Report to Congress

Biennial Report to Congress on the Food Safety and Food Defense Research Plan Submitted Pursuant to Section 110(g) of the FDA Food Safety and Modernization Act, Public Law 111-353

U.S. Department of Health and Human Services

Food and Drug Administration

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Executive Summary

While the responsibility for food safety and the protection of the nation's food and agriculture supply against unintentional and intentional contamination and other emerging threats is an important responsibility shared by federal, state, local, tribal, and territorial governments and private sector partners, the principal responsibility lies with the Department of Health and Human Services (HHS), the United States Department of Agriculture (USDA), and the Department of Homeland Security (DHS). Research to support food safety and food defense efforts is primarily conducted by several agencies within these departments (e.g.,at HHS, the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), at USDA, the Agricultural Research Service (ARS), the Economic Research Service (ERS), the Food Safety Inspection Service (FSIS), and the National Institute of Food and Agriculture (NIFA), as well as by various academic institutions primarily funded through federal grants and collaborative agreements. These agencies are positioned to contribute science-based information to public health decision making and policymaking that will ultimately help improve the safety and security of the food supply. In addition to federally supported research, private industry, particularly food companies, conduct research to assist them in developing and updating their company-based food safety programs. However, there is a clear need for the development of additional public-private research partnerships with regards to identifying priorities and mitigation strategies aimed at improving food safety.

Identifying and conducting science and research to support foodborne illness reduction strategies is a challenging and constant undertaking due to the complexity and continual evolution of food production and processing practices, food distribution, and consumer preferences and practices. In addition, new foodborne hazards continue to emerge, and others that have existed for some time are being found in foods not historically associated with a particular foodborne pathogen. Recent data on foodborne illness from CDC illustrate that many food safety issues persist and that we need to be alert to new challenges since many hazards can be transmitted through a variety of foods.

As food safety knowledge and activities have evolved, the objectives of the federal agencies responsible for the safety and security of the food supply have as well. The research goals across these agencies provide both broad and focused strategic approaches depending on their respective regulatory responsibilities. Their range provides the opportunity to develop research applicable to addressing current recognized foodborne microbial and chemical contaminant risks and threats, while also providing the framework for research and extension activities to address long-term, as well as emerging needs. As technologies and methods advance, new pathogens and chemical contaminants are often identified. Examples of salient issues of concern, both existing and emerging, include produce food safety, the detection and characterization of chemical (or bio-threat) contaminants, the potential effects from climate change on food safety, and the development and evaluation of prevention and intervention strategies along the food production continuum. Bringing this to the forefront was the enactment in 2011of the FDA Food Safety and Modernization Act (FSMA or the Act) (Public Law 111-353), which calls for a science-based, public health, prevention-oriented food safety system, and the establishment of an infrastructure needed to support such a system as well as identifying and highlighting the critical role science and research will continue to play in ensuring a safe food supply.

Continuation of mission critical research is essential for supporting science-based prevention standards, understanding and detecting foodborne hazards, and developing intervention strategies to protect the U.S. food supply and consumers. This report highlights food safety research being conducted or supported by individual agencies as well as collaborations among federal agencies. For the purposes of meeting the reporting requirement in section 110(g) of FSMA, research projects have been divided into eight general categories:

- Prevention, intervention, and control of foodborne hazards;
- Detection of microbial, chemical, and radiological hazards in food, feed, and dietary supplements;
- Molecular characterization of foodborne pathogens as it relates to research on the mechanism of disease and/or epidemiology of foodborne disease;
- Antimicrobial resistance/susceptibility of foodborne microorganisms;
- Designed epidemiologic studies of foodborne illness/associated organisms;
- Risk assessment, modeling, management, and communication;
- Safety assessments of foodborne hazards, including toxicological studies; and
- Economic analysis.

The federal government has invested significant resources to maintain and build its scientific foundation in these areas and each agency is increasingly prioritizing food safety research needs in alignment with their respective public health goals and mission.

Of note is the FSMA requirement in section 108 that calls for the development of a National Agriculture and Food Defense Strategy (NAFDS) that details specific food and agriculture defense goals, objectives, key initiatives, and activities to be accomplished by HHS (primarily FDA), USDA, DHS, and other stakeholders. The Act also mandates that the NAFDS shall include a coordinated research agenda for use by the Secretaries of HHS and Agriculture in conducting research to support its goals and activities. To avoid duplication of efforts, the agencies have agreed that the food defense research activities and plans will be captured as part of the FSMA section 108 requirement and the section 110(g) Biennial Food Safety and Food Defense Research Plan and will focus on broad food safety activities, with cross-reference to the NAFDS for the food defense research activities, as appropriate.

The federal agencies engaged in food safety research are involved at times in overlapping areas of research, albeit tailored to the individual needs and applicable laws under which each agency exists and operates. These needs are largely dictated by the different applications of research outcomes for public health regulation or public service. Hence, there are differences in the scope of the research, the technologies employed, and the ultimate use of the knowledge gained from research activities. Nevertheless, it is recognized that the FSMA legislation emphasizes more active engagement by the agencies in coordinating and integrating food safety research.

This report to Congress is the first step in documenting progress toward a coordinated, risk-based, and mission-critical federal food safety research strategy. Implementation of an enhanced, integrated approach to research will position the federal agencies to more effectively address the issues threatening the food supply. Strategically and operationally linking research

needs to the regulatory goals of the agencies will create focused synergy and momentum and will increase the ability of the agencies to meet their public health goals. Fostering a culture of collaboration with other research and health agencies in the federal government, state government agencies, academia, private industry, and foreign regulatory counterparts will expand scientific capability and permit all stakeholders to benefit from the great strides being made nationally and internationally. With transparent, collaborative processes for prioritizing science and research needs, HHS, USDA, and DHS can collectively move forward strategically and achieve a clear and consistent focus on mutual goals while leveraging the regulatory and research capabilities of partners to help meet the highest priorities. These efforts will require significant investments in cutting edge technologies and expert human capital that strengthen science and technology infrastructure with the future in mind. Future reports will expand on progress made in prioritizing research areas, allowing for greater collaboration and coordination among the agencies, promoting integrated capacity-building among stakeholders, and facilitating more efficient leveraging of existing and future research resources.

Introduction

On January 4, 2011, the President signed FSMA into law (Public Law 111-353). Pursuant to section 110(g) of FSMA:

> *Biennial Food Safety and Food Defense Research Plan - The Secretary [of Health and Human Services], the Secretary of Agriculture, and the Secretary of Homeland Security shall, on a biennial basis, submit to Congress a joint food safety and food defense research plan which may include studying the long-term health effects of foodborne illness. Such biennial plan shall include a list and description of projects conducted during the previous 2-year period and the plan for projects to be conducted during the subsequent 2-year period.*

The following report is the first biennial report in response to this mandate since the signing of FSMA.

This report on the food safety research portfolio and plan of HHS and USDA was developed as an interagency collaborative effort. Food defense research conducted or supported by DHS, HHS, and USDA is not included because it is covered under section 108 of FSMA and will not be repeated in this report.[1] Research conducted by the National Institutes of Health (NIH) is also not included in this report as it is primarily basic in nature with regards to studies on anaphylaxis, allergic inflammation, and food allergies. However, it is anticipated that there will be increased

[1] With regards to food defense, the collective description used by the federal agencies, including HHS, USDA, and DHS, encompasses activities associated with protecting the nation's food supply from deliberate or intentional acts of contamination or tampering. This term encompasses other similar verbiage (e.g., food protection, food security, bioterrorism, and counterterrorism). Section 108 of FSMA, which calls for a National Agriculture and Food Defense Strategy (NAFDS), has a research agenda component. The section 108 interagency workgroup (HHS, USDA, DHS, and EPA) has taken the lead regarding the food defense research agenda and the section 110(g) workgroup has focused primarily on food safety research aspects and cross-references the section 108 NAFDS report.

collaboration and coordination within HHS between FDA and NIH and this information will be captured in future reports.

Background

American consumers have high expectations with regards to the safety of the food they eat. A strong and coordinated federal research portfolio is critical to meeting these expectations. Industry has similar high expectations that regulations and guidance issued by government agencies will provide effective standards for food safety and be based on sound science.

Congress has recognized the unique challenges in ensuring the safety of the food supply in the 21st century and has provided the first major overhaul of food safety legislation in more than 70 years by enacting FSMA. The Act directs the building of a new food safety system based on the public health principle of comprehensive prevention, an enhanced focus on risk-based resource allocation, and partnership across the public and private sectors to minimize hazards from farm to table. Implementation of an integrated approach to research will allow federal agencies to more efficiently address the issues threatening the safety of the food supply in the decades to come.

The federal inventory of research projects funded and active during fiscal years (FY) 2011 and 2012 along with a summary of collaborative interagency strategic planning for future years are presented in this report.

The federal agencies involved in drafting this report are listed below.

Department of Health and Human Services (HHS)
Centers for Disease Control and Prevention (CDC)
Food and Drug Administration (FDA)

Department of Agriculture (USDA)
Agricultural Research Service (ARS)
Economic Research Service (ERS)
Food Safety Inspection Service (FSIS)
National Institute of Food and Agriculture (NIFA)

The listed agencies support both intramural and extramural research programs, with the exception of NIFA, which funds exclusively extramural research. The inventory includes information for projects primarily within eight general categories:

- *Prevention, Intervention, and Control of Foodborne Hazards*

  Food is generally not a sterile product and often can be contaminated with foodborne hazards. Therefore, production and processing practices should be used that minimize the presence of such hazards to avoid causing foodborne illness in humans. Because hazards can enter a food product at multiple points in the food production chain, an effective strategy requires that we have control or intervention procedures at several places in that chain. We

need to ensure that all foodborne hazards are receiving an appropriate level of attention in the research programs in proportion to the level of risk that they generate in the food production chain. In addition, an important component of all control programs is the education of producers, processors, food handlers, and consumers about proper food handling techniques and the implementation of hazard analysis and critical control points (HACCP) type programs, where appropriate.

- *Detection of Microbial, Chemical, and Radiological Hazards in Food, Feed, and Dietary Supplements*

The desired goal is to have detection and quantification methods that are highly reliable, rapid, cost effective, and can be used at the production or processing site with a minimum of expense. Effective use of such detection methods requires an understanding of the sampling of animals, plant products, and food products. These detection systems would support implementation of HACCP and HACCP-like programs in processing facilities or on-farm requirements for produce safety and the implementation of Good Agricultural Practices/Good Manufacturing Practices in the farm or production setting. Another critical need is the sampling and testing of both domestic and imported fresh fruits and vegetables to ensure a safe food product for the American public. It is also important to provide user-friendly detection methods that are rapid and can be used in a variety of settings within the food distribution system.

- *Molecular Characterization of Foodborne Pathogens as it Relates to Research on the Mechanism of Disease and/or Epidemiology of Foodborne Disease*

Emerging molecular-based technologies and methods are transforming laboratory detection and surveillance of foodborne contaminants and related infections. They have been instrumental in recent local and large scale multi-state outbreak investigations, efficiently pinpointing their sources and genetic origins. Also, critically important is the identification of virulence factors, epidemiological markers, and other determinants that influence the ability of pathogenic microorganisms to use foods as a vehicle for disease transmission, thereby providing enhanced epidemiological investigation, earlier interventions, and more accurate product attribution.

- *Antimicrobial Resistance/Susceptibility of Foodborne Microorganisms*

The development and transfer of antimicrobial resistance are not new issues; however, the current magnitude of the problem with respect to resistance to antibiotics used in human medicine and the speed with which new resistant bacterial strains have emerged has elevated its public health significance. The non-judicious use of antibiotics in both human medicine and in food producing animals has been cited as a contributor to this problem, along with other environmental contributors. An improved understanding of the relationship between non-judicious use of antibiotics in multiple settings and the increasing number of resistant bacterial pathogens will permit more informed regulatory decisions. A particular issue that needs to be resolved is the contribution of the sub-therapeutic usage of antibiotics in food producing animals to the emergence of drug resistant zoonotic bacterial pathogens. An

understanding of how antimicrobial resistance develops and is transferred from one organism to another is also crucial to finding solutions to this problem.

- *Designed Epidemiologic Studies of Foodborne Illness/Associated Organisms*

Credible microbial risk assessments require substantive data on the epidemiology and ecology of these organisms. Population-based studies of foodborne or waterborne illnesses in humans provide vital information about routes of exposure, hazardous food consumption patterns (behavioral factors and handling practices), and other aspects that reduce the uncertainties about these problems. Epidemiologic and ecologic studies about these human pathogens in the production and processing environments are also very important in determining the potential sources of contamination and possible control points.

