Introduction and Rationale

While a healthy diet for consumers depends on plentiful consumption of fresh fruits and vegetables, the science necessary to provide procedures that assure safe production, processing and handling is inadequate. The spinach crisis of 2006 demonstrated the critical necessity for better measures to assure a safe and reliable food supply. Since then, the leafy greens industry has developed and implemented extensive new food safety standards, metrics and compliance programs. These were developed with the best available experience and judgment. However, it became clear during this development that the science to provide a definitive basis for these standards and programs is insufficient. For these reasons, it was important to bring together the best international representatives to identify those essential research needs.

The first International Lettuce and Leafy Greens Food Safety Research Conference, comprised of invited academic scientists, state and federal regulators, and industry representatives, U.S. and international, was held September 20 and 21, 2007 in the Washington, D.C. area, with the charge to develop a prioritized list of research needs to address the issue of \textit{E. coli} O157:H7, \textit{Salmonella} and other human pathogens in and on lettuce and leafy greens. The major goals were to identify solution-driven research objectives, to build consensus on needs and to build the working relationships and collaborative partnerships necessary to achieve success.

Priority Research Needs for Lettuce and Leafy Greens:

1. Intervention Strategies, Technologies and Validation of Standards for Field Operations.
   - Develop transfer coefficients, as affected by field environmental factors, for irrigation water.
   - Develop verification criteria for composted soil amendments.
   - Conduct research to provide a scientific basis for current intervention metrics.
   - Quantitatively identify extrinsic factors affecting survival/demise of pathogens in field soil, and develop methods to verify field suitability.
   - Evaluate environmental impacts and sustainability of technologies for treating/disinfecting irrigation water.
   - Develop technologies to enhance the effectiveness of wash water systems.

2. Assessment of Risk and Risk Reduction.
   - Develop the science necessary to evaluate, rank and minimize risks posed by environmental factors (e.g., airborne contamination, water, wildlife, geography, seasonality, weather) and actual practices in field preparation, production, harvest and post-harvest handling.

   - Determine the microbial ecology of pertinent pathogens (e.g., \textit{E. coli} O157:H7) and factors affecting their survivability, amplification or demise in various environments, in production and throughout the leafy greens supply chain.
• Determine the factors and practices affecting the potential for pathogen internalization from pre-harvest to packaging.
• Develop the tools needed to conduct meaningful research in microbial ecology and to verify interventions
  a. develop validated rapid microbiological methods for pathogens (including sampling) and
  b. identify an acceptable pathogen surrogate for E. coli O157:H7.

Research Conference Report

The Process:

Over 70 invited academic scientists, state and federal regulators, and industry representatives, U.S. and international and already involved in leafy greens food safety, were asked to review several documents prior to the conference, including the Lettuce Industry Research Agenda from a California Lettuce Research Board meeting in 2005, recent fresh produce food safety research summaries provided by both USDA ARS and USDA CSREES, and a bibliography of microbial food safety of lettuce and leafy greens publications prepared by Drs. Robert L. Buchanan and Steven Gendel of FDA. Consideration of these documents, along with presentations on current industry practices and research knowledge, provided a foundation for the participants, divided into four Working Groups, to formulate specific priority research needs that could be conducted with the limited funding available for fresh produce food safety research.

The Working Groups were asked to identify whether needed research was short or long term, to consider previously conducted studies, and to focus on those studies that could reasonably be expected to result in risk reduction. The groups were challenged to consider the entire production chain from the field to retail, to consider the practical and economic realities of implementing the research results as well as to consider any methodologies, scientific disciplines or research sectors that could successfully address the issues.

Scope of Issues:

The Working Groups sequentially considered production/preharvest, harvest/cooling/processing/value-added, and finally the distribution/food service/retail/end user steps of the leafy greens supply chain. The initial research prioritization effort revealed research needs primarily in the preharvest and production area and then later in post harvest and processing; focusing efforts on prevention prior to distribution/food service/retail sectors. For future sessions, increased representation in the distribution/food service/retail area will be sought.

