CENTER for PRODUCE SAFETY

2024 Research Priorities

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OVERVIEW AND FORMAT

The Center for Produce Safety has since its creation, utilized collaborative and consensus-building approaches of continued improvement and openness to identify key funding priorities and to attract, encourage, and guide the best science was applied to this 2024 Request for Proposals. Stakeholder input has led CPS to adopt a modified format to simplify the RFP while laying out the research concept priority areas and more focused problem-solving or data-gap-filling needs in a manner designed as guidance to the research community for proposal submissions. This new format retains the foundational tenets that have guided the CPS mission: Fund the Science, Find Solutions, and Fuel the Change. The narratives that guided past RFPs have equally been based on the substance of the challenges and opportunities to address the constants associated with well-recognized hazards. The 2024 RFP format was developed to provide a clear expressed desire, by CPS, to specifically attack the known unknowns in risk characterization and risk mitigation. This year, CPS is experimenting with an approach provided as Hazard-Risk Statement tables, found below. The purpose is to provide a targeted but still non-limiting overview of the priority research opportunities that conceptually guide applicants to a desired outcome to "fuel change" across different anticipated timeframes.

In the current format, the key element stems from the central "Guiding Hypothesis Statement" in resetting core scientific methodologies (Observation, Question, Research, Hypothesis, Experiment, Data Analysis, Conclusion, and Communication) to fulfill and move closer to optimizing the development of actionable or adaptive research outcomes. CPS encourages open and creative approaches to restating or refining the Hypothesis Statement as best fits the proposed objectives consistent with the described priority area. The background and narrative around each Hazard-Risk Statement is intentionally brief. Interested applicants are strongly advised to engage with CPS on a strategic area(s) of interest to better understand the risk-based research needs, industry observations, experiences, perspectives, and supporting rationale. These pre-submission interactions are an established CPS process and have been invaluable for many PIs who have been awarded CPS project funds. PIs and stakeholders interested in better understanding the format and "Critical Review Elements" are encouraged to contact proposals@centerforproducesafety.org.

As in each annual CPS RFP solicitation, other concepts, risk-defining and investigative research questions, and novel "game-changing" technologies, which may not readily or clearly do not fit within a priority Hazard-Risk and Guiding Hypothesis Statement, will be accepted for review. While not a separate strategic area, CPS is acknowledging its continuing interest to explore potentially "game-changing" solutions or paradigm-shifting knowledge. However, solutions that appear to force adoption into produce safety applications are frequently unsuccessful within the review process, for various reasons. A key reason is that, while the quality of the concept and experimental design may be well structured, the fit to current or foreseeable industry practice sufficient to "fuel change" is judged to be well below the threshold for award recommendation. It is in the best interest for any PI considering a proposal, not convincingly aligned with the 2024 RFP format, to engage with CPS well in advance of submission. Strict confidentiality for this engagement process is always maintained. This interaction is often most helpful in connecting the research team with a produce industry or affiliated service provider, as may be described as a "Critical Industry-Fit and Outcome Review Element."

How to Use the Hazard-Risk Statement Tables: As stated above, the central focus for the required expanded rationale and justification, specific objectives, experimental design narrative, methods and materials, methods of analysis, and measures of success (i.e., statements of anticipated actionable outcomes rather than professional accomplishments and outputs) should be in terms of applying appropriate scientific methodology

to support or disprove the Guiding Hypothesis Statement (or an accepted revision). Critical Review Elements are provided for simplified clarity in priority technical and industry expectations, and also will be used as instructional guidance for CPS reviewers. The elements listed should not be considered all-inclusive, and additional proposal requirements are available in supplementary documents detailing policies, formatting, types of awards available, and application guidance. Included in these Critical Review Elements is the expectation that a proposal must clearly demonstrate the distinct concepts of hazard and risk and how they direct anticipated outcomes. CPS reviewers will be directed distinctly to assess each proposal for evidence of a solution offered to a hazard-related risk *that does exist and not to an abstract assessment of whether a risk may exist without sufficient practical context.*

Office Hours: *It is highly recommended* that all interested parties, and certain priority concepts found below may require, contact proposals@centerforproducesafety.org with pre-proposal development preparation questions or to arrange for a confidential pre-submission virtual conference with CPS Technical Committee members, industry representatives, subject matter experts, and the CPS Executive Director.

