and regularly monitored "to identify any conditions that are reasonably likely to introduce known or reasonably foreseeable hazards into or onto produce or food contact surfaces" *(178)*.

Throughout the PSR, the FDA uses the phrase, "known or reasonably foreseeable" to describe hazards and defines it as, "a biological hazard that is known to be, or has the potential to be, associated with the farm or the food" *(177)*. The FDA does not define biological hazards in the PSR, but in their guidance for industry issued for the *Current Good Manufacturing Practice, Hazard Analysis and Risk-Based Preventive Controls for Human Food* (HARPC) rule, the agency describes biological hazards as "parasites, environmental pathogens and other pathogens" and further states that their focus is on bacterial pathogens for HARPC and that viral and parasitic biological hazards (referred to herein as microbiological or microbial hazards) are primarily addressed by following proper worker hygiene and disease control practices per the PSR and current good manufacturing practices *(171)*. In communications with industry, the FDA emphasized that "known or reasonably foreseeable" hazards carry enhanced urgency that should prompt growers to routinely evaluate, on a case-by-case basis, potential sources and routes of contamination followed by implementation of preventive controls *(86)*. As was summarized in Part I of this series addressing agricultural water used on produce crops *(100)*, this includes bacterial (e.g., *Salmonella*, pathogenic *E. coli*, *Campylobacter*), viral (e.g., norovirus), and parasitic (e.g., *Cyclospora cayetanensis*) human pathogens that originate primarily in animals (domesticated, wildlife, humans) as well as a few pathogens such as the bacterium, *Listeria monocytogenes* that is more typically described as environmental (e.g., soil, water) *(30, 37, 61, 79)*.

When assessing "known or reasonably foreseeable" microbiological hazards, knowing what to look for and understanding how to evaluate what is observed are key to adequately and effectively inspecting and monitoring produce production areas. How growers assess what they see during inspection and monitoring activities is heavily influenced by their knowledge and understanding of what constitutes potential sources of microbiological hazards. Because microbial hazards are not visible with the naked eyes, a grower must be able to recognize potential sources of microbial hazards and know where microbial hazards are more likely to occur. Some sources of hazards (e.g., a pile of raw manure) are very evident and visible, while others (e.g., a leaking septic system) are less visible and more likely to remain unrecognized *(108, 200)*. The person conducting visual inspections of an agricultural water system should have knowledge of the property's history and an understanding of visual cues or signs of a potential microbiological hazard. After identifying potential sources of microbial hazards, growers must then make a decision or judgment about whether the hazard is likely to result in contamination of the agricultural water system. Evaluating the likelihood of contamination (risk) from sources of microbial hazards will be further discussed below.

In a white paper addressing microbial contamination of drinking water systems, the U.S. EPA discusses a variety of routes through which human pathogens can enter a water distribution system including leaking pipes, valves, joints, and seals; treatment break-through; cross-connections and backflow; finished water storage vessels; improper treatment of materials or equipment; and inadequate distribution system security *(170)*. These routes of contamination, to varying degrees and circumstances, are also applicable to agricultural water distribution systems and should be considered during the inspections of those systems. When agricultural water is used during various production and harvesting activities, it may be contained in or have contact with numerous types of equipment including holding tanks or cisterns; equipment-mounted application tanks; manifold and boom lines and nozzles associated with aerial application equipment; and pipes, tape, or hoses associated with irrigation systems *(194, 195)*. Because agricultural water delivery systems are often in contact with the ground or in extensive subsurface conveyances, the integrity of system components is crucial to preventing contamination. For

treated agricultural water distribution systems, treatment breakthroughs may involve faulty disinfectant levels, filtering systems, or defective or absent airgaps *(145)*. System components may fail if equipment is not well-maintained or worn from extensive use *(145)*.

Agricultural water system maintenance is the natural follow-up activity to system inspection and monitoring: when deficiencies are found during inspection or monitoring, they should be corrected. The FDA's agricultural water system maintenance requirements include maintenance of the areas near water sources and distribution systems (i.e., free of debris, trash, and domesticated animals) and proper storage of equipment to prevent or reduce the potential for contamination *(178)*. Due to their temporary usage or sequential uses in multiple and often separate farm areas, how agricultural water distribution and application systems are stored is crucial to preventing microbial contamination. Water, sediment, and biofilms remaining in pipes between irrigation events, crop cycles, and even seasons, can support the survival and growth of microorganisms increasing the likelihood of contamination if pathogens are present, even in subsurface drip systems *(47, 170)*. Studies measuring *Salmonella* in irrigation systems detected the pathogen at the end of the delivery system but not (or below the detection limit) at the source *(6, 7)*. Benjamin et al. *(12)* also reported a higher mean concentration of *E. coli* in water from irrigation pipes than from reservoir sources. Measurements of total coliform at the end of line water samples were also higher than source groundwater samples for Pagadala et al. *(128)* and Shelton et al. *(149)*. Enciso-Medina et al. *(47)* studied 18 subsurface drip irrigation systems and reported that approximately half of the systems were inadequately backflushed and received little to no chlorinated cleaning. In their study of the effects of biofilm in irrigation pipes on the microbial quality of irrigation water, Pachepsky et al. *(127)* recommended flushing an irrigation system prior to use in the field – a practice that study participants in Pagadala et al. *(128)* reported conducting infrequently or not at all. Irrigation equipment should be stored in such an elevated manner that allows air circulation but protects from moisture accumulation, soil intrusion, and pest access.

The extent of control

As stated in the PSR (subpart E), agricultural water system inspections and maintenance must be conducted "to the extent they are under your control, to identify any conditions that are reasonably likely to introduce known or reasonably foreseeable hazards into or onto covered produce or food contact surfaces" *(178)*. One of the most frequently discussed topics related to "the extent of control" when assessing the likelihood of contamination from microbiological hazards, especially to flowing surface water such as rivers, creeks, irrigation canals, and water distribution laterals, is related to distance. Where does "the extent they are under your control" begin and end? Does it mean to the border of one's property or beyond? If the assessment requirement is not defined by property borders, how far upstream must one inspect for potential hazards? Determining how far upstream one should inspect involves numerous considerations and challenges beginning with having access. Irrigation canals or waterbodies may run through urban or rural municipalities, including associated restricted access roadways, or may be surrounded by private property that may not be accessible for inspection. Even if one had access, mitigating a hazard may not be within one's control or any practical control measure may be prohibited by various federal, state, or local ordinances (i.e., endangered species protection).

In the standards for agricultural water (subpart E), the FDA acknowledges this very issue and qualifies monitoring and inspection of agricultural water systems "to the extent that the system is under the farm's control" (§ 112.42) *(178)*. However, as explained in their preamble, for the agricultural water assessment of preharvest water applied directly to produce crops (§ 112.43), the agency still requires covered farms to assess possible sources and routes of contamination, including "those over which they may not have control" when determining the safe use of agricultural water on unharvested covered produce *(178)*. Otherwise, a grower is responsible for the microbial quality of preharvest water applied directly to produce crops. It is not entirely clear how the FDA expects the water's microbial quality to be ascertained, especially when irrigation water may travel for miles before it reaches a field, but the FDA lays this responsibility squarely on the user's shoulders.

Assessing how far human pathogens can travel in flowing surface water from a point source or storm-influenced, non-point source is difficult due to the highly variable conditions. Despite the challenges, researchers have attempted to define and quantify the probability of contamination in particular locations with hopeful applicability to similar scenarios. In their research using tracer molecules to study microbial transport in lined and unlined irrigation canals in Maricopa and Yuma counties, Arizona, and Imperial County, California, Lothrop et al. *(111)* observed a 2-$\log_{10}$ reduction by 966 m downstream of the tracer seed point. Based on their findings, Lothrop et al. *(111)* recommended exploring irrigation canals up to 950 m (1,038 yards or 0.6 miles) upstream to check for potential sources of pathogen contamination. In their survey of agricultural water on produce farms in California's Central Coast region, Benjamin et al. *(12)* reported that generic *E. coli* concentrations in stream water decreased by 3% for every 10 m interval as sampling moved downstream away from rangeland. In a more generalized study conducted in New Zealand, Buck et al. *(23)* studied the effects of land use on water quality and

