CPS 2023 RFP – FINAL PROJECT REPORT

Project Title:
Development of a risk ranking tool for evaluating hazards and risks related to agricultural water Subpart E

Project Period:
January 1, 2024 – December 31, 2025 (extended to January 31, 2026)

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Objectives:

1. Identify and gather historical laboratory and field-based data on the presence and persistence of microbial pathogens and indicators in produce production systems, with a specific focus on agricultural production water; utilize the Delphi technique to build qualitative data to assess potential risk. (We aim to bring together experts from produce growing regions and commodities across the country to evaluate hazards and relative risks with respect to the proposed language in Subpart E.)

2. Using historical and newly generated data, develop a quantitative microbial risk assessment (QMRA) tool that will encompass probabilistic assessment of factors influencing pathogen survival and risk to produce.
3. Integrate the Objective 1 pathogen and indicator datasets into the QMRA tool to quantify relative risk implications of spatial, temporal, and crop-specific variables.
4. Develop tailored case studies for agricultural water use that represent diverse growing conditions across the country. (These case studies will allow for broader dissemination of the tool into the fresh produce community, offer a preliminary assessment of risk for the individual users, and provide a framework for the users to modify their scenarios as their existing/proposed production practices evolve over time.)

Funding for this project was made possible by a grant from the U.S. Department of Agriculture (USDA) Agricultural Marketing Service. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA.

Additional funding for this project was provided through the CPS Campaign for Research.

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Summary of Findings and Recommendations

Findings:

- **Impact of Sample Type:** Artificially contaminated composite lettuce samples consistently exhibited higher *E. coli* concentrations compared to whole heads, and/or *E. coli* was detectable for a longer duration in composite samples under field conditions.
- **Effect of Initial Contamination Level:** Initial bacterial load influenced persistence. At higher inoculum levels, minimal *E. coli* die-off was observed on romaine lettuce up to 14 days post-application, but at lower levels, decreased concentrations were observed within 1-day post-application under real-world growing conditions.
- **Commodity-Specific Die-off Patterns:** The spray treatment had minimal impact on bacterial contamination of lemon fruits compared to romaine lettuce under field conditions, indicating a commodity-specific difference in survival dynamics or die-off.
- **Pathogen and Pathogen Surrogate Comparability:** In greenhouse trials, *E. coli* TVS353 and pathogenic O157:H7 strains demonstrated comparable die-off patterns across all tested commodities, supporting the use of TVS353 as a suitable organism for field studies.
- **QMRA Ag Water Risk Tool:** The risk tool frames priority scenarios identified by subject matter experts into a consistent, user-friendly, and transparent platform. The selected combinations of pathogen, crop type, and agricultural water use practice were directly translated into model outputs in terms of risk, allowing scenario-specific estimates and simulations.
- **Model and Tool Outputs:** Outputs highlight the importance of source water and agricultural water use practice as potential drivers of risk when source water is contaminated. For example, scenarios with greater contact between irrigation water and harvestable portion of the crop produce higher estimated risks to produce contamination and ultimately consumers.

Recommendations:

- **Survival is Variable:** Bacterial survival is influenced by many factors, including initial contamination load, growing environment, weather conditions, and commodity type. Careful consideration is required for the scientific justification used in a Subpart E Pre-harvest Agricultural Water Assessment.
- **Data Gaps in Die-off:** Major data gaps remain for pathogen attenuation or die-off under realistic pre-harvest field conditions for many crop-pathogen combinations. Similarly, treatment combinations (e.g., for *Listeria* for peracetic acid [PAA] and chlorine) were less available, constraining scenario coverage. The quantification of pathogens (e.g., $\log_{10}$ CFU/g over time) rather than presence/absence is of great interest to develop reduction rates for use in dynamic and risk modeling. Therefore, mitigation benefits in quantitative models depend strongly on the availability and quality of evidential treatment and attenuation for diverse sets of conditions. Risk benchmarks and tool use are recommended to support structured agricultural water assessments and inform grower practices, rather than as a stand-alone determination of compliance. The results can be useful to compare relative risk across growing scenarios, crop types, and use of water treatment or other mitigation strategies to guide grower/irrigation practices in a user-friendly manner supported by science.

Abstract

Recent revisions to the Food Safety Modernization Act Produce Safety Rule Subpart E shift away from testing water quality to a risk-based approach of assessing potential hazards to agricultural water. In the revision, the farm must determine "safe and of adequate sanitary quality" by conducting an Agricultural Water Assessment. While the rule offers opportunities for increased flexibility for growers, concerns have been raised related to the expectation for growers and ultimately how can/will the rule be implemented and enforced given its subjective nature. Unless better tools are developed, risk-based decisions will be driven largely by perception of risk rather than by scientific data/assessment. This project utilized historical laboratory and field data to develop a quantitative microbial risk assessment tool to quantify the impact of microbiological risk due to growing practices outlined by the U.S. Food and Drug Administration. Outputs highlight the importance of source water and agricultural water use practice as potential drivers of risk when source water is contaminated. The results can be useful to compare relative risk across growing scenarios, crop types, and use of water treatment or other mitigation strategies to guide grower practices in a user-friendly manner supported by science.

Background

Recent revisions to the U.S. Food and Drug Administration (FDA) Food Safety Modernization Act Produce Safety Rule Subpart E shift away from traditional water quality testing of indicator bacteria to evaluate water quality, to a more risk-based approach of assessing potential hazards to agricultural water used in a pre-harvest environment. Historically, the FDA defined "adequate sanitary quality" using quantitative standards for microbial water quality and required growers to calculate their Geometric Mean and Statistical Threshold Value for each water source (<126 CFU/100 ml and <410 CFU/100 ml generic *E. coli*, respectively). In the revision, the farm must determine what is "safe and of adequate sanitary quality" by conducting an Agricultural Water Assessment or "AgWA" to determine if water is safe for its intended use. An AgWA may include an evaluation of the following factors: Animal Impacts & Activities, Soil Amendments, Human Waste, Other Water Users, Other Potential Sources of Hazards, Crop Characteristics, Ag Water Use Practices, Environmental Conditions, and Other Relevant Factors. Of these factors, Crop Characteristics (including in the context of Ag Water Use Practices and Environmental Conditions) is a relatively novel consideration in the context of prior risk evaluation schemes applied to agricultural water (IFPA/Harmonized, WG/LGMA). While the revised language offers opportunities for increased flexibility for growers to meet the rule, concerns have been raised related to how industry will systematically move through an assessment, the expectation for growers to understand concepts of hazards versus potential risks, and ultimately how can/will the rule be implemented and enforced given its subjective nature. While the FDA published the Agricultural Water Assessment Builder (https://agwaterassessment.fda.gov) to support industry, the builder is simply a set of questions with no risk ranking of growing practices or crop types, quantitative data, or survey logic built into the questionnaire. To put it simply, unless better tools are developed, risk-based decisions will be driven largely by perception of risk rather than by scientific data/assessment.

Research Methods and Results

Die-off Assessment: To support growers in implementing a Subpart E mitigation measure based on the time interval between final water application and harvest, *E. coli* die-off was evaluated across various crops through both field and greenhouse experiments. Commodities, bacterial strains, and sampling timepoints are defined in **Table 1** (see Appendices).

Field Trials: Four independent field trials were conducted using romaine lettuce (2) and lemon fruit (2). Romaine lettuce was grown in one-acre plots following standard industry practices at the Yuma (Trial 1) and Maricopa (Trial 2) Agricultural Centers. Four-year-old lemon trees from the Yuma Agricultural Center (Trials 1 and 2) were also obtained. At or near maturity, produce was directly contaminated using backpack sprayers, mixed with surface water and pathogen surrogate, green-fluorescent protein-tagged (GFP) *E. coli* TVS353. No additional irrigation or water application was applied for the remainder of the studies.

Romaine Lettuce: Whole-head and composite samples (N = 150) were collected up to 336 hours (14 days) post-inoculation and analyzed using a Most Probable Number (MPN) technique. Whole-head samples were weighed into individual bags following a three-by-three dilution scheme of 250, 25, and 2.5 g, while composite samples were weighed at 375, 37.5, and 3.75 g (**Figure 1**). Samples were enriched and plated on selective media. Plates were considered positive when colonies fluoresced under UV light. *E. coli* concentrations were estimated using an FDA BAM-based MPN calculator (https://pub-connect.foodsafetyrisk.org/microbial/mpncalc/). For Trial 1, a high bacterial load was evaluated (~4.40 log CFU/mL). Head concentrations decreased by 0.52 ± 0.57 log MPN/100g by 336 hours (**Figure 2**). Composite samples showed no *E. coli* reductions (4.46 ± 0.00 log MPN/100g) by the final timepoint (192 hours, 8 days), with only a transient decrease of 0.34 ± 0.46 log MPN/100g at 48 hours. In Trial 2, a lower bacterial load was evaluated (~2.69 log CFU/mL). Concentrations fell below the limit of detection by 70 hours for heads (<1.0 MPN/g) and by 190 hours for composites (<0.68 MPN/g), corresponding to >2.00 and >3.00 log MPN/100g reduction, respectively.

Lemon: Whole lemon fruits (N = 205) were harvested up to 24 hours post-inoculation, and several cultural techniques were used to assess *E. coli* presence, including standard spread plating, IDEXX Colilert QuantiTray, membrane filtration, and fruit enrichment. For Trial 1, immediately after spray application (0 hour), mean *E. coli* concentrations on lemons were 3.23 ± 0.79 log CFU/fruit. By 1 hour, all samples were below the limit of detection via spread plating (<20 CFU/fruit) and IDEXX processing (<1 CFU/fruit) and remained non-detectable at subsequent timepoints (2, 3, and 24 hours). Following enrichment, 19% (8/42) were positive at 1 and 2 hours; however, no samples were positive at 3 and 24 hours, 0% (0/33). Due to the rapid initial *E. coli* die-off observed in Trial 1, Trial 2 assessed die-off up to 3 hours to capture the rapid decline in bacterial concentration. Immediately after spray application (0 hours), mean *E. coli* concentrations on lemons were 3.44 ± 0.73 log CFU/fruit. All fruits fell below the limit of detection by 1.5 hours (90 minutes) via standard spread plating (<50 CFU/fruit) and by 2.5 hours (150 minutes) via membrane filtration (<2 CFU/fruit) (**Table 2**). At 3 hours, only 10% (1/10) of the fruits were positive post-enrichment.