HAZARD-RISK STATEMENT TABLES: PRIORITY RESEARCH TOPICS

1. Agricultural Production Water

1a. Non-treated agricultural water

Rationale for priority topic: Antimicrobial treatment of agricultural water, as may be allowed under the Food Safety Modernization Act (FSMA) Produce Safety Rule, required by a commodity-specific agreement or marketing order, or established as a supplier approval requirement, may invariably not achieve full dose levels within and throughout the full conveyance and distribution system and to emitters. This system pressurization, treatment sufficiency charging, and stabilization interval results in non-treated well or surface water contacting the crop. Source ag-water or residual water from this source(s) remaining in the system or attached to biofilms within the surface or subsurface conveyances has been shown to periodically contain non-conforming levels of required performance-defining fecal indicators or foodborne pathogens. This non-treated water represents an inadequately defined risk during each irrigation event, whether routinely applied or applied according to a prescribed interval before a scheduled harvest.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Agricultural Production Water	Non-treated agricultural water applied directly to foliar and fruit surfaces of row crops or tree crops (microenvironment modification) may deposit contaminants before antimicrobial water treatment is initiated.	Antimicrobial treatment of overhead-applied surface water is effective in significantly reducing the likelihood of the secondary phase of risk exposure due to survival of pathogen contamination on crop surfaces prior to treatment system stabilization.	• Clear evidence of a scientifically valid surrogate(s) or ubiquitous naturally occurring index microbe(s). • Experimental design incorporates a broad range of seasonal and commodity practices. • Quantitative and qualitative “die-off” and amplification evaluations must plan for a progressive increase in sample number.	• Experimental objectives and design address the likelihood of risk (frequency of occurrence and levels when contamination does occur). • Antimicrobial formulations, dose, and application variables are consistent with selected system practices. • Anticipated outcomes transferable to related commodities, related regional profiles, and non-interfering with crop management practices required for market quality.

1b. Wells and groundwater sources

Rationale for priority topic: Wells and groundwater/aquifer sources vary in inherent water quality and/or the quality of known protective system design and construction. Grower-friendly protocols are needed to assess the potential for seasonal or chronic surface influences of groundwater, the unsaturated soil horizon zone, and the perched groundwater saturation zone waters on fresh produce safety. Strict reliance on known aquifer and borehole depth may not be adequate to ensure seasonal uniformity of agricultural water quality and system integrity. Additional research is needed to develop improved insights of measurable well or groundwater source design attributes that could be recommended to growers to better enable characterization and management of wells potentially under these influences and how these attributes ultimately impact risk.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Agricultural Production or Postharvest Water	Groundwater, well zone-of-influence and drawdown source waters are negatively impacted by contaminants originating from diverse surface factors, including well construction or maintenance.	Chronic or episodic non-compliance of source waters, extracted by wells, with current indicators of acceptable quality can be resolved by detailed large volume testing and isolation of breaches to provide protection from surface influences.	• Detailed assessment casing and grout integrity, known or suspected well screen number and depths, check valve integrity, surface water pooling or storm-related intrusion, and other relevant factors. • Comprehensive analysis of at least three situationally different groundwater extraction systems.	• Experimental design includes a grower accessible option(s) for practice adoption or adaptation. • Experimental design uses a scientifically valid surrogate, field-safe tracer, or practical and affordable isotope tracking methodology.

1c. Acute or chronic contamination of water systems

Rationale for priority topic: Short-term comparative project to develop science-based outcomes available to industry that will support the development of best practices in risk assessment, preventive programs, and realization of risk exposure events in sanitary management of agricultural-water distribution systems. Improved management practices are needed to address the establishment of biofilms or association of foodborne pathogens with established biofilms composed of nonpathogenic microbes within agricultural-water equipment and distribution. Biofilms within these surface and sub-surface conveyance and application systems are recalcitrant to disassembly (of the core biofilm). Evidence of effective treatment options to manage risk or eliminate characterized chronic or acute event (such as flooding or contaminated fertigation) contamination is needed. A priority emphasis is on large networked systems and treatments compatible with organic crop production.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Agricultural Production Water	Acute or chronic contamination of water conveyance and distribution systems.	Preventive and corrective management of ag-water conveyance and delivery systems are achieved within the treatment conditions and criteria of the Safe Drinking Water Act.	• Microcosm, mesocosm, and other model system experimental objectives must be developed from a primary characterization of a potentially impaired commercial ag-water distribution system. • Measures of success must follow a robust quantitative log-reduction and qualitative plan for a progressive increase in sample number to validate functional elimination.	• It is highly recommended for PIs and research teams to meet virtually with CPS to ensure an understanding of the needs and parameters of any model system development. • Close collaboration with and cooperation from industry in proposal design and planned outcomes.