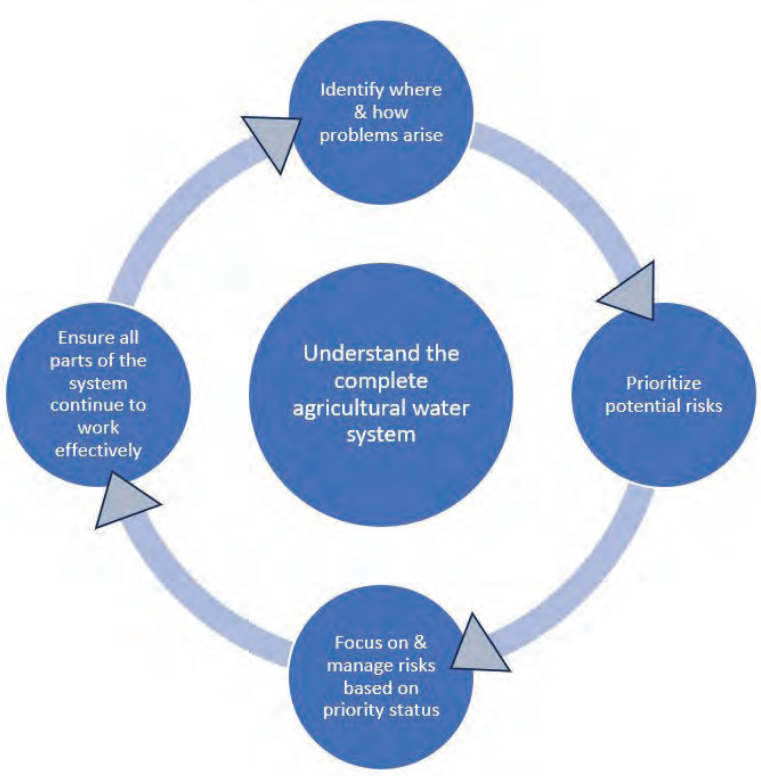

Figure 1. Water safety plan to understand the agricultural water systems.

concluded that larger streams are more likely to be influenced by upstream land use whereas smaller streams are more susceptible to local land use. However, because of the highly variable conditions and limited temporal and spatial scope of the studies, caution in applying these experimental results is warranted as they may not be applicable under dissimilar conditions even at the same location.

Assessing contamination risk in agricultural water systems

In addition to the more familiar requirements to inspect, monitor, and maintain an agricultural water system, subpart E also requires an agricultural water assessment specifically to address water that is applied directly to crops during growing activities (per § 112.43) *(178)*. In subpart E *(178)*, the FDA defines an agricultural water assessment as "an evaluation of an agricultural water system, agricultural water practices, crop characteristics, environmental conditions, and other relevant factors (including test results, where appropriate) related to growing activities for covered produce to: (1) Identify any condition(s) that are reasonably likely to introduce known or reasonably foreseeable hazards into or onto covered produce or food contact surfaces; and (2) Determine whether measures are reasonably necessary to reduce the potential for contamination of covered produce or food contact surfaces with such known or reasonably foreseeable hazards." To break down the FDA's definition, the agricultural water assessment builds on the system inspection findings and subsequent maintenance of the agricultural water system ("based in part on the results of any inspections and maintenance you conducted under § 112.42") with additional elements addressing how and when the water is used (practices), the crops on which it is used, the environmental conditions in which it is used, and other relevant factors such as microbial water quality testing *(178)*. The definition also states the purpose of this evaluation: to identify conditions that may introduce microbiological hazards into/onto produce or food contact surfaces (contamination) and to determine whether actions are necessary to prevent or reduce the likelihood of contamination posed by the microbiological hazards (mitigation) *(178)*.

In their publication outlining their system for analyzing irrigation water quality during foodborne illness outbreak investigations, Gelting and Baloch *(59)* from the Center for Disease Control and Prevention recommended the World Health Organization's (WHO) *Water Safety Plan Manual – A Step-by-Step Risk Management for Drinking-Water Suppliers* as a source of helpful information on conducting an agricultural water assessment *(87)*. The WHO's manual *(87)* outlines a systematic process *(Fig. 1)* that focuses on four basic steps: 1) identify the risks, 2) decide which one is the highest priority, 3) determine how the risk can be managed, and 4) establish

how the ongoing effectiveness of the management approach can be confirmed. This process mirrors the FDA's risk-based process in the HARPC rule and could be restated in HARPC terms: 1) identify the hazards and assess the contamination risk, 2) implement controls and/or mitigation measures, and 3) monitor the controls to verify they are effectively reducing the contamination risk *(171)*.

In addition to evaluating the irrigation water sources, Gelting and Baloch *(59)* recommended assessing the surrounding watershed(s) to identify potential contamination sources and routes that may reach the irrigation water source or extraction point *(59)*. They also emphasized the significance of seasonal changes or changes caused by other circumstances or events in both surface and ground water sources *(59)*. Gelting and Baloch's *(59)* recommended system was directed toward assessing the cause of a foodborne illness outbreak and was used not only to identify "possible sources of contamination, but also the conditions in the environment that facilitated or created that contamination" (the root cause). Unlike an environmental assessment to identify a root cause of an outbreak, the objective of an agricultural water assessment is to identify potential sources of microbiological hazards in the environment that are likely to cause contamination and prevent them from doing so or in the absence of a practical method for prevention, use of routine monitoring to reduce the likelihood of contamination. Although they serve different purposes, both activities (determining the cause of an outbreak and conducting an agricultural water assessment) are assessing sources of microbiological hazards that pose a contamination risk to agricultural water systems.

In order to conduct an agricultural water assessment, growers must have a comprehensive understanding of their agricultural water systems including where the system may be vulnerable to contamination from microbiological hazards. Although some conditions under which microbial hazards may cause contamination are easily recognizable, many are more obscure and less straightforward (i.e., further upstream, unobservable, hidden from view) or subject to compounding influences. These more subtle or obscure conditions that have the potential to result in contamination may require additional instruction and education to increase awareness and provide a basis for situational responses. Assessing whether an identified source of microbial hazard is likely to result in contamination of crops also requires skill, practice, and many times, the benefit of data-informed assessment resulting from various approaches to testing. Education and training are essential for food safety personnel to adequately evaluate the probability that microbiological hazards will contaminate agricultural water systems during assessments. Numerous written materials and in-person training sessions are available from trade associations, private companies, the Produce Safety Alliance, the national and regional coordination centers, and the cooperative extension system operated through the U.S.' land-grant universities *(85, 137, 160, 174)*. It is prudent for industry members to use these training opportunities, in combination with peer-to-peer experiential sharing, to increase their knowledge of identifying and managing contamination risk in their operations.

Agricultural water source – location and nature

Determining whether a microbiological hazard is likely to contaminate an agricultural water system is influenced by the type *(Fig. 2)*, inherent or seasonal condition of the water source, topography surrounding the water source, weather-related conditions, and conditions of and activities on adjacent and nearby land, especially in relation to animal presence and activity *(75, 130, 140)*. Although not all human pathogens originate from animal feces, most contamination risks to agricultural water are from exposures to animal and human feces *(88, 130, 185, 189)*. In their meta-analysis of *Salmonella* prevalence in water, Rocha et al. *(140)* reported that source water was the most important variable influencing the frequency of *Salmonella* detection. When considering the risk of pathogen contamination posed by various types of agricultural water sources as depicted in *Figure 2*, untreated or inadequately treated wastewater presents a high risk whereas potable water sources present a low risk of contamination *(57)*. The vulnerabilities of agricultural water sources can be countered by features (e.g., proper construction and maintenance) and practices (e.g., routine inspection and maintenance) that protect agricultural water.

Groundwater

Groundwater (water that exists beneath the earth's surface) largely consists of surface water that has been absorbed and dispersed through soil and rock layers before reaching the water table *(24, 55, 82)*. Because of the processes involved in moving through soil and rock layers, groundwater is generally considered to less likely be microbially contaminated than surface waters when used on and in proximity to unharvested produce crops *(7, 12, 100, 165, 175)*. As surface water infiltrates the soil, contaminants, including human pathogens, are removed from water by physical, chemical, and biological processes *(2)*. How fast water moves through the soil (percolation and filtration rate) influences how effectively these processes remove microorganisms, by absorption or adsorption, before the water reaches the water table *(2)*. A comprehensive review of the numerous factors and conditions affecting the filtration rate of microorganisms to groundwater is beyond the scope of this article, but Abu-Ashour et al. *(2)* provides a detailed review of factors that affect microbial transport through soil. In brief, under normal circumstances, the transport of microorganisms through the earth to groundwater is affected primarily by the properties of the soil and rock layers (e.g., soil type and structure, porosity, moisture content, organic materials) *(2, 126)*.

TYPE OF IRRIGATION WATER	RISK RANKING
Raw or inadequately treated wastewater	Highest Risk
Surface waters	
Groundwater collected in shallow wells, due to inadequate installation or improper maintenance	
Groundwater collected from deep wells	
Potable water	Low Risk



Figure 2. The risk associated with various types of irrigation water.

