APPENDIX B:
Technical Basis Document for *Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens*

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Introduction

This document serves as a supplementary source of information to the *Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens (Leafy Green Guidelines)*. In the *Leafy Green Guidelines*, metrics and action levels were established for a variety of process areas judged to be potential contributors to the risk of microbial contamination.

During the guideline development process, many stakeholders identified a need for a document that provided the basis and rationale for the choice of metric; this document is intended to serve that need. Since its first publication, the fresh produce industry has updated the *Leafy Greens Guidelines* on numerous occasions to keep abreast of changes in industry practice and new regulations and research. The rationale for major changes related to research and industry practices are explained in this document.

This document discusses the technical basis for the metrics and action levels. In general, a three-tier approach was used to identify appropriate metrics:

1. A comprehensive literature review was conducted to establish whether a scientifically valid basis for establishing a metric has been published.
2. If the literature review did not identify published scientific support for an appropriate metric, existing standards or metrics supported by authoritative or regulatory bodies were adopted.
3. If neither scientific studies nor existing standards or metrics from authoritative bodies supported adoption of a specific metric, consensus among industry representatives and/or other stakeholders was sought.

The following sections provide a detailed explanation of the processes and rationale for derivation of each metric.

Agricultural Water Sources and Uses

The *Leafy Green Guidelines* were originally focused on periodic water testing for generic *E. coli.* Several improvements have been made since then to detect and correct any potential issues that may be associated with irrigation water quality in a more holistic manner by considering the integrity of the entire irrigation water distribution system. Late in 2018, the leafy green industry learned from the U.S. Food and Drug Administration (FDA) outbreak investigations associated with leafy greens, that two clinical *E. coli* strains from people who became sick in these outbreaks were found in irrigation water used on leafy greens. These findings prompted additional changes to practices for irrigation water quality and safety. Based on the Arizona metrics' version 12 and California metrics' October 24, 2019 version of the *Leafy Greens Guidelines*, best practices and metrics for agricultural water were determined based on water quality, how the water is applied, and when it is applied. Because of this, the focus of the best practices and metrics shifted primarily from source water to the entire agricultural water system.

In relation to microbial water quality, agricultural water was divided into two categories – Type A and Type B. Type A agricultural water systems are unlikely to contain indicators of fecal contamination either due to natural hydrogeologic filtration or through controlled U.S. EPA and state regulated treatment regime as demonstrated by an agricultural water system assessment as outlined in Appendix A, microbial testing, and when applicable, treatment verification. Type B systems include all other agricultural water systems. Type B water can become Type A water if it is treated with U.S. EPA approved chemical treatments and / or filtering systems.

When agricultural water is used in overhead applications, three types of microbial water quality assessments are generally required; however, assessment requirements vary depending on: 1) the water source and 2) when the assessment occurs. Baseline assessment of source water is required in most instances before water is used within the 21-days-to-scheduled-harvest window. Initial assessments, conducted at the end of the delivery system prior to the 21-days-to-scheduled-harvest window, are to ensure microbial water quality is being maintained throughout the delivery system. Throughout the growing season, maintenance of microbial water quality is routinely verified at the end of the system.

The decision to increase the stringency of microbial water quality standards within 21 days to scheduled harvest is based on the preponderance of evidence in the scientific literature. Field study reports vary on the length of time *E. coli* survives on plants after being applied via overhead irrigation water. More studies supported a 21-day survival period than studies showing lower survival rates (Fonseca *et al*., 2010; Gutierrez-Rodriquez *et al*., 2012, 2019; Koike *et al*., 2009; 2010; Moyne *et* al., 2011; Suslow *et al*., 2010; Wood *et al.*, 2010). As more research is conducted on *E. coli* survival in various growing regions, this time period may be modified.