- *Risk Assessment, Modeling, Management, and Communication*

Quantitative risk assessment requires both solid data and specialized tools, e.g., biological and mathematical models. To facilitate the ability of regulatory agencies to determine critical points for regulatory effectiveness, risk mitigation modeling must be performed. This analysis helps identify points where intervention might have the greatest impact on reducing the risk of an adverse human health outcome, rather than determining the points of greatest risk. A high quality risk assessment model will also inform the researchers about the gaps in the information base where research could be effective in filling those gaps and improving decision making about food safety issues. Identified risks must also be effectively managed and communicated.

- *Safety Assessments of Foodborne Hazards, including Toxicological Studies*

Safety assessments for foodborne hazards are designed to determine exposures that meet a reasonable certainty of no harm to the human consumer. Toxicological studies can serve to identify hazards, the nature of the toxicity expressed by those hazards, and characterize the dose-response relationship between exposure and effect. Exposure studies, consisting of analysis of the nature and concentration of foodborne hazards, are combined with the results of the toxicological studies to provide an overall risk assessment to determine whether that risk exceeds a reasonable certainty of no harm.

- *Economic Analyses*

Economic analyses are an important component of the food safety effort because they can provide: 1) information on the benefits and costs of regulatory intervention and alternatives; 2) information about the effect of economic incentives that might induce industry to undertake certain new practices; and 3) an understanding of the societal cost of foodborne illness in humans and the societal cost of eliminating it. Therefore, economic studies need to be done in parallel with other research, so that the economic benefit or cost is clearly understood from the beginning and can be weighed against the probability of foodborne illness occurring.

In general, these categories were modified from the July 1999 *Federal Food Safety Research: Current Programs and Priorities* report compiled by the National Science and Technology Council Committee on Science Interagency Working Group on Food Safety Research[2]. That report focused on topics directly related to microbial food safety research, while this report expands the scope to include other food safety hazards (e.g., chemical hazards and radiological hazards).

Food safety is generally described as the condition which ensures that food will not cause harm to the consumer when prepared and/or eaten according to its intended use. It refers to the conditions and practices that preserve the quality of food to prevent contamination and foodborne illnesses as well as assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. As a scientific discipline, it is described as handling, preparation, and storage of food in ways that prevent foodborne illness.

For the purposes of this report, research is generally described as "…a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge…." (45 CFR 46.102). As an example, ARS's National Program 108: Food Safety (animal and plant products) Strategic Vision[3] describes food safety research as seeking "… ways to assess, control, or eliminate potentially harmful food contaminants, including both introduced and naturally occurring pathogenic bacteria, viruses and parasites, toxins, and non-biological-based chemical contaminants, mycotoxins and plant toxins." This characterization is appropriate for the basis of the food safety research described herein. While it is acknowledged that research related to nutrition, health benefit, consumer behavior, market surveys, consumer education, and labeling are important, they do not fit within this definition of food safety research and are therefore not covered in this report.

Agency Strategic Planning, Research Prioritization Processes, and Partnerships

To help ensure the safety of the U.S. food supply, it is imperative that federal agencies maintain a strong science infrastructure. Robust and effective research programs are needed to provide the science base for regulatory decisions and actions. Each agency has in place a process which determines research priorities and aligns them with department- or agency-specific strategic plans. Those processes are described in the sections below.

HHS

[2] The Federal Food Safety Research Report: Current Programs and Future Priorities, compiled by the National Science Technology Council Committee on Science, Interagency Working group on Food Safety Research, July 1999 is available online at http://www.whitehouse.gov/sites/default/files/microsites/ostp/FoodSafetyResearch1999.pdf

[3] USDA/ARS National Program 108: Food Safety (animal and plant products) Strategic Vision is available online at http://www.ars.usda.gov/research/programs/programs.htm?np_code=108&docid=836

Centers for Disease Control and Prevention (CDC)

CDC, one of our nation's public health agencies, helps to protect America from health, safety, and security threats in the United States and overseas. CDC fights diseases—whether chronic or acute, curable or preventable, started at home or abroad, or caused by human error or deliberate attack. CDC supports communities and citizens in doing the same.

CDC's unique role in food safety is to provide the vital link between illness in people and the food safety system by: 1) tracking emerging foodborne illness trends; 2) identifying and investigating outbreaks to stop illnesses and identify gaps in the food safety system; 3) providing information to FDA, FSIS, and the food industry to guide and prioritize interventions for food safety prevention; 4) providing expert advice, guidance, training, and education to state and local governments, other partners, and consumers; and 5) tracking whether prevention efforts are working. CDC conducts applied research in the prevention, detection, and control of foodborne illness. It also oversees and participates in national networks and interagency working groups of state and local public health agencies, in coordination with FDA and USDA.

Applied Research

CDC's applied food safety research and related studies focus on:

- Causes and trends in foodborne outbreaks and illness;
- Antimicrobial resistance in human foodborne illness;
- Environmental factors contributing to foodborne and waterborne illness and outbreaks; and
- Effectiveness of prevention, detection, and control strategies and population-based interventions.

National Networks and Interagency Working Groups

CDC collaborates with FDA and USDA on the development of research agendas for foodborne disease and participates in several interagency working groups and national networks. Using these critical data, these networks and work groups assess risks associated with different foodborne pathogens—results of which have informed policy and program decision-making.

Subject Matter Expertise and Leadership

CDC scientists, including those working on foodborne disease, generally work in organizational units based on the hazard or infectious agent. These scientists come from multiple divisions within different centers within CDC.

The following table lists these organizational units and their areas of expertise in foodborne disease.

CDC Center	Division	Areas of Expertise
National Center for Emerging & Zoonotic Infectious Diseases	Division of Foodborne, Waterborne, & Environmental Diseases	Bacterial pathogens
	Division of High-Consequence Pathogens & Pathology	Variant Creutzfeldt-Jakob disease & other prion diseases
National Center for HIV/AIDS, Viral Hepatitis, STD, & TB Prevention	Division of Viral Diseases	Hepatitis A
National Center for Immunization & Respiratory Diseases	Division of Viral Diseases	Norovirus & other enteric viral diseases
National Center for Global Health	Division of Parasitic Diseases	Foodborne illnesses & outbreaks caused by parasites
National Center for Environmental Health	Division of Emergency & Environmental Health Services	Contributing factors & Environmental antecedents of foodborne illness.
	Division of Environmental Hazards & Health Effects	Non-infectious causes of foodborne illness such as pesticides, mycotoxins, & marine toxins
	Laboratory Sciences (DLS)	Rapid, mass spectrometry-based laboratory methods for detecting, quantifying, & differentiating botulinum neurotoxin serotypes, subtypes, & toxin variants

CDC draws on many sources when developing or periodically revising programs, including foodborne-illness surveillance systems, program evaluations, applied research, as well as stakeholders and partners from government, industry, academia, and professional organizations. These data sources enable CDC to address emerging national needs and incorporate new, cutting-edge, scientific methods in their food safety programs and applied research.

Food and Drug Administration (FDA)

FSMA, the first major overhaul of the food safety law in over 70 years, is transforming FDA's food safety program by providing it with new public health mandates and enhanced tools for ensuring the safety of the food supply in the 21st century. FSMA emphasizes a new food safety system based on prevention, enhanced focus on risk-based resource allocation, and partnerships with state, local, and foreign government agencies and the private sector to minimize hazards from farm to table. To effectively implement this new food safety mandate, it is imperative that FDA ensures a strong science infrastructure, clearly identifies its research needs, and collaborates with other public health and research agencies in the federal and state government, academia, foreign regulatory counterparts, and private industry.

FDA's Office of Foods, now called the Office of Foods and Veterinary Medicine, was created in 2009 to lead a functionally unified foods program and enhance the Agency's ability to meet today's great challenges and opportunities in food and feed safety, nutrition, and other critical areas. It encompasses the wide range of responsibilities and activities of the Center for Food Safety and Applied Nutrition (CFSAN) and the Center for Veterinary Medicine (CVM), and involves coordination with the foods-related field activities of the Office of Regulatory Affairs (ORA).

The 2012-2016 Foods and Veterinary Medicine (FVM) Program Strategic Plan, released in 2012, identifies key goals and objectives to advance food safety, nutrition, and animal health. In working to meet the goals of this plan, the FVM Program created the Science and Research Steering Committee (SRSC), which includes science and research leaders from the operating units of the FVM Program, including CFSAN, CVM, ORA, the National Center for Toxicological Research, the Office of the Chief Scientist, and the Office of International Programs. The SRSC was charged with and has developed the components of both the FVM and FDA regulatory science strategic plans aimed at strengthening and maintaining core science and research capabilities[4].

SRSC has also developed and is implementing a common framework for prioritizing FVM Program research that ensures alignment with FVM strategic direction and goals. On an annual basis during the last quarter of the fiscal year, SRSC, along with other senior level programmatic leadership within FDA's FVM Program, identify expected research outcomes (EROs), encompassing both cross-cutting and center-specific research needs, to be accomplished in the subsequent fiscal year. This includes identification and prioritization of immediate and longer term FVM science and research needs using public health and risk-based ranking matrices. Ranking of EROs includes such factors as regulatory impact, benefit to internal and/or external stakeholders, potential cost and complexity, time-to-results, availability of facilities, equipment and expertise, scientific merit, and alignment with strategic plans. Once approved, EROs are translated into an operational plan, including specific research projects, to be implemented by each center or in collaboration with other centers for cross-cutting projects. SRSC provides oversight and monitoring of the operational plan, to ensure cross-center collaboration, where appropriate, as well as counsel and support for accomplishing research objectives. Progress on the identified research priorities are tracked at least twice a year. The process is in place to ensure that the FVM Program's research is directed at the important strategic and regulatory goals and is collaboratively executed to maximize: 1) impact on public health, including health of food producing animals and companion animals; 2) linkage to the Agency's mission; and 3) research capacity across the FVM Program. The process also takes into account the Federal Food Safety Research Enterprise, including extramurally-funded research and leveraging of collaborative research projects and capabilities of other federal agencies.

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[4] Foods Program Science and Research Strategic Plan reflected in FDA's Strategic Plan for Regulatory Science (http://www.fda.gov/ScienceResearch/SpecialTopics/RegulatoryScience/ucm267719.htm) and the Foods and Veterinary Medicine Strategic Plan (http://www.fda.gov/AboutFDA/CentersOffices/OfficeofFoods/ucm273269.htm)

An integrated science/research program based on the principles of prevention and risk-based priority setting provides research solutions to support current and evolving FDA regulatory issues and science priorities. Additionally, implementation of an integrated approach to research positions FDA to more effectively and efficiently address the issues threatening the food supply and animal health in the decades to come.

FDA's Centers of Excellence

FDA is actively involved in high-visibility formalized endeavors with several academic institutions through its Centers of Excellence (COE) Program. COEs are formal FDA partnerships supported in part through cooperative agreements. They serve to build dynamic and diversified channels for developing and disseminating new ideas and knowledge among government, academia, industry, and consumer groups. Their specific goal is to help FDA address complex scientific, public health, and regulatory issues. These collaborations yield critical information that enhances FDA's ongoing efforts to protect the food supply. The four COEs are as follows:

- *Joint Institute for Food Safety and Applied Nutrition*

  The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is funded in part by a cooperative agreement between FDA and the University of Maryland, College Park. The Institute, established in 1996, is a jointly administered, multidisciplinary research, education, and outreach program. JIFSAN provides multiple forums for the food safety and nutrition communities to interact and exchange information. The program supports collaborative research projects related to food safety including projects on antimicrobial resistance, foodborne pathogens, genome sequencing and genomic analysis, nanotechnology and nanosafety in food, and risk assessment modeling. Additionally, the program includes an undergraduate internship program that supports FDA's intramural research programs.

- *Western Center for Food Safety*

  The Western Center for Food Safety (WCFS) is a collaborative partnership between FDA and the University of California, Davis, supported by a cooperative agreement. The WCFS focuses on the development of research approaches and collection of research data critical to understanding the risks associated with the interface of production agriculture and food safety. Such knowledge is important in the development of scientifically validated "best practices" for mitigating those risks at the production (versus processing) level. The WCFS has access to farm plots in the Salinas Valley, providing the opportunity to conduct field trials in order to address current knowledge gaps associated with produce safety in an area of the United States that produces high volumes of leafy greens along with other produce commodities.

- *National Center for Natural Products Research*

  The National Center for Natural Products Research (NCNPR) is supported in part by a cooperative agreement between FDA and the University of Mississippi (UM). The

cooperative research programs developed by UM-NCNPR addresses scientific issues related to the safety of botanical dietary supplements and botanical ingredients. Through its research program, NCNPR acquires, validates, and characterizes authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to FDA, for evaluation of their safety. Through this collaboration, FDA has access to authentic botanical reference materials, rare chemical standards, and unique laboratory approaches for the assessment of new botanical dietary ingredient safety. Since these standards form the basis of analytical methods, toxicological studies, and regulatory enforcement strategies, a reliable source of these materials is important to the Agency's mission.