1d. Antimicrobial treatment of source water: impact on soil health

Rationale for priority topic: Recent research has provided short-term insights to the absence of or sustained detectable alterations in the microbiome and metagenomic abundance, diversity, and functional profiles of soil under the influence of antimicrobial treatment of irrigation source water. However, from a risk-benefit perspective, it has always been apparent that long-term studies are needed to optimally determine the effects of routine and continual application of antimicrobials to ag-water on the soil microbiome and overall soil health. Anecdotal and empirical evidence for deleterious effects on soil health, crop productivity, and equipment have circulated within the industry. The primary water treatments of uncertainty include hypochlorite and peracetic acids. The need for longer-term studies has been identified as a priority. A structured research approach should be focused on understanding these issues from an organized scientific-methodology approach over a sustained period and inclusive of a cross-section of soil textures and commodity management and rotational practices. While overall agricultural water quality is essential to manage, the volumes associated with irrigation represent the greatest recognized risk of survival, persistence and, potentially, growth (specific to bacterial foodborne pathogens close to scheduled harvest). Frequency, dose to meet performance criteria for indicators, dose to meet log-reduction criteria for pathogens, antimicrobial form, secondary reaction products, and other factors have been questioned for their long-term impacts on soil productivity and current concepts of soil health.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Agricultural Production Water	(Long-term study) Antimicrobial treatment of source water used for irrigation to mitigate foodborne pathogen risk may result in risks which undermine soil health and sustainability goals.	Optimized residual dose management of antimicrobials, even if continually applied to irrigation source water, does not result in a measurable or irreversible negative impact on soil health, textural, and aggregate structure.	• Strong evidence for a secure multisite, comprehensive, and standardized systems approach. • Stable institutional access to study sites within relevant regional areas to commercial production of the chosen system. • Relevant fidelity to crop rotational practices and land management.	• A clear plan for near-term actionable “off-ramps” for study outcome adoptions within the long-term project design. • At combination of at least three paired trait and three divergent study site selection criteria.

2. Soil Amendments of Biological Origin

Rationale for priority topic: Whether composted or thermally treated, current soil amendments of animal origin systems operating under Process to Further Reduce Pathogens (PFRP) management and control plans have proven inconsistent in adequately protecting growers from water, soil, and product contamination. Moreover, input testing is not a fully reliable approach to mitigating the risk of contaminant transference to crops from adjacent land, post-application, and, potentially, in-use amplification during or after a crop production cycle.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Soil Amendments of Biological Origin	Stress-adapted pathogens in raw material or feedstock, process validation or control inadequacies, post-process contamination, or any combination of the preceding are a high risk of soil, water, and environmental crop production and harvest operational product sources of contamination.	1. Post-application incorporation of biological soil amendments and granular biofertilizers into sub-surface soil prevents the realization of the risk from multiple potential modes and vectors of contaminant transference. 2. Pre- or post-process treatment with an environmentally benign material is a cost-effective PFRP for pelletized biological fertilizers of animal origin. 3. Amplification of post-process survival or recontamination by bacterial pathogens in compost and biological fertilizers occurs between <u>leafy greens</u> crop cycles.	• Clear evidence of a scientifically valid surrogate or ubiquitous naturally occurring index microbe. • Experimental design incorporates a broad range of seasonal and commodity practice variations. • Quantitative and qualitative "die-off" evaluations must plan for a progressive increase in sample number. • Leafy greens are the priority for 2024 as risk potential, severity, and consequences are well known, but other commodities/categories may be considered.	• Experimental design must include a comprehensive plan for commercial field verification of any microcosm or mesocosm studies. • Proposals must address regulatory and industry acceptance, especially NOP considerations, for any post-process treatments.