Greenhouse Trials: All commodities were cultivated in pots under greenhouse conditions at the University of Arizona WEST Center and spot-inoculated individually with each *E. coli* strain (GFP TVS353, REPEXH01, TW14359) per fruit or leaf. *E. coli* O157:H7 strains REPEXH01 (2018 romaine lettuce) and TW14359 (2006 spinach) were previously associated with fresh produce outbreaks. Tomato fruits (N = 51), spinach leaves (N = 122), and romaine leaves (N = 60) were collected up to 10 days post-inoculation. For each sampling event, one tomato, two spinach leaves, or one romaine leaf was collected per sample bag. Using standard spread plating on selective media, the presence of *E. coli* was evaluated. Results indicate that bacterial strain did not significantly affect *E. coli* survival across commodities ($p > 0.60$) (**Figures 3-5**). By 1 day, >3 log CFU/produce mean reduction was achieved on tomato and romaine, and by 2 days on spinach. At the final sampling time point (7 or 10 days), mean reductions were 4.95 ± 1.07, 4.39 ± 0.61, and 4.39 ± 0.43 on tomato fruits, spinach leaves, and romaine leaves, respectively.

Risk Survey: To better support risk tool development and supplement die-off data generated by the research and extension team, subject matter experts (SMEs) in academia with expertise in fresh produce food safety and water quality were engaged with an online Qualtrics™ survey. The survey was also modified to a Mentimeter™ presentation to accommodate live participation by food safety industry professionals in Yuma, Arizona. The survey consisted of 46 questions (see **Appendix A**) and included questions related to factors identified by the FDA as critical for pre-harvest agricultural water assessment, as well as risk ranking (27/46), identification of research gaps (7/46), and factor-specific questions (12/46). The factors called out by FDA that were evaluated in the survey were agricultural water degrees of protection (degrees of protection, hereafter), agricultural water distribution system (distribution system), agricultural water source (source), agricultural water use practices (use practices), crop characteristics, and environmental conditions. Participants ranked agricultural water assessment factors based on conceptual (13/27) and scenario (14/27) formatted questions. For example, participants were asked to rank said factors based on their impact on biological hazard (e.g., *Salmonella, E. coli*) introduction to the pre-harvest agricultural water (i.e., conceptual) and based on their impact on the likelihood of leafy greens overhead irrigated becoming contaminated if surface water was used (i.e., scenario).

Nine responses from academia SMEs were collected for the initial subset of questions (Q1-13), with only seven participant responses recorded for the remainder of the survey (Q14-46). A total of thirteen industry responses were recorded, with industry professionals identifying as grower/food safety (10/13) and allied industry (3/13). At least one of the top factors for 100% (27/27) of ranking questions aligned between academia and industry, and in 40.7% (11/27) of questions, all top factors aligned (**Figure 6**). This indicates agreement between SMEs and industry on their individual assessment of factors that contribute to elevation of risks associated with agricultural water. Top-ranked factors in scenario-based questions with surface water (7/7; 100%) varied more than those with groundwater (1/7; 14.3%). This demonstrates that risk concepts surrounding surface water are not as easily understood or agreed upon between SMEs and industry. When the top three factors did not align, degrees of protection were primarily ranked by academia (13/16; 81.3%), while environmental conditions were identified by industry (7/16; 43.8%) (**Figure 7**). This indicates that the academia SMEs may be more concerned with how a water source may or may not be protected while industry members believe that environmental conditions may more greatly impact water quality (risk). Academia and industry identified use practices, source, and distribution system as areas to focus agricultural water assessments, with crop characteristics and environmental conditions as low-priority areas. Across surveyed groups, crop characteristics was one of the top selected factors for lacking scientific research. This is an important finding considering recent regulatory attention given to the impact of crop characteristics on pathogen survival/transfer to produce and human health risk.

Identification of Relevant Risk Scenarios: After academia and industry survey responses were collected, a follow-up virtual meeting was held with academia SMEs to discuss survey findings. SMEs then met in person to discuss tool development, and input was provided to scope risk assessment scenarios for the tool. **Table 3** summarizes the priority scenarios identified based on pathogens of concern, categories of commodity/crop according to different growing nature, and modes of irrigation. These scenarios provided the basis for the model and tool development. The primary pathogens of interest identified were *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica*.

Quantitative Microbial Risk Assessment Framework: In order to aid growers in decision making using scientific data generated by this project coupled with input from academia SMEs and others in industry, a quantitative microbial risk assessment (QMRA) approach was used to create a web application for computing the risks from multiple produce risk scenarios based on the high-priority scenarios identified (**Figure 8**). The web application was developed using R Shiny (Version 4.3.2 R Core Team, 2023). The web application provides users with selections on pathogen, crop type, water source, irrigation method, and options for mitigation. The model output provides the probability of infection to the consumer both with and without various mitigation strategies and is designed to provide users with information to support informed decision making.

Exposure Assessment for QMRA Models: Multiple exposure scenarios were modeled. A generalized exposure equation for consumption of raw produced irrigated with water that is treated via a variety of methods is represented in Equation 1 for the dose of pathogenic *E. coli*, *Salmonella enterica*, or *Listeria monocytogenes* (CFU d-1) was estimated using Equation 1. Data from the current work as well as the published literature was used to parameterize model variables as described in the sections that follow.

$$Dose = C_w \times 10^{-\lambda} \times W \times M \times \%_{cons} \times BW \times 10^{-k(t)} \qquad \textbf{Eq. 1}$$

Table 4 lists the definitions and units of the parameters for **Equation 1**, which includes produce consumption rates per person per day, water transfer to the crop based on irrigation practices, potential pathogen log reductions through applied chemical water treatment, and inactivation over time due to delayed harvest timing. The identified scenarios and priorities (**Table 3**) were directly used to inform the development of an exposure and risk model and thus the options for the user to select to define their scenario in the risk tool, illustrated in **Figure 9**.

Due to the broad categories for crop growing nature (**Table 3**), subcategories were identified based on categorization for food intake and fruit/vegetable type by the United States Department of Agriculture (USDA, 2024). From these sub-categories (**Table 5**), specific crops were chosen to cover a broad range of crop types and characteristics while representing each of the sub-categories and growing natures. From this list, the model for consumer exposure was constructed beginning with parameterization of each specific crop where available, either from the literature or from this study. **Tables 5-7** list the parameter estimates and distributions for consumption rates per capita per day based on bodyweight (*M* and *f*) (**Table 5**), the water transfer *W* (**Table 6**), and reduction rates for applying chemical treatment (λ) (**Table 7**).

Log reduction from applying chemical sanitizers to the irrigation water was estimated for commercially available sanitizers based on studies where reductions were quantified. Two studies applied peracetic acid (PAA) and chlorine (Cl) in the field to reduce concentrations of *E. coli* (Murphy et al., 2023b) and *Salmonella* (Murphy et al., 2023a) in pre-harvest applications, and thus were directly applicable to this study. Based on log-linear models, log reductions were estimated and utilized as a range of probable values (uniform distribution) for model simulation in the tool under different dosing concentrations of the sanitizer. These ranges and dosages were utilized to extrapolate reductions between ranges. Reductions were unavailable for *Listeria* at this time (**Table 7**).

The inactivation rate over time (k(t)) was estimated based on field measurements in this study and in literature. A two-phase model was selected based on literature findings of in-field die-off on produce (**Equation 2**). Where applicable, inactivation rate values, k, were estimated according to faster early (phase 1) exponential decay and slower (phase 2) exponential decay, if no additional contamination occurs during the experiment. Thus, k(t) was calculated from experiments as a distribution to account for uncertainty for each phase (**Equation 2**):

$$k(t) = \begin{cases} k_1 \text{for } 0 \le t \le 2 \text{ days} \\ k_2 \text{ for } t > 2 \text{ days} \end{cases} \qquad \textbf{Eq. 2}$$

A literature review was conducted to find and analyze pre-harvest, in-field inactivation over time of the three bacteria on the full list of crops in this study to complement the data collected in this study. The majority of crop-bacteria combinations were not found, due to the lack of relevant studies or for a misalignment of conditions, for example, where studies focused on contamination or inactivation during post-harvest conditions, such as during storage, processing, or transport (Akomea-Frempong et al., 2023; Al Daour et al., 2024; Fatica & Schneider, 2011; Grounta et al., 2013). Therefore, **Table 8** only includes estimated reduction rates for *E. coli* on five of the identified crops: lettuce, spinach, tomatoes, apples, and citrus. Derivation of $k(t)$ in this study points to a major research gap in this topic.

Dose-Response Assessment: To estimate the risk of infection following exposure to a pathogen, dose-response relationships were selected based on clinical studies. The best-fit model for each study was the beta-Poisson model (**Equation 3**), and the best-fit parameters for each model and pathogen are listed in **Table 9**. For *E. coli*, the model is based on an infection probability after exposure to *E. coli* O157:H7. To account for this in the exposure model, an additional fraction of *E. coli* O157:H7 to total *E. coli* was added as a normal distribution of 10^Normal(μ = 1.9, σ = 0.6) truncated at 0 (Ottoson et al., 2011; Pang et al., 2017).

$$P_{\text{inf}} = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha} \qquad \textbf{Eq. 3}.$$

Example Results: To demonstrate the tool (**Figures 10-16**), a scenario (**Table 10**) was simulated. The initial concentration of total *E. coli* was set to a normal distribution with a mean of 1 $\log_{10}$ CFU/mL and a standard deviation of 0.1 $\log_{10}$ CFU/mL for this example. The tool used these inputs to simulate the infection risk over time and compared it with the user-selected risk benchmark.

Exploration of Leveraging External Data with Large Language Models: To complement the development of the risk ranking tool, our team evaluated if large language models (LLMs) can effectively predict the presence of coliform bacteria in water using physicochemical and sanitizer residual features, providing a rapid and low-cost alternative to conventional laboratory testing (**Figure 17**). This question is significant because coliform detection is a key public health concern, yet current methods are resource-intensive and not always feasible in real-time monitoring contexts. For data, we used experimental measurements from irrigation water that were collected from coliform-inoculated lettuce trials above. The purpose of the experiment was to see if coliform bacteria could be detected (in any detectable concentration) after no irrigation treatment, peracetic acid (PAA) treatment, and calcium hypochlorite (Cl) addition to water. In this study, we tested the importance of other water quality attributes for this binary presence/absence prediction. To address this, we tested five LLMs; ChatGPT-4o, Claude 4, Llama 4, Grok 3, and Gemini 2.5 under both zero-shot conditions, where predictions relied solely on heuristic reasoning without labeled data, and few-shot conditions, where limited labeled samples guided learning.