General Recommendations

Research Scaled to Real World. All participants felt that research should be conducted on a pilot-scale or field operational basis and that we must get beyond the classical laboratory research of the past. It was noted that research on microbial ecology and field interventions should focus on actual field conditions and what the pathogens are doing, not what they can be made to do.
Access to Private Sector Data. Critical information exists in both industry-conducted data collection and industry-funded research. These private sector operational data must be mined in such a way as to allow access with confidentiality. Collaborative partnerships between industry, academia and government must be facilitated to ensure research is focused on solutions for real world practices and data gaps.

Kill Step Technology. While there is a crucial need for a pasteurization process for lettuce and leafy greens, it is recognized that no current technology has a significant potential to accomplish this in the near future. Therefore, significant expenditure of research funds here is unlikely to result in a meaningful intervention until an innovative approach is found.

Working Groups’ Detailed List of Identified Research Needs:

Each Working Group developed a listing of prioritized research needs that after examination can be grouped into three key sectors:

1. Intervention Strategies, Technologies and Validation of Standards
2. Assessment of Risk and Risk Reduction
3. Microbial Ecology

The individual proposed priorities are listed below for each sector. Many of the identified research priorities involve the need for environmental controls from the field through the processing plant.

The four Working Groups often identified the same research needs although expressed in varied terms. Insufficient time was available to reconcile such differences as a combined group.

1) Intervention Strategies, Technologies and Validation of Standards
   • Define what needs to be achieved through a clearly delineated Food Safety Objective.
   • Develop a lethal kill step for pathogens that can be effective in a high organic load (post harvest).
   • Determine how to use cumulative hurdles and practical interventions since a single kill step may not be attainable.
   • In place of current water standards, develop science-based water standards directly applicable to lettuce and leafy greens production, or scientifically substantiate the applicability of recreational water standards for the field.
   • Determine the validity of buffer distances from potential contamination sources such as intensive animal operations and sources of wildlife, and determine if other intervention strategies are available.
   • Develop Integrated Pest Management procedures for fields that provide practical, economical, sustainable, legal and effective deterrents (or exclusion or redirection) for currently uncontrollable animals that may be pathogen vectors.
   • Develop better strategies and uniform procedures to verify microbiological suitability of fields, particularly after potential contamination events.
   • Conduct intensive raw material sampling and analysis on representative farms and fields that represent specific conditions to evaluate effectiveness of current metrics and standards.
• Scientifically validate cleaning procedures, including equipment sanitation standards, based on transfer coefficients for pathogens from potential field sources to equipment.
• Validate methodologies for cleaning and sanitizing procedures for all produce contact surfaces (bins, totes, hands, equipment, and utensils).
• Develop an effective or new technology for treating/disinfecting irrigation water that is practical, sustainable, economical, and legal in face of state and federal regulations.
• Develop efficacious wash water treatment with rapid methodology to substantiate effectiveness.
• Optimize water disinfectant efficacy and prevent disinfectant failure due to factors such as pH, temperature, time, concentration, product flow rate (product to water ratio), etc.
• Research possible antimicrobial treatments for retail and food service.
• Identify interventions to minimize risk of airborne contamination in fields with adjacent compost storage/operations, etc.
• Develop protocols and standards to minimize airborne or waterborne contamination.
• Develop lot-specific microbiological verification protocols and standards to measure the efficacy of intervention strategies.
• Research and develop additional interventions beyond the wash step - vacuum, antimicrobial film, chlorine dioxide, MAP, probiotics, etc. based on naturally occurring, field contamination events.
• Examine how to adjust intervention strategies due to seasonal and geographical changes in animal populations, temperatures, etc.
• Develop harmonized criteria to evaluate new and emerging intervention technologies.