3. Adjacent and Nearby Land Features and Activities

Rationale for priority topic: Siloed projects on aerosols, AFO/CAFO, wildlife, and adjacent land features and activities have not yet been integrated into adequately science-based, comprehensive, and clear guidance for growers, handlers, and food safety professionals developing standards and operational policies. Regional codependent and sequentially dependent hazard analysis represent a high priority need. The aim is to support a long-term program (a CPS conceptual commitment tied to project performance) to develop both fundamental and practical knowledge and integrated risk-based data. The anticipated outcomes, over the course of the research program, would result in an actionable dataset to functionally simplify this complex, challenging, and known multifaceted risk. Federal programs, not accessible to the public at this time, have advanced efforts in this area, but these datasets (such as the migration of wildlife, risk prediction based on shifts in weather, extreme weather events, and climate change) have not currently been effectively utilized in an integrated manner within publicly available research domains. CPS foresees that a successful award will take advantage of current and emerging risk assessment tools and concepts including digital twin models, machine learning and artificial intelligence, and econometric behavior. The recognized opportunity will be to leverage different approaches to data analytics, building rationale scenarios and options for risk exposure and severity prediction. The intended applications would be used, initially, in key growing regions of the United States, considered to have the highest potential for contemporaneous or near-term transferability and extension across the Americas. The emerging solutions would help guide decision-making at local and regional scales for domestic and imported goods.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Adjacent and Nearby Land Features and Activities	Multiple and potentially codependent risk of dispersal, transport, and deposition of viable and infectious foodborne pathogens from adjacent and nearby land to specialty crop production, accumulation, and handling sites.	An integrated regional-scale, multiple stressors, and codependency risk assessment and predictive modeling will identify and prioritize leading opportunities for industry resource and risk management of hazards associated with adjacent lands.	• A detailed and coordinated plan for multidisciplinary teams. • A combined and integrated plan for accessing existing datasets and databases with novel site and risk factor data acquisition. • Strong justification for selection and methods of study of the hazard.	• Convincing industry support in private land access consistent with proposed experimental design. • Assertions of end-user simplicity in model use must be described graphically.

4. Flooding

Rationale for priority topic: A reassessment of current guidance and established metrics for post-flood setback distances from the visible leading edge of the flood waters will benefit from scenario-specific data.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Flooding	Flood waters from point and non-point sources may bring diverse pathogens to crop soil and deposit viable contaminating populations in a generally heterogeneous pattern. The survival and persistence of pathogens is dependent on many factors not generally predictable on a site-specific basis. To mitigate risk uncertainties, since regional floods along the CA Central Coast in April 2006, setback distances from the visible leading edge of flooding have been based on the radius of field equipment turning (30 ft).	The subsurface horizontal and preferential flow of flood waters within a defined lot area require a prudent setback metric greater than 30 ft for public health and grower business protection.	• Comprehensive experimental design for simulating a flood event including but not limited to diverse soil texture, structure, grade, aggregate structure, and macro-pore channeling. • Clear evidence of a scientifically valid surrogate, field-safe tracer, or isotope tracking methodology.	• Priority emphasis on comparative and differential risk assessments on-site. • Close collaboration with and cooperation from industry in proposal design and planned outcomes.

5. Environmental Source Deposition of Foodborne Pathogens

Rationale for priority topic: A diversity of environmental point and nonpoint sources of pathogens of public health risk can be deposited on produce surfaces and may survive and persist to the point of consumption. Expanded knowledge of preharvest risks associated with survival post-deposition, in-orchard persistence of pathogens post-arrival, and the risk potential (could include risk exposure) for postharvest persistence is desired for currently underrepresented Rosaceous tree fruit as well as more commonly studied category varieties and systems. A diversity of environmental point and nonpoint sources of pathogens of public health risk can be deposited on produce surfaces and may survive and persist to the point of consumption. Proposals would address the following priority focus on bacterial pathogens in diverse orchard management style and climate/weather. The priority focus is on orchard environments of the PNW including pear, sweet cherry varieties, and apple varieties under overhead watering for microclimate management.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Environmental Source Deposition of Foodborne Pathogens	Deposition of foodborne pathogens during production survive into postharvest environments and potentially to the point of consumption.	The post-deposition "die-off" kinetics are rapid and the persister population survival of bacterial pathogens on tree fruit surfaces is sufficiently limited to meet expectations as a natural system of risk reduction and preventive control.	• Clear evidence of a scientifically valid surrogate or ubiquitous naturally occurring index microbe. • Experimental design incorporates a broad range of seasonal and orchard variation as well as fruit position within the canopy. • Challenge inoculations must address diverse forms of contaminant source/vector origin. • Quantitative and qualitative "die-off" evaluations must plan for a progressive increase in sample number.	• Experimental design must include open environment verification of "die-off" kinetics under diverse systems. • Log reduction models must be from realistic initial population loads. • Anticipated outcomes should be described in Logic Model with predicted, practical standards development as the endpoint.