Determining water quality metrics for water sources and uses must consider (1) which microorganisms to test for and the test methods, (2) action levels to apply, and (3) appropriate corrective actions when standards (e.g., acceptance criteria) are not met. An ideal test method would detect all pathogenic organisms present; however, this is not scientifically or economically feasible for many reasons:

- Concentrations of microbial pathogens can vary widely in fecal matter. Hence, if testing focuses on specific pathogens, the presence of fecal contamination may not be detected even if significant contamination is present (Ashbolt *et al.* 2001; World Health Organization 2004). While continuous monitoring or daily testing for pathogens might more reliably detect these microbes, this approach is economically unfeasible.
- Existing test methods may not be able to detect the wide variety of pathogenic organisms that might be present (World Health Organization 2004). Even if water is routinely tested for the more common pathogenic organisms, this does not guarantee other pathogens are not present.

Given the reasons above, and guidance and/or comments from various regulatory agencies (US EPA 1986; California Department of Health Services (CDHS) and California Department of Food and Agriculture (CDFA) 2006; US FDA 2006), use of an "indicator" microbe was determined to be the most effective and efficient testing approach. Testing for generic *E. coli* is considered the best available indicator of a fecal contaminated water source.

Generic *E. coli* is generally non-pathogenic; thus, using this as an indicator organism results in action levels that are not necessarily health risk-based. Although increasing levels of generic *E. coli* in a water source are likely to correlate with increasing health risk, "bright line" levels of generic *E. coli* above which health risks are unacceptable are difficult to established. Because this is true, action levels based on generic *E. coli* concentrations should not be considered as separating "safe" or "unsafe" levels—they should only be considered as indicators of fecal contamination or increasing bacteriological densities.

To set generic *E. coli* action levels for water used in agricultural applications, it was decided that it would not be possible to use one set of levels for all uses. For instance, water that contacts edible portions of plants have more stringent standards than water that does not contact edible portions of plants. In order to address this issue, use-specific standards were created for the following uses determined to be most critical to lettuce and leafy green food safety:

- Pre-harvest non-foliar applications. Where edible portions of the crop are not contacted by water (e.g., ground chemigation, furrow or drip irrigation, dust abatement water).
- Pre-harvest foliar applications applied greater than 21 days to the scheduled harvest date where edible portions of the crop are contacted by water (e.g. overhead sprinkler irrigation, pesticides/fungicide application, etc.).

- Pre-harvest foliar applications applied within 21 days to the scheduled harvest date where edible portions of the crop are contacted by water (e.g., overhead sprinkler irrigation, pesticides/fungicide application, etc.).
- Post-harvest direct contact applications. (e.g. re-hydration, core in field, harvest equipment cleaning, bin cleaning, product cooling, product washing).

For water used for non-foliar applications and for foliar applications prior to the 21-days-to-scheduled-harvest-window, a rolling average and single sample maximum metric was set. These metrics were based on water quality standards developed by the U.S. EPA in their risk assessment of *E. coli* in recreational waters were used to establish action levels (US EPA 1986;2003). U.S. EPA determined that the geometric mean of *E. coli* in recreational water systems should not exceed 126 MPN *E. coli*/ 100 mL to protect against unacceptable risk of waterborne diseases. In addition to this geometric mean value, they also determined single sample maximum values for various beach-use types. These single sample maximums are based on certain confidence levels of the geometric mean value of 126 MPN. For a "Designated Beach," U.S. EPA used the 70% confidence level, which is a value of 235 MPN/100 mL. For rarely used beaches, they used the 95% confidence level of 576 MPN/100 mL. These three guidelines were used to establish action levels for non-foliar applications and for pre-harvest water used prior to the 21-days-to-scheduled-harvest-window.

Pre-harvest water used on crops prior to the 21-day-to-scheduled-harvest window and non-foliar pre-harvest applications must meet the geometric mean requirement of 126 MPN/100 mL, but foliar applications must adhere to the lower 235 MPN/100 mL metric while non-foliar applications use the less strict 576 MPN/100 mL standard.

