2022 CPS Research Priorities

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Priority Foundational, Methods Validation, On-site Verification, and Proof-of-Concept topics

As has been the practice and policy of CPS since its creation, research concept priority areas and focus are provided within the narrative of the RFP. Some are open and broad, while others are more targeted and narrow in scope. Proposals outside of these topics will be accepted for review. For either category of hypothesis-framed investigation, researchers are strongly encouraged to engage with CPS and its Technical Committee to explore experimental question and design concepts. 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 contact or collaborator.

> NOTE TO APPLICANTS: Proposals involving assessments of harvest spray-washes, aqueous cooling, water flumes, spray + brush wash beds, or other systems to achieve quantitative and qualitative log-reduction of pathogens, indicators, index microbes, or applied surrogates must focus on removal and/or lethality in relation to product surfaces. For experimental design procedures involving challenge inoculations, post-inoculation populations densities, following a pre-treatment stabilization interval, should be ≤ log 5 CFU/product unit or a specific spot-inoculation site (log 3 ≤ plaque-forming units for viral surrogates; ≤ log 2 oocysts for parasites or parasite surrogates). If an immersion inoculation procedure is used for tree fruit or fruit vegetables, recovery from product surfaces must include a comparative analysis from primary areas including pedicel or peduncle zone, equatorial zone, and blossom-end zone.

The rationale for this proposal development expectation is that CPS feels the scientific literature, including multiple system studies funded by CPS, provide ample evidence of the performance of antimicrobial water amendments in reducing or preventing cross-contamination. Sub-objectives within these topic-focused proposals would benefit from co-assessments of prevention of cross-contamination to non-inoculated product and food contact surfaces, but these should not be the sole anticipated results.

Proposals involving antimicrobial treatments to achieve microbial log-reduction, as well as objectives evaluating environmental viability reduction, must include a progressive, three-step semi-quantitative or qualitative assessment of viability, once the calculated limit of detection results in non-detection, to establish the evidence for lethality. Experimental design objectives are encouraged to include an assessment of the role non-detection results due to the induction of a viable but not culturable (VBNC) state for bacterial pathogens, indicators, or surrogates but CPS discourages this from being the dominant objective of a study.

1. Cyclospora

a. *Cyclospora cayetanensis* sporulation rates – Current and future preventive actions and root cause analysis investigative efforts by industry, as expected by the FDA, are based on and dictated by the state of the scientific knowledge surrounding expectations for extra-host environmental

maturation and sporulation kinetics and dynamics leading to post-consumption infectivity. Confirmation or corrections to our understanding of these critical biological facts is sorely needed to address apparent inconsistencies in temporal induction and sequential steps leading to the infectivity of oocysts shed into diverse production environments and, potentially, food contact surfaces. Equally, more evidence is needed to characterize the range of maturation and sporulation kinetics under variable and multifactorial environmental conditions, from growing and harvesting through short-term storage, processing, distribution, and point of service or consumer handling and holding. A specific priority outcome within this fundamental research topic is to determine the potential impact of long-standing methods for oocyst harvesting, processing, and stabilization protocols applied to the study of *C. cayetanensis* biology and ecology. A particular interest is to clarify the potential for current protocols to influence induction or suppression of maturation, cellular developmental, oocyst surface traits, physiological responses to stress, and viability. This objective may be included in a broad proposal or as a standalone project. Comparative studies with reasonable surrogates, such as Eimeria spp. may be included to provide directional knowledge for later phases with *C. cayetanensis* Regardless, a clear demonstration of sourcing-commitment to obtain human specimens of *C. cayetanensis* oocysts for these studies is essential,

b. Qualitative and quantitative *Cyclospora cayetanensis* (Cc) risk and risk assessment contributors – This priority is largely but not exclusively focused on the issue of Cc as an emerged and endemic public health risk within the U.S. The range of potential risk amplifiers and modifiers is open, but examples of interest include quantitative assessments of persistence and transference in soil, water, crop, on filtration devices (including temporal recovery during backflush protocols), and product handling fomites and environments. Clearly, these objectives may be combined and integrated with the priority described in Cyclospora 1. This priority is considered by CPS as more appropriate for proposal where a surrogate, such as *Eimeria* spp., are deployed to facilitate and provide initial assessments of filtration, transference, methods development, or other model ecological studies, when comparative studies based on a secured source of Cc are not available.