6. Hygienic and Sanitary Design

Rationale for priority topic: Several lines of evidence from multiple outbreak investigations, sanitary surveys, and risk assessments have demonstrated that a key risk-vulnerability in produce grading, sorting, quality-keeping treatment, handling, and packing occurs at points of contact with brushes and rollers. The ability for industry to execute effective and consistent cleaning may be limited by the inherent design and fabrication. Various CIP and COP strategies have been developed by the industry, but performance remains a challenge. These challenges are particularly acute whenever fruit lusters or waxes are included at the operational packing line unit. A priority area was requested to solicit new designs for brushes, to convey produce through a packinghouse, which are easier to clean and sanitize than what is currently available.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Hygienic and Sanitary Design	Various brush rollers, brush applicators, and dewatering brushes and rollers, and conveyance rollers used for tree fruit and fruit vegetable packing are a recognized issue in "cleanability" and demonstrated risk factor in the establishment of environmental pathogens.	Application of the principles of hygienic design applied to packing-line brushes, fully embracing concepts of novel sanitary operational performance, will support the level of routine and periodic deep cleaning necessary to prevent the establishment of resident environmental pathogens.	• Proposed hygienic design, fabrication, and improved sanitary performance elements must anticipate principles reflecting access for cleaning, material compatibilities, durability, and tolerance of associated proposed thermal and non-thermal procedures for soil and wax removal.	• Studies should include enhanced methods for removing wax from brush and other food contact surfaces. • Close collaboration with and cooperation from industry in proposal design and planned outcomes. • A clear plan for an actionable "hands-off" for novel brush design to a brush manufacturer and cleaning options.

7. Field Harvest Equipment as a Contaminant Source

Rationale for priority topic: As an extension of daily cleaning and pre-operational sanitation, the establishment of periodic cleaning and prioritized sanitation of potential points-of-accumulation for indicators and index microbes during harvest is an additional step to support a defensible "clean-break" for field harvest equipment. As this equipment typically moves from lot to lot, field to field, and within or between production regions, traceability (as it relates to recall or other responses to detection on product, packing or processing facilities, or equipment) becomes highly problematic. Practical and logistically reasonable protocols and formulations are anticipated to assist the industry in continual efforts to improve and standardize a set of effective options to implement such practices.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Equipment as a Contaminant Source (Field harvest)	Limitations in hygienic design and sanitation practices for field harvest equipment may result in the establishment of environmental pathogens leading to food contact surface and, subsequently, product contamination. Additionally, a Quantitative Microbial Risk Assessment (QMRA) framework would help understand bacterial pathogens' amplification, survival, and potential for cross-seasonal movement with equipment.	Targeted CIP cleaning and sanitation of defined elevated risk areas for potential pathogen accumulation during harvest operations represent a significant advance in developing guidance for a defensible "clean-break" on field harvest equipment.	• Development of a baseline dataset for index and/or indicators which define general vs. elevated or sentinel risk sites. • At least two regions, commodities, or types of field harvest equipment are involved in the study proposal.	• It is highly recommended for PIs and research teams to meet virtually with CPS to ensure an understanding of a practical "clean-break." • Close cooperation with industry in proposal design. • A clear plan for near-term actionable "off-ramps" for study outcome adoptions within the long-term project design.