Macropore channels in soil have more influence on how surface water impacts shallow groundwater and wells but are typically less common in frequently cultivated soils for row crop production *(31, 42, 52)*.

Although the likelihood of contamination may be reduced in groundwater compared to surface water, groundwater may become contaminated if the well is not properly constructed or maintained or degrades over time *(15, 45, 53, 71, 72, 105)*. The microbial quality of groundwater can also be affected by a rising or falling water table or surface water bodies as happens during times of drought or flooding. Of 190 groundwater samples taken from monitoring wells (small wells constructed with 3–6 meter screens below the water table to observe conditions and measure water quality down-gradient from potential contamination sources) in eight concentrated animal feeding operations (CAFOs) in California's Central Valley, *Salmonella* and *E. coli* O157:H7 were each detected in two samples (1.1%), 46 were generic *E. coli*-positive (24.2%), and 185 were positive for *Enterococcus* spp. (97.4%) *(83, 105)*. Domestic wells (water-production wells constructed for domestic and agricultural use with screens extending 10 or more meters below the water table) at the CAFOs were also tested, and 4.2% and 87.5% were positive for generic *E. coli* and *Enterococcus* spp., respectively *(83, 105)*. Duffy et al. *(45)* reported finding low concentrations of generic *E. coli* (0.3 – 1.1 log CFU/mL) in wells on produce farms in Texas. Generic *E. coli* and *Enterococcus* spp. concentrations were significantly associated with well depth, season, and the type of adjacent land use *(45)*. The researchers also took samples from 200 regional domestic and community service district wells that had positive test results for generic *E. coli* (4.5%) and *Enterococcus* spp. (10.3%), but not for pathogenic bacteria *(45)*. *Enterococcus* concentrations were significantly associated with proximity to CAFOs, seasons, and potassium concentration in the water *(45)*. In their study of *Salmonella* prevalence in irrigation water on Virginia's eastern shore, Gu et al. *(71, 72)* conducted weekly sampling of well water (n = 400) used for irrigation on four tomato farms during 2014 and 2015 growing seasons. Over the 70-week sampling period, *Salmonella* was detected on 13 occasions (3.25%) in three of the four wells (average depth of 60 m) *(71, 72)*. In a U.S. survey of drinking water wells, 4.1% of groundwater samples (n = 539) and 4.8% of wells (n = 442) in 35 states tested positive for enteroviral infectivity in cell culture *(1)*. Bacteria are more readily removed from water than viruses as they filter through the soil. Viruses, being ten times smaller than bacteria, can more easily penetrate soil, especially soil with high sand or gravel content that have larger pore sizes *(1, 15, 167)*.

How a well is constructed and maintained also affects the microbial integrity of its water *(83)*. Well construction is regulated at the state and local level, but specific aspects of a well are integral to maintaining its microbial water quality. These include: a sanitary well cap or seal vented with an intact screen that is sufficient to prevent entry of insects, small mammals, or surface water and a grout seal in the uppermost section of the annular space (the space between the bore hole and the well casing) *(83, 166)*. If other wells are drawing water from the same aquifer, the condition and construction of those wells also affects the microbial water quality. Abandoned but improperly decommissioned and sealed wells can result in groundwater contamination, especially during storm and flood events *(165)*.

Well depth is also a factor in microbial quality of its water. Groundwater from deep wells is less likely to have microbial contamination than water from shallow wells *(72, 105)*. Some wells may be designed to tap groundwater

that is seasonally available closer to the surface above the impervious clay layer covering an underlying aquifer by the placement of slots or screening along the casing *(165)*. These casing perforations designed for water capture at shallower than typical depths may inadvertently allow surface water access *(165)*. Knowledge of a well's design and the soil profile are critical for managing the safe use of groundwater on produce crops.

Where a well is located can significantly affect the water's microbial quality *(82, 83)*. Surface water may infiltrate and contaminate wells even when they are well constructed and maintained *(15, 58, 59, 66, 72, 165)*. Aquifers may be covered and protected by a dense impervious clay layer, but if, for example, breaks form in the clay layer, surface water can enter the aquifer *(165)*. In other instances, infiltration from surface waterbodies may be enhanced when well water levels fall below the level of nearby surface waterbodies such as rivers and lakes. When well water levels fall below nearby surface water levels, a gradient towards the well occurs. Sustained, rapid pumping of water in large volumes from wells may create large gradients of water toward a well *(15, 58, 59)*. Large produce farms that rely on well water for irrigation often pump thousands of gallons of water per minute from wells for an entire day *(82, 83, 165)*. An enhanced gradient increases water flow and reduces filtration as travel time through the soil increases resulting in a more rapid well refilling with water that may not be appropriately filtered *(52, 53)*. When drawdown of well water occurs rapidly and the incoming water from a nearby surface waterbody is contaminated, increased filtration rates resulting from the increased gradient of water flow can lead to elevated pathogen levels in well water *(15, 58)*.

When circumstances deviate from normal conditions, the probability that groundwater may become contaminated increases. Being aware of how the probability of contamination increases with unusual and/or abnormal circumstances (e.g., excessive rain or extreme drought) is essential to the safe use of groundwater in growing and harvesting produce crops. When precipitation occurs in larger than normal volumes over an extended time period, the soil may become saturated, and the filtration rate can be dramatically altered *(2, 14, 16)*. Microorganisms are more readily transported through saturated soil compared to unsaturated soil *(14, 16)*. Therefore, in some instances, flooding may be as much a danger to groundwater as it is to surface water.

Surface water

How land adjacent to or near surface water sources and distribution systems is used has a major effect on the water's microbial quality. Depending on the location, surface water sources may be exposed to contaminants ranging from naturally occurring rain events causing surface runoff to completely manmade activities like illegal dumping *(51, 138)*. *E. coli* concentrations in sediment from roadside ditches next to agricultural land (mean of 886.0 MPN/g) were significantly higher than *E. coli* concentrations in sediment from ditches next to forested land (mean of 646.0 MPN/g) *(51)*. Surface water contamination may occur when animals have direct contact with or when their feces are directly deposited into agricultural water *(4, 182)*. More often, contamination occurs when runoff from feedlots, fields, pasturelands, sewer overflows, or defective septic systems contaminates the agricultural water source or system *(4, 32, 63, 77, 89, 112)*. For example, in a river basin in eastern Ontario, upstream livestock pasture density was associated with *E. coli* O157:H7 detection in stream water samples as were homes with documented septic system issues and increased human *Bacteroidales* markers *(196, 198)*.

Surveys of microbial water quality in agricultural and non-agricultural watersheds using source tracking methods indicate that the probability that surface waters are contaminated is location-specific depending on the size of the waterways, the type and number of animals present, geological and topographical aspects of the landscape and terrain, and human activities *(23, 65, 108, 112, 131, 161, 185, 198)*. Environmental conditions that affect human pathogen survival in agricultural water are discussed further below. Strawn et al. *(161)* reported that most of the *L. monocytogenes* and *Salmonella*-positive samples in surface water sources on New York produce farms were collected from small, low-order waterways with widths below three meters. Lyautey et al. *(112)* also found similar results for *E. coli*: greater density in smaller tributaries vs. larger watercourses. In a two-year survey of central California coastal waterbodies, *E. coli* O157:H7 occurrence was positively associated with agricultural land use whereas fecal indicator bacteria (FIB) and *Salmonella* concentrations and the occurrence of *E. coli* harboring *stx* genes were positively associated with urban land use *(185)*. In an agriculturally dense county in Wisconsin, dairy feeding operations were associated with a 79% increase in the relative abundance of a bovine source tracking marker in surface water *(196)*. In a multi-year study comparing a mountain stream with a pastural stream, the average *E. coli* concentration (607 CFU/100 mL) in the stream running through agricultural land was an order of magnitude higher than in those measured in the mountain stream (14 CFU/100 mL) *(131)*.

The degree of protection

The likelihood that agricultural water systems will become contaminated includes, not only the existence of potential microbiological hazards in the surrounding environment, but also how water systems are protected from microbiological hazards. In subpart E, the FDA specifically calls out protection from "other water users, animal impacts, and adjacent and nearby land uses related to animal activity (e.g., grazing or commercial animal feed operations), application

of biological soil amendments of animal origin, or presence of untreated or improperly treated human waste" *(178)*, all of which are commonly found in rural agricultural settings. However, as urban areas expand, produce fields may be near recreational sites or surrounded by industrial properties and residential communities that carry the potential for waste discharge containing human pathogens *(85)*. Beyond the circumstances cited in subpart E, growers should also consider how their agricultural water systems are protected from other potential contamination events and circumstances such as regional flooding, storm-triggered releases from wastewater treatment facilities, or localized runoff.