- *Institute for Food Safety and Health*

In 1988, FDA/CFSAN and Illinois Institute of Technology (IIT) established the National Center for Food Safety and Technology (NCFST) to serve as a link to the food and food-related industries and to house FDA/CFSAN's Division of Food Processing, Science, and Technology (DFPST). Now part of IIT's Institute for Food Safety and Health (IFSH), NCFST continues to operate under this unique cooperative agreement as a place where university, government, and industry scientists work side-by-side on the development, validation, and implementation of food processing and packaging technologies critical for food safety. IFSH's NCFST is the only center in the nation where FDA scientists are located onsite and are able to work collaboratively on projects with industry and academia.

NCFST's unique partnership with FDA enhances food safety and nutrition for the benefit of the public by: 1) providing cooperative research that provides fundamental food safety information, in the public domain, for use by all segments of the food science community in product and process development; 2) serving as a critical resource to FDA to ensure that policy and new regulations for food processing and packaging are based on sound science; 3) facilitating the evaluation, validation, and commercialization of new technologies that show promise in improving aspects of public health related to food; 4) addressing path-to-market issues relating to technology-based innovation that are key to ensuring the safe application of new technology; and 5) conducting research in food processing and packaging that allows CFSAN to address technical regulatory issues as they arise and to respond rapidly when a new food-related public health or food defense issue arises.

USDA

Agricultural Research Service (ARS)

ARS has a 5-year research program cycle. Each presidential administration sets its own goals and priorities, which USDA then incorporates into a departmental strategic plan. ARS, in turn, fulfills its role as a federal agency by ensuring its research addresses the goals and priorities outlined in the USDA Strategic Plan. ARS begins this process with the development of its own strategic plan, which provides the framework for presenting the ARS budget and reporting accomplishments and for tracking ongoing activities. Specific protocols are in place to ensure that the work of ARS is relevant to either immediate or long-term scientific efforts, and that it is

of the highest quality. These protocols involve extensive planning by ARS, and input from staff, the external scientific community, stakeholders, customers, and other government entities.

The Office of National Programs defines and directs the ARS National Programs (NP). Near the end of the 5-year cycle, the work conducted under the current NP Action Plan is evaluated via a retrospective review panel (RRP). NP teams provide external retrospective review peer panels with accomplishment summaries for each NP, using the aggregate information contained in the annual reports and projects aligned with that NP. After evaluating the aggregated accomplishment summaries, the retrospective review panel assesses the value of the research that has actually been conducted as it compares to the accomplishments that had been projected in the NP Action Plan. The panel also makes recommendations for future research priorities that begin with a National Program Workshop (NPW), which defines the purpose and goals of the NP. At this point, the current 5-year NP Action Plan is concluded, and the planning process for the next 5-year NP Action Plan begins.

In the NPW, a cross section of customers and stakeholders participate in identifying and prioritizing research needs for each program. To ensure ARS research is relevant, input is obtained from several groups (including the RRP) to help identify the major issues confronting American agriculture and related industries. In this ongoing interaction with stakeholders, ARS obtains the perspective of the current presidential administration and Secretary of Agriculture. While the core research activities remain relatively constant from year to year, changing administrations highlight different priorities. A wide array of customers, stakeholders, and partners, who represent the national agricultural enterprise, identify their key issues/problems requiring technological solutions, which are then taken into consideration during the program planning process that produces the NP research agenda. The scientific community – national and international, public, and private – is part of the discussion about where to most effectively and efficiently deploy national research capabilities. ARS scientists and managers bring their background and experience into this decision-making process. Their knowledge of what ARS has done or is doing, coupled with their understanding of related work being conducted by universities, private research laboratories, and other institutions, helps in the melding of input from all the identified sources into a coherent research agenda that addresses the highest priority issues. Beneficiaries such as American citizens provide input about agricultural issues they rank as research priorities through communication with their congressional representatives or through direct communication with ARS staff. In developing 5-year Action Plans, National Program Leaders (NPLs) evaluate and assess the needs and priorities exchanged at the NPWs and identify research priorities for each NP. Once these priorities are identified, the NP team responsible for each program develops the 5-year Action Plan to guide the overall research for that program. After the research priorities, goals, and 5-year Action Plans have been developed for each NP, NPLs assign one or more of the specific research objectives and allocate funds to lead scientists or research teams.

Project plans are the foundation of ARS research. All project plans must meet stringent criteria for scientific purpose and methodology. The primary purpose of the project plan must be to address a research challenge outlined in one of the NP 5-year Action Plans, using the best available science to do so. After the lead scientist has completed the project plan, it is reviewed internally and then submitted for peer review. Peer review is an independent, external, and

expert evaluation of the scientific and technical merit of each prospective ARS project plan to ensure scientific quality and enhance its chance of success. Peer review provides an additional level of assurance, transparent to stakeholders and policy officials, that ARS research will be conducted appropriately and in line with accepted scientific methods.

Economic Research Service (ERS)

ERS conducts relevant, objective economic research and policy analyses that inform program and policy decisions throughout the federal government. The agency's mission is to anticipate food, agricultural, agri-environmental, and rural development issues that are on the horizon, and to conduct sound, peer-reviewed economic research. The research findings are available when those issues make it to the "front burner," demanding short-term decisions by USDA or related government agencies. ERS collects input on research needs from the program agencies on both a periodic formal and ongoing informal bases, and responds to numerous direct requests for specific research and analysis from USDA.

In planning its research program, ERS considers opinions from multiple internal and external sources (both inside and outside USDA). ERS uses an annual internal workplan development process to identify key research topics, develop projects, identify products and services that meet the needs of clients and stakeholders, and set priorities for allocation of resources across program areas. ERS also combines internal and external input to develop priorities. ERS leadership periodically meets with each USDA Under Secretary's office and the heads of the agencies within the mission area, as well as with staff offices (such as the Office of the Chief Economist). At these meetings, ERS asks what issues they foresee on the horizon that could benefit from economic research. ERS considers this important stakeholder input, and it has a strong influence on the agency's research priorities.

ERS shapes its program and products principally to serve key decision-makers who routinely make or influence public policy and program decisions. The research program is designed to both anticipate and respond to decision makers' information needs through direct analyses and the development of analytic methods. ERS provides program support and policy analysis to USDA and other federal policy officials through responses to customized requests for analysis and information ("staff analysis"). ERS also conducts special analyses at the request of the USDA policy officials, from quick turnaround requests to more in-depth research efforts. The agency is currently undertaking a strategic planning process to identify opportunities for increased program efficiency and to identify strategic opportunities for investment in high-priority topics for research, market analysis, and data development. As part of this process, ERS is seeking input from a diverse array of clients and stakeholders to ensure that its programs meet customer needs and to solicit ideas to further strengthen the relevance and impact of the agency's products and services. Recommendations from the strategic planning process will be delivered in late 2014.

Food Safety and Inspection Service (FSIS)

To facilitate the accomplishment of the FSIS-required research and data, FSIS developed and maintains a list of research priorities identified by FSIS Program Offices and stakeholders. The

list of research priorities is posted on the FSIS website[5] and is communicated to USDA research agencies and universities. FSIS is the public health agency in USDA that is responsible for the safety of meat, poultry, and egg products. FSIS prevents foodborne illness by understanding and influencing activities along the farm-to-table continuum, empowering its employees, and strengthening the food safety infrastructure in the United States. FSIS applies the latest research to: 1) understand foodborne illness and emerging trends; and 2) develop, maintain, and use innovative methodologies, processes, and tools to protect public health efficiently and effectively. To encourage scientific engagement, FSIS works closely with its federal partners to address FSIS research priorities. FSIS also works collaboratively with the ARS researchers and/or NIFA supported researchers to develop foundational analytical chemistry and microbiology techniques and methods. FSIS validates and/or extends these analytical methods to adopt them for use in FSIS laboratories to monitor hazards in meat, poultry, and egg products.

The data required to complete food safety risk assessments are also frequently generated by USDA and/or university researchers. FSIS uses the data to develop risk assessments for microbial and chemical hazards in FSIS regulated products. To develop and maintain the list of FSIS research priorities, a panel of food safety experts is identified to comprise the FSIS Research Priorities Review Panel. All FSIS programs are invited to appoint members to this Panel. The Panel currently consists of representatives from the Office of Public Health Science, the Office of Program and Policy Development, the Office of Data Integration and Food Protection, the Office of Field Operations, the Office of International Affairs, the Office of Outreach, Employee Education and Training, the Office of Public Affairs and Consumer Education, and the Office of Program Evaluation, Enforcement, and Review. FSIS Research Priorities Review Panel members are charged with soliciting potential revisions (additions, modifications, or deletion of priorities) to the list. Panel members also are encouraged to reach out to FSIS stakeholders to identify research and data needs. Proposed revisions are presented to the Panel, discussed, and voted on by the members. Proposed revisions receiving a majority of affirmative votes are subsequently presented to the FSIS Data Coordinating Committee, which reviews FSIS data and policy prior to public dissemination. Revisions that are approved by the Data Coordinating Committee are vetted through the Management Council and subsequently applied to the FSIS Research Priorities list. The FSIS Research Priorities Review Panel reviews the research priorities list every 6 months. The highest ranked research needs are reflected on the FSIS Research Priorities. The revised FSIS research priorities list is posted on the FSIS website and communicated to FSIS food safety partners, including USDA research agencies.

One component of FSIS research consists of validating and/or extending analytical methods for use in FSIS laboratories to monitor hazards in meat, poultry, and egg products. FSIS also conducts risk assessments for microbial and chemical hazards in FSIS regulated products. These activities only partially fulfill FSIS' data gaps and research needs. FSIS has a formal procedure for identifying its entire suite of research priorities. These research priorities are communicated to USDA research agencies (e.g., ARS, ERS, NIFA) as well as other research organizations (e.g.,

[5] USDA/FSIS's list of research priorities is available online at http://www.fsis.usda.gov/science/Food_Safety_Research_Priorities/index.asp

universities, state agencies). FSIS monitors research activities that address FSIS research priorities and outcomes are often adopted.

National Institute of Food and Agriculture (NIFA)

NIFA is USDA's primary extramural research funding agency. Its mission is to advance knowledge for agriculture, the environment, human health and wellbeing, and communities. NIFA is one of four USDA agencies in the Research, Education, and Economics (REE) mission area. It is unique in that it employs food safety strategies that require an integrated approach to solving complex food safety problems. Integrated food safety programs include components that address basic and applied research, formal classroom education, and extension or outreach to groups outside of the formal classroom setting. An integrated approach focuses on food safety as a continuous process from production, through harvesting and processing, to retail, and finally to consumers.

NIFA food safety grant programs also support grants that focus solely on mission-driven, basic research as part of the overall research portfolio. REE and NIFA research provides the means to ensure that the food supply is safe and secure for consumers, and that food and feed have met foreign and domestic regulatory requirements. Since many foods are imported and exported from the United States, food safety has been considered a global issue. The REE and NIFA food safety research programs have involved both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are used to inform national and international strategies delivering research results to regulatory agencies, commodity organizations, industry, and consumers.

The framework for defining NIFA research priorities was established in the 2008 Farm Bill and is consistent with the REE Action Plan. The REE Action Plan was based on the 2008 Farm Bill, which directed the REE mission area agencies to develop "A Roadmap for USDA Science." The Roadmap articulated a vision for delivering the research, tools, and statistical data needed to meet the needs of USDA agencies and the country. That vision also reflected the growing needs domestically and globally for a comprehensive approach to food and agriculture. The REE Action Plan further developed the vision outlined in the Roadmap and provided focus, direction, and accountability in all REE programs. The Farm Bill also directed NIFA to gather input from stakeholders to help determine program priorities, goals, and objectives. Stakeholders are any groups or individuals that are users of NIFA competitive programs and the knowledge generated by those programs. In FY 2010, a stakeholder listening session was held in Washington, DC, to solicit stakeholder input for NIFA programs. More recently, a stakeholder listening session was held in FY 2012, also in Washington, DC. This listening session was followed by a series of seven webinars designed to solicit additional input from those not able to personally attend the on-site listening session. All of the food safety programs at NIFA have been developed and modified based on extensive stakeholder input from partners and collaborators.