8. Controlled and Contained Facility Environmental Contamination

Rationale for priority topic: Limited information and scientifically developed hazards assessment and characterization of the overall and operational unit specific risk profile for biological, chemical, and toxigenic hazards (e.g., in water, growth substrate/media, seed sourcing, seed treatments, chemical fertilizers, other inputs, GMP elements of facility design and management) among diverse controlled environment agriculture (CEA) systems. Included in this priority area is an expectation for commercially relevant data on practical transfer coefficients for risk exposure onto or into produce during growing, harvesting, packing, and holding. Both incremental and comprehensive approaches to developing a systems-based programmatic inputs and environmental sampling plan for CEA systems, which supports evolving guidance and emerging industry standards.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Controlled and Contained Facility Environmental Contamination	Design and operational factors representing risk of food contamination associated with production and processing facilities may have points of commonality and unique aspects in prerequisite programs, GMPs, and preventive controls within CEA systems.	A systems approach to quantitatively and qualitatively addressing potential contaminant sources and their transfer coefficients in CEA facilities resolve current uncertainties regarding hazards and risk.	• Experimental design is focused on characterizing risk potential within a multi-year plan associated with evidence for routes and modes of potential establishment, dispersal, persistence, and transference of contaminants. • Strong justification for the choice(s) of contaminant categories included for models. • Development of a representative digital twin for each facility enrolled in the proposal.	• Clear intent for close cooperation from at least three CEA firms in proposal design, facility access, and planned outcomes. • A well-developed data management and release plan for detection of any hazard presence and contaminant transference events.

9. Regional Known or Reasonably Foreseeable Recurring Pathogen

Rationale for priority topic: Non-host environmental reservoirs of the REP *E. coli* O157:H7 have not been unequivocally elucidated at a level sufficient to represent an actionable root-cause mitigation. Industry knowledge and experience over several years of field surveys, sentinel sites, and on-farm research trials have not identified a clear and definitive mode(s) of transport from adjacent and nearby lands. Recent federal surveillance testing programs have not identified these REP subtypes on leafy greens products during the seasonal period most associated with past illnesses. More importantly, the apparent limited diversification of the REPEXH02 sub-clade cluster, over a span of time potentially expected to result in greater than observed host-based or environmentally determined accumulation of SNPs, is not fully understood. Understanding the basis for chromosomal and accessory genome stability may provide insights to these non-host reservoirs and opportunities for mitigation.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Regional Known or Reasonably Foreseeable Recurring Pathogen	(Long-term study) Determine the non-host reservoirs and evidence for environmental persistence and amplification of REPEXH02 and identify genomic factors which favor environmental and competitive fitness of these recurring subtypes of EHEC.	REPEXH02 is genomically and phenotypically characterized by differential traits, relative to other STEC, which, once resolved, will identify non-cattle host(s) and/or environmental harborage sites which constitute the root cause of recalcitrant barriers to prevention of crop contamination.	• A detailed description of access to and integration of archival isolates and comprehensive longitudinal effort at isolation of novel strains from a relevant study area. • Clear plan for non-duplicative experimental design relative to recent highly related longitudinal studies in a relevant priority region, genomic characterization of archival strains, and evolutionary genomic analysis previously reported.	• Close collaboration with and cooperation from industry in proposal design and planned outcomes. • A clear plan for near-term actionable “off-ramps” for study outcome adoptions within the long-term project design.

10. Human, Domesticated Animal, and Wildlife Fecal Matter

Rationale for priority topic: A proof-of-concept (POC) or short-term discovery and demonstration study – Evidence of potential risk exposure to fecal contamination is generally screened by bacterial indicator species. While several fecal-origin specific options are available from commercial service labs, industry has been using total fecal coliforms and generic *E. coli* (for these purposes considered the defining fecal indicator in the coliform group) for decades. This is largely a reflection of lower cost and short time-to-result. Various industry standards, specifications, performance criteria, and other risk identification and safety metrics continue to rely on solely fecal coliforms as a broad fecal contamination indicator. The diverse fecal coliform group includes many non-*E. coli* bacteria and ubiquitous types with no or unreliable connection to a fecal source. This uncertainty regarding association of quantitative screening outcomes with actual fecal origin and product risk has long led to serious and often costly consequences in data interpretation. The term thermotolerant coliforms is preferred to remove the term "fecal" from how this group would relate to risk or process control performance verification testing. As the retention of "fecal coliforms" as a risk-indicator group for fresh produce appears likely in the near or long term, new options are needed to remove much of the uncertainty in decision-making following a noncompliant test result within a given product specification, standard, or metric.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Human, Domesticated Animal, and Wildlife Fecal Matter	Diverse sources of fecal material or fecal matter waste streams may contaminate production, handling, and distribution environments and inputs, fomites, equipment, or products. Modern technologies provide opportunities to differentiate non-*E. coli* of likely fecal origin or association from those which are best adapted to environmental sources, including roots, shoots, flowers, and fruit.	Genomic sequence-determined or other real-time protocols effectively differentiate between fecal coliforms of true fecal origin from warm-blooded animals and those thermotolerant but non-*E. coli* coliforms ubiquitous to water, soils, and specialty crop tissues.	• Test methods must use 44.5 to 45.5°C incubation temperatures for foundational and verification cultural studies. • Experimental matrices for sourcing thermotolerant coliforms must originate from environmental, inputs, production, and product sources encompassing specialty crop agriculture.	• It is highly recommended for PIs and research teams to meet virtually with CPS to ensure an understanding of the needs and parameters of any prospective test method.