Waterbodies are protected from the likelihood of contamination to varying degrees. Protection from contamination may be due to the location of the waterbody and the topography of the surrounding land *(85)*. For example, a reservoir may be located at the base of a hill but is surrounded by a graded 5-ft berm that protects it from runoff *(83)*. In their study of how landscape structures affect foodborne pathogen detection in stream water used as agricultural water on New York produce farms, Weller et al. *(189)* concluded that strategies to decrease overland flow from animal operations to agricultural water help protect agricultural water from contamination.

Protection may come from vegetation such as hedgerows, trees forming a windbreak, or a fence that keeps animals from gaining access *(46, 68, 85)*. Protection effectiveness and sustained efficacy involves the properties and construction of your system. Subpart E specifically mentions whether a system is open or closed; a closed system would obviously be more protected than an open system. For example, a well may be located in a poor location such as a flood plain but is well-constructed with an elevated concrete seal that prevents water from infiltrating at the wellhead *(83)*. Conditions that offer agricultural water systems protection from contamination are best documented with photos and verified by testing under "stress conditions" (e.g., rain events > 4 cm in 24 h).

Animal access and intrusion

In subpart E, especially in section § 112.43, the FDA focuses extensively on contamination risk from animals – "animal impact and adjacent and nearby land uses related to animal activity" *(178)*. Produce production areas with nearby wildlife habitat and/or animal husbandry, where animals are in proximity to and may even intrude into water used as agricultural water on produce crops, increase the probability of contamination *(65, 101, 106, 182, 197)*. In the San Joaquin Valley at sites selected for proximity to potential fecal sources (e.g., concentrated animal feeding operations and rural communities), nearly 5% of irrigation water from canals (n = 83) tested positive for *E. coli* O157 *(144)*. Water from four reservoirs in the Salinas Valley chosen for reported animal intrusion and their proximity to a CAFO and composting operation tested positive for *E. coli* O157 (18.75%; n = 16) and *Salmonella* (50%; n = 4) (144). However, none of the water sampled from components of irrigation systems linked to the contaminated sources tested positive for the pathogens *(144)*.

Studies evaluating the impact cattle have on microbial water quality when they have access to streams in pastures consistently show increased levels of FIB such as *E. coli*, human pathogens, and microbial source tracking markers *(41, 50, 65, 106, 142, 163, 182, 197, 199, 200)*. *E. coli* increased 36-fold in water samples collected in central Indiana during the summer/fall when cattle on pasture had access to the in-pasture stream *(182)*. Water samples collected downstream, over a 25-month period, from a pasture stream accessed by dairy cows in southwestern North Carolina had 300% greater fecal coliform and enterococci levels than samples collected upstream *(106)*. In a survey of a rural Virginia watershed, Graves et al. *(65)*, reported that source tracking methods indicated cattle were the dominant fecal contamination source in creek water whereas deer and geese were minor contributors. Stream water sampled in Nebraska, where cattle grazed for two-week periods during late spring, recorded downstream *E. coli* levels that increased by 7.76 MPN/100 mL, and *E. coli* O157 was detected in downstream water up to 2.5 months after grazing was discontinued *(142)*. In a two-year study, Cooley et al. *(41)* sampled water from lakes, streams, rivers, and ponds used for commercial produce production in California's Central Coast and reported the highest prevalence of *E. coli* O157:H7 occurred at sites close to cattle operations. Wilkes et al. *(199)* reported increased odds of detecting *Salmonella* spp. or norovirus in water samples from two agricultural watersheds in eastern Ontario with substantial dairy production when a ruminant source tracking marker was present relative to when the ruminant marker was absent. Human pathogens and higher levels of ruminant *Bacteroidales* markers were present in water samples from Ontario streams where cattle had unrestricted access compared with streams where cattle access was restricted *(197, 200)*. In a 5-year study of water quality in a stream running through pastureland in eastern Ontario, mean loads of bacteria (total and fecal coliforms, *Enterococcus*, *Clostridium perfringens*) in stream water were compared at three locations: 1) where water entered the pasture where cattle were excluded from the stream (cattle restricted areas), 2) where stream water flowed out of the cattle restricted areas and into the pasture where cattle had unrestricted access, and 3) where stream water flowed out of the unrestricted pastures *(163)*. Bacterial loads in stream water decreased as stream water passed through the cattle restricted areas and increased as water passed through the unrestricted pasture *(163)*. *E. coli* loads were reduced in both the cattle restricted and unrestricted areas but to a lesser extent in the unrestricted area (6%) than in the restricted area (30%) *(163)*. In a survey of surface waters used for

irrigation in lower mainland British Columbia, the density of upstream livestock was positively correlated with detection of *L. monocytogenes* and pathogenic *E. coli (50)*.

Animals other than cattle can also affect the microbial quality of surface water sources. Oocysts and cysts from the waterborne protozoa, *Cryptosporidium* and *Giardia* that were isolated from wild canids' feces had higher potential to infect humans than did strains isolated from cattle feces *(123)*. *E. coli* (n = 1,961) isolated from 52 creek water samples in southeastern North Carolina were analyzed using microbial source tracking methods to identify sources *(108)*. An average of 64% and 27.1% of isolates were associated with lagoon effluent from swine operations and cattle manure, respectively *(108)*. In a study of microbial source tracking markers in agricultural watersheds in eastern Ontario, the odds of detecting *Arcobacter* spp. were positively correlated with the presence of a wildlife marker relative to its absence but negatively correlated with the presence of a ruminant marker *(199)*. In their research at the USDA research center in Nebraska, Hansen et al. *(77)* found that waterfowl density significantly affected *E. coli* concentrations in a stream running through cattle pastures following autumn rainfall events. Based on these and other such studies of animal-associated risks to agricultural water sources in produce-growing regions, an understanding of location-specific animal risks and, where possible, management of those risks are integral to preventing contamination of surface water sources.

Risks related to agricultural water practices

In alignment with the FDA's definition of agricultural water (water that has direct contact with the produce crop), apart from crops (e.g., carrots, radishes, onions) that grow in the soil, subpart E only addresses contaminated preharvest agricultural water applied to the crop and not to soil (i.e., via furrow and subterranean drip irrigation methods). How and when agricultural water is applied to crops affects the probability of contamination *(162, 192)*. The application method and the time interval between agricultural water application and crop harvest are relevant to the probability of crop contamination, and per subpart E, are to be addressed in agricultural water assessments *(178)*. The likelihood that application of agricultural water containing microbial hazards directly to plants may cause contamination is fairly straightforward, but assessing that risk becomes more complicated when considering the time of application in relation to when the crop is harvested.

Application method

When assessing the likelihood of contamination from agricultural water use, whether water or agronomic inputs mixed with water are applied directly to plants via overhead sprinklers, to the base of plants via micro-sprinklers, or to the plant roots via furrow systems has bearing on the potential transfer of human pathogens to edible portions of the crop. For example, subsurface and furrow irrigation systems are less likely to contaminate row crops than overhead sprinkler systems *(3, 8, 56, 118, 119, 150, 156, 201)*, and subsurface irrigation is less likely to contaminate row crops than furrow *(151)*. Solomon et al. *(150)* compared lettuce contamination via spray irrigation of 32 lettuce plants with *E. coli* O157:H7-contaminated water to irrigation of the soil surface around the lettuce plants with *E. coli* O157:H7-contaminated water and reported more lettuce contamination from direct application to the foliage (90.6%) than with irrigation of the surrounding soil (18.8%). Fonseca et al. *(56)* compared transfer of *E. coli* from water to lettuce when plants were irrigated via furrow, overhead sprinkler, or drip irrigation systems and reported greater *E. coli* contamination in lettuce irrigated by overhead sprinkler systems than in lettuce irrigated with drip or furrow irrigation systems. Williams et al. *(201)* also reported more bacteria on lettuce irrigated by overhead sprinkler systems than plants irrigated by drip systems and suggested this was the result of increased available moisture on those plants irrigated via overhead sprinklers.