Predictions were generated across three scenarios that excluded specific features: Date and Time, pH, and Water Temperature, and the outputs were compared against ground-truth labels as well as across models (**Figure 18**). We tested both zero-shot prompting, by giving it no related observations from our data (such as using another irrigation treatment) and few-shot prompting using data from another irrigation treatment. We also conducted variable importance assessments by excluding some variables from the prediction (such as pH, time of sensor measuring residual, etc.) For example, removing time of residual information would reduce the model's ability to predict the microbial load reduction as a function of time. Results revealed that Llama4 with few-shot prompting information was strongest when deprived time/date information depending on the metric with 80% accuracy and 78% F1 of precision-recall; Grok 3 consistently performed best when deprived pH information, achieving the highest accuracy (78% zero shot/81% few shot), macro F1 (77% zero shot/80% few shot) F1 of the precision-recall; and while all models suffered loss with the deprivation of water temperature information, Gemini 2.5 delivered the top overall results, particularly in macro F1 (in the 70s % for both zero- and few- shot). ChatGPT-4o performed well in heuristic zero-shot reasoning but adapted poorly in few-shot learning, while Claude 4 and Llama 4 delivered steady midrange performance across most scenarios. We conclude that although no single LLM dominated in every case, Grok 3 emerged as the most reliable across conditions, and Gemini 2.5 demonstrated context-dependent strengths in the few-shot setting.

These comparative findings further revealed that while all models relied on similar heuristic cues such as turbidity and sanitizer residuals, their ability to balance precision and recall varied across experimental conditions. Also, we found that date/time and pH can confound performance and lower the predictive performance, while water temperature (in degrees Celsius) and residual turned out to be the most important variables that we tested. These findings highlight the potential of LLMs as decision-support tools for water quality monitoring, and the importance of cross-model evaluation to improve robustness and reliability in environmental applications. This suggests that combining or triangulating outputs from multiple LLMs could provide a more robust and reliable framework than relying on a single model in isolation.

LLM Results: Results by LLM are shown in **Figure 19.** Variable importance ranking: 1) Sanitizer Residual Concentration (Chlorine/PAA), 2) Water temperature, 3) Turbidity, 4) pH.

Outcomes and Accomplishments

- New data was generated on microbial die-off as well as the impact of agricultural water treatment on die-off for new pathogen/commodity types and combinations to aid in industry risk assessments.
- An extensive literature review aggregated and synthesized published literature on pathogen occurrence, persistence, and mitigation measures for different crop types
- The scenarios covered by the web application identified scenarios of overhead irrigation and crops eaten raw such as leafy greens as higher risk based on inputs from the current newly generated field data and the published literature.
- Many large language models (LLMs) were tested and their capacities to predict pathogenic microbial presence after treatment was documented.
- A multi-scenario QMRA web application was developed with grower input to assist growers with understanding risk management opportunities for different hazard-treatment-crop scenarios. The app is flexible to accommodate user selections for pathogen, crop type, water source, water use practices, and mitigation measures.

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APPENDICES

Publications and Presentations

Presentations:

- Center for Produce Safety Research Symposium 2024 and 2025
- International Association for Food Protection 2025
- Southwest Ag Summit 2025
- Association for Computing Machinery’s (ACM’s) Bioinformatics and Computational Biology (BCB) 2025

Budget Summary

This project was awarded $417,963 in research funds, and the majority of funds were spent.

Tables 1–10 and Figures 1–18 and Appendix A (see below)

Table 1. Commodities, *E. coli* strains, and sampling timepoints assessed in field and greenhouse trials.

Environment	Commodity	Trial	Strain	Number of Samples	Sampling Timepoints
Field	Romaine Lettuce	1	TVS353[a]	84	1, 24, 48, 70, 192, and 336 hours
		2	TVS353	66	1, 24, 48, 70, 190, and 334 hours
	Lemon	1	TVS353	115	0, 1, 2, 3, and 24 hours
		2	TVS353	90	0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes
Greenhouse	Romaine Lettuce	1	TVS353, REPEXH01[b], and TW14359[b]	60	0, 1, 2, 3, 5, 7, and 10 days
	Spinach	1	TVS353, REPEXH01, and TW14359	122	0, 1, 2, 3, 5, 7, and 10 days
	Tomato	1	TVS353, REPEXH01, and TW14359	51	0, 1, 2, 3, 5, and 7 days

[a] Pathogen-surrogate green-fluorescent protein tagged *E. coli*
[b] Pathogenic O157:H7 *E. coli*

Table 2. Cultural results from Trial 2 lemon fruits.

Timepoint (minutes)	Plate Counts (log CFU/fruit)	Filter Counts (log CFU/fruit)	Enrichment Positive (# of fruits)
0	3.44 ± 0.73[a]	-	10/10[c]
15	2.71 ± 0.34	-	10/10
30	1.91 ± 0.64	1.55 ± 1.00	9/10
45	1.71 ± 0.44	1.33 ± 1.00	7/10
60	1.59 ± 0.41	0.58 ± 0.94	5/10
90	1.40 ± 0.00	0.14 ± 0.29	4/10
120	1.40 ± 0.00	0.11 ± 0.34	3/10
150	-[b]	0.00 ± 0.00	3/10
180	-	0.00 ± 0.00	1/10

[a] Mean ± standard deviation
[b] No samples were processed.
[c] Number of fruits positive / Total number of fruits enriched

Table 3. Priority scenarios identified from subject matter experts.

Hazard/ Pathogen	Commodity	Irrigation Mode	Exposed Population	Mitigation Option 1 - Treatment	Mitigation Option 2 - Time Interval (Die-Off)
E. coli O157	Low/ Underground	Drip	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
E. coli O157	Bush/ Elevated/ Vine/ Staked	Spray	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
E. coli O157	Tree	Overhead	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
Listeria	Low/ Underground	Drip	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
Listeria	Bush/ Elevated/ Vine/ Staked	Spray	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
Listeria	Tree	Overhead	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
Salmonella	Low/ Underground	Drip	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
Salmonella	Bush/ Elevated/ Vine/ Staked	Spray	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest
Salmonella	Tree	Overhead	Consumer	Irrigation water disinfection (Y/N)	Time interval from water application to harvest

Table 4. Exposure model parameters and definitions.

Definition	Parameter	Units
Concentration of pathogens in irrigation water	C_w	CFU/mL
Water transfer to crop	W	mL/g
Mass crop consumed per day	M	g/kg/d
% of population who consumes crop	f	Unitless
Bodyweight per person	BW	kg
Inactivation constant (die off)	k	d^{-1}
Additional inactivation (chemical treatment)	λ	Unitless
Time since application	t	d

Table 5. Crop-specific ingestion rates from (USEPA, 2018)

Crop Growing Nature	Sub-category	Crop	Consumption rate, *M* (g/kg/d)	Fraction of the Population that Ingests the Crop, *f* (Unitless)
Low/ Underground	Leafy greens	Lettuce	μ = 0.26, σ = 0.01	0.62
	Leafy greens	Cabbage	μ = 0.05, σ = 0.01	0.13
	Leafy greens	Spinach	μ = 0.00857, σ = 0.001[a]	0.13
	Root/ Tuber	Carrot	μ = 0.11, σ = 0.01	0.46
	Fruit vegetables (thin skin)	Cucumber	μ = 0.09, σ = 0.01	0.45
	Melon/ Squash (thick rind)	Melon	μ = 0.26, σ = 0.03	0.52
	Berry/ Soft fruit	Strawberries	μ = 0.06, σ = 0.01	0.36
Bush/ Elevated/ Stalk	Flower/ Head crops	Broccoli	μ = 0.09, σ = 0.01	0.16
	Flower/ Head crops	Cauliflower	μ = 0.03, σ = 0.01	0.16
	Legume/ Pod	Peas	μ = 0.05, σ = 0	0.18
	Fruit vegetables (thin skin)	Peppers	μ = 0.08, σ = 0.01	0.869
	Fruit vegetables (thin skin)	Tomatoes	μ = 0.66, σ = 0.02	0.89
Tree	Pome fruit	Apple	μ = 0.21, σ = 0.02	0.28
	Pome fruit	Pear	μ = 0.04, σ = 0.01	0.06
	Tropical fruit	Bananas	μ = 0.2, σ = 0.01	0.48
	Citrus fruit	Citrus	μ = 0.11, σ = 0.01	0.2
	Stone fruit	Peaches	μ = 0.04, σ = 0.01	0.43

[a] These values were estimated from 0.6 g/d reported in the 1997 version of the USEPA Exposure Factors Handbook (USEPA, 2018)

Table 6. Water transfer to crop (*W*, mL/g) from (Stine et al., 2005).

Irrigation Practice	Water Transfer (mL/g)
Drip	0.00000088
Furrow	0.00011
Overhead	0.011

Table 7. Reduction from treatment from (Murphy et al., 2023b, 2023a).

Bacteria	Disinfectant	Dose (ppm)	LR range
E. coli O157:H7	PAA	6	0 to 0.37
		10	0.09 to 3.07
	Cl	2-4	3.59 to 5
		10-12	3.43 to 5.65
Salmonella enterica	PAA	6	0.2
		10	0.2 to 1.4
	Cl	2-4	3.6 to 4.2
		10-12	4.1 to 4.3
Listeria monocytogenes	PAA	6	NA
		10	NA
	Cl	2-4	NA
		10-12	NA

Table 8. Inactivation rates, reduction of *E. coli* from pre-harvest time in-field after contamination.

Crop	k_1	k_2	Source
Lettuce	Normal (μ = 2.451, σ = 0.0545)	Normal (μ = 0.08, σ = 0.0632)	(Moyne et al., 2011)
Spinach	Normal (μ = 1.883, σ = 0.103)	Normal (μ = 0.107, σ = 0.0445)	This study
Cabbage	NA	NA	
Carrot	NA	NA	
Cucumber	NA	NA	
Melon	NA	NA	
Strawberries	NA	NA	
Broccoli	NA	NA	
Cauliflower	NA	NA	
Peas	NA	NA	
Peppers	NA	NA	
Tomatoes	Normal (μ = 3.76, σ = 0.08)	Normal (μ = 0.191, σ = 0.124)	This study
Apple	Normal (μ = 3.963, σ = 0.0632)	Normal (μ = 0.575, σ = 0.0542)	(Murphy et al., 2025)
Pear	NA	NA	
Bananas	NA	NA	
Citrus	Normal (μ = 50, σ = 10)	NA	This study
Peaches	NA	NA	

Table 9. Dose-response models and parameters for a clinical endpoint of infection.