11. Wildlife Fecal Deposition

Rationale for priority topic: Avian species remain a concern for contamination of specialty crops typically consumed raw, regardless of the commodity or region. The overall risk potential may be low in many situations, but the specific risk remains shrouded in uncertainty for growers. In general, the most common bird hazing for diversion from farms, such as techniques based on general disruptive or mimic-specific sound waves, have a limited span of effectiveness against those species most recognized as sources of zoonotic pathogen deposition during on-farm intrusion. Most prevalent and "affordable" methods are deployed on a simplistic, frequent, and constant time interval. The longevity of effectiveness may be greatly extended by integrating digital camera surveillance and species category with autonomous triggering of deployment when risk of consequential contamination is greatest.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Wildlife Fecal Deposition	Avian species typically standout as the most challenging wildlife of site-specific and seasonally variable sources of risk for zoonotic pathogen contamination of specialty crops.	Diverse bird hazing and harassment techniques may be greatly improved in effectiveness and longevity of function by shifting from continual frequency-based applications (preset time interval) to activity, species/type, and population density thresholds developed by on-farm digital surveillance and machine learning.	• Compare the degree of priority bird species acclimation to hazing with and without digital camera autonomous control of pyrotechnic, distress call mimics, sonic waves, or other common devices already in use. • Clear and established science-based system for quantifying avian prevalence and risk in a farm setting.	• Proof-of Concept (POC) or short-term project objectives. • Anticipated outcomes should be described by a Logic Model with predicted, practical protocols, economic assessments, and performance criteria standards development as the end-point. • Leveraging plan for continuity of preliminary data and expanded field validations.

12. Postharvest Condition or Defect

Rationale for priority topic: Postharvest arrival post-packing and processing may have visually apparent quality, condition, or signs of incipient decay (for simplicity these will be termed *conditions*), which has long been recognized to have the potential to increase the risk of foodborne illness and severity. Naturally, foodborne pathogens must be present or acquired at some point from preharvest to arrival at distribution or foodservice points of preparation or service. There is an interest to modernize and reassess the quantitative risk potential during the "last mile" in the supply chain at foodservice and retail. These conditions, noticeable and assessed for acceptance at receiving, may be a consequence of preharvest, harvest, pre-shipping postharvest management, and post-processing storage and distribution, including time and temperature of the production history. Collectively, these factors may contribute to reduced product surface cleanability, preharvest or harvest contaminant entrapment, internalization, and increased likelihood of preharvest to postharvest amplification of pre-existing bacterial pathogens or infectivity (including triggering of sporulation) of concern for foodborne illness. Some of these *conditions* may trigger risk-elevating metabiotic interactions between foodborne pathogens and the associated microbiome; a mode of interaction between two or more microorganisms, in this use, in which one organism (the pathogen) depends on the activities of another on a substrate or a host (produce tissue) to precede the internalization and/or amplification of the pathogen to release otherwise inaccessible nutrients, free moisture, or favorably modifications to the tissue environment, such as altered tissue pH or inactivation of inhibitory molecules.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Postharvest Condition or Defect	Condition, quality, fractional spoilage, or incipient decay may increase the risk of illness upon consumption by amplification, pre-adaptive or induced stress tolerance, hypervirulence, or other modes of interactions with the product, the environment, subsequent handling practices, or the associated microbiome.	Post-contamination risk potential at point-of-sale and point-of-purchase is strongly influenced by product quality, condition, or metabiotic interactions.	• Focused model studies which broadly address a key list of categories or commodities (informed by retail industry interaction). • Sound preliminary data which supports any proposed risk models using metagenomic profiles, validated index microbiome, or metagenomic consortia, or individual index microbe or surrogate.	• QMRA outcomes must include a sensible and practical design supported by a clear Logic Model for how results would improve QA/QC assessments on arrival and acceptance decision-making of product.