c. *Cyclospora* practical risk reduction in seepage irrigation – This very specific applied research priority seeks to encourage creative approaches to minimize or prevent transference of Cc oocysts from open, exposed intra-cropping seepage irrigation ditches, associated with either horizontal or "subbing-up" irrigation methods. Studies addressing this priority must reflect the specific soil type/texture (i.e., muck/peat or highly sandy soils with underlying hardpan layer) amenable to this multi-row sub-irrigation method is used. Splash transference during intense rainfall, from these intra-block lateral ditches, commonly 12 beds between ditches, is a key mode of potential cross-contamination targeted for a preventive control under this RFP priority. Currently, there is no publicly available information to indicate that horizontal or vertical transference from a primary canal, contaminated with Cc oocysts, to within-crop irrigation ditches occurs and results in transference to the aerial portion of the crop or surrounding soil at levels reasonably likely to be transferred during harvest operations. Farm-centric applied research is needed to address the potential Cc risk associated with seepage irrigation and develop "readily implemented and economical practices for risk reduction. The experimental design for large-scale model systems

and/or on-farm studies are recognized not to, necessarily, require the use of Cc oocysts in challenge studies or using naturally contaminated water sources.

2. Growing

a. Risk analyses related to adjacent land features and uses – While a recognized hazard for decades, the potential for dispersal, deposition, persistence, and secondary cross-contamination of foodborne pathogens [and growth potential for bacterial pathogens] from adjacent land features, uses, and operations has recently received elevated awareness and attention as a significant source of risk. Recent unequivocal evidence of zoonotic pathogens in adjacent, surrounding, and nearby areas to fresh produce production and related farming operations is important information for food safety system design. However, there remain significant knowledge gaps specifically, quantitative data that will lead the scientific and public health and environmental regulatory agencies towards a greater capacity to predict those parameters/conditions under which transfer may occur. Dispersal and deposition which results in consequential persistence in the soil, and/or on the crop, equipment, or packing materials.

Therefore, CPS seeks research proposals to provide outcome-based food safety controls for growing operations that are nearby or adjacent to animal operations or other sources of foodborne pathogens. Additionally, development, validation, and on-site verification of risk assessment tools and predictive models of adjacent land use risk factors. A multipurpose risk assessment tool(s), and associated food safety controls for different end-users (i.e., grower, research, investigative) to monitor, detect, and efficiently and economically assess the risk of adjacent land contaminant transference remains a research priority for CPS.

Adjacent land use (i.e., CAFO, AFO, composting, other specialty crops, non-food crops) or land feature (i.e., wildlife refuge, food processing facility attracting animal vectors, low use or abandoned on-farm buildings, urban settings) is very broad. Less recognized examples of concern include elucidating the risk profile of shared traffic corridors and roads between animal production, composting facilities, rangeland, and specialty crop farms.

These potential adjacent land risk factors are also of interest in relation to controlled environment agriculture.

NOTE TO APPLICANTS - CPS invites several types of research proposals within this priority area but feels many of the current knowledge gap areas are most reasonably addressed, initially, as Proof-of-Concept or short-term projects to define the targeted system and site(s) of proposed study. Characterizing the risk potential and a preliminary risk exposure in advance of a future, more detailed and well justified experimental design is strongly encouraged. Highly speculative hypotheses, unsupported by preliminary data, establishing a reasonable foundation for anticipated results and solutions-directed outcomes, are strongly discouraged.

Recognizing that a variety of factors will influence the risk of contamination of fresh produce from adjacent land use features and activities, CPS has prioritized field-based

research leading to user-friendly models which better inform the industry of on the aerosolization conditions and modifiers which lead to short-range and long-range transport of indicator/pathogen-associated particulates. Research and predictive models must combine particulate dispersal and deposition on crops, relevant contact surfaces, and agricultural water sources. As a priority, field-based research may be focused on a particular adjacent land feature but should include multiple locations within that risk category. For example, experimental design should include a radius or justified set of vectors around various animal operations (beef, dairy, swine, poultry), manure and compost handling operations, or non-point source wildlife habitat for the prevalence of predictive indicators or pathogen-specific markers at varying distances from the target source. If viable pathogen recovery is proposed, all isolates must be subjected to whole genome sequencing (WGS) for comparison to an identified or selected focal point source or non-point source to support qualitative and/or quantitative tracking of target microorganism dispersal. These data, along with relevant metadata (e.g., weather data preceding each sampling; wind direction and speed; conditions where the animals are kept, and concentrations of animals; presence of natural or man-made wind blocks and effects on altering particulate dispersion and settling distances; presence of insects, birds or other animals that can vector pathogens), can help field-validate whether rapid detection will help growers make informed decisions.
Research may be conducted on dedicated research farms or experimental field locations, but proposals must include research objectives conducted with industry cooperator participation. Sites must be selected within production locations and an extended and defined perimeter of risk-relevant adjacent land uses and features.

b. Bioaerosol heat map from animal operations/ Risk associated with animal operations – Dust and bioaerosols are a specific subset of the research priorities described in *Risk analyses related to adjacent land features and uses* above. CPS anticipated research outcome interests are broad and encompass innovative approaches to integrated qualitative and quantitative experimental system design incorporating row crop, vine crop, tree fruit, packing facilities, or controlled environment production systems. Parameters including point source and non-point source site topography, environmental, aerosolized particulate size distribution, quantitative particulate viable and non-culturable indicator, index target, and/or pathogen particulate content, and a comprehensive array of climatic metadata.