Pathogen	Model	Parameters	Source
E. coli O157:H7	Beta-Poisson	$\alpha = 0.1778$ $\beta = 1.7796e6$	(Haas et al., 2014)
Salmonella enterica	Beta-Poisson	$\alpha = 0.3126$ $\beta = 2884$	(Haas et al., 2014)
Listeria monocytogenes	Beta-Poisson	$\alpha = 0.253$ $\beta = 19.12649$	(Golnazarian et al., 1989; Haas et al., 1999)

Table 10. Chosen scenario details for simulation.

Parameter/Option	Choice
Water source	Surface water
Water use practice	Overhead
Pathogen	*E. coli* O157:H7
Crop type	Lettuce
Mitigation measure 1: Treatment	PAA (10 ppm)
Mitigation measure 2: Time to harvest	7 days
Benchmark	10^{-6}

Figure 1. Whole head romaine lettuce collection post inoculation.

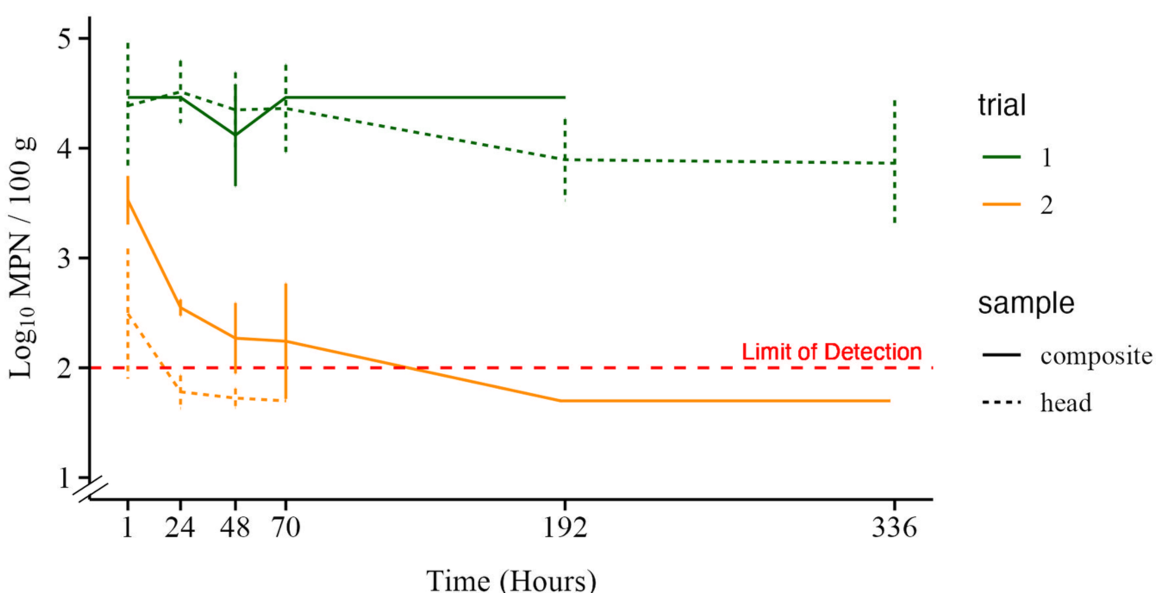

Figure 2. Mean *E. coli* concentrations on field-cultivated romaine lettuce by sample type from Trials 1 and 2 (error bars represent standard deviation).

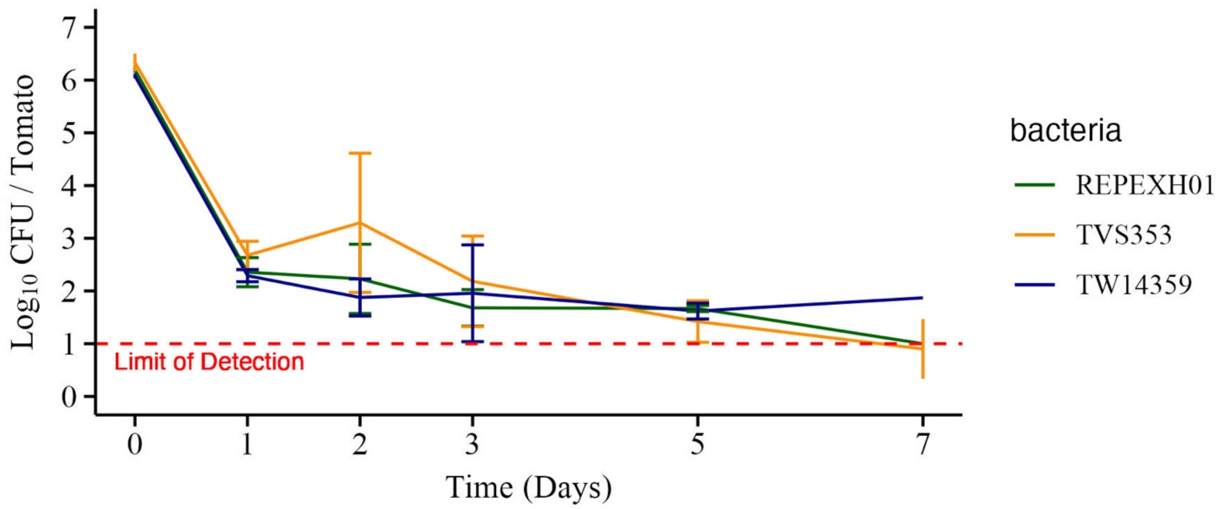

Figure 3. Mean concentrations of greenhouse-cultivated tomato fruits by *E. coli* strain (error bars represent standard deviation).

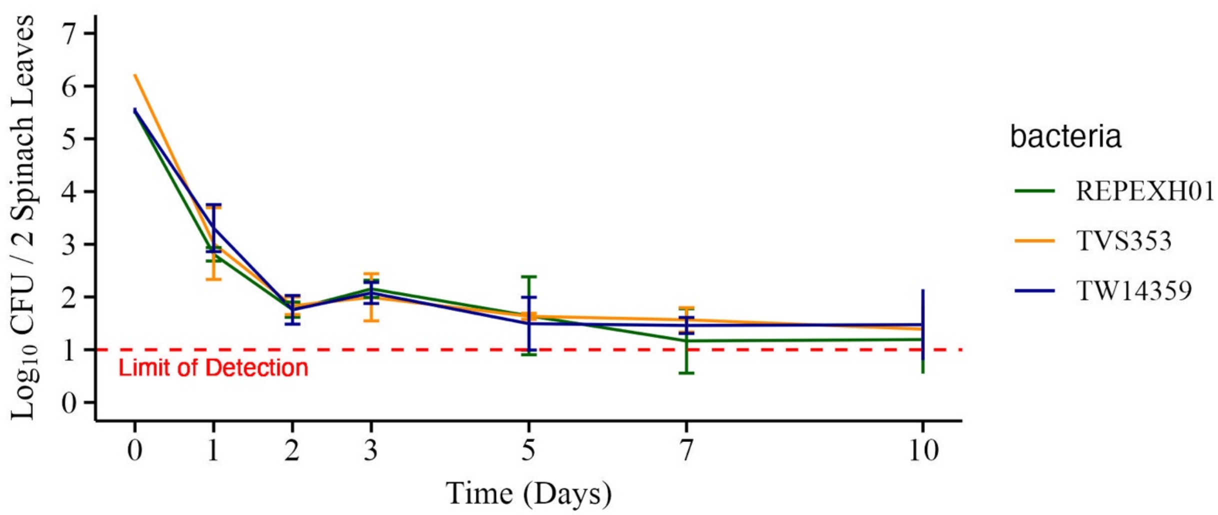

Figure 4. Mean concentrations of greenhouse-cultivated spinach leaves by *E. coli* strain (error bars represent standard deviation).

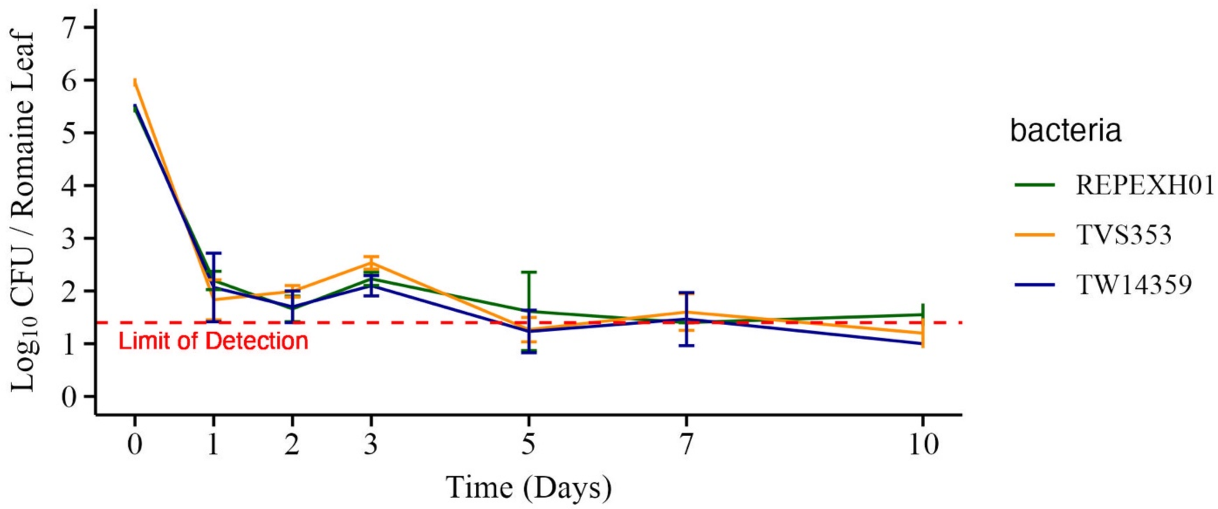

Figure 5. Mean concentrations of greenhouse-cultivated romaine lettuce leaves by *E. coli* strain (error bars represent standard deviation).