Both formal and informal procedures are used to obtain stakeholder input. These have included stakeholder workshops, symposia, listening sessions, public hearings, technical reviews, peer panel recommendations, white papers, NIFA departmental review reports, presidential directives, interagency strategic plans for research and development, regulatory policies impacting food

quality and safety, and industry plans and priorities. Unsolicited comments, suggestions, and recommendations from the general public are welcomed via a stakeholder's webpage developed by NIFA.[6] In addition, all NIFA Requests for Applications (RFAs) specifically solicit stakeholder input as dictated by the requirements of the Agricultural Research, Extension, and Education Reform Act of 1998 (7 U.S.C. 7613c(2)). This section requires the Secretary of Agriculture to solicit and consider input on all current RFAs from persons who conduct or use agricultural research, education, and extension. Input gathered must be considered during formulating of future RFAs for competitive programs. These processes and networks have helped NIFA ensure the relevancy of its programs to local, state, regional, and national needs.

NIFA programs and activities focus on critical scientific issues. To further ensure that NIFA's competitive grant programs have focused on critical needs, priorities are identified with input from Congress and from the National Agricultural Research, Education and Extension Advisory Board (NAREEAB). In addition, NIFA National Program Leaders have identified research priorities by monitoring national, international, and global food safety trends. Each has built and maintained strong collegial relationships with government and industry-wide networks of food safety professionals. All of the competitive programs managed and administered by NIFA use relevance and quality as criteria for pre-award evaluation of grants awarded. Relevancy is established taking into consideration the industry and/or consumer needs and priorities. The quality is assessed based on the scientific merit, proposed procedure, and potential to succeed. Programs are dynamic and change periodically to address emerging national needs consistent with cutting edge science.

Highlights of Interagency Food Safety Research Areas

There are a number of ongoing, food safety research areas in which multiple federal agencies are collaboratively engaged. Additionally, some of the research conducted or funded by one agency may address the needs of other agencies. Highlights of these interagency research areas, which fall within the eight general categories, are provided below. The extensive lists of research projects covering FYs 2011 and 2012 are provided in the Appendices.

Prevention, Intervention, and Control of Foodborne Hazards

Environmental Health Specialists Network (EHS-Net)

EHS-Net is a collaboration involving CDC, FDA, USDA, and six state and local health department sites (comprised of all or some parts of California, Minnesota, New York, New York City, Rhode Island, and Tennessee). These partners have come together in an effort to better understand environmental factors that impact food and water safety. Research activities are designed to: 1) identify and understand environmental factors associated with food and water-borne illness and outbreaks, and 2) identify and understand the strengths and weaknesses of

[6] USDA/NIFA's stakeholder webpage is available online at http://www.nifa.usda.gov/nea/stakeholder.htm

environmental public health regulatory programs responsible for food and water safety. The EHS-Net program was established in 2000. EHS-Net food-related research activities for FYs 2011 and 2012 include the Listeria Retail Deli Study and the Kitchen Manager Certification Study (more specific descriptions of these two studies are included in Appendix A).

Veterinary Laboratory Investigation and Response Network (Vet-LIRN)

The Veterinary Laboratory Investigation and Response Network (Vet-LIRN) works with veterinary diagnostic laboratories nationwide to document, investigate, and diagnose animal feed or drug-related illnesses. FDA provides funding to veterinary diagnostic laboratories in order to further response capacity, establish protocols for reporting to FDA, and conduct proficiency testing to demonstrate network readiness. Vet-LIRN speeds FDA's response to high priority chemical and microbial feed and drug contamination events by sampling and analyzing veterinary diagnostic specimens. Veterinary diagnostic specimens are collected in response to consumer complaints involving pets, 'exotic' animals, and horse or farm animals. Current examples of the network's activities are collaborations with CDC on the recent *Salmonella* Infantis outbreak and Vet-LIRN laboratory investigations into cases of animal food-related illness. These efforts can contribute to overall food safety as animal feed events could signal potential issues in the human food system.

Salmonella Thermal Resistance during Desiccation and Rehydration in Low Water Activity Foods

Salmonella has been implicated in numerous multi-state outbreaks associated with the consumption of dry foods, such as peanut butter, chocolate, cereal, and grains. The high risk of *Salmonella* in dry foods is largely attributed to its ability to survive the long-term stress of starvation and lack of free water, while the majority of other pathogens cannot. Furthermore, the desiccated *Salmonella* cells are very resistant to thermal and other inactivation treatments. This research, sponsored by a grant from NIFA and conducted by FDA's Center of Excellence at the Illinois Institute of Technology's Institute for Food Safety and Health, will use microbiological challenge studies and new molecular tools to study the stress response of *Salmonella* during desiccation and rehydration prior to and during industrial thermal processing. This work will provide important data on the heat resistance of *Salmonella* after different treatments of desiccation and rehydration, and it will provide a mechanistic understanding of the response to *Salmonella* under conditions of desiccation stress prior to heat stress that will allow the design of effective validation studies. Understanding the mechanisms by which *Salmonella* survives conditions of desiccation, as well as processing and sanitation treatments, will lead to more effective control strategies and reduced disease.

Investigation of Naturally-Occurring Botulism Events

The CDC National Botulism Laboratory Team (NBLT/EDLB) investigates cases of suspect botulism, in partnership with state health department laboratory and epidemiology staff and the Enteric Diseases Epidemiology Branch. Since the early 1960s, NBLT has identified contaminated food products (both commercial and home-prepared) that have caused foodborne botulism outbreaks. Identification of botulinum toxin-contaminated commercial food products

by NBLT has many times resulted in manufacturer investigations and other actions by FDA and/or USDA on the products they regulate. Some recent examples include identification of botulinum toxin in commercially prepared carrot juice, refrigerated soup, frozen manufactured chili, and canned hot dog chili sauce. NBLT conducts studies to determine the conditions that resulted in botulinum toxin contamination of foods to assist regulatory agencies and public health agencies in developing improved guidance for consumers. Specific NBLT activities include: 1) conducting laboratory tests to identify the presence of botulinum toxin in remnants of foods known to be consumed by botulism patients; 2) investigating, in conjunction with EDEB epidemiology staff, food handling practices of effected consumers, restaurant food handlers, and in some cases food distributers; and 3) conducting laboratory studies to simulate contamination of foods with *Clostridium botulinum* spores and determining conditions (temperature, incubation time, etc.) for germination and toxin production.

Detection of Microbial, Chemical, and Radiological Hazards in Food, Feed, and Dietary Supplements

Food Emergency Response Network

The Food Emergency Response Network (FERN) is a secure laboratory system. Its mission is to integrate the nation's multilevel food-testing laboratories to detect, identify, respond to, and recover from a bioterrorism or naturally-occurring public health emergency or outbreak involving the food supply. Under section 202(b) of FSMA, FDA provides a biennial report to Congress on FERN[7]. Co-managed by joint National Program Offices within FDA/ORA and FSIS, FERN plays a critical role by providing a nation-wide food testing network that is able to respond to emergencies involving biological, chemical, or radiological contamination of foods and feed. Currently, FERN is composed of 172 federal, state, local, tribal, military, and academic laboratories. The network's strengths lie in its surveillance and emergency activation capabilities and capacity, its training and preparedness strategies, and its comprehensive research and methods development programs. With regard to the latter, FERN funds and manages cooperative agreement programs to investigate new technological applications, develop and extend analytical methods, and ensure method performance. Most notably, research programs within FERN have resulted in the development and validation of more than 18 microbiological, 29 chemical, and 3 radiological methods with varying degrees of validation based on fit-for-purpose. FERN also developed a multi-agent screening method for use by laboratories with significant capacity and capability for detection of Biosafety Level 3 organisms and toxins of concern. Such contributions have been instrumental in support of multiple nationwide surveillance, emergency, and food safety events. Over 2,700 samples have been analyzed to date through FERN directed activities that include *E. coli* O157:H7 outbreak in spinach, 2006; *Salmonella* in peppers, 2008; melamine contamination in pet food & infant formula, 2007-2009; *Salmonella* in peanut butter, 2009; Deepwater Horizon Oil Spill, 2010; Radiological Crisis in Japan, 2011; FERN/Method Development Program Joint Produce Surveillance, 2011; Arsenic in Juice and Rice Surveillance, 2012; and Political Event Surveillance Assignments (e.g., the

[7] http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm375711.htm

Republican and Democratic National Conventions of 2008 and 2012, and the Presidential inaugurations in 2009 and 2013). FERN also provides a vehicle by which participating government agencies can compare, share, and coordinate laboratory analytical findings. It also strengthens the capacity of state and local laboratories, facilitating the ability of these laboratories to serve as first responders during food emergencies. In a larger context, FERN also interacts with other laboratory networks through the Integrated Consortium of Laboratory Networks, which aims to improve coordination of laboratory response to incidents and to identify gaps in laboratory preparedness and response.

Development of Methods and their Validation for Detection, Identification, and Characterization of Salmonella and other Pathogenic Foodborne Microorganisms

CDC estimates that *Salmonella* causes approximately 1.4 million illnesses per year. One third of bacterial outbreaks are associated with the consumption of foods contaminated with *Salmonella*, resulting in salmonellosis, a serious infection that can lead to hospitalization and, in some cases, death. There are over 2,500 serotypes of *Salmonella*, many of which cause human disease. A number of research efforts within HHS and USDA are focused on developing methods using advanced DNA-based biotechnologies that will help speed up detection, identification, and characterization of these pathogens in commodities such as produce, spices, milk, poultry, and eggs and egg products, and validating these methods, with the forward vision of applying these technologies in federal regulatory laboratories, state health agencies, and private industry. This will enable more rapid trace back to the source of the contamination. These technologies include whole genomic sequencing, microarray, multiplex polymerase chain reaction (PCR) that can identify more than one pathogen in one screening, rapid molecular phage typing, molecular serotyping using PCR, as well as metagenomics (identifying specific pathogen DNA) where recovery of an isolate is not necessary to identify the contaminant. As new technologies are established, agencies will further refine and streamline newly developed methods not only for *Salmonella*, but also for other foodborne pathogens with the aim of ensuring the safety of the nation's food supply.

Development of Analytical Methods to Screen for Chemical Contaminants and Economic Adulterants in Priority Products

A critical role of both USDA's and FDA's food safety programs is to analyze foods for the presence of chemical contaminants and economic adulterants that would be harmful to the consumer. Because there are thousands of chemical hazards that could potentially contaminate diverse food types, the development of methods to detect, identify, quantitate, and confirm chemical contamination of food presents many challenges. The need to ensure the safety of imported foods, which have doubled in the last decade, makes this responsibility more urgent. Research projects in this area involve improvement of current procedures for chemical analysis or development of new, validated methods to be used for regulatory monitoring and surveillance programs. Currently, analyses focus on various classes of chemicals (e.g., pesticides, toxins, metals, etc.) in specific commodities using state-of-the-art instrumentation for detection and characterization. However, this approach is limiting in that contaminants or adulterants not included on the target list are not identified. Therefore non-targeted methods that will detect any chemical hazard not ordinarily found in food are also being developed. The utilization of these

technologies and tools for rapid and accurate data processing, will reduce discovery time allowing the federal regulatory agencies to focus on identification and the eventual remediation of the contaminant. Once validated, these methods could be used in federal laboratories, state health agencies, and private industry. The outcome of this work should provide the consumer with safer foods and other regulated products such as dietary supplements, as well as help to ensure accurate labeling of food contents.

Interagency Residue Control Group

The mission of the Interagency Residue Control Group (IRCG) is to coordinate efforts in the testing of meat for veterinary drug residues, pesticides, and other chemical contaminant between agencies. IRCG, which is co-chaired by FSIS and FDA, also has representation from CDC, ARS, the U.S. Environmental Protection Agency (EPA), and the USDA Agricultural Marketing Service. The group was created to implement the memorandum of understanding originally signed by FDA, FSIS, and EPA in 1984. The IRCG meets monthly and works to develop and improve mechanisms to exchange information, address emerging issues, coordinate efforts in regulatory testing, address procedural and regulatory issues-between the different agencies, facilitate and prioritize methods development research, and provide guidance on the implementation of new technologies into regulatory testing. The efforts of the IRCG help to ensure that laboratory results generated by the laboratories of FSIS can be used to support regulatory actions at FDA and that efforts are focused on those who routinely sell animals for slaughter that contain unsafe levels of antibiotics and other drugs.