2) **Assessment of Risk and Risk Reduction**

• Perform a risk ranking for environmental factors in field preparation, production and harvest.
• Identify level of risk from airborne contamination.
• Identify whether natural barriers reduce or increase risk of contamination.
• Develop a means for rapid response research to address transient real world research questions (research ready SOPs).
• Develop Food Safe Compost standards. Whatever method of composting is used, develop performance and sampling standards, and determine if compost is a deterrent to survivability of *Salmonella* and *E. coli* in amended soils.
• Determine whether processing in the field contributes to contamination of product. Examine field coring, sanitization of equipment, time/temperature at the time of harvest, crop residue left in the field, chlorine levels, water temperature, internalization, moisture levels at the time of harvest and determine the contribution of each factor to risk.
• Examine agricultural water standards in relationship to risk, based on transfer coefficients, presence and levels pathogens and factors such as amplification, die-off, type and age of leafy green.
• Examine potential and risk for pathogen (*E. coli* O157:H7) amplification between harvest and cooling under operational conditions.
• Develop cleanability/sanitary design standards for produce contact equipment (production and harvest) including detection technology for equipment contamination.
• Determine the effect of retail practices such as misting, temperature control, commingling, crisping, etc., on risk.
• Evaluate the risk of norovirus in lettuce and leafy greens at retail and in food service.

3) **Microbial Ecology**

• Determine survivability of pathogens (i.e., *E. coli* O157:H7) under various production conditions and as affected by background microflora, UV light, temperature, pH, etc.
• Determine how pathogen survival and growth on lettuce and leafy greens is affected by commodity, leaf morphology, varieties, maturity, seasonality (i.e. spring, summer, fall, winter crops).
• Determine the ecology of pathogens internal to the plant structure preharvest. Determine the potential for internalization of pathogens into the leaf subsurface tissue and how it may be affected by commodity type, variety, season and maturity.
• Determine how the ecology of *E. coli* post harvest is affected by varietal differences, maturity, internalization, etc. particularly on injured and cut surfaces.
• Determine if internalization of pathogen occurs with vacuum cooling, hydrovac and washing in post harvest and fresh cut produce handling situations.
• Determine how *E. coli* moves through soils comparing vegetated versus cultivated operations, various soil types, etc.
• Identify an acceptable *E. coli* surrogate for real world studies.
• Examine whether and how distribution of *E. coli* from a contamination source can be mathematically modeled.
• Conduct research on the factors affecting survivability and growth of *E. coli* post-harvest or for the entire production supply chain from 'cut to cool'.
• Develop effective rapid microbiological methods for *E. coli* O157:H7 and other pathogens to be able to conduct needed research.
• Determine effects of temporal changes in water quality, soil and plant surfaces on plant pathogen growth and survival in the production environment.
• Determine transfer potentials for pathogen transference from adjacent land use practices (e.g. air dispersion from soil, air dispersion from water use, etc.).
• For postharvest and fresh cut produce handling, determine pathogen - food contact surface transfer coefficients.
• Evaluate the effect of time and temperature on pathogen survival and growth from farm to fork (harvest, cooling, process plant, in bag, distribution, retail and food service).
• Examine the potential for application of 'sniffer' technology used in counterterrorism for field air monitoring.
• Study the microbial ecology of irrigation water sources and determine the persistence/growth/survival factors affecting pathogens.
• Determine temperature effects on virulence of *E. coli* O157:H7.
• Determine the mechanism of resistance of pathogens on produce to antimicrobial treatments.
• Research the environmental factors that contribute to the transport and transfer of pathogens in the field and post harvest.
Next Steps:

This report will be provided to industry, research funding agencies, Congressional leaders, professional/scientific organizations, and research entities. A subsequent meeting will be organized in 2008 to assess progress and update research priorities.

Conference Organizers and Support
The Organizing Committee included:

- Tom Farewell, Dole Food Company
- Jim Lugg, Fresh Express
- Robert L. Buchanan, FDA Center for Food Safety and Applied Nutrition
- James Lindsay, USDA Agricultural Research Service
- James R. Gorny, University of California - Davis, Postharvest Technology Research and Information Center
- Martha Roberts, University of Florida IFAS
- David Gombas, United Fresh Produce Association

Financial support for the meeting was provided by a donation to the United Fresh Research and Education Foundation by Fresh Express and Dole Food Company.

Available Documents and Information

This conference report, along with the following supplemental information, is publicly available at www.unitedfresh.org:

- Microbiological Safety of Leafy Greens Bibliography
- USDA CSREES research summaries
- USDA ARS research summaries
- Leafy Greens: Practices, Possibilities and Controls
- Working Group Reports

For additional information about the Conference and its proceedings, please contact Dr. David Gombas, United Fresh Produce Association, (202) 303-3411, dgombas@unitedfresh.org.