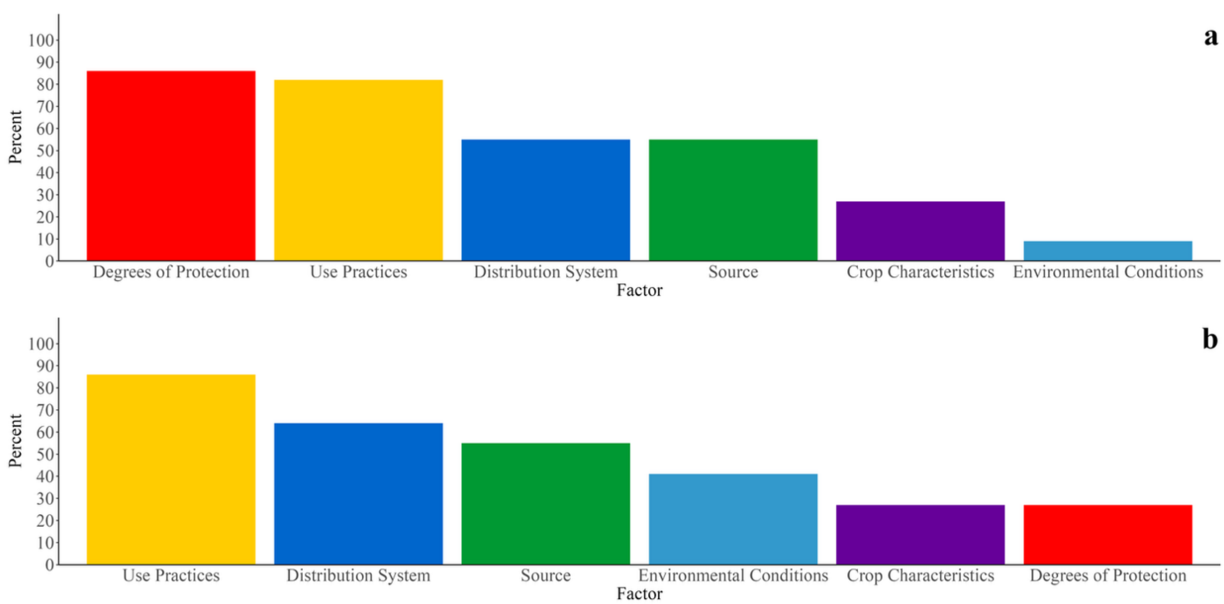

Figure 6. Frequency of factors ranked among the top three for academia (a) and industry (b) in biological hazard impact questions (N = 22).

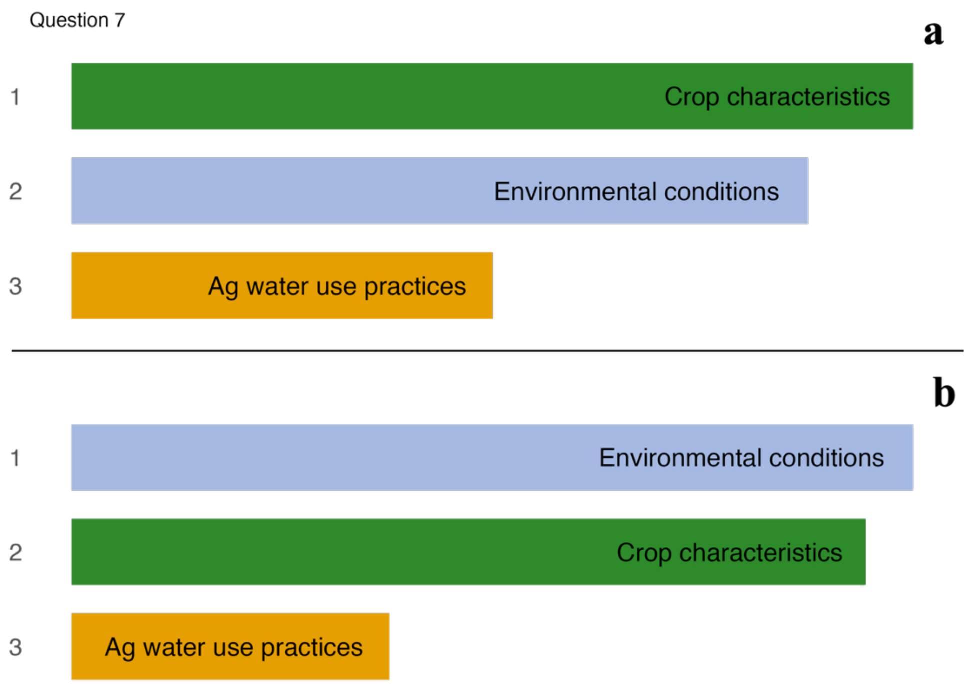

Figure 7. Example of when academia (a) and industry (b) agreed on the top three factors. Question 7: Rank the factors of an agricultural water assessment based on their impact on biological hazard survival and persistence on the harvestable portions of the crop, with 1 representing the highest impact and 6 representing the lowest impact.

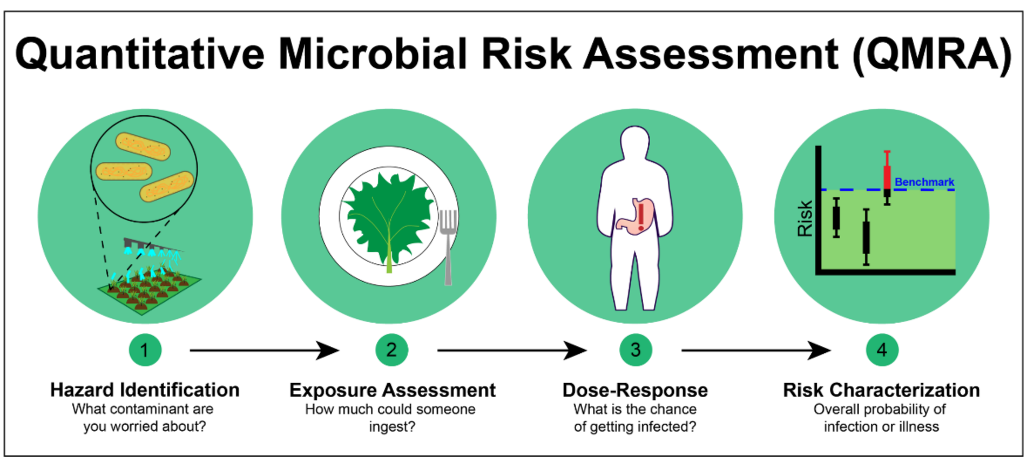

Figure 8. Quantitative microbial risk assessment (QMRA) framework

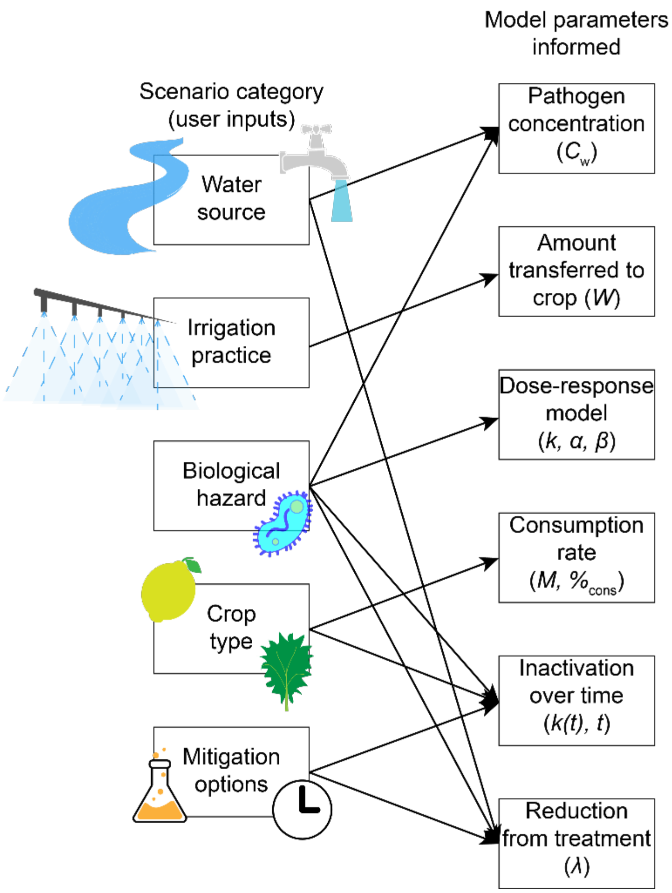

Figure 9. Illustration of which user-selected scenario options inform the selection of values or distributions for each model parameter.