Molecular Characterization of Foodborne Pathogens as It Relates to Research on Mechanism of Disease and/or Epidemiology of Foodborne Disease

Development and Utilization of Molecular Subtyping and Next Generation Serotyping and Sequencing for Foodborne Outbreaks

PulseNet is a national network of public health and food regulatory agency laboratories coordinated by CDC, with the involvement and participation of FDA and FSIS along with state and local health departments. Participating laboratories perform standardized molecular subtyping of foodborne disease-causing bacteria by pulsed-field gel electrophoresis (PFGE). PFGE can be used to distinguish strains of organisms such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Listeria*, or *Campylobacter* at the DNA level. Even though PFGE is the current "gold standard" for the subtyping of foodborne bacterial pathogens, it has some limitations. However, clinical laboratories are increasingly using culture independent methods to diagnose infections and it can be anticipated that cultures will not be available for surveillance within a foreseeable future. Hence, there is a need to prepare for scenarios where cultures may no longer be available. Several approaches are being explored to address this:

- *Enhanced, next-generation subtyping methods for foodborne bacteria:* Enhanced assays for subtyping *Salmonella, E. coli, Vibrio, Campylobacter, Listeria monocytogenes* and *C. botulinum* are being developed. Assays that have been or are under development include DNA-microarrays, amplicon sequencing, molecular serotyping, clustered regularly interspaced short palindromic repeats (CRISPR) typing, multilocus variable number of

tandem repeats analysis (MLVA), and PCR-based assays of markers associated with virulence and antimicrobial resistance. An example of a molecular serotyping method currently being used for enhancing the response time for outbreak investigations is the one called Bioplex based on the Luminex liquid microarray. This method was recently used to successfully identify *Salmonella* Bareilly in tuna associated with the 2012 foodborne outbreak of salmonellosis in which this novel application was able to confirm epidemiological evidence that linked the outbreak of illness to contaminated tuna scrape and allowed investigators to have several days advance notice of the target serotype for this outbreak.

- *Implement whole genome sequencing (WGS) of foodborne bacteria:* Comparative analysis of WGS of foodborne pathogens will reveal potential new virulence mechanisms and new subtyping markers and create a reference for future surveillance. Real-time WGS of STEC and *Salmonella* clusters done side by side with PFGE analysis help in assessing the utility of the technology in outbreak investigations. In July 2012, FDA, CDC, and FSIS, in partnership with the University of California, Davis, and Agilent Technologies, announced a public-private partnership to create a public database of 100,000 foodborne pathogen genomes to help speed identification of bacteria responsible for foodborne outbreaks. The database will provide a roadmap for the development of tests to identify pathogens and provide information about the origin of the pathogen. The tests have the potential to significantly reduce the typical public health response time in outbreaks of foodborne illness to days instead of weeks. Open access through the NIH's National Center for Biotechnology Information (NCBI) database will allow researchers to develop tests that can identify the type of bacteria present in a sample within a matter of days or hours, significantly faster than the approximately 1 week it now takes between diagnosis and genetic analysis. The collaboration will be a 5-year effort to sequence the genetic code of approximately 100,000 important foodborne pathogens and make this information free and publicly available. The sequencing will include the genomes of important foodborne pathogens such as *Salmonella*, *Listeria,* and *E. coli.* With the goal of making the food supply safer for consumers, the new database will significantly speed testing of raw ingredients, finished products, and environmental samples taken during investigation of foodborne illness outbreaks. This type of information also enables scientists to make new discoveries that drive the development of new methods to control disease-causing bacteria in the food chain.

- *Metagenomic analysis of clinical specimens:* A study is currently underway to obtain critical experience with the use of metagenomics for surveillance. This initial research will determine the level of subtyping information that can be obtained by whole-sample sequencing (WGS) of specimens, and metagenomics approaches will be applied to outbreaks of unknown etiology.

These new methods will transform laboratory surveillance of foodborne infections. For example, they have already been instrumental in several local and large multi-state outbreak investigations efficiently pinpointing their sources and genetic origins. These methods will also improve microbiological food attribution efforts.

Molecular Characterization and Virulence Determination of Shiga toxigenic and Enterohemorrhagic Escherichia coli Strains Isolated from Foods and Produce

Escherichia coli O157:H7 remains the primary *E. coli* pathogen that causes foodborne outbreaks worldwide. Atypical O157:H7 strains are causing a portion of these illnesses, yet these variants are not detected by existing assays. Additionally, other shiga toxin producing *E. coli* (STEC) have also emerged as important pathogens. Aside from the six serogroups recently focused on by the USDA for meats, there are many others that have been found in foods and these variants may also be pathogenic. Research efforts are being aimed at characterizing atypical O157:H7 as well as non-O157 STEC strains isolated from foods to determine their virulence potentials and to develop specific detection assays. If successful, this work would enable the identification of these pathogens in FDA- and USDA-regulated foods.

Antimicrobial Resistance/Susceptibility of Foodborne Microorganisms

National Antimicrobial Resistance Monitoring System (NARMS)

The National Antimicrobial Resistance Monitoring System monitors trends in antimicrobial resistance among foodborne bacteria from humans, retail meats, and food animals at slaughter. NARMS is a collaboration among CDC, FDA, and ARS established in 1996. Agencies collaborate on epidemiological and microbiological analyses to understand the nature and magnitude of resistance in the food supply, in order to help formulate policies on the safe use of antimicrobial agents. Research activities include:

- Antimicrobial susceptibility testing of bacterial isolates collected for FoodNet, outbreak characterization, and other special focus studies;
- Enhancements in uniformity and timeliness of susceptibility testing methods and reporting with domestic and international partners;
- Characterization of bacterial subtypes and genetic mechanisms responsible for antimicrobial resistant human infections, including the development and validation of new technologies that provide comprehensive genomic data for high-resolution strain typing; and
- Laboratory experiments to determine the antimicrobial use conditions that promote the development and spread of resistant strains.

Value-added studies that help the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) establish interpretive criteria for antimicrobial susceptibility/resistance of zoonotic bacterial pathogens are also conducted under the NARMS program.

Designed Epidemiologic Studies of Foodborne Illness/Associated Organisms

FoodNet – Foodborne Diseases Active Surveillance Network

FoodNet was established in July 1995 and is a collaborative program among CDC, 10 state health departments, FSIS, and FDA. The Network conducts surveillance for *Campylobacter*,

Cryptosporidium, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157, *Shigella*, *Vibrio*, and *Yersinia* infections diagnosed by laboratory testing of samples from patients. The goals are to determine the burden of foodborne illness in the United States, to monitor trends in the burden of specific foodborne illness over time; and to attribute the burden of foodborne illness to specific foods and settings. At CDC, the Enteric Diseases Laboratory Branch (EDLB) participates in epidemiological studies with FoodNet and the Enteric Diseases Epidemiology Branch (EDEB) aimed at identifying microbiological risk factors for STEC infections and adverse outcomes of such infections (hemorrhagic colitis and HUS) and developing tools that are useful to determine the most important sources of foodborne infections by comparing the subtypes of pathogens found in food, veterinary, and environmental sources, with clinical isolates (microbiological attribution). FoodNet determines the incidence of laboratory-confirmed infections for bacterial pathogens transmitted commonly through food. However, these reports represent only a subset of the true number of cases of diarrheal illness that occur in the community; most diarrheal illnesses are undiagnosed and, therefore, not reported. To more precisely estimate the burden of acute diarrheal illness and to describe the frequency of important exposures, FoodNet has conducted population-based telephone surveys of persons residing in the surveillance area. Although foodborne outbreaks are common, approximately 95 percent of foodborne infections occur as sporadic (non-outbreak) cases. It is difficult to determine what specific exposure caused a person with a sporadic infection to become ill; however, risk factors can be explored through population-based studies. Studies are conducted to examine the importance of various possible risky exposures (such as specific foods) and practices (such as food preparation and handling practices) as contributors to illness caused by specific pathogens.

Identification and Control of Microbiological Hazards in Fruits and Vegetables: A Field Epidemiological and Intervention Study in Mexico

The goal of this study is to understand how fruits and vegetables become contaminated on farms. This interagency collaborative NIFA-funded project involves scientists from ARS, FDA, and WCFS and will study crops that are at high risk of contamination like jalapeno peppers, cantaloupes, and tomatoes. Researchers will test these fruits and vegetables for bacteria and viruses that cause disease. They will also use new laboratory methods to find where these fruits and vegetables became contaminated (e.g. water, hands, soil). The study area will include several U.S.-Mexico border states and engage the U.S. and Mexican agricultural communities. This study, and the information it generates, will help to better characterize safe fruit and vegetable practices and therefore reduce the number of outbreaks associated with fruits and vegetables.

Risk Assessment Modeling, Management, and Communication

Interagency Food Safety Analytics Collaboration (IFSAC)

The Interagency Food Safety Analytics Collaboration (IFSAC) was created in 2010 by CDC, FSIS, and FDA, to address complex analytical issues that require cross-agency cooperation and agreement, beginning with pathogen-food source attribution. By working together to address these challenges, FDA, CDC, and FSIS can ensure that methods and results produced as a result

of this collaboration are appropriate, meaningful, and useful to all parties. A specific project is aimed at providing data and expertise to FDA to generate a list of "Most Significant Contaminants" for targeted control in foods (required under FSMA). To address FSMA needs, FDA will produce economic estimates and rank pathogen-food pairs using published results.[8] CDC will provide the starting food-pathogen attribution data set needed for the FDA analyses, and the IFSAC project team will provide subject matter expertise regarding the interpretation of attribution data and methods being used by the FDA Contaminants Working Group (FDA-CWG). The FDA-CWG analysis will generate the costs related to illness, hospitalization, and death for each food-pathogen pair as well as quality-adjusted-life-years (QALYs) for each FDA-regulated food-pathogen pair. With this information, contaminants will be ranked for each food group according to which are associated with the greatest cost and loss of QALYS. IFSAC input may help identify methods and data for updating estimates in 2 years, and IFSAC participation will provide the opportunity to anticipate how the contaminants list may affect other attribution work. More specific descriptions of IFSAC-related projects are included in Appendix A.

Interagency Risk Assessment Consortium (IRAC)

The Interagency Risk Assessment Consortium (IRAC)[9] consists of representatives from U.S. government agencies (inclusive of various agencies, institutes, and centers within HHS, USDA, DHS, the Department of Commerce, the Department of Defense, and EPA), with food safety responsibilities. The consortium was established in 1998, in response to Presidential Executive Order 13100 and subsequent planning and implementation documents of the President's Food Safety Council. IRAC was re-chartered in 2011 to implement a recent recommendation from the President's Food Safety Working Group on coordination of risk assessment among federal agencies. IRAC aims to improve risk assessment research, enhance the development and use of risk assessment tools, and serve as a forum to communicate about risk assessment and related research issues. IRAC accomplishes many of its goals through the work of its Policy Council and Technical Committee, both of which include representatives from each of the 21 federal agencies and offices that constitute the consortium's current membership. Over the past 14 years, IRAC has expanded the range of issues that it addresses beyond food-safety (microbial and chemical) risk assessment. Research issues addressed by IRAC include data quality, peer review, nutrients, susceptible subpopulations, nanotechnology, integration of genomics data into dose-response analysis, and leveraging of epidemiologic and risk assessment methods. The consortium also addresses clarification of various approaches to assessing risks.

Pathogen Persistence and Processing Optimization for Elimination in Foods

Although significant efforts have been taken to control *Listeria monocytogenes* (Lm) in ready-to-eat (RTE) foods over the last decade, a study was conducted to determine whether changes occur

[8] Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ, et al. 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg Infect Dis. 19

[9] A more comprehensive overview of the Interagency Risk Assessment Consortium can be found at http://foodrisk.org/irac/

in the "true" prevalence and levels of the pathogen and to provide current data to assess the relative ranking of higher risk foods. This multi-agency (CDC, FDA, FSIS, ARS), multi-disciplinary study was undertaken to determine the current prevalence and levels of Lm in deli-packaged versus prepackaged RTE foods purchased at retail establishments in four FoodNet sites. Phase I of the study included 10 product categories: smoked seafood, seafood salad, low acid cut fruits, soft cheese, deli salads (non-meat), raw milk, sandwiches, deli meats, deli salads containing meat, and dried/fermented sausage. Samples were collected in both supermarket chain and independent grocery stores in California, Maryland, Connecticut, and Georgia over a 12-month period beginning in December 2010. Collaborations between FDA, FSIS, ARS, and several universities enabled the collection of a representative and sufficient number of samples. Samples were analyzed using FDA's Bacteriological Analytical Manual (BAM) method and enumeration of positive samples by the most probable number (MPN) method and direct plating. Of the approximately 7,500 FDA regulated samples tested, the observed prevalence ranged from about 0% to 1.0% for seven product categories. Of the approximately 3,500 FSIS-regulated products tested, the observed prevalence ranged from about 0% to 0.1% for three product categories. For the samples testing positive in screening, Lm levels ranged from approximately <0.3 - 2.4 log colony forming units per gram. This is the most comprehensive survey of Lm in retail RTE foods in the past decade. The findings provide data to assess changes in Lm prevalence and levels in RTE foods and will be used to revise the 2003 Interagency Lm Risk Assessment.