However, irrigation systems do not always work as expected or according to design – systems may become damaged, emitters may become clogged, and leaks may occur *(128)*. In addition, weather conditions (e.g., wind) may affect the intended delivery method (e.g., cause windblown spray or misting) *(128)* or may cause counter-flow backup of water in furrows resulting in crossing-over of the raised planting bed and contact with lower leaves or hanging fruit. For example, Choi et al. *(35)* observed more viral contamination of lettuce grown in subsurface drip irrigated fields than furrow irrigated fields because cracks in the soil allowed water to penetrate the soil surface where it had contact with lettuce plants. Because water applied via drip systems or reaching produce via spray drift is not intended to directly contact crops, growers may not consider this water to be "agricultural water" as defined by the FDA *(178)*. Nonetheless, per FDA's definition, in addition to considering whether water "is intended to" contact crops, growers must also consider whether water "is likely to" contact produce. In their preamble to subpart E, the agency addressed drip tape leaks stating that growers should consider whether the water may cause the crop "to become adulterated under section 402 of the FD&C [Food, Drug, and Cosmetic] Act" *(178)*. Although growers may not conduct an agricultural water assessment for micro-irrigation or drip irrigation systems because they believe that water does not fit the FDA's definition of agricultural water, in their decision-making process, growers should consider the probability that water from leaks in the mainlines (lay-flat tubing) or drip lines and from windblown water sprays may result in crop contamination.

Application-to-harvest interval

When evaluating contamination risk, the amount of time between the application of agricultural water to produce

crops and the harvest of those crops affects the likelihood that the pathogen is still present when the crop is harvested. If water contaminated with human pathogens is applied to crops, but the crop is not harvested until days or weeks later, the crop may no longer be contaminated because the pathogens have died from exposure to UV light from the sun, desiccation, competition with or predation by plant native microbiota, etc. *(11, 19)*. However, if contaminated agricultural water has contact with edible portions of the crop, reaches protected sites on aerial surfaces of the crop, and/or has contact with the crop close to the time of harvest, the probability that contaminated produce will cause disease in consumers is considerably elevated *(12, 33, 118, 132, 164)*. Although the use of application-to-harvest time intervals as mitigation for reducing the likelihood of contamination is allowed by the FDA in subpart E, the AZ and CA LGMA guidelines for leafy greens are presently the only food safety guidelines that restrict the time interval between application to edible portions of leafy green crops and scheduled harvest to 21 days.

Numerous studies have been conducted to determine how long human pathogens or fecal indicator organisms survive on produce crops after contaminated water has been applied, and study findings have been highly variable *(11, 22, 125, 136, 147, 191)*. What is generally known from study findings and accepted about many pathogens such as pathogenic *E. coli* and *Salmonella* is that they tend to have a bi-phasic die-off that begins with a steep decline followed by a tailing phase when population die-off slowly tapers off *(11, 13, 22, 113, 118, 147, 191)*. As *Table 1* details, study findings show that die-off or survival is affected by numerous factors related to agronomic practices, weather, the crop, and the pathogen.

Crop characteristics

The third component of the FDA's requirements for an agricultural water assessment relates to crop characteristics; namely, the properties of a crop and how it is grown that make it susceptible to pathogen contamination if contaminated water is applied *(178)*. In subpart E's preamble, the FDA states that growth characteristics such as growing near to the ground and surface properties such as porosity "affect the probability and degree of contamination" *(178)*. Because crop characteristics do not directly relate to the microbial quality of the water itself (i.e., whether it is "safe"), some have argued that it should be excluded from subpart E *(116, 143)*. Because the focus of this article is on the contamination of agricultural water, we consider crop characteristics as outside the scope and will not be addressing it here. Nevertheless, an abundance of studies, too numerous to list here, have addressed commodity-specific vulnerabilities to human pathogen contamination *(9, 17, 25, 26, 78, 205)*, and many have reviewed contamination of produce commodities, in general *(19, 20, 21, 168, 203)*.

Risk factors related to environmental conditions

In conducting the agricultural water assessment, growers must identify environmental conditions that may impact the agricultural water system or unharvested produce crops during growing activities *(178)*. In the codified language, the FDA includes air temperatures, sun exposure, and the frequency of heavy rain or extreme weather events as types of environmental conditions to be evaluated, citing stirred sediments and damage to edible leaves as examples of how the water system and produce crops may be affected by extreme weather *(178)*. In the preamble to subpart E, the FDA also mentions airborne transmission and changes in temperature and seasonality as environmental conditions that are expected to affect human pathogen persistence *(178)*. Again, this article focuses on how environmental conditions affect human pathogen introduction and survival in agricultural water systems and, as such, does not specifically address how crop condition (e.g., damage from disease or weather) affects the likelihood of crop contamination when contaminated agricultural water is directly applied. In Part I of this series, we address the effects of season, sediments, aquatic plants, and agrochemicals on human pathogen persistence in agricultural water. Here we note numerous studies investigating the effects of precipitation and runoff on water systems and the specific aspects of water (e.g., physicochemical properties) that affect the survival of human pathogens in the water. Many of the study findings, as noted below, are applicable across produce growing regions, but some are specific to the study location (i.e., the specific site or site region) and may not be applicable to other produce production areas.

Precipitation/Rainfall

Rainfall and, more generally, wet conditions are routinely noted in studies as a risk factor for detecting high levels of and more diverse microbial source tracking markers, elevated levels of fecal indicator organisms, and/or the presence of human pathogens in water from both agricultural and urban areas across North America. Numerous studies have found that high concentrations of fecal indicator organisms or detection of human pathogens are related to the amount of rainfall over time or the occurrence of a rain event in relation to when the water samples were taken *(32, 50, 62, 69, 92, 104, 152, 158, 161, 185, 189, 190, 202)*. Rainfall was positively correlated with FIB and *Salmonella* concentrations in a study of the impact of urbanization and agriculture on bacterial pathogen occurrence in coastal waterbodies of central California *(185)*. *Salmonella* detection was positively correlated with significant rain events in water accessed at public sites in the produce growing area along California's Central Coast *(62)*. In a study of irrigation ponds on produce farms in Suwannee River watershed (Georgia), *E. coli* O157 concentrations increased after rainfall, and rainfall and temperature were the two most important factors positively

TABLE 1. Risk factors affecting pathogen or fecal indicator die-off/survival on produce crops

Risk Factor		Effect	Reference
Agronomic practices	Irrigation method	Iceberg and romaine samples were positive for *E. coli* up to 7 days when using sprinkler irrigation, whereas only one product sample was found positive for *E. coli* when using subsurface drip and surface furrow irrigation methods.	*(56)*
		Although the irrigation method did not impact population size through day 7, on later sampling days the irrigation method did influence the percentage of lettuce plants positive for *E. coli* O157:H7. The number of positive samples was significantly greater among plants irrigated by overhead sprinkler than plants irrigated by drip at day 28 during spring 2008 and at days 7 and 14 during spring 2009.	*(118)*
		E. coli-positive onions (n = 50) were detected at days 1 (8%) and 7 (2%) with subsurface drip inoculation, and at days 1 (14%), 7 (4%), and 14 (4%) with surface drip inoculation.	*(119)*
	Pesticide application	A single in-field pesticide (acibenzolar-S-methyl, peracetic acid, copper hydroxide, streptomycin) application to *Salmonella*-contaminated tomato plants significantly reduce *Salmonella* concentrations at 2 days post inoculation but no significant difference in concentrations were observed at 6 and 9 days post inoculation.	*(70)*
		In the field, when a bacterial cocktail containing *E. coli* O157:H7 and *Salmonella* Enteritidis was added to Bravo 500 at 6 log CFU/ml, both organisms were recovered from leaves and fruit skins of sprayed tomato plants after the recommended 1 day-to-harvest interval. *E. coli* and *Salmonella* survived longer on tomato leaves when sprayed in saline (at least 26 and 56 days, respectively) than when sprayed in Bravo 500 (>45 h and <15 days, respectively).	*(73)*
		Salmonella applied to field grown tomatoes through pesticide application was able to survive up to 15 days in up to 80% of the collected samples.	*(109)*
Weather	Available moisture	Die-off occurred more rapidly on unharvested leafy greens as relative humidity decreased.	*(11)*
		Water availability appeared to be the determinant factor in survival of *E. coli* O157:H7 on spinach.	*(74)*
		Warm temperatures and limited rainfall over 5 days resulted in a 2.2-log reduction in *E. coli* counts on lettuce plants.	*(10)*
		The increases in *E. coli* O157:H7 population size in the phyllosphere of young romaine plants ranged from 16- to 100-fold under conditions of warm temperature and the presence of free water on the leaves.	*(18)*
		All of the *Listeria*-positive spinach leaf samples in the study were associated with rain events.	*(193)*
	Temperature	Warm temperatures and limited rainfall over 5 days resulted in a 2.2-log reduction in *E. coli* counts on lettuce plants.	*(10)*

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TABLE 1. Risk factors affecting pathogen or fecal indicator die-off/survival on produce crops (cont.)