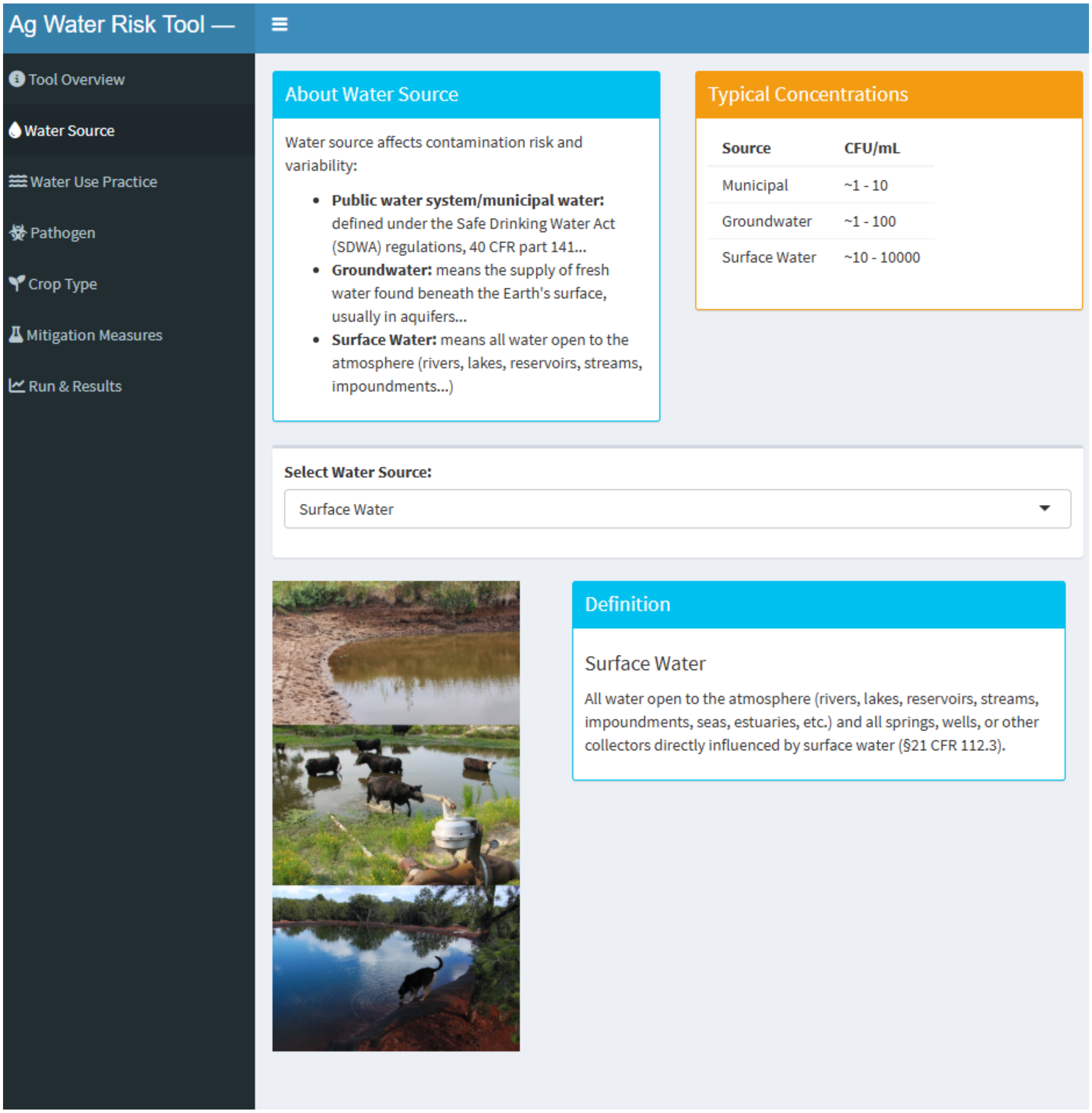

Figure 10. Tool Selection One: A user selects a water source (i.e., surface water, groundwater, or municipal water).

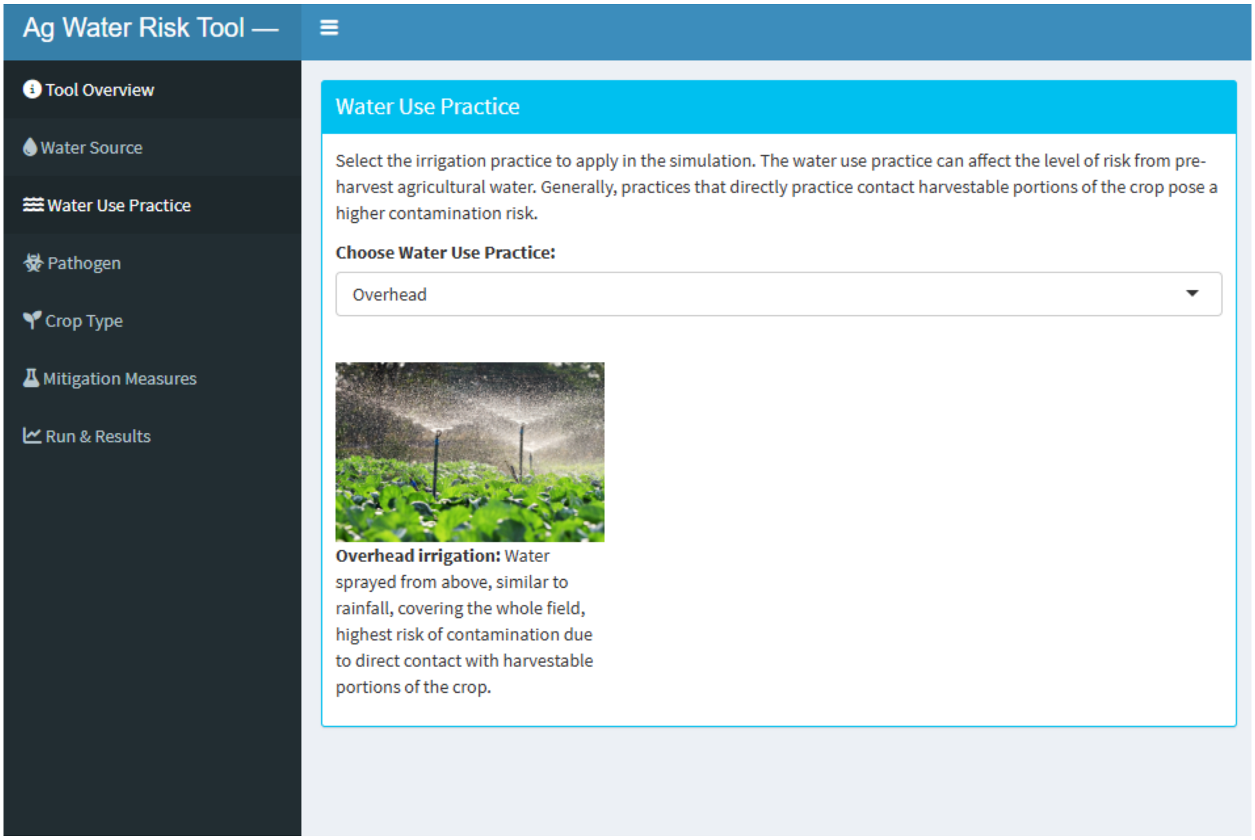

Figure 11. Tool Selection Two: Water use practice is selected (i.e., overhead, drip, or furrow).

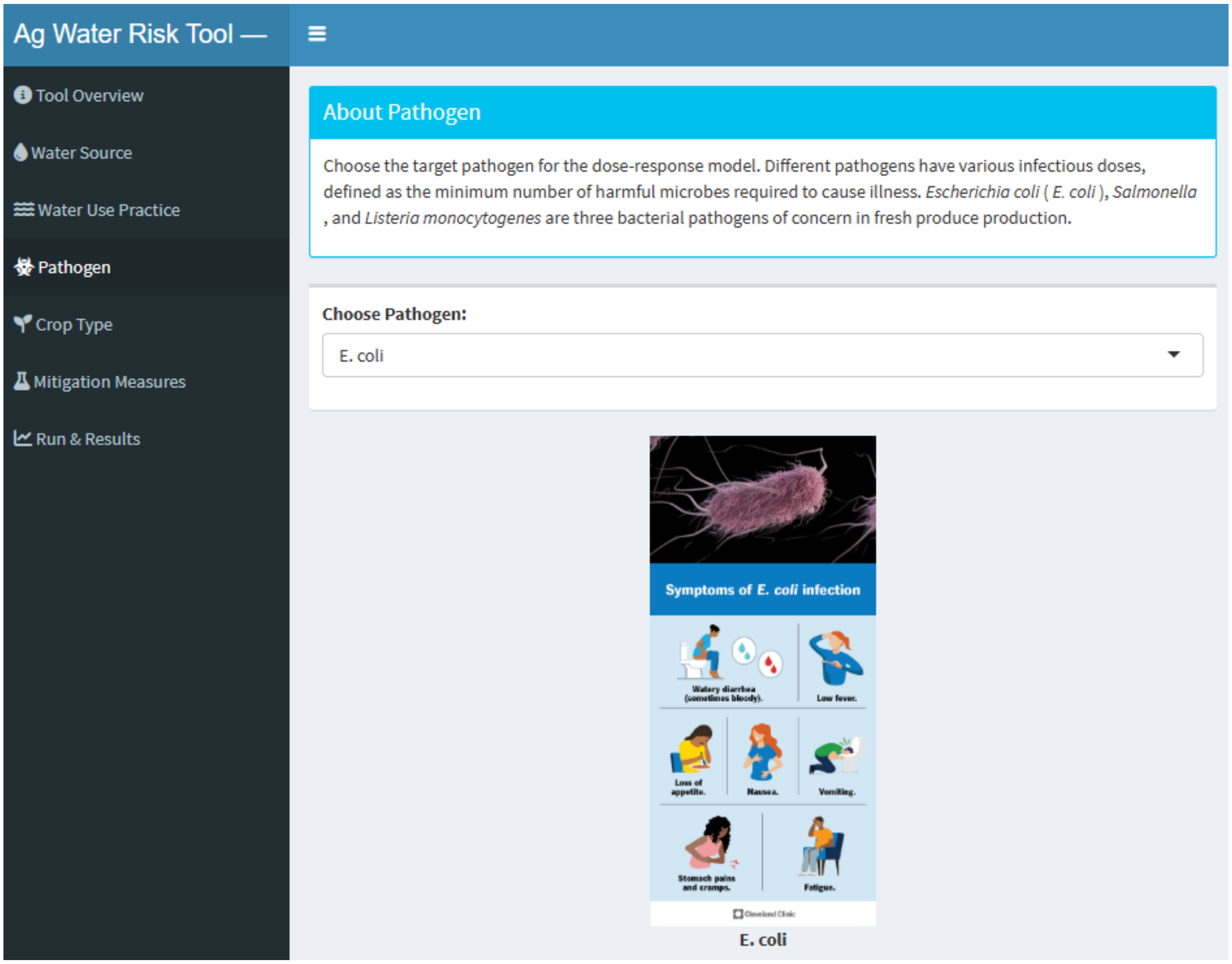

Figure 12. Tool Selection Three: The pathogen of interest is selected (i.e., *E. coli, Salmonella, Listeria*).