Spatio-Temporal Mapping of Livestock and Wildlife Locations and Populations for Risk Models: Predicting the Distribution of Feral Swine to Improve Risk Management of Foodborne Enteric Pathogens

Under an interagency grant from FDA, scientists at USDA-APHIS are developing a predictive model of feral swine occurrence for the United States and collecting data necessary to develop a model of pathogens found in feral swine. Specifically, USDA-APHIS will: 1) collect, compile, evaluate, and validate data on key ecological features influencing the abundance and distribution of feral or wild swine; 2) develop models to predict feral swine distribution in the United States using standard techniques for building species-distribution models with presence-absence data (e.g., generalized linear models, generalized additive models); 3) validate models of feral swine distribution by cross-validation of data used for model development or compilation of additional datasets not used for model development; and 4) evaluate feral swine disease surveillance and monitoring data that could be used to support modeling of prevalence and distribution of pathogens. Focal pathogens for this study include *E. coli*, norovirus, and *Salmonella*. Determining the temporal and spatial distribution of feral swine in the United States and evaluating the likelihood that swine carry enteric pathogens will enable FDA to utilize its geospatial models to predict the location specific likelihoods of produce contamination by feral swine. This model also has the potential to be adaptable for other wildlife species.

Food Allergen and Gluten Sensitivity Research Program

Based on CDC monitoring and surveillance data, an estimated 12 million Americans have a food allergy or sensitivity to gluten. Currently, the only way for these consumers to prevent a pathogenic response is to avoid the allergenic or gluten-containing food. Since sensitive

consumers rely on accurate and complete food labels to prevent potentially life-threatening reactions, it is critical that food labels accurately describe the contents of the food they purchase. Without science-based standards and methods to measure allergens and glutens in food, however, it is not possible to implement appropriate risk management practices; to assess the effectiveness of allergen and gluten preventive control and management systems; to take effective enforcement action; or to evaluate industry petitions and notifications of food contents. The Food Allergen and Gluten Research Program addresses these needs through research to develop accurate and sensitive assays for their detection and through evaluation of available clinical data followed by effective risk assessment modeling.

Safety Assessments of Foodborne Hazards, including Toxicological Studies

Toxicology and Toxinology of Mycotoxins in Foods

Fumonisin B1 (FB1) is a mycotoxin produced by *Fusarium verticillioides* and *F. proliferatum*. It is found in corn and evidence suggests it is a possible risk factor for neural tube defects (NTD) in populations consuming large amounts of contaminated corn-based foods. A series of chemical and mechanism-based bioassay studies were conducted collaboratively with scientists from USDA, FDA, the University of Nebraska, and the Texas Woman's University. These studies demonstrated the effectiveness of extrusion cooking alone or with glucose supplementation to reduce the FB1 concentrations and related toxicity using an in vivo model. It was shown that extrusion alone and, more effectively, extrusion with glucose supplementation significantly reduced FB1 levels in and toxicity of the cooked product. Toxicity and elevated levels of biomarkers were correlated with FB1 concentrations in the cooked products indicating that degradation products, including "hidden" matrix-associated forms, did not significantly contribute to toxicity. Dose response data from these studies were used in the fumonisins safety evaluation conducted by the 74th Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA). The studies formed the basis for the Committee's conclusion that studies with *Fusarium verticillioides* culture material, when compared to studies conducted with pure FB1, indicate that the complex mixture of toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB1. This work was used in the international fumonisins safety assessment by JECFA to support the Committee's conclusion that studies conducted with highly purified FB1 may underestimate the risk associated with consumption of naturally-contaminated corn and corn products. The results were also used by risk managers worldwide to support risk management decisions designed to minimize the health risks associated with fumonisin exposure.

Plant and Soil Factors that Influence Bioavailability of Heavy Metals in Crops

Lead levels in produce have recently come under serious consumer and medical scrutiny particularly as nutritious snack foods for children. USDA-ARS identified higher than normal levels of lead in carrots grown on old orchard soils where lead-arsenate insecticide had been used before 1950. Peeled carrots were shown to have higher carrot lead, showing that the contamination pathway was not due to soil adherence to the roots. Lead accumulates in the xylem portion of the root with very little lead in the rest of the storage root. Additional root crops (beet, turnip, and radish) were similarly tested and lead accumulation was observed, but

considerably lower than that found in carrot. This appears to result from the long xylem through the carrot compared to the wider diameter of the other root crops. Potato had very low lead when grown on the same soils, showing that phloem-fed tissues such as tubers, fruits, and grains accumulate very low levels of lead even on high lead soils. The findings support FDA's goal of understanding how carrots can be contaminated with lead, and the industry's need to limit production of crops with high lead levels. The results are also critically important for the USDA Agricultural Marketing Service which provides food through the School Lunch Program.

Economic Analysis

Food recall study entitled: "*Food Recalls: Why Bother?"*

Food recalls have been a cornerstone of the U.S. food safety policy for many years and have been commonly viewed as a way to prevent illnesses. Yet, food recalls may serve other purposes as well. It has been reported that safety failures motivate firms to invest in safety precautions to reduce their liability costs; others have shown that market structure and reputation affects investment. The goal of this research, conducted by ERS using data from FSIS and CDC, is to examine the extent to which: 1) recalls reduce illnesses, and 2) industry responds to the economic consequences of recalls. Results show that recalls prevent few illnesses and that liability, recall, and recovery costs are small relative to investment. Investment in food safety programs occurs before recalls in plants engaged in industries dominated by a few large firms because in these industries recalls can be directly linked to a specific firm. In competitive industries, however, producers remain anonymous, even after a recall. Thus, the firm-level fear of a business failure is diminished. As a consequence, there is little investment in food safety programs in competitive industries unless there is a drop in industry demand due to the recall. In this case, the entire industry may be motivated to collectively raise investment in food safety programs. Results from this research will provide a better understanding of the impacts of recalls on illness and of how competitive pressures on firms affect the incentives recalls create for investment in prevention of foodborne illness.

Comparison of Recently Published Estimates of Cost of Foodborne Illness

In 2012, two major studies published estimates of the cost of foodborne illnesses in the United States. The results of these studies appear to differ greatly. This study, conducted by ERS, compares recent cost-of-foodborne-illness estimates with each other and with 2001 ERS estimates. The results show that the two primary drivers of differences in the cost-of-illness estimates are the change in CDC estimates of the incidence of foodborne disease and differences in the number of diseases each study evaluates. Decisions on whether to include monetized QALY loss are also a source of differences in the cost-of-illness estimates. Once these factors are accounted for, differences in cost-of-illness estimates are small relative to uncertainty in disease incidence estimates. Relative rankings are affected more by the pathogens included in the analysis than by the method used to estimate the cost of illness. Quantified estimates of the public health and economic burden of foodborne illness provide important information for federal food safety policy makers. This research clarifies why recent estimates of the cost of illness differ and assesses how those differences may affect the use of estimates in food safety policy analysis.

FDA Import Refusals for Food by Exporting Country

Imports are an increasingly important source of supply in the United States, and there is increasing public concern about the safety of imported food. This research, conducted by ERS, analyzes data on FDA's food import refusals from 2005 forward. The study focuses on those exporting countries with the most refusals plus select countries with FDA international posts. Regression analysis is used to investigate factors associated with refusals due to adulteration versus misbranding. Factors considered include food type, container type, level of processing, and year. Preliminary results show adulteration violations as a share of total violations have declined over time and that the type of container and level of processing are significant indicators of whether or not the violation is classified as an adulteration or misbranding. Findings should help inform FDA's risk-based management of food import safety.

Collaborative Interagency Strategic Planning

HHS, USDA, and DHS will continue to focus efforts on comprehensive prevention by introducing new safety standards and practices aimed at preventing all foods and feed from being contaminated. These standards will be grounded in the latest food safety research and science, and will be informed by experts from within the federal agencies; the food and feed industry; and the academic, animal health, and public health arenas. By setting science-based preventive control standards for the way industry produces, processes, distributes, stores, transports, and markets food and feed items, regulators will define the responsibility of the operators throughout the food system to ensure the safety of products that enter into commerce. Given the risk of intentional contamination, federal agencies will also continue to play an important role in food defense by developing analytical tools for detection and standards for protecting the food and feed supply from tampering or other deliberate actions. Research and evaluation on the effectiveness of preventive control standards will continue to be an area of focus, along with partnering with agricultural and industry suppliers, distributers, and marketers to improve their knowledge of regulations, guidance, hazards, and mitigation strategies.

Implementation of an enhanced, integrated approach to research—one where education and outreach are integral components of the research portfolio—will position the federal agencies to more effectively address the issues threatening the food supply. Strategically and operationally linking research needs to agency regulatory goals will create focused synergy and momentum and will increase the ability of agencies in meeting those goals. Fostering a culture of collaboration with other research and health agencies in the federal government, state government agencies, academia, private industry, and foreign regulatory bodies will expand scientific capability and permit all stakeholders to benefit from the great strides being made across the country and globally. With transparent, collaborative processes for prioritizing science and research needs, HHS, USDA, and DHS can collectively achieve a clear and consistent focus on mutual goals and leverage the regulatory and research capabilities of partners to help meet the highest priorities. These efforts will require significant investments in cutting edge technologies and expert human capital that strengthen the science and technology infrastructure with the future in mind.

Conclusion

American consumers have high expectations of those responsible for ensuring the safety and security of the U.S. food supply. They expect that the federal agencies, as well as state, local, territorial, and tribal regulatory and public health authorities will work together to protect the food supply from both unintentional and intentional hazards.

Federal agencies engaged in food safety and food defense research support a diverse portfolio of research projects aimed at improving the safety and security of our nation's food supply, as evidenced in this comprehensive report. Enhanced coordination and collaboration amongst the agencies will be a key component of ensuring a continued safe food supply. This can be done by utilizing an array of tools to accommodate various kinds of collaborations among the three primary departments responsible for food safety and food defense: HHS, USDA, and DHS. The tools may include exploratory workshops, conferences, networking programs, and collaborative research schemes. Implementation of a more integrated approach to research will position the federal agencies to more efficiently address the issues threatening the food supply. Strategically and operationally linking research needs to each agency's regulatory goals will create focused synergy and momentum and will increase the likelihood of achieving these goals. Fostering a culture of research collaboration between federal and state government, academia, private industry, and foreign regulatory bodies will expand scientific capacity and provide benefits both within the United States and globally. These efforts will require significant investments in cutting edge, innovative technologies that strengthen the science and technology infrastructure and are rooted in the guiding principles of:

- Public health is the first priority – all activities are carried out with the end goal of protecting the public and animal health;
- Partnering with others to build science-based prevention systems is the key to success – federal agencies must partner with a variety of stakeholders to ensure that safety is built into food production and processing from farm-to-table so that we prevent foodborne illness before it begins;
- Scientific expertise and innovation are fundamental to the federal agency research enterprise – maintaining current mission-critical science and research capabilities and investing in emerging disciplines, sciences, and technologies to address emerging needs will help mitigate future risks to food safety; and
- Government transparency is the national standard – federal agencies are committed to operating with transparency and providing other stakeholders with the opportunity to provide input.

The global food safety arena is in an extraordinarily dynamic phase. Change is underway, and it is change that is grounded in our scientific understanding of foodborne illness, its causes, and how it can be prevented. New food technologies mean that the knowledge needed to ensure the safety of these new types of foods must keep pace. The process of ensuring that our federal science and research agenda mirrors our public health and regulatory priorities will be a continual process.

With the establishment of transparent, collaborative processes for prioritizing science and research needs, federal agencies can achieve a clear and consistent focus on their food safety goals and leverage the regulatory and research capabilities of partners to help meet the highest priorities of protecting the U.S. food supply. It is critical that federal agencies invest the necessary resources needed to carry out these activities, but they also must plan strategically and coordinate across the federal research enterprise to make the best use of limited resources.

Appendices

The appendices for each agency [HHS (CDC, FDA) and USDA (ARS, ERS, FSIS, NIFA)] contain the extensive lists of food safety research by category. The above examples in the body of the report highlight food safety research areas in which multiple federal agencies are collaboratively engaged. The content of the appendices is limited to descriptions of research projects funded and active during FYs 2011 and 2012 and were written in accordance with that timeframe. During the time between the composition of this report and the release to Congress, the status of specific studies may have changed. Because FSMA mandates reporting on a biennial basis, updates will be provided in future reports.