Risk Factor		Effect	Reference
Weather	Temperature	A study using mixed-effect logistic and linear regression models to identify the climate and management variables that are associated with *E. coli* contamination (presence and concentration) in leafy green vegetables reported a robust positive correlation between *E. coli* presence on preharvest leafy greens and the minimum temperature of the sampling day: for a 1°C increase in daily minimum temperature, the odds of having *E. coli*-positive samples on leafy green vegetables increased by a factor of 1.48. The maximum temperature during the 3 days before sampling was positively correlated with *E. coli* concentration on leafy green vegetables .	*(107)*
		The results indicated that there was a significant association between the average daily temperature and both log aerobic plate count and log *Enterococcus* for approximately 5 days prior to sample collection.	*(188)*
	Exposure to UV light	*E. coli* O157:H7 survival was higher on spinach leaves sprayed on the abaxial (lower) side than on leaves sprayed on the adaxial (upper) side.	*(49)*
		E. coli O157:H7 survived for at least 25 days on leaf surfaces of iceberg, romaine and spinach, with survival greater on the abaxial side of the leaves than on the adaxial side.	*(204)*
Crop	Type	*Salmonella* survived longer on spinach than on lettuce.	*(11)*
		More microorganisms survived significantly longer on cantaloupe than on lettuce and bell peppers.	*(157)*
	Variety/ cultivar	In the field study, five days after spraying the plants, *Salmonella* contamination was significantly affected by cultivar. Nine days after spray contamination of plants in the field, no effect of cultivar was exhibited due primarily to the low prevalence of contamination observed for *Salmonella* (8 of 300 plant samples positive by enrichment culture) and *E. coli* O157 (4 of 300 plant samples positive by enrichment culture).	*(48)*
		Field survival of *Salmonella* and *E. coli* O157:H7 was affected by lettuce cultivar.	*(110)*
	Plant characteristics/ tissue components	Certain tissues of the spinach plants were observed to be more supportive to populations of *E. coli* than others. Recovery of *E. coli* and *E. coli* O157:H7 was greatly enhanced at the base of the shoot and at the petioles of the leaves. A 2-fold increase in the number of positive samples for petioles when compared to leaves was observed.	*(74)*
		The *E. coli* O157:H7 population size was consistently ca. 10-fold higher on the young (inner) leaves than on the middle leaves.	*(18)*
	Growth phase	Recovery of *E. coli* and *E. coli* O157:H7 was greatly enhanced in developing tissue.	*(74)*
		The increases in *E. coli* O157:H7 population size in the phyllosphere of young romaine plants varied significantly with leaf age.	*(18)*

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TABLE 1. Risk factors affecting pathogen or fecal indicator die-off/survival on produce crops (cont.)

Risk Factor		Effect	Reference
Crop	Growth phase	When 2-week-old plants were inoculated, *E. coli* O157:H7 could not be recovered from lettuce plants at 28 days post inoculation. When *E. coli* O157:H7 was inoculated onto 4-week-old plants, the organism could be detected by enrichment throughout the experiment through harvest for three of the four trials.	*(118)*
	Physical condition (injured / damaged vs. undamaged)	In iceberg lettuce irrigated with contaminated water, injury immediately prior to inoculation and harvest significantly increased persistence of *E. coli* on lettuce plants.	*(10)*
		E. coli O157:H7 grew on damaged spinach leaves held for 3 days at 8 and 12°C, with the population increasing by 1.18 and 2.08 log CFU per leaf, respectively. *E. coli* O157:H7 did not grow on damaged romaine leaves at 8 or 12°C. Damaged cilantro and Italian parsley leaves held at 8°C for 4 days did not support the growth of *E. coli* O157:H7.	*(95)*
	Crop's natural microbiota (epiphytic bacteria)	Two epiphytes were isolated from pathogen-contaminated plants that interact differently with *E. coli* O157:H7. *Wausteria paucula* enhanced the survival of *E. coli* O157:H7 six-fold on lettuce foliage grown from coinoculated lettuce seed. In contrast, *Enterobacter asburiae* decreased *E. coli* O157:H7 survival 20- to 30-fold on foliage	*(40)*
		Survival of immigrant cells of *Salmonella enterica* on plants appears to be strongly context dependent, and the presence of common epiphytic bacteria on plants can protect such immigrants from at least one key stress (i.e., desiccation) encountered on leaf surfaces.	*(136)*
Pathogen	Type	In a controlled environment study of the effects of relative humidity on bacterial and viral pathogen survival, hepatitis A, coliphage PRD1, and *C. perfringens* were found to have the lowest inactivation rates on cantaloupe, lettuce, and bell peppers, whereas feline calicivirus and *E. coli* ATCC 25922 tended to become inactivated most rapidly.	*(157)*
		Significantly greater populations of *Salmonella* than *E. coli* O157:H7 on lettuce surfaces were observed including a larger number of survivors after populations dropped below the limit of detection. This suggests an effect during field survival associated with individual bacterial strains that could alter initial establishment of pathogen on the plant phyllosphere. This difference may also be attributed to individual strain differences associated with physiological and genetic traits or, has been reported, a general observation that *Salmonella* has superior environmental fitness relative to *E. coli.*	*(110)*
	Strain	*E. coli* strains isolated from the same approximate geospatial location, but different spinach-associated environment, appeared to decline in population at different rates over time despite having identical inoculum doses.	*(74)*
		L. monocytogenes strains vary in the capacity to grow while attached to lettuce.	*(64)*
	Initial concentration	Inoculum dose under the tested conditions does not appear to have an impact on the overall equilibrium survival of bacteria on the surface of spinach, irrespective of growing conditions.	*(74)*

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TABLE 1. Risk factors affecting pathogen or fecal indicator die-off/survival on produce crops (cont.)

Risk Factor		Effect	Reference
Pathogen	Initial concentration	Irrigation water containing *E. coli* O157:H7 at 10^2, 10^4, or 10^6 CFU/ml was applied to spinach 48 and 69 days after transplantation of seedlings into fields. *E. coli* O157:H7 was initially detected after application on the surface of plants dosed at 10^4 CFU/ml (4 of 20 samples) and both on the surface (17 of 20 samples) and internally (5 of 20 samples) of plants dosed at 10^6 CFU/ml. Seven days post-spraying, all spinach leaves tested negative for surface or internal contamination.	*(49)*
	Characteristics that allow it to adapt to a stressed environment	Dormancy allows organisms to enter a reversible state of low metabolic activity when faced with unfavorable environmental conditions.	*(94, 102)*
		When lettuce plants were inoculated with *E. coli* O157 in the laboratory, the greatest persister fraction (cells which are in a dormant state that arise naturally in bacterial populations) was observed during population decline on dry leaf surfaces.	*(120)*

associated with the pathogen's detection in water samples *(69)*. Haley et al. *(76)* also found a statistically strong correlation between *Salmonella* levels and average daily rainfall for days 1 and 2 prior to sampling water from the Upper Suwannee Basin. In the Etobicoke Creek watershed in Toronto, Ontario, more diverse animal DNA markers were present in water samples from a creek and beach after an extremely heavy rain event than samples taken on drier days *(152)*. In a river traversing an agricultural watershed in southern Alberta, *Salmonella* spp., *Campylobacter* spp., and *E. coli* O157:H7 presence was positively associated with total rainfall on the day of sampling and three days prior to sampling *(92)*. Weller et al. *(190)* also reported an increased likelihood of *L. monocytogenes*, *Salmonella*, and *Listeria* spp. isolation as rainfall increased 3–4 days before sample collection in their New York agricultural water study. Overland flow, subsurface flow (e.g., tile drainage), and resuspension of sediment can all contribute to microbial levels in surface waterbodies during rainfall events.

However, studies assessing the relationship between rainfall and bacterial levels in waterbodies have not consistently reported a positive relationship. The association between rainfall and microbial levels or pathogen presence in agricultural water is also influenced by other spatial and temporal factors (e.g., type of waterbody, when and how much precipitation occurs) and agricultural practices in response to rain events *(63, 93, 115, 202)*. Won et al. *(202)* found that rainfall greater than 20 mm 24 hrs before sampling was significantly associated with total coliform and *E. coli* counts in 100 mL of irrigation canal water but not in reservoir water. This discrepancy may be related to the degree of protection for each of these waterbodies.