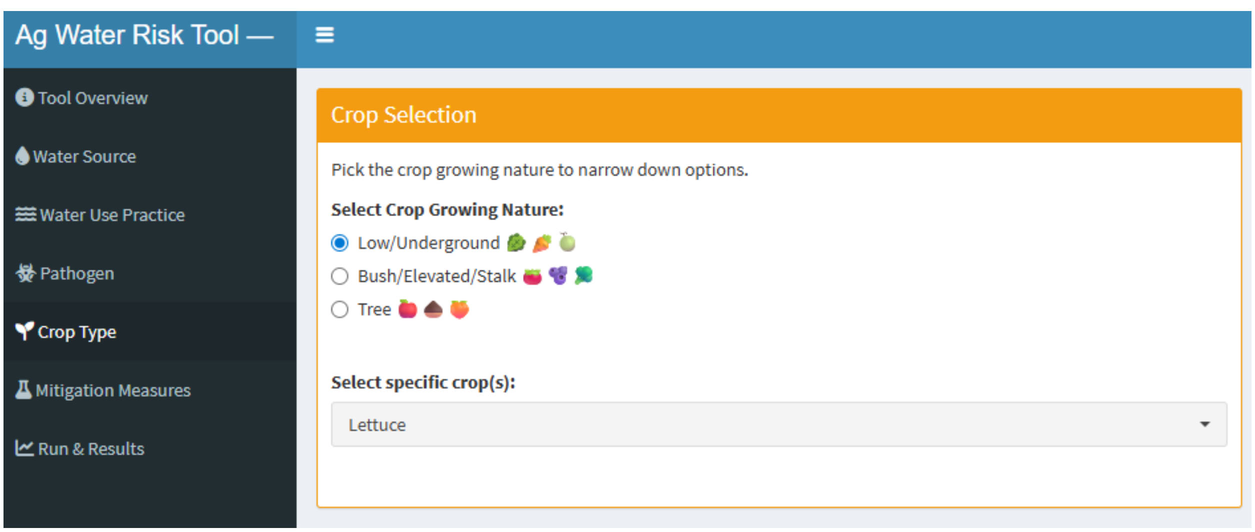

Figure 13. Tool Selection Four: Crop type(s) are selected.

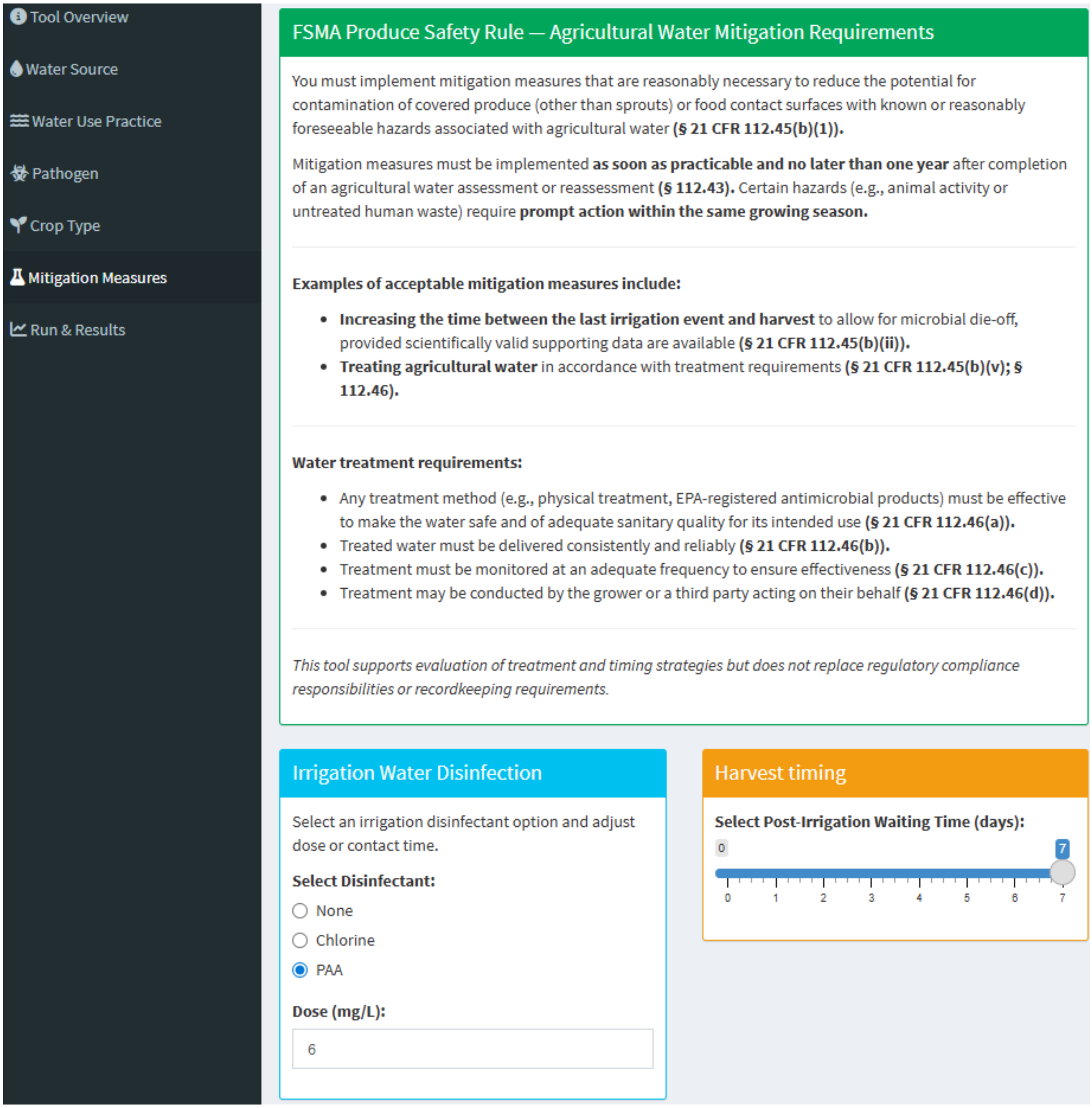

Figure 14. Tool Selection Five: Mitigation measures (water disinfection and/or harvest timing) are selected.

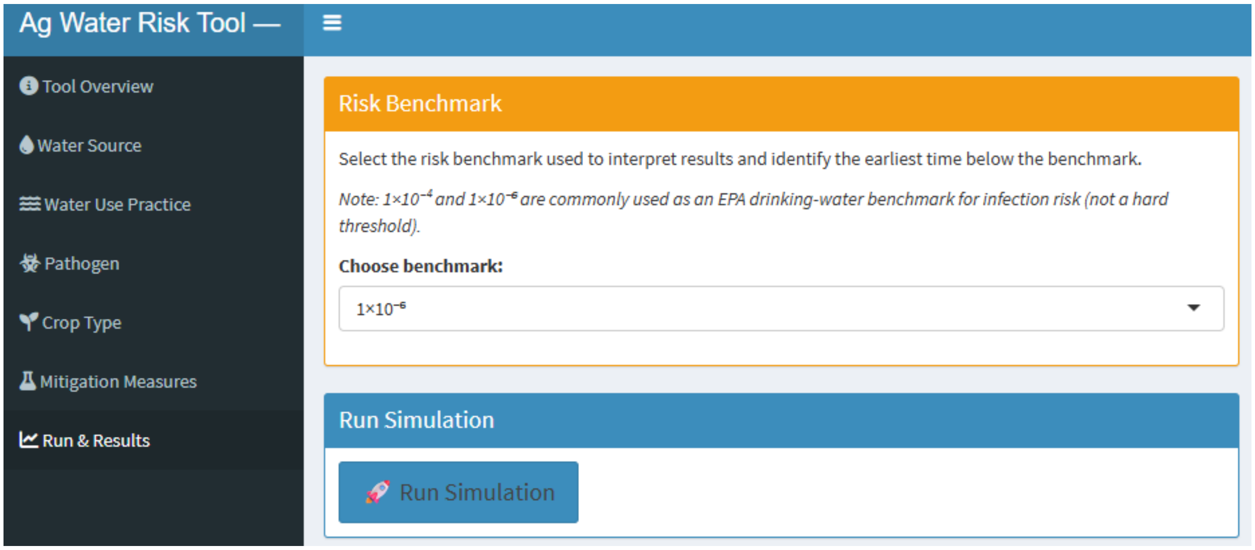

Figure 15. Tool Selection Six: Risk Benchmark is selected, and the simulation is run.

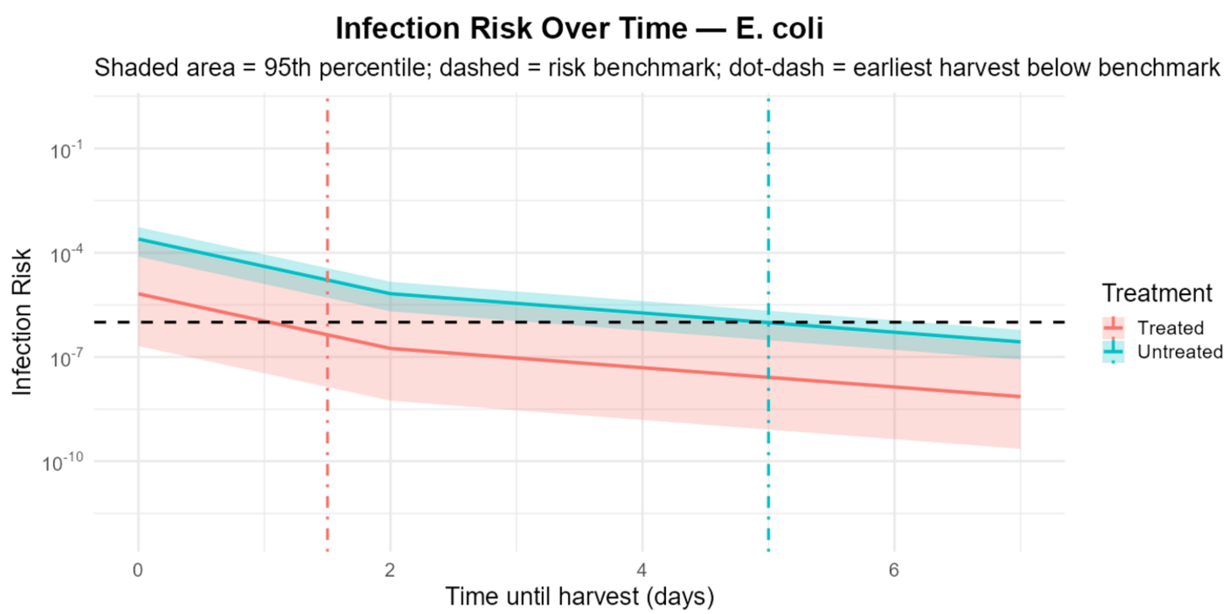

Table 1. Probability of infection at the selected harvest time (median and 95th percentile) under untreated and treated scenarios.

Crop	Untreated (initial)	Treated (initial)	Untreated (day t)	Treated (day t)
Lettuce	2.45e-04 (7.70e-05–5.41e-04)	6.56e-06 (2.07e-07–2.08e-04)	2.71e-07 (8.49e-08–5.97e-07)	7.23e-09 (2.29e-10–2.29e-07)

Interpretation and Recommendation

Without water treatment, the median infection risk falls below the risk benchmark after **5 days** of post-irrigation waiting time.