APPENDICES

Biennial Report to Congress on the Food Safety and Food Defense Research Plan

Submitted Pursuant to Section 110(g) of the FDA Food Safety and Modernization Act,

Public Law 111-353

FSMA Section 110(g) Report
Appendix A [CDC Food Safety Research List for FY 2011 and FY 2012][10]

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[10] *Disclaimer: Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its approval, endorsement, recommendation, or favoring by the United States Government or any department, agency, office, or branch thereof.*

Prevention, Intervention, and Control of Foodborne Hazards

Environmental Health Specialists Network (EHS-Net)-Related Studies

Listeria Retail Deli Study

According to the FDA/FSIS 2003 *Listeria monocytogenes* in ready-to-eat foods risk assessment[11], deli meats pose the greatest risk of listeriosis per year and per serving when compared to other ready-to-eat foods previously linked to *L. monocytogenes*-contamination. Although *Listeria's* infection rate is low in comparison to other major foodborne pathogens, it has a high case fatality rate of 19%. Since 2002, the number of cases of listeriosis in the United States is not continuing to decline. To address this issue, this research study will focus on determining how delis' characteristics, managers' and workers' characteristics, and delis' food safety policies and practices individually and collectively correlate with the risk of cross-contamination of ready-to-eat foods with *L. moncytogenes*. This study is based on the FDA/JIFSAN observational study published in the *Journal for Food Protection*.[12] Descriptive data on individual deli workers' actions involved in performing work-related tasks, such as slicing deli meat, will be collected through notational observation. Data on both physical and operational characteristics of each retail deli's environment will be collected including information on the deli's layout and food safety policies such as equipment (cutting boards, knives, etc.) usage and cleaning. Thirdly, data on managers' and workers' characteristics, including food safety practices (e.g., hand washing practices), knowledge, training and certification will be collected and evaluated.

Kitchen Manager Certification Study

Research indicates that eating outside the home is associated with foodborne illness, and epidemiological research has found that over half of reported foodborne illness outbreaks are associated with restaurants. Recent data has suggested that the presence of a certified Kitchen Manager (KM) in restaurants reduces the risk of a foodborne illness outbreak. However, little is known about the relationship between KM certification and food safety knowledge or about how certification may work to reduce foodborne illness outbreaks. The study will explore the relationship between KM certification, food safety knowledge, and control of foodborne illness outbreak risk factors by comparing the food safety knowledge levels and attitudes of certified KM person-in-charge (PIC) and non-certified KM/PIC and examining the relationships to foodborne illness outbreak risk factors in restaurants that have a certified KM/PIC to those without a certified KM/PIC, as well as comparing food safety knowledge of food workers in restaurants with a certified KM/PIC to those without a certified KM/PIC. Results of this study can be used to promote kitchen manager certification.

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[11] http://www.fda.gov/Food/ScienceResearch/ResearchAreas/RiskAssessmentSafetyAssessment/ucm183966.htm

[12] Lubrean et al, Observational Study of Food Safety Practices in Retail Deli Departments: *Journal of Food Protection*, Volume 73, Number 10, October 2010 , pp. 1849-1857(9)2010)

Detection of Microbial, Chemical, and Radiological Hazards in Food, Feed, and Dietary Supplements

Botulinum Neurotoxin Detection and Differentiation by Mass Spectrometry

Rapid methods that can detect botulinum neurotoxins able to detect and differentiate toxin serotypes in less than one day in food, stool, and blood are needed. Methods that can distinguish toxin subtypes and strains or variants are also beneficial to determine commonality or differences between concurrent outbreaks and to help trace sources of infection. CDC has developed a rapid test (capable of running samples in 6-8 hours in a 96 well plate format) for botulinum neurotoxins that cause botulism in humans (BoNT/A, /B. /E, and /F) called Endopep MS. The method is highly selective (toxin extraction with monoclonal antibodies, enzymatic cleavage and mass spectrometry detection) and is more than 10 times more sensitive than the mouse bioassay. Subtyping of the toxin has recently been added. The method has been validated analytically in serum, stool, gastric juice, and representative panels of foods. The subtyping method has also been applied in many outbreaks during the last 2 years. The Endopep MS method is a rapid method that compliments the mouse bioassay and genetic testing. Subtyping is generally available in less than 24 hours.

Risk Assessment, Modeling, Management, and Communication

Interagency Food Safety Analytics Collaboration (IFSAC)-Related Studies

Provide Data and Expertise to FDA to Generate List of Most Significant Contaminants for Targeted Control in Foods (required under Food Safety Modernization Act)

To address FSMA Section 104(a) needs, FDA will produce economic estimates and rank pathogen-food pairs using results from the Painter paper (currently under review for publication). CDC will provide the starting food-pathogen attribution data set needed for the FDA analyses, and the IFSAC project team will provide subject matter expertise regarding the interpretation of attribution data and methods being used by the FDA Contaminants Working Group (FDA-CWG). The FDA-CWG analysis will generate the costs related to illness, hospitalization, and death for each food-pathogen pair as well as quality-adjusted-life-years (QALYs) for each FDA-regulated food-pathogen pair. With this information, contaminants will be ranked for each food group according to which are associated with the greatest cost and loss of QALYS. IFSAC input may help identify methods and data for updating estimates in 2 years, and IFSAC participation will provide the opportunity to anticipate how the contaminants list may affect other attribution work.

IFSAC Outbreak Bias Project: Evaluate Potential Limitations of Using Attribution Estimates Obtained from Outbreak Reports

Data from foodborne disease outbreak surveillance are used to determine the best attribution estimates currently available to regulatory agencies for decision-making. Outbreak-associated illnesses account for less than 25% of human foodborne illness; therefore, we do not know how well attribution estimates based on outbreak reports characterize the common causes of foodborne illness and food vehicle exposures in the general population. This project uses data from multiple human illness surveillance systems and survey results from the National Health and Nutritional Examination Survey (NHANES 2007-2008)[13] to evaluate how likely the foodborne pathogens and food risks identified in outbreak investigations reflect those of the entire population. State health departments submit to CDC about 800 reports each year about foodborne disease outbreaks that they investigate. The reports contain information on the number of people ill, the food that made them ill, and much other data. CDC, regulatory agencies, and other stakeholders use these data to make estimates about the number of illnesses in the United States caused by various food products. However, most illnesses are not part of recognized outbreaks, and we do not know whether the ill people and foods involved in outbreaks are similar to those not in outbreaks. This project uses data from several human illness surveillance systems and survey results from the National Health and Nutritional Examination Survey (NHANES 2007-2008) to evaluate how likely the persons, pathogens, and foods identified in outbreak investigations reflect those of the rest of the population.

IFSAC Hald Model Project: Adapt Hald Attribution Method to FDA and FSIS Data Sources

[13] http://www.cdc.gov/nchs/nhanes/nhanes2007-2008/nhanes07_08.htm

CDC, FDA, and FSIS have many data sources that may be used to determine estimates of foodborne illness source attribution. Specifically, a method has been developed in Denmark (Hald, T. et al, A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis, *Foodborne Path and Dis*, 2004) that uses information about food contamination, consumption patterns in the population, and number of foodborne illnesses to estimate the number of illnesses associated with specific foods. This project will use food contamination data collected by FDA and FSIS, food consumption data collected by the USDA and from the National Health and Nutrition Examination Survey (NHANES), and human illness data collected by CDC to evaluate how effectively these data may be used to estimate food source attribution using the Danish method.

IFSAC Attribution Estimates Project: Develop Estimates of Foodborne Illness Source Attribution for Four Priority Pathogens

Currently, CDC, FSIS, and FDA, do not have a standardized method to derive outbreak-based attribution estimates and confidence bounds. This project will develop (1) attribution estimates based on data reported to the Foodborne Disease Outbreak Surveillance System from 1998-2010 commoditized using the new hierarchy developed in Project I above, and (2) criteria to identify and explore uncertainty issues. The attribution estimates will be based upon outbreaks with simple foods implicated (i.e., a single contaminated ingredient is implicated as the cause of illnesses, or all ingredients in the implicated food can be assigned to a single commodity group). This project will also determine approaches for attributing complex implicated foods and generating uncertainty bounds for outbreak-based attribution estimates.

FSMA Section 110(g) Report
Appendix B [FDA Food Safety Research List for FY 2011 and FY 2012][14]

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[14] Because of the 2 year period that this section covers and the way FDA captures project descriptions', there may be some projects that appear similar in scope and design, however they are in fact separate studies or continuations of the same study. *Disclaimer: Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its approval, endorsement, recommendation, or favoring by the United States Government or any department, agency, office, or branch thereof.*

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Prevention, Intervention, and Control of Foodborne Hazards

Chemical Contaminants – Food Additives

Food Irradiation: Chemical Changes in Food and Food Contact Substances due to the Absorption of Ionizing Radiation

Irradiation treatment has been shown to generate furan in some food products and furan is listed in the U.S. Department of Health and Human Services list of carcinogens. The goal of this research is to analyze various foods after irradiation treatment to determine if furan is generated by the radiation process and compare this data to heat treated foods. Additionally, the chemical changes that occur when food contact materials are treated with radiation will be investigated. FDA will utilize this information in developing regulations for materials that may be used during the irradiation treatment of foods**.** Because irradiation reduces microbiological contamination in foods, success of this work could permit more uses of irradiation for processing food and help reduce foodborne illness.

Migration Database of Additives and Contaminants in Food Packaging Systems for use in Predictive Migration Models

To predict additive migration from plastic packaging at various conditions of use, FDA maintains a migration database of specific migrants, polymers, and food simulants at specific temperatures. However, gaps in diffusion data exist for organic migrants in commercially important polymer packaging materials, including polyesters and nylons. The goal of this research is to collect diffusion data for a series of organic surrogate compounds in these polyester and nylon polymers. These new migration data would enhance FDA's confidence in assessing industry submissions for new packaging materials and calculating exposures to additives within packaging by providing a simple means to ensure that migration data submitted for FDA review are scientifically sound.

Effect of High Pressure Processing on Migration Characteristics in Flexible Packaging Materials

High pressure processing (HPP) is an alternative food preservation technology that has an increasing presence in the commercial marketplace. The use of polymer-based flexible packaging materials has allowed the application of HPP to pre-packaged food products. Many of these materials have been shown to withstand HPP without a significant loss of physical or mechanical properties; however, limited published data exist for the migration behavior of chemical additives in packaging materials after treatment by HPP. The goal of this research is evaluate consumer dietary exposure to additives from packaging treated with HPP, especially if this process promotes such migration behavior into foods. These results from this research would help FDA in determining if any potential safety risks result from increased consumer exposure to migrating packaging constituents.

Chemical Contaminants – Other

Development of a Protocol for Use of Portable Gloveboxes for Safe and Effective Sample Preparation in Emergency Situations

The goal of this research was to conduct experiments using powdered infant formula to devise a protocol for safe and effective handling of powdered samples in portable gloveboxes. Studies included representative sampling, cleaning procedures to prevent cross-contamination between samples, and sample decontamination prior to removal from the glovebox. Relatively inexpensive portable gloveboxes used inside an exhausting chemical fume hood have also been investigated as a possible option for laboratories that are not equipped with permanently installed gloveboxes. Safe ways to improve the speed and ease of handling samples in a confined space were also explored. From the results of this study, a harmonized protocol is being developed.

Toxins

Methods for Reducing Aflatoxin Contamination of Almonds and Corn

Aflatoxins are toxic compounds formed in foods contaminated by specific mold species. They are a threat to human and animal health since some forms are potent liver toxins and carcinogens. The goal of this research is to determine ways to reduce aflatoxin in susceptible foods such as corn and almonds. The first approach aims to control the growth of mold in food, thus limiting toxin production. The second approach is directed at destroying or removing aflatoxins from food or feed after they have been formed. If successful, this research would benefit the food industry and consumers by reducing aflatoxin levels in susceptible commodities such as corn and almonds.

Development of Reference Materials for Caribbean Ciguatoxins in Fish

Ciguatera fish poisoning (CFP) of seafood consumers is caused by eating tropical and subtropical fish that have concentrated neurotoxins (ciguatoxins: CTXs) from microscopic algae (*Gambierdiscus* spp.). Many fish species (e.g. grouper, amberjack, and barracuda) have been reported to cause CFP, however, the distribution of toxic fish is sporadic and not all fish from a CFP prone area are toxic. There are no commercial sources of CTXs, which are reference standards needed for the development of testing programs to prevent toxic fish from entering commerce. The goal of this research is to produce pure CTX reference standards. If successful, these standards could be used by FDA and States to validate tests, and support efforts to prevent CFP of seafood consumers.

Discovery and Application of Biomarkers for Seafood Decomposition

Seafood decomposes when it is subjected to time and temperature abuse, and becomes unfit for human consumption. Some species of fish can become toxic when decomposed, causing scombrotoxin (histamine) fish poisoning. The goals of this research are to evaluate seafood processing practices that prevent decomposition and to develop tests to assure that decomposed

seafood is not distributed to the public. If successful, the improved practices and tests would be used by FDA to improve the effectiveness of guidance for the seafood industry.

Allergens and Gluten

Cleaning and Validation to Prevent Allergen Cross-Contact

Cleaning is an important tool for controlling allergens in food processing facilities. There is a need for more information on effective ways to clean allergens from food processing equipment. The goals of this research are to identify procedures that can be used by the food industry to remove allergenic foods residues from different food-contact surfaces and to identify or improve methods for measuring the presence of allergens in foods and on equipment surfaces. If successful, this research would provide information on how the industry can control allergens in food processing facilities through improved cleaning procedures and methods for detecting allergens.