   Proposals should be prepared to address the knowledge gain limitations of these complex studies and avoid overly limited or simplistic dust/bioaerosol capture array deployment schemes. CPS has identified an industry priority need in validation and verification of efficacious but practical sentinel-alert systems for field and on-farm deployment. The broadly adoptable system would establish or lead to continual or seasonally targeted monitoring of source setback-breaching and crop location relevant dust/bioaerosol risk exposure. Proposals which combine methodology development addressing model dust/bioaerosol capture, quantitative compositional biomarker analysis, and predictive risk modeling in relation to terrain, land features, distance, and meteorological conditions are also encouraged.

3. Harvest

At-harvest risk profile for equipment contamination, amplification, and cross-contamination – Recent root cause analysis has identified the need for quantitative assessments of the conditions potentially leading to pathogen contamination and amplification potential (bacterial pathogens) of stationary or mobile/mechanized harvest equipment and aides as well as packing bins, totes, and other packaging units. Associated cross-contamination transference from any exposed product contact or adjacent surfaces, internal entrapment and harborage sites, and other sites, forms, or operational functions (i.e., in-field trimming, conveyance to a bulk transport bin or container). The priority focus includes *Salmonella*, *E. coli, and Listeria* persistence and amplification potential under diverse product type, harvest and field-prep practices, environmental conditions, clean-to-clean timeframes, sanitation procedures (including differential formulation and application methods), overall hygienic design of equipment, contact surface fabrication and equipment condition and wear. Of critical interest are research proposals that include detailed descriptions of factorial transfer coefficients across the previously mentioned parameters. Similar objectives to elucidate sources, persistence (including evidence of retained infectivity), and transfer coefficients specific to human enteric viruses and *Cyclospora cayetanesis*, or validated surrogates, are also encouraged. Clearly described harvest system-specific descriptions, with at least an initial stepwise analysis of current cleaning and sanitation systems, or preliminary data identifying key and recurring sanitation failure points, are expected for all proposals.

4. Agricultural

a. Identification of potentially beneficial or deleterious impacts of ag-water treatment – CPS invites proposals from diverse specialty crop production regions which establish a comparative physicochemical and metagenomic profile baseline of soil health in relation to treatment of agricultural water, used in preharvest crop production, with antimicrobials. Comparative analysis should include matched soil texture, compositional characteristics, and recent (minimally 2 years) cropping history where treated and non-treated water applications from the same source are conducted. Experimental design may include a single or multiple antimicrobial formulation or device treatments, which are recognized by FDA under the current Produce Safety Rule provisions or have satisfied EPA ag-water treatment validation protocol specifications. Studies may be proposed to be conducted on a large-scale model system or within a commercially grown crop and following up to two annual seasons at the same site. Proposals should include objectives incorporating a factorial frequency of application to the crop and achieve industry-defined microbiological performance criteria for surface water treatment. Data objectives must include basic soil inorganic and organic compositional analysis, including primary soil aggregate structure and porosity, at key incremental timepoints across the project period.

b. Impact of preharvest water treatment on established foodborne pathogen persistence – Industry has requested expanded evidence for the potential for ag-water treatments to reduce the risk of preharvest contamination introduced to a crop during irrigation events or crop management foliar applied treatments prior to the first required or selected water treatment regime prior to harvest. Additionally, this research priority seeks to address the risk reduction potential, by

concurrent or subsequent treated irrigation water events with an antimicrobial, for contamination occurring during system pressurization and stabilization prior to treatment injection or uniformity or at any time during an injection which may result in "break-through" of indicator species during an ag-water treatment application. Similar assessments which assess the potential for log-reduction of contamination from environmental sources, other than the water applied during crop irrigation, following an antimicrobial amendment to ag-water. This priority may be combined with the RFP topic described in 4c - *Microbial risk characterization of leafy green commodities which are harvested multiple times*. All proposals should be designed to provide detailed quantitative log-reduction outcomes applied to or suitable for QMRA when combined with other research on pathogen persistence on the target crop. Persistence in soil and secondary cross-contamination to the crop prior to and during harvesting is encouraged.