13. Human-Specific Waste

13a. Screening assay for HAV

Rationale for priority topic: Proof-of-concept (POC) or short-term project for future leveraged funding**.** Detection of hepatitis A virus (HAV) in non-clinical samples may be conducted by several recognized methods of varying sensitivity and specificity. Real-time PCR, including LAMP and bioluminescent techniques, qPCR protocols can be highly specific and sensitive in detection of the single-stranded virion, but the detection targets do not differentiate virulent (infectious) from avirulent (non-infectious). Although not a produce-specific issue, economic losses are likely to have resulted from positive detection of non-infectious forms.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Human-Specific Waste	(Method development to address current limitations to functional and practical changes to HAV detection) Develop a first-level screening assay for specific detection of an intact/ functional hepatitis A virus capsid protein as a precursor to detection and confirmation of infective HAV RNA. What is the non-host environmental persistence and infectivity of the intact HAV? Of the detectable RNA?	Detection of an intact/ functional encapsulating capsid protein is essential to any decisions regarding risk exposure, persistence, development of mitigation effectiveness, and sensible application of tools and methods for environmental and product screening.	• Strong justification and rationale for the proposed solution and how the innovation is anticipated to address industry concerns for false association of infectious HAV with product. • Proposal narrative and methods balance descriptive completeness for technical reviewers, with a respectful effort towards readability and comprehension by non-specialists charged with the CPS review process.	• Anticipated outcomes should be outlined in a Logic Model with predicted economic of use and performance standards. • A clear plan for near-term actionable “off-ramps” for study outcome adoptions within the long-term project design. • Support from American Frozen Food Institute.

13b. Recovery efficiency for *Cyclospora*

Rationale for priority topic: Proof-of-concept (POC) or short-term comparative study to validate an improved protocol to disrupt/dissolve oocysts of *Cyclospora cayetanensis*, and related human pathogenic *Cyclospora* species, for improving DNA yield for detection protocols and platforms. Regardless of the associated advancements and predictions for simpler, faster, more robust, and less costly *C. cayetanensis* testing for the parasite DNA, any lab must 1) crack open the oocysts and 2) demonstrate and document optimized, repeatable, and reproducible % DNA purification and recovery efficiency. Current scientific literature, and estimates from SMEs in the field, suggest the current DNA recovery efficiency at less than 30% of known density gradient and flow cytometry purified oocyst samples. Improving recovery efficiency is essential for any detection platform utilized to conduct risk potential and risk exposure with low oocyst numbers (e.g., water, soil, tissue, non-zoonotic animal hosts or vectors by mechanical transfer). Substantially improved DNA recovery efficiency is also seen as an important POC outcome for genomic sequencing and strain characterization from non-clinical samples. Even recent advancements in PCR detection offer little functional and meaningful use for testing environmental and product contamination in detection and source-tracking.

HAZARD	Known/Presumptive Risk Factor	Guiding Hypothesis Statement	Critical Technical and Design Review Elements	Critical Industry-Fit and Outcome Review Elements
Human-Specific Waste	*Cyclospora cayetanensis*, *C. ashfordi* and *C. henanensis* are signature human-specific parasites which are a significant endemic public health burden in exporting and domestic regions.	Wholly novel or sequentially combined procedures for release and purification of DNA from human pathogenic *Cyclospora* species can be optimized to achieve a minimum of 45% recovery efficiency.	• POC or short-term research proposals must have a clear commitment for access to or reliable sourcing of oocysts. • A clear and concise protocol for documenting the composition and recovery efficiencies from non-clinical samples. • Demonstrated PI and/or Co-PI initial proficiency in reproducible DNA recovery efficiency ≥25%.	• As a POC or limited duration research project, the anticipated outcomes must include a convincing description of the longer-term research directions and benefits to the industry beyond the understood benefits to research and public health.