When water is applied to crops pre-harvest within 21 days to the scheduled harvest date and for post-harvest direct crop contact or food-contact surfaces applications, more stringent requirements are to be met due to the lack of additional steps to remove or reduce contamination and the potential for cross-contamination. For these applications, the water quality standard has been set at non-detectable generic *E. coli* in 100 mL, and if generic *E. coli* is detected, the water must be treated. For pre-harvest use of treated water, growers are required to also test for total coliforms as a measure of treatment effectiveness, but test results are for monitoring purposes only and do not have enforceable thresholds. In addition to the one-point-in-time sample and test for routine verification of microbial quality, water treatment parameters are also routinely monitored while in use – continuously with periodic manual verification unless the system has been shown to have a low degree of variation. Flow-rate in addition to treatment-related parameters such as residual antimicrobial levels, pH, dose settings, etc. are also monitored. For treated agricultural water systems, guidelines for monitoring treatment parameters during pre-harvest overhead applications and continuous treatment monitoring in post-harvest systems are also provided in the *Leafy Greens Guidelines* and Appendix A to facilitate meeting this standard.

Water sampling locations prescribed in the *Leafy Green Guidelines* vary depending on the type of irrigation water system. For treated water, it's important to know the water quality and treatment parameters of water near the source, but it is most critical to know the microbial quality and treatment parameter values of the water contacting the crop (i.e., at output locations).

A complete list of the various sampling requirements and action levels are outlined in Table 2A, 2B, 2C, 2D, 2E, 2F, and 2G in the *Leafy Greens Guidelines*, while decision trees explaining their use are shown in Figures 1, 2A, 2B, 3A, 3B, 3C, 4, 5, and 6.

Soil Amendments

Many regulatory bodies have set guidelines for production of soil amendments as well as acceptable levels of microbial organisms in finished products. A complete list of the metrics is provided in Table 3. Decision trees are found in Figures 7A and 7B.

The Leafy Green Guidelines address the use of manure, composted soil amendments and heat-treated soil amendments.

Manure

The application of raw manure or soil amendments containing untreated animal by-products, un-composted / incompletely composted animal manure and/or green waste, or non-thermally treated animal manure to lettuce and leafy green production fields is thought to be a high-risk practice, and the *Leafy Green Guidelines* do not permit these practices. Initially, allowing use of manure in fields for production of lettuce and leafy greens with a suitable application interval (120 days as suggested in the National Organic Program guidance) (USDA 2002) was considered; however, this use was prohibited after discussion and comments received from multiple stakeholders. Given the long survival period of bacteria in raw manure (over 120 days in some references), it was determined that the 120-day period was not acceptable, and that raw manure should not be used in the production of lettuce and leafy greens (Islam, 2004a, 2004b, 2005). However, in order not to completely restrict the use of land that has at some point had raw manure applied, a one-year waiting period prior to planting lettuce and leafy greens was considered appropriate.

Composted Soil Amendments

Due to the existence of California state regulations regarding the production of compost (CCR Title 14 – Chapter 3.1 – Article 5 2007), these guidelines were essentially adopted “as is” for the *Leafy Green Guidelines*, with the addition of *E. coli* O157:H7 testing as an additional safeguard.

These guidelines largely rely upon fecal coliforms as the pathogen indicator organism. Testing for generic *E. coli* as opposed to fecal coliforms was considered; however, because fecal coliforms are hardier, and guidance does not exist for *E. coli* levels in compost, tests for fecal coliform were considered more technically feasible and conservative relative to testing for generic *E. coli* (Jin *et al.* 2004; Entry *et al.* 2005).

A three-hurdle process was considered to be sufficient for safe application of composted soil amendments to lettuce and leafy green crops. The first hurdle requires use of a validated process for compost production; the second requires microbial testing, and the third requires applying an application interval to minimize risk from remaining pathogenic microorganisms.
A 45-day application interval was deemed appropriate due to the three-hurdle metric design. Raw manure must be composted with an approved process and pass testing requirements before an application interval is observed. Some commenters supported the use of the National Organic Program’s 120-day waiting period for use of raw manure. However, because the 120-day period is specific to raw (uncomposted) manure, it was judged reasonable to shorten this period to 45-days.