5. Postharvest

Optimizing stonefruit brush wash beds and hydrocoolers – The process of washing, defuzzing (peaches), and waxing stone fruit on brush wash beds varies greatly among packing facilities in California's Central Valley. Brush wash, wax, and dry brush beds are potentially where the greatest risk of cross-contamination by foodborne pathogens exists within a facility. Recirculated hydrocooler water represents a similar potential for risk. Decisions regarding process variables are often influenced by concerns related to the effects of antimicrobial agents on fruit quality and condition, which in turn, may have a negative effect on the efficacy of sanitizing agents. Proposals are invited to define best practices for washing, defuzzing, waxing and hydrocooling that can be adopted by industry to reduce the potential risks associated with *Listeria*, *Salmonella*, and *E. coli* while preserving product quality. This may also include postharvest interventions such as antimicrobial additions to fruit waxes and lusters and verification of the efficacy of commercially available edible antimicrobial fruit coatings or other finished product treatment(s).

6. Packing

Listeria management and control in dry avocado packing operations – Special Request: Mexican Hass Avocado Importers Association has been working with CPS for over a year to contribute to the CPS research program. As required by the USDA approval, the initial funding start date for this project category will need to be initiated by April 2022.

Background: Domestic and imported Hass avocados have been subject to FDA surveillance sampling for *Listeria monocytogenes* since the FDA FY 14-16 Avocado Sampling Assignment revealed the presence of *L. monocytogenes* on over 17% of all avocados sampled from all producing areas, including the United States, Mexico, Chile, and Peru. The avocado industry was generally unprepared for this finding, and this has resulted in high-profile recalls of fresh avocados and the investigative detection of widespread *L. monocytogenes* in a packing facility within the U.S.

There exists a wealth of practical information on cleaning and sanitation of fresh produce packing facilities but most of this represents "wet" operations, i.e., facilities that use water for cleaning and/or transport of the products that they pack. Most Hass avocados packed in the U.S. and

Mexico, the primary market source, are packed without hydrocooling, fluming, or washing which is referred to as a dry packing process. The antimicrobials applied to single-pass and recirculated water, added to cooling and wash water, are not present in dry packing processes. The day-to-day and in-process sanitary conditions of dry packing are less characterized for diverse produce, including avocados, as compared to wet operations in the fresh produce industry.

L. monocytogenes is considered ubiquitous and transient populations of *Listeria* present on harvested avocados, equipment, pallets, and footwear may be introduced into packing facilities daily. Clearly, as in recent studies in other systems, this poses a risk of cross contamination and could lead to these transient populations of *L. monocytogenes* becoming established and representing a business and public health risk. The following priority objectives have been identified by the MHAIA:

- Determine the prevalence and critical harborage site potential of *Listeria* spp. in dry avocado packing facilities
- Determine the potential for short or persistent wet areas around or at avocado contact surfaces
- Determine the effectiveness of current industry cleaning and sanitation programs (includes routine or periodic wet cleaning in a "dry-operation") with dry avocado packing operations.
- Determine how to improve on current cleaning and sanitation practices in dry avocado packing operations.
- Establish how clean breaks can be effectively accomplished in dry operations. This should include transfer coefficients for different types of food contact materials and may include strategies to minimize time needed between defensible lot-to-lot clean break intervals.

7. Storage

Influence of associated tree-fruit microbiota of food safety during storage – Tree fruit packing may involve short, medium, or long-term postharvest storage. The associated microbial communities on the surface, at natural openings, at harvest or postharvest handling induced openings or micro-wounding sites, and in sub-epidermal tissues or entry points (i.e., lenticels) may influence foodborne pathogen persistence and growth potential. This priority area is open to diverse systems of research, but the current priority is on long-term storage systems, such as with many varieties of apple. An additional purpose of this research would be to develop directional knowledge on how orchard management decisions may influence pathogen survival, positively or negatively, due to modifications of the associated microbiome. The industry seeks a scientifically valid assessment of risk potential as influenced by orchard management systems, and integrated with postharvest handling systems, on the fruit microbial community and metagenomic succession under long term storage.

One such integrated system of interest is pears. The pear industry in the Pacific Northwest of the U.S, is prepared to assist researchers collaboratively developing approaches to characterize the predicted persistence and die-off kinetics of pathogens, or validated surrogates, on pears under various commercial storage conditions (such as cold storage, packaging type, and whether wax has been applied to the pear) and as influenced by the associated microbiota. Building upon

existing research, the anticipated outcomes should be developed to determine how the typical culturable microbiota, with emphasis on yeasts, molds, and lactic acid bacteria, affect pathogen survival and amplification potential during long-term storage conditions. Assessments may include characterizing the non-culturable microbiome community and development of a metagenomic profile during long term storage, primarily as a foundation for future leveraged studies of tree fruit associated microbiome on positive or deleterious impacts on food safety.