In a Central Florida study, *Salmonella*, coliform, *E. coli*, or aerobic plate count levels in surface water were not associated with the average rainfall over the previous day, week, and month before sampling *(115)*. One explanation for the discrepancies among studies of rainfall's effect on microbial levels is that the effect on the microbial quality of agricultural water may be indirectly related to the rain event, such as a rain event affecting agricultural practices or other environmental conditions that, in turn, affect the microbial quality of water. For example, irrigation water from surface water sources in a New York produce growing region was more often positive for *Salmonella* when, three days before sampling, rainfall was less than 0.64 cm than when it was above 0.64 cm *(93)*. This association between *Salmonella*-positive samples and low precipitation (e.g., rainfall below 0.64 cm) was attributed to growers' use of surface water to irrigate crops when precipitation was insufficient *(93)*. *Salmonella* presence and levels may have been elevated in the irrigation water ponds from lower water levels and stirred up sediment when water was pumped out for irrigating crops *(93)*. This may also have been the situation in Monterey County, CA, where Gorski et al. *(63)* also reported an increase in *Salmonella*-positive samples a month after precipitation dropped to nearly 0 cm.

Runoff

Directly related to rainfall is the elevated probability of contamination posed by overland flow or runoff transporting human pathogens to agricultural water following rain events or irrigation. Rainfall increases microbial contamination of agricultural water from contaminated runoff and/or resuspension of pathogens in sediment or decreases contamination by dilution *(90, 126, 129)*. Runoff occurs when rain falls faster than it can be redirected or absorbed into the ground. Runoff most likely plays a greater role in

contaminating agricultural water than rainfall without runoff *(169, 181)*. In waterbodies that are elevated or otherwise protected from runoff, rainfall may have little effect on microbial quality *(181, 196)*. In a survey of agricultural water on produce farms in Georgia, *Salmonella* concentrations in storm flow from streams and ditches were an average of 16 times higher than concentrations before storm events *(80)*. In a survey of storm runoff from pastures on working farms and ranches, fecal coliforms concentrations were more than 80% lower in storm runoff from livestock pastures where manure had been applied more than two weeks in advance of a storm when compared to runoff from pastures with manure applications within two weeks of a storm *(103)*. In New Zealand, Collins et al. *(39)* used a rainfall simulator to study *E. coli* concentrations in overland flow running off a steep grazed hillside pasture into a headwater stream. The time lapse since the last grazing episode was a statistically significant predictor of both *E. coli* load (max 10^8 per m^2 of hillside) and concentration (max 10^7/100 mL) in the overland flow *(39)*.

Extreme rain events or storms may lead to flooding, contaminated floodwaters can contaminate inadequately or improperly protected ground and surface agricultural water. In surveillance of irrigation water for pathogen contamination following a flood, Castro-Ibanez et al. *(29)* reported four presumptive positives and one culture-confirmed positive for *Salmonella* one week after flooding. During flooding of a stream, Nagels et al. *(121)* measured *E. coli* levels in an area where dairy and beef cattle grazed and reported *E. coli* concentration nearly doubled with turbidity and peaked during maximum flow acceleration rather than peak flow. In their study of complex interactions between weather and microbial water quality, Weller et al. *(190)* concluded that rainfall is an indicator of conditions such as increased surface run-off and flooding that facilitate pathogen movement from environmental sources into surface water.

Land use and land cover may considerably affect bacterial loads in runoff events *(67)*. Most studies of runoff from agricultural land address how agrochemicals or nutrients contaminate surface water sources whereas studies of runoff from urban land more frequently address microbial contamination of surface water *(5, 103, 124, 155, 185)*. Information pertinent to managing the microbial water quality of surface water can be gleaned from both agricultural and urban land runoff studies. In their study of storm drain outfalls in Tampa Bay, Florida, Ahmed et al. *(5)* evaluated the influence of precipitation on bacterial levels and found that the mean concentrations of culturable *E. coli* and *Enterococcus* spp. were ten-fold higher in wet compared to dry conditions. The water samples also contained sewage-associated marker genes along with antibiotic resistance genes associated with sewage suggesting that aging sewer infrastructure contributed to the contamination *(5)*. In Milwaukee, large spikes of *E. coli* (e.g., ~1,300–8,000 CFU/100 mL) and enterococci (e.g., ~2,500 – 3,500 CFU/100 mL) followed high rates of discharge into the surrounding rivers and estuary after rainfall events *(124)*. Rainfall that exceeded two inches in 24 hours was associated with large quantities of sewage contamination *(124)*.

Return flow water (surface and subsurface water that leaves the field following irrigation water application) is a specific type of runoff that could be captured and reused depending on its microbial safety *(206)*. *E. coli* levels and STEC, *L. monocytogenes*, and *Salmonella* presence were measured in irrigation return flow water at three sites on Arizona vegetable ranches *(206)*. *E. coli* was present at levels as high as 530 CFU/100 mL; one sample was positive for *E. coli* O145 and nine samples were positive for *Salmonella* whereas all samples were negative for *L. monocytogenes (206)*. Tile drains, often used in agricultural areas to drain spent irrigation water from fields, may facilitate pathogen transfer to surface water. Givens et al. *(60)* examined gene markers for livestock-related bacteria and viruses in surface water within an extensively tiled drained area of the South Fork Iowa River basin before and after swine manure application. They reported significantly higher levels of hepatitis E virus, fecal coliform, *E. coli*, enterococci, and *Staphylococcus* in surface waters after swine manure application *(60)*. In a follow-up study, Rieke et al. *(139)* also found a higher number of samples containing genetic markers for *Escherichia/Shigella, Serratia,* and *Enterobacter* spp., all of which include opportunistic human pathogens, in drainage water than in surface water samples.

Animal husbandry may have a major impact on the probability that agricultural water is contaminated from runoff. In cattle production areas of Texas, Wagner et al. *(183)* compared *Bacteroides* marker levels in runoff when cattle were present or absent in pasture and found that the levels in runoff were significantly affected by the timing of runoff events in relation to when cattle were grazing. Over time pathogens die-off in manure, however, aged animal feces still may pose a substantial risk of contaminating agricultural water. Devane et al. *(44)* found a substantial release of *E. coli* (10^4/100 mL) from runoff that encountered aging cowpats in a pasture setting protected from wildlife and birds *(44)*. *E. coli* concentrations initially dropped sharply with a continued low-level release, which remained above the detection limit for the 160-day sampling period *(44)*. This study suggests that feces in the environment may serve as a reservoir for *E. coli* even as they decompose over months in the outdoor environment.

Animal behavior enhances the likelihood of contamination from feces. Animals habitually follow the same routes when navigating pastures and riparian areas. These paths are typically compacted and devoid of vegetation creating conduits for more rapid runoff flow and diminished infiltration into soil *(114, 153)*. Agricultural water sources and delivery systems with sloped embankments or next to sloping terrain are susceptible to runoff from wildlife and/or domestic animal paths. Creation of sinks for runoff from

animal paths facilitates infiltration into soil and prevents runoff from reaching agricultural water *(114, 153)*.

Parameters associated with microbial water quality

After human pathogens or fecal indicator organisms enter an agricultural water system, their ability to survive is challenged by an array of environmental factors including UV light exposure, sediment and suspended particulates, interactions with indigenous microbes that may be predators or competitors, nutrient availability, and physicochemical properties of the water (see *Table 2*) *(43, 81, 96, 97, 98, 99, 148, 180, 186, 187)*. Harris et al. *(81)* listed the physicochemical properties of water that may affect human pathogen survival in water as water temperature, pH, oxidation-reduction potential, turbidity, conductance, total dissolved solids, total organic carbon, and nitrogen content. Dean et al. *(43)* reviewed 61 studies of human pathogen reduction in surface waters and reported that sunlight exposure, temperature, and predation generally increased reduction, and turbidity increased survival. Numerous studies of the correlation between human pathogens and/or indicator organisms and environmental factors report that turbidity, dissolved oxygen, pH, and conductivity exerted the most influence over *E. coli* populations *(71, 133, 159)*.

Because microorganisms often attach or absorb to the surface of particulates and elevated flow generally stirs up sediment resulting in greater turbidity (i.e., elevated particulate levels in the water), the microbial quality of surface water is greatly affected by the amount and size of suspended particulates and the water's flow rate *(36, 100, 129, 138, 190)*. The more rapid the flow rate, the more suspended particulates from sediment are typically carried in the water *(138, 179, 180)*. This effect is especially noticeable when surface runoff increases, or flooding occurs *(179, 190)*.