Heat-Treated Soil Amendments

Due to limited information related to the process and expected microbial populations found in heat- treated soil amendments, metrics were primarily based on the composting metrics described above. Some processes are discussed in the literature (US EPA 1994; Bellows and Baker 2005); this information was used to set some metrics for temperature and contact times. Most of these U.S. EPA based requirements are for biosolids but are also considered to be appropriate for application to raw manure. Because the process for heat-treating manure is much more controlled than composting, a stricter requirement for fecal coliform (negative) was considered reasonable for heat-treated soil amendments. In addition, based on the recommendations of expert reviewers, *Listeria monocytogenes* was added to the list of target microorganisms with an acceptance criterion of non-detect (<1 CFU per 5 grams). Recently there have been several *L. monocytogenes* outbreaks linked to fresh produce commodities including packaged leafy green salads. Studies demonstrate that *L. monocytogenes* can persist in soil amendments and soil (Erickson, 2015; Vivant, 2013).

Due to the stricter testing requirements and more tightly controlled process used with heat-treated soil amendments, if a validated process is used no application interval is required for these types of amendments. If the process is not validated, a 45-day application interval was deemed appropriate due to the three-hurdle metric design. A longer application interval such as the National Organic Program's 120-day waiting period for use of raw manure was considered; however, because the 120-day period is specific to raw (uncomposted or heat treated) manure and in absence of more definitive research a waiting period of 45- days is utilized in the interim to provide an extra hurdle.

Non-Synthetic Crop Treatments

Due to limited information related to the process and expected microbial populations found in non-synthetic crop treatments, metrics were primarily based on the composting metrics described above. However, due to the foliar application of many of these types of treatments, a more stringent guideline was considered to be appropriate for microbial testing (i.e., negative for fecal coliform, *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp.). Specific metrics are found in Table 4 of the *Leafy Green Guidelines*, and a decision tree for these treatments can be found in Figure 8.

Due to the stricter testing requirements and used with non-synthetic crop treatments and their intended use as foliar applicants, if a validated process is used no application interval is required for these products. If the process is not validated, a 45-day application interval was deemed appropriate due to the three-hurdle metric design.

Flooding

The flooding definition applied in *Leafy Green Guidelines is* based on the definition accepted in the first CSG document. Although some comments related to possible changes in this definition, since there is no consensus at this time, the original definition was retained.

The distance not to be harvested from the high-water mark of any flood event was selected to be 30 feet, based on the turn-around distance of farm equipment to prevent cross- contamination. This distance may be increased if there is the uncertainty about the location of the high-water mark or if some equipment has a greater turning radius— whether to increase this distance is to be determined by an appropriately trained food safety expert, with possible consultation with other experts as necessary.

The required waiting period after flooding prior to planting (60 days) was selected based on comments from regulatory bodies when this document was first developed; these comments were consistent with original time periods based on USDA NOP guidance on use of manure (i.e., it was assumed that the worst-case flooding event would be equivalent to use of raw manure on fields) (USDA 2002). This 60-day-prior-to-planting time period is roughly equivalent to 120-days prior-to-harvest depending on the specific growing season of the crop and was considered to be easier to implement in the field. An option to reduce this time period to 30 days is provided if growers can demonstrate, through a valid sampling program, that soil microbial levels are lower than those required for composted soil amendments. The development of the soil sampling plan and the sampling itself must be undertaken by a reputable third-party environmental consultant or laboratory.

Regardless of the use of the standard 60-day period or the 30-day period, all decisions related to use of flooded land should be made with the consultation of a qualified food safety professional. This person should have the same qualifications as described in the Environmental Assessments section below.

Environmental Assessments

In order to maintain vigilance over the conditions associated with the production of lettuce and leafy greens, periodic monitoring of production fields is required. This monitoring requires visual observation of field conditions with focus on animal activity and neighboring land uses. This monitoring should begin one week prior to planting and continue through the growing cycle. In addition, two formal assessments must also be conducted, one within one week prior to harvest and the other at harvest.