Foundational, short-term applied research

(≤1 Year Project period; may include Proof-of-Concept proposal)

Please read ATTACHMENT A - "End to end" supply chain risk models

1. Compost Microbiological Criteria – Standards for ensuring the safety of compost developed with animal manure feedstock, as one category of biological soil amendments of animal origin, are recognized to depend more on process controls and measurement of critical time: temperature parameters during composting than end-point microbiological testing. However, though recognized as an imperfect parameter for safe-use assurance, current standards and metrics require lot-to-lot sampling and microbial testing for an indicator group and pathogens. Recently, it has been proposed that the quantitative measurement criteria for the long-standing fecal indicator group (total fecal coliforms < 1000 MPN / gram of dry compost) is too permissive, especially for row crops such as leaf greens, herbaceous culinary herbs, root crops, and use in producing agricultural teas. As the fecal indicator and pathogen starting point of the composting process is rarely, if ever, determined in commercial systems, the end-point analysis has limited informative value. It has been suggested that based on the typical proportional logarithmic content of indicators to pathogens in animal manures and associated typical proportional time: temperature logarithmic die-off of these groups, a single decimal reduction standard (total fecal coliforms < 100 MPN / gram of dry compost) would provide significantly greater assurance of protective finished and cured compost. CPS invites short-term, creative research proposals to challenge this testable hypothesis using a combination of naturally contaminated feedstock and, minimally, a pilot-scale but full commercial compost process. Proposed objectives may include a comparative assessment of alternative indicators of a properly managed composting process Experimental design parameters should include sampling regime analysis and a quantitative assessment of variability of microbiological targets within a composted lot beyond the currently prescribed minimal lot-qualifying sampling plans.

2. Pathogen persistence on fruit surfaces – Post-arrival/cross-contamination persistence of pathogens on tree fruit and soft fruit (various berries) have been studied in several systems and regional locations and using various pathogen surrogates or indicators. The available scientific evidence is that the process kinetics leading to measurable net survival is highly variable and subject to wide influence by many factors. Orchard topography, canopy structure, orientation relative to incident sunlight, microclimate, variety, crop inputs, and orchard management (e.g., stand density, trellising, shading structures) are recognized variables. CPS seeks preliminary, foundational research leading to QMRA strategies. This directional research should address and

incorporate modern approaches and scientific knowledge which build upon past research, or parallel approaches with under-represented crops, with greater attention and detail devoted to the influence of edaphic factors in determining the persistence of contamination 1) in the field, orchard, or grove; 2) During harvest and initial postharvest handling; 3) During long-term storage; and 4) In relation to potential interactions with spoilage and decay pathogens during long-term storage The justification, rationale, and detailed description of proposal objectives needs to have a central risk-based focus and clearly defined over-arching research question with a significant anticipated translational relevance to risk management and practical interventions.

3. State of the Science and Current Research Scoping White Papers – CPS invites short duration projects to develop issue briefs which provide a technical state of the science, an assessment of practical research investment opportunities, and a projected timeframe for adoption or a maturing technological innovation. Scoping of research activity should emphasize recent publications and technical abstracts which include an analysis of the coverage of knowledge and knowledge gaps, rather than a simple list of cataloging of citations. Priority topics include:
    a. Potential for plant breeding to provide a seed or vegetatively propagated source of foodborne pathogen preventive control management.
    b. Update on the produce safety and sustainable farming practices nexus
    c. Qualitative Risk Assessment of indoor produce production and handling systems with emphasis on the diversity of growth matrix sources and management, irrigation and nutrient film or hydroponic systems.

4. Short-term Projects
    a. Contrast dry transfer coefficients of harvest container contact surfaces to product, managed to a “visually clean” standard, in comparison to wet cleaned, sanitized, and dried container
    b. Develop an initial risk profile and key knowledge gap assessment of wholesale markets and repack operations
    c. Microbial risk characterization of leafy green commodities which are harvested multiple times (kale, cilantro, parsley, cabbage, some baby greens, etc.). How does potential risk change from the first harvest to the second or the third? How do inputs (water/soil amendments), environmental conditions (adjacent land use/wind speed/wind direction/topography, etc.), crop characteristics (dense cabbage head v. open contoured kale leaves), and harvest practices impact the potential for contamination?