Studies of variables associated with elevated bacterial levels in water routinely find turbidity as a positive predictor of indicator levels and pathogen presence *(36, 77, 84, 134, 154, 190)*. In their study of irrigation districts in Washington and California, Partyka et al. *(134)* reported that, for every log increase in turbidity, the odds of detecting *Salmonella* increased three-fold. Hansen et al. *(77)* reported correlation between increasing *E. coli* concentrations and increasing turbidity in an agricultural water system consisting of treated groundwater pumped to the surface and discharged into a 7-mile stream that emptied into a reservoir. The treated groundwater feeding the system contained no generic *E. coli* and minimal amounts of suspended particles with very low turbidity; however, as the water flowed through the system, sediment accumulated increasing the water's turbidity and generic *E. coli* levels, especially following rainfall events *(77)*.

Combination of factors

Indicator organism levels and pathogen presence in agricultural water systems are related to multiple seasonal factors such as ambient temperature, water temperature, avian and wildlife migratory patterns, precipitation levels, and agricultural practices *(38, 77, 100, 198)*. Studies investigating microbial quality of agricultural water sources frequently look at risk factors in isolation to evaluate the association or effects. However, in reality, it is more likely that a combination of numerous factors or a sequence of events affects microbial presence and/or levels than it is the result of solely one risk factor. In a study of the association between the likelihood of human pathogens in stream water used in New York produce production and landscape and weather factors, the likelihood of *L. monocytogenes* detection in stream water samples increased with upstream dairy farm density in combination with recent rain events *(189)*. Weller et al. *(190)* suggested that synergistic interactions of flooding or runoff increases bacterial concentrations in sediment and subsequent sediment disturbances then resuspends particles with attached bacteria into the water column. Elevated levels of *E. coli* in a pastoral stream following precipitation were attributable to different sources depending on the season *(77)*. In the fall, elevated *E. coli* was primarily due to waterfowl density, which specifically affected slow flowing portions of the stream, and in the summer, they were attributed to cattle density within 50 m of the stream up to 30 days before precipitation *(77)*. The co-detection of two or more human pathogens (*E. coli* O157:H7, *Salmonella* spp., and *Campylobacter* spp.) in stream water samples from a river basin in eastern Ontario was positively associated with total rainfall over 27 mm the week prior to sampling when water temperatures were below approximately 14°C *(198)*. When water temperatures were higher than 14°C, detection of 2 or more of these pathogens was positively associated with rainfall greater than 42 mm the week prior to sample collection *(198)*. Broad generalizations about the likelihood of contamination based on one environmental risk factor may oversimplify the complexity of pathogen fate and transport in the environment. However, over complication may also be an obstacle to assessing the likelihood of contamination. In most cases, increased probability of contamination is intuitive (i.e., runoff from a pasture where cattle are grazing presents more risk than runoff from non-grazed grasslands). In times of drought, more human pathogens may become airborne from a CAFO than become airborne in a wet environment or heavy rains create rapid runoff over the bare, dry ground. Otherwise, the likelihood of contamination generally increases when environmental conditions increase human pathogen survival and provide an easy conduit to rapidly transport them to agricultural water systems. An understanding of relationships between and interactions among environmental risk factors allows for a more realistic assessment of the probability of contamination in each distinctive production location.

TABLE 2. Physicochemical properties of agricultural water affecting the microbial quality

Condition	Effects on the microbial quality of water	Reference
UV light	Ultraviolet light can damage cellular DNA and destroy cellular components, but light can only penetrate water to a depth of about 22 cm.	*(91)*
	Sunlight accelerates decay of microorganisms.	*(97)*
	The presence of artificial or natural sunlight was frequently one of the main drivers of inactivation for fecal indicator bacteria, pathogenic bacteria, viruses, and bacteriophages.	*(43)*
	The magnitude of decay from ambient sunlight differed over time and across viral targets.	*(117)*
	Viruses that are not susceptible to exogenous inactivation are only inactivated if UVB wavelengths (280–320 nm) are present, such as in very clear, open waters or in containers that are transparent to UVB. Bacteria are susceptible to slightly longer wavelengths. Some viruses and bacteria (especially Gram-positive) are susceptible to exogenous inactivation, which can be initiated by visible as well as UV wavelengths.	*(122)*
Suspended particulates (turbidity)	Turbidity had a strong effect on the predicted probability of *Salmonella* presence as a function of *E. coli* level. Microorganisms attach or absorb to the surface of particulates.	*(84)*
	FIB concentrations were significantly greater when … water was turbid.	*(141)*
	Dissolved oxygen, turbidity and pH were the only environmental variables that showed association with bacterial concentrations.	*(133)*
	Suspended particulate matter (SPM) concentration had a significant effect on the decay rates of both *E. coli* and enterococci from ovine feces in brackish microcosms, but decay rate was greater at low SPM concentrations for *E. coli*, whereas the opposite was observed for enterococci, whose decay rates increased as SPM concentration increased.	*(135)*
	For NY streams, one of four top-ranked factors associated with elevated *E. coli* levels was turbidity. On average, *E. coli* levels in the sampled streams increased as turbidity increased from 0 to 50 NTUs.	*(190)*
Microbial composition (competitors and predators)	Protozoan grazers consume bacteria and viruses.	*(97, 117)*
	The presence of indigenous microbiota and matrix significantly influenced *E. coli* decline.	*(99)*
	The presence of predation typically was found to increase decay for fecal indicator bacteria, pathogenic bacteria, viruses, and bacteriophages.	*(43)*
	Water disinfection significantly increased survival of *E. coli, E. coli* O157:H7 and *Ent. faecalis* in the water column. In the competition experiments where only the natural bacterial biota was manipulated, the addition of kanamycin did not affect the survival of *Ent. faecalis* but resulted in greater survival of *E. coli* in water and sediment. Bacterial species/type influenced survival more than the level of competition in this experiment. This study demonstrates the complexity of interactions of fecal indicator bacteria and pathogens with indigenous microbiota and location in aquatic habitats and argues against over-generalizing conclusions derived from experiments restricted to a particular organism or habitat.	*(187)*
	Predation and competition had significant negative effects on *E. coli* survival.	*(186)*

Continued on the next page.

TABLE 2. Physicochemical properties of agricultural water affecting the microbial quality (cont.)

Condition	Effects on the microbial quality of water	Reference
Nutrients	Higher nutrient levels increased *E. coli* survival …The highest nutrient level (5x) mitigated the effect of predation on *E. coli* survival.	*(186)*
Conductivity	Enterococci were positively correlated with conductivity.	*(76)*
Flow rate	Elevated streamflow resulted in greater levels of *E. coli* in the water column.	*(129)*
	Significantly greater fecal coliform and *E. coli* levels under no-flow conditions than when the river flowed.	*(33)*
	FIB concentrations were significantly greater when stream flow was low or stagnant.	*(141)*
	For NY streams, one of four top-ranked factors associated with elevated *E. coli* levels was flow rate; on average, *E. coli* levels in the sampled streams increased as flow rate increased from 0.0 to 1.0 m/s.	*(190)*

CONCLUSION

Growers have much to consider when inspecting, monitoring, and assessing their agricultural water systems, as mandated by subpart E of the PSR. To make effective decisions based on these activities necessitates an understanding of the sources of microbiological hazards in the production area, knowledge and experience to assess the probability of contamination posed by each microbial hazard, and decision-making and problem-solving skills to prevent or reduce the likelihood of contamination. To this end, the studies summarized herein explore how animal presence and activity, weather, topography, agricultural practices, and various other conditions in the surrounding area, increase or reduce the potential of microbial hazards to contaminate agricultural water systems. Numerous studies provide ample evidence that surface water is more likely to become microbially contaminated than groundwater. Nevertheless, groundwater is not without contamination risk, and well location, construction, and maintenance play significant roles in maintaining well water's microbial quality as does the volume pumped at any one time, especially during unusual weather events (i.e., heavy rains or flooding). Microbial water quality surveys in agricultural and non-agricultural watersheds using source tracking methods indicate that the size of the waterways, geological and topographical aspects of the landscape and terrain, the type and number of animals present, and human activities influence the likelihood that surface waters are contaminated. How a water system is protected from potential contamination relates to geographical and hydro-geographical features of the surrounding land, animal presence and activity, and other users of the water system. Environmental conditions and how and when agricultural water is used also affect the potential transfer of human pathogens to edible portions of the crop. Deciding the distance upstream or from a water source/system that one should inspect cannot be determined by a formula and will vary with kinetic factors largely depending on upstream users of the water and the activities and conditions of nearby and adjacent land. Because every agricultural water system has unique aspects, study findings may not be completely applicable under different conditions or at different locations. To effectively evaluate the likelihood of contamination, it is important for growers to consider the "whole picture", because rarely does one factor stand alone as the sole cause of contamination. Experience gained from routine monitoring and awareness coupled with consistent and thorough training provides a good foundation for effectively assessing the likelihood of contamination from microbiological hazards when they are present.

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