With water treatment, the risk benchmark is achieved after **1.5 days** .

Figure 16. Example simulated results for the scenario outlined in **Table 10.** The two-phase model of in-field die-off reduced the per-person infection risk for lettuce consumption below the 10^{-6} benchmark after 1 day with and without additional treatment of 10ppm of PAA.

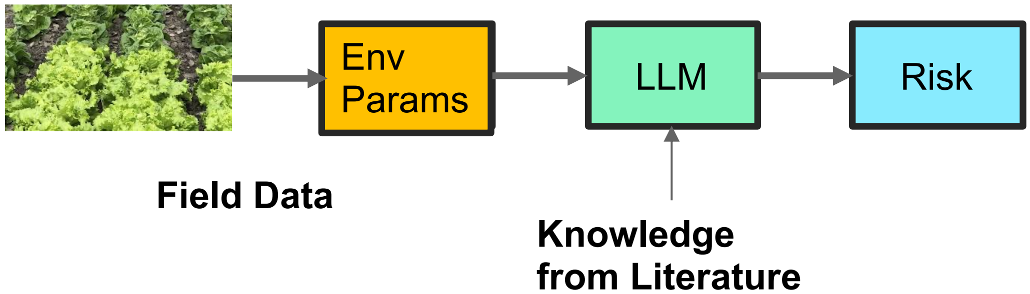

Figure 17: Proposed pipeline of assessing microbial risk using existing knowledge from the scientific literature.

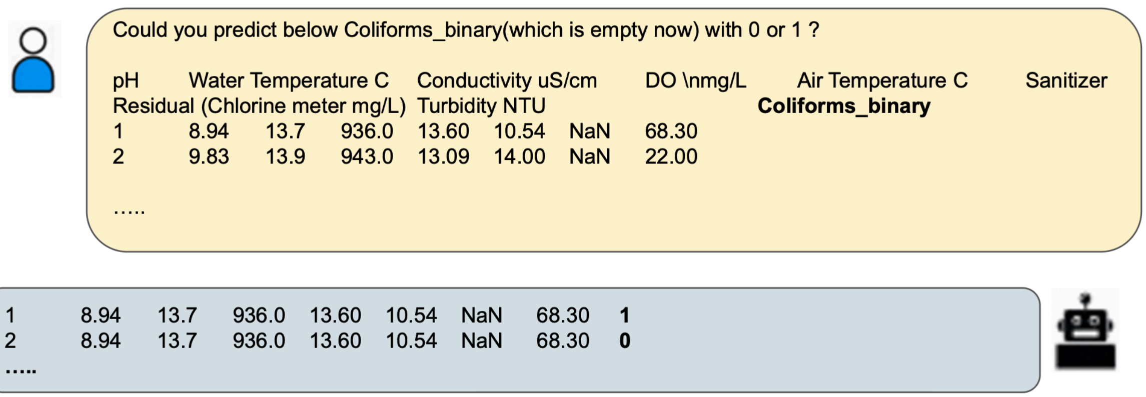

Figure 18: An example LLM prediction using Zero-shot Prompting

Model	Setting	Accuracy	Macro F1	Macro Precision	Macro Recall
ChatGPT-4o	Zero-shot	0.5797	0.5412	0.7542	0.6282
Claude 4	Zero-shot	0.5507	0.5367	0.6062	0.5833
LLaMA 4	Zero-shot	0.4783	0.4445	0.5309	0.5192
Grok 3	Zero-shot	0.6812	0.6795	0.7155	0.7026
Gemini 2.5	Zero-shot	NA	NA	NA	NA
ChatGPT-4o	Few-shot	0.5652	0.3611	0.2826	0.5000
Claude 4	Few-shot	0.5507	0.5095	0.6958	0.5987
LLaMA 4	Few-shot	0.5652	0.3611	0.2826	0.5000
Grok 3	Few-shot	0.7391	0.7065	0.7866	0.7077
Gemini 2.5	Few-shot	0.5652	0.3611	0.2826	0.5000

Figure 19: Model performance for all features – Grok was one of the best performing models, most likely exploiting the data structure.

Appendix A. Risk Ranking – Survey Questions

Q1: Rank the following components of an agricultural water assessment based on their **impact on biological hazard introduction to the pre-harvest environment**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q2: Rank the following components of an agricultural water assessment based on their **impact on biological hazard introduction to pre-harvest agricultural water**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q3: Rank the following components of an agricultural water assessment based on their **impact on biological hazard introduction to the harvestable portion of the crop**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q4: Rank the following components of an agricultural water assessment based on their **impact on biological hazard transfer to the pre-harvest environment**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q5: Rank the following components of an agricultural water assessment based on their **impact on biological hazard transfer to the harvestable portions of the crop**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q6: Rank the following components of an agricultural water assessment based on their **impact on biological hazard survival and persistence in the pre-harvest environment**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q7: Rank the following components of an agricultural water assessment based on their **impact on biological hazard survival and persistence on the harvestable portions of the crop**, with 1 representing the highest impact and 6 representing the lowest impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q8: Rank the following components of an agricultural water assessment based on the **availability of hazard mitigation measures for grower implementation**, with 1 representing the most mitigation measures and 6 representing the least mitigation measures. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q9: Rank the following components of an agricultural water assessment based on **confidence in the effectiveness of hazard mitigation measures**, with 1 representing the greatest confidence and 6 representing the least confidence. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q10: Rank the following components of an agricultural water assessment according to **available scientific research**, with 1 representing the most scientific research and 6 representing the least scientific research. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q11: Rank the following components of an agricultural water assessment according to **confidence in available scientific research**, with 1 representing the greatest confidence and 6 representing the least confidence. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q12: Rank the following components of an agricultural water assessment based on their **negative impact on overall pre-harvest agricultural water sanitary quality**, with 1 representing the most negative impact and 6 representing the least negative impact. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Q13: If you had to advise a grower tomorrow on where to focus their food safety efforts for pre-harvest agricultural water, how would you rank the following components of an agricultural water assessment in terms of **priority?** With 1 representing the greatest priority and 6 representing the lowest priority. Drag factors to reorder as desired.

Agricultural water source
Agricultural water distribution system
Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
Agricultural water use practices
Crop characteristics
Environmental conditions

Rank the following components of an agricultural water assessment (top row) based on their **impact on the likelihood of [crop x; first column] becoming contaminated if *surface water* is used**, with 1 representing the highest impact and 6 representing the lowest impact. Each number (1 through 6) must be used once per row (left to right).

	Agricultural water source	Agricultural water distribution system	Agricultural water degrees of protection	Agricultural water use practices	Crop characteristics	Environmental conditions
Q14 [carrots furrow irrigated]	☐	☐	☐	☐	☐	☐
Q15 [leafy greens furrow irrigated]	☐	☐	☐	☐	☐	☐
Q16 [leafy greens overhead irrigated]	☐	☐	☐	☐	☐	☐
Q17 [bush tomatoes drip irrigated]	☐	☐	☐	☐	☐	☐
Q18 [bush tomatoes spray applied]	☐	☐	☐	☐	☐	☐
Q19 [apple trees drip irrigated]	☐	☐	☐	☐	☐	☐
Q20 [apple trees spray applied]	☐	☐	☐	☐	☐	☐

Please ensure that each ranking number (1 through 6) is used only once per row before clicking next.

Rank the following components of an agricultural water assessment (top row) based on their **impact on the likelihood of [crop x; first column] becoming contaminated if *groundwater* is used**, with 1 representing the highest impact and 6 representing the lowest impact. Each number (1 through 6) must be used once per row (left to right).

	Agricultural water source	Agricultural water distribution system	Agricultural water degrees of protection	Agricultural water use practices	Crop characteristics	Environmental conditions
Q21 [carrots furrow irrigated]	☐	☐	☐	☐	☐	☐
Q22 [leafy greens furrow irrigated]	☐	☐	☐	☐	☐	☐
Q23 [leafy greens overhead irrigated]	☐	☐	☐	☐	☐	☐
Q24 [bush tomatoes drip irrigated]	☐	☐	☐	☐	☐	☐
Q25 [bush tomatoes spray applied]	☐	☐	☐	☐	☐	☐
Q26 [apple trees drip irrigated]	☐	☐	☐	☐	☐	☐
Q27 [apple trees spray applied]	☐	☐	☐	☐	☐	☐

Please ensure that each ranking number (1 through 6) is used only once per row before clicking next.

Q28: What, if any, components of an agricultural water assessment lack scientific research to adequately perceive risks of biological hazards? Select all that apply.

- ☐ Agricultural water source
- ☐ Agricultural water distribution system
- ☐ Agricultural water degrees of protection (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins)
- ☐ Agricultural water use practices
- ☐ Crop characteristics
- ☐ Environmental conditions

Q29: Please expand on gaps in scientific research relating to the **agricultural water source**.

____

Q30: Please expand on gaps in scientific research relating to the **agricultural water distribution system**.

____

Q31: Please expand on gaps in scientific research relating to **agricultural water degrees of protection** (e.g., adjacent land use/animal impacts and activities and/or biological soil amendments of animal origins).

____

Q32: Please expand on gaps in scientific research relating to **agricultural water use practices** (including application methods and the time interval between direct application and harvest).

____

Q33: Please expand on gaps in scientific research relating to **crop characteristics**.

____

Q34: Please expand on gaps in scientific research relating to **environmental conditions**.

____

Q35: Should different crops be grouped by common or similar characteristics?

- Yes
- Maybe
- No

What crop characteristics, if similar, would you recommend to a grower to group in an agricultural water assessment?

		Group	Do Not Group
Q36	Agronomic growing practices (e.g., spacing, bed height)	○	○
Q37	Antimicrobial properties (e.g., cruciferous vegetables)	○	○
Q38	Blossom fruit (e.g., cucurbit crops, night shade crops)	○	○
Q39	Epidermal layer quality (e.g., fragile or tough)	○	○
Q40	Growing nature (e.g., on the ground)	○	○
Q41	Root depth or structure	○	○
Q42	Surface adhesion susceptibility	○	○
Q43	Surface area	○	○
Q44	Surface texture	○	○

Q45: What additional crop characteristics, if similar, would you recommend to a grower to group in an agricultural water assessment?

____

Q46: What other relevant components should be included in an agricultural water assessment?

____

End of Survey.