The *Leafy Green Guidelines* focus on two key areas: animal activity in a field and adjacent land use.

Animal Activity in Field (Wild or Domestic)

The metrics developed for assessing animal intrusions in production fields were based on best professional judgment about proper assessment and corrective actions. In general, it was assumed that continuous monitoring for this type of event was not feasible, so periodic monitoring as well as pre-harvest and harvest formal assessments were determined to be a viable alternative.

In general, due to the likely subjective issues in determining whether or not an animal intrusion is significant and presents a risk of contaminating lettuce or leafy green produce, the *Leafy Green Guidelines* specifies that a trained food safety professional or personnel be involved in decisions related to animal intrusion. In order to best conduct environment assessments focused on animal intrusion, the following is recommended:

- A solid understanding of the principles of food safety as applied to agricultural production in addition to the successful completion of food safety training at least equivalent to that received under standardized curriculum recognized as adequate by the FDA.
- Each fresh produce production operation involved in growing, harvesting, and / or packing should have a dedicated food safety professional whose primary job function is development, implementation, and supervision of a comprehensive food safety program.
- At a minimum the individual will have some training in relevant fields of science including but not limited to biology, microbiology, food science, chemistry, and botany. Experience in actual food safety operations especially those related to fresh produce is strongly recommended.

These requirements recognize the fact that food safety in the fresh produce industry is an endeavor based on scientific principles and that significant formal training is required to prepare individuals for food safety management responsibilities in the industry.

In the case of animal intrusion events, each situation has unique aspects and too many variables to definitely outline metrics for all of them. The food safety professional will use their best professional judgment to determine whether to harvest product, how much buffer distance should be assigned for various intrusions, and whether remedial options might reduce or eliminate risk from intrusions. The only established metric for this area is that crop with any evidence of fecal material may not be harvested, and if fecal material is found, the produce surrounding the fecal material shall not be harvested. Originally, a minimum 5-foot radius buffer distance from the spot of contamination was established based on best professional judgment, and research findings have confirmed that this buffer distance is adequate (Koike, 2008 & 2009).

Crop Land & Water Source Adjacent Land Use

Developing metrics related to acceptable distances from production fields to various adjacent land and water uses was difficult due to a dearth of scientific literature on the topic, and the many different environmental factors that might be encountered in the field. In order to provide some basis for determining these distances, the various types of land uses were first characterized according to their relative risk (the land uses of possible concern were first selected during various grower/processor meetings in the fall of 2006). For instance, active composting operations were considered to have a relatively high risk, while normal water ways were considered to have a lower risk.

Once the relative risk associated with each type of land or water was agreed upon, acceptable proximate distances from the land/water were determined. The use of a "proximate" metric instead of a defined lower or upper boundary was considered appropriate due to the countless factors that might be found in a particular environment. A "one size fits all" strategy did not seem reasonable. Due to the lack of suitable science for defining "safe" distances, almost all of the distance metrics were determined by best professional judgment between the document authors, growers/producers, and the expert reviewers of the document. Following the 2018 *E. coli* O157:H7 outbreak in romaine, the distance recommended for productions areas next to CAFOs was increased from 400 to 1,200 feet based on a 2015 study at USDA's Nebraska research facility demonstrating that leafy greens 600 feet from a feedlot had contamination (Berry, 2015). Stakeholders in Arizona agreed to double this study's maximum distance where contamination was found as a precautionary starting point for leafy green production next to a CAFO; therefore, it adopted 1,200 feet as a precautionary distance. California took the same distance into consideration but also the number of cattle held in the feedlot. The California standard is 1,200 feet if more than 1,000 head are present and increases to a mile if more than 80,000 head are present in the feedlot. Several factors were also identified that might necessitate increasing or decreasing some of the distances (see Table 7 for the complete list). As additional science is brought to bear on this issue, it is anticipated that the metrics will change accordingly.

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