Adulteration and Misbranding

Retail survey of pet food to determine species identity of label claims

Within the last two years, FDA has analyzed pet food to determine the accuracy of the label claims about the types of meat in the pet food in an effort to determine if the meat in the pet food was something other than what was stated on the label. Based on the results, a survey of pet food samples was conducted to determine the accuracy of label claims of pet food. The goal of this research is to determine the accuracy of label claims concerning the types of meat in retail pet food. This study would provide useful data to assist FDA's mission to protect animal health by determining which pet food may pose a potential risk due to the use of unexpected animal sources of the meat.

Microbial Pathogens

Salmonella

Effect of Pre and Pro-biotics on the Immune System Modulation and Clearance of *Salmonella* Enteritidis in Laying Hens

Eggs remain a primary source of *Salmonella* Enteritidis (SE) infections. *Salmonella* infection in chicken is partially controlled by the immune system, especially macrophages. The goal of this research is to evaluate if feeding pre- and probiotics to chickens can improve chicken macrophage survival, thereby increasing chicken resistance to *Salmonella*. Many probiotic bacteria, which are generally recognized as safe, could be incorporated into chicken feed to enhance chicken resistance to SE as an alternative to the use of antibiotics in chicken feed. This may be a viable method to increase resistance of laying hens to *Salmonella* infection and decrease egg contamination, thus improving egg safety.

Identification of Potential Naturally Occurring Antagonist to *Salmonella* Newport in the Virginia Eastern Shore Tomato Growing Area

Foodborne illnesses have been associated with consumption of fresh tomatoes cultivated on the Eastern Shore of Virginia. These tomatoes have been found to be contaminated with *Salmonella* Newport. FDA has identified some naturally occurring antagonistic microbes from a tomato-growing environment in a previous study. The goal of this research is to use these microbes as biocontrol agent(s) to decrease pre-harvest contamination of tomato crops with *Salmonella*. If successful, this work could provide strategies to prevent the inadvertent introduction of *Salmonella* into the tomato supply, thereby reducing the possibility of associated foodborne illnesses.

Salmonella Desiccation Resistance and Survival in Extremely Low Water Activity Foods

Salmonella has caused numerous outbreaks of illness linked to consumption of dry foods, such as peanut butter, chocolate, cereal, and grains. Most other pathogens cannot live in dry foods due to the lack of water, but *Salmonella* can live for extended periods of time. The goal of this research is to evaluate the mechanisms by which *Salmonella* survives conditions of low humidity and water content, as well as some processing and sanitation treatments. The knowledge gained from this research would provide FDA with basic understanding of the physiology of *Salmonella* in dry foods and help in developing more effective interventions to minimize *Salmonella* contamination in dry foods.

Validation of Microwave Pasteurization of Multiple Shell Eggs

The current infection level of *Salmonella* Enteritidis (SE) in eggs is estimated to be 1 in 20,000; meanwhile approximately 65 billion eggs are produced in the U.S. annually. Thus there are potentially millions of SE infected eggs produced every year. The goal of this research is to evaluate the ability of microwave heating to produce a pasteurized in-the-shell egg quickly and with quality comparable to that of un-treated or non-pasteurized eggs. If successful, this in-the-shell egg pasteurization process could provide the consumer with an additional alternative that offers increased safety and protection when consuming eggs.

Processing and Sanitation of Botanicals and Spices Used as Food Additives or Supplements

Contaminated spices and botanical products have been the cause of numerous recalls and foodborne illness outbreaks. Despite these recalls and outbreaks, little information is available on the normal microbial loads associated with these products, or how commonly they become contaminated with foodborne pathogens. The goals of this research are to determine the microbial load of a variety of spices and to evaluate the effects of processing and storage conditions on survival of *Salmonella*, a cause of many outbreaks, in spices and plant-derived ingredients. The information gathered in this study could enable development of appropriate treatments for the destruction of pathogens and appropriate storage conditions to prevent contamination or further growth of microorganisms.

Potential for Cross-Contamination in the Spice Processing/Packing Environment

Salmonella contaminated pepper has been linked to large outbreaks in the U.S. Because spices are so widely used in food manufacturing, contamination with harmful bacteria has the potential to result in widespread illness. Root cause investigations provided evidence that cross-contamination via spread of the bacterium in the pepper's manufacturing environment, played a key role in these recent outbreaks. The goal of this research is to evaluate the cross-contamination potential in spice processing and packing environments in order to provide new insights that can be useful in improving spice safety.

Sanitation Guidelines for Almond and Nut Butter Processing

Historically nuts and nut butters have not been considered a hazardous food with respect to microbial contamination; however, recent outbreaks of salmonellosis have challenged that view. Recent outbreaks involving peanut butter products have shown that current cleaning and sanitation protocols for nut butter processing are inadequate. The goals of this research are to evaluate the contamination of food-contact surfaces with *Salmonella* via transfer studies and to validate a two-step cleaning and sanitation method for pilot-scale processing equipment that can be used as guidance for the nut butter processing industry. The results from this research would provide industry and regulators with information on ways to improve the safety of nuts and nut butter products.

E. coli O157:H7 and STEC

Analysis of Gene Function in *Escherichia coli* O157:H7 from Outbreaks Associated with Fresh Produce

Foodborne illnesses have been increasingly related to fresh produce consumption. As fresh produce has become more popular in healthy lifestyle diets, it is important to develop ways to improve its safety and prevent illnesses. Freshly harvested produce is often cleaned with sanitizers during washing, and the most common sanitizer is chlorine. Yet, bacteria such as *E. coli* O157:H7 can survive, through unknown means, the cleaning and sanitization process. The goal of this research is to determine how *E. coli* O157:H7 genes switch on and off during exposure to chlorine to understand the survival mechanisms and design better treatments. The data would provide industry and regulators with information on best practices to improve the safety of fresh produce.

Listeria

Adaptation of *Listeria monocytogenes* in High Osmolarity and Refrigeration Temperature

Listeria monocytogenes causes listeriosis, a disease with significant risks of fatality for at risk populations, and can contaminate and grow in ready-to-eat foods. There is limited information on how *Listeria* grows in refrigeration and in the presence of high salt, which complicates control of this organism at food processing plants and within certain food products. The goal of this research is to evaluate *Listeria* mutants with altered growth characteristics to better

understand the genes that play a role in its ability to grow at refrigeration temperatures and/or high salt concentrations. This characterization will help FDA identify new methods to reduce or prevent *Listeria* contamination in foods.

Vibrio

Evaluation of High Salinity Relaying of Gulf Coast Oysters for the Reduction of *Vibrio vulnificus* to Non-Detectable Levels

The naturally occurring marine bacteria, *Vibrio vulnificus,* are responsible for the majority of deaths associated with eating raw oysters in the U.S. These bacteria are abundant in warm coastal waters, including areas where oysters are harvested for human consumption. To reduce the health risk from these bacteria, FDA recommends treatment of shellfish harvested during the warmer months to eliminate these bacteria. The goal of this research is to evaluate the practice of relocating oysters to high salinity water, where *V. vulnificus* are unlikely to be found, to purge these harmful bacteria from the oysters. If successful, this process could be used by the seafood industry to provide a safer product to consumers.

Clostridium

Inactivation of *Clostridium botulinum* Spores using High Pressure Processing

Clostridium botulinum spores are widely distributed and present in nature. If low-acid foods containing these spores are not properly processed, surviving spores in the product can grow and produce toxin during room temperature storage. This scenario can potentially result in a botulism outbreak. The goal of this research is to evaluate the effects of high pressure in combination with high temperature processing as a means of destroying *C. botulinum* spores while maintaining a high quality product. The results of this research would help FDA and the food industry in determining if high pressure processing has the potential to produce safe low-acid extended shelf-life foods.

Inhibitory Growth Boundary Conditions for *Clostridium botulinum*

Clostridium botulinum spores are widely distributed in raw food materials. The International Commission on Microbiological Specifications for foods has proposed the use of the Food Safety Objective system for managing food safety. The hazard of *C. botulinum* continues to exist with low-acid shelf-stable foods which are expanding rapidly in the marketplace. The goal of this research is to evaluate the boundary conditions of intrinsic properties of foods such as pH, water activity, salt and others that are inhibitory to *C. botulinum* growth and toxin production. The information obtained from this research could be modeled and incorporated into the calculation of the Food Safety Objective.

Other Bacterial Pathogens

Investigations Focused on Promoting the Safety of Fresh Produce: Fate of Pathogens Following Fresh-cut Produce Preparation with Kitchen Utensils

Based on epidemiological data from Europe, North America, Australia and New Zealand, a substantial proportion of foodborne disease has been attributed to improper food preparation practices in consumers' homes. Efforts to convince consumers to change inappropriate food-handling practices, particularly with regard to ready-to-eat-foods, have met with only minor success due in part to consumers' failure to associate their food-handling practices with foodborne illness. Results of this project are expected to be most valuable as supportive evidence when drafting or editing consumer recommendations. Such information would be adopted in home kitchens in order to reduce the likelihood of contamination or spread of harmful microbial organisms during the preparation of fresh-cut and ready-to-eat fruit and vegetable items. Results that may be expected of this study could have a more direct FDA impact when considering procedural and regulatory implications for retail preparation of product, to include hand preparation (by knife, etc.) and automated preparation (slicers, dicers, etc.).

Strengthening Good Agricultural Practices (GAPS) for Reducing Bacterial Contamination of Produce

The goal of this research was to conduct extensive field sampling at enrolled produce farms and livestock operations in California's central coast to identify potential sources and risk factors for microbial contamination of fresh leafy greens. Results from the multi-year (2008-2010) longitudinal study indicated that *E. coli* O157:H7 and *Salmonella* occur rarely on plants or soil during field production, but these pathogens are transiently prevalent in regional surface water, sediments, livestock, and wildlife populations. Generic *E. coli* concentration of pre-irrigation water was found to be positively associated with 24-hour cumulative rainfall and negatively associated with increasing distance between sample location and nearby vegetation or riparian habitat. Generic *E. coli* concentration in all water and sediment samples did not correlate with the presence of pathogens (*E. coli* O157, *Salmonella*). These findings are being used to refine GAP metrics for fresh produce related to irrigation water and environmental assessments.

Development and Analysis of a Protocol to Assess Survival of Fecal Organisms in Agricultural Soils Amended with Raw Manure

In accordance with the FSMA mandate, FDA issued a proposed produce rule that will contain standards for agricultural practices used in the production of fresh produce, including the use of raw manure. There is limited and often widely varying data in the scientific literature regarding the prevalence, survival, growth and transference of human pathogens on fresh produce and in the growing environment. Survival, depending on the environment, manure type, and pathogen considered, has been reported to be from a few days, to over a year. The protocols used to generate the existing data are also widely varied, are often very specific to particular regions or conditions, and cannot be extrapolated to make overarching conclusions. FDA has initiated the development of a protocol and research is needed to evaluate its appropriateness for use by the produce industry to develop safe handling practices for the use of manure as a soil amendment

that would comply with existing safety standards of the Federal Food Drug and Cosmetics Act, and the mandates put forth by FSMA. In order for such a protocol to be scientifically valid, it must be standardized for use. ARS will help in such validation, by evaluating and implementing a field application, monitoring and sampling scheme to observe the survival and persistence of various human pathogens (or surrogates) present in raw manure applied to fresh produce production fields. The results of this study will influence both FDA thinking on the safe use of raw manure, and will provide for future research in unique environments to be conducted which will in turn inform local growers about safe alternative approaches to the safe use of raw manure.

Microbial Analysis of Bagged and Fresh Produce

Ready-to-eat (RTE) foods have been a concern for FDA since there is no further processing between the time of purchase and consumption. Consequently, RTE foods that come into contact with various pathogens during production become vehicles for disease. The purpose of the tomato and leafy green task orders is to increase our knowledge on the incidence and prevalence of pathogens associated with tomatoes (Salmonella) and leafy green vegetables (Salmonella, Shigella, and E. coli O157:H7) and soft cheeses. Organic, non-organic, imported and domestically grown products will be sampled during this survey. Samples will be collected from retail locations (grocery stores or equivalents) located at widely distributed geographic locations across the U.S. A greater understanding of the distribution and frequency of contamination of fresh-cut tomatoes, RTE leafy greens, and soft cheeses will greatly enhance our understanding of the epidemiology of enteric diseases caused by consumption of contaminated products. Such an understanding may result in the development of interventions that will reduce or prevent the occurrence of related foodborne outbreaks.

Isolation and Identification of Yeasts with Antagonistic Activities against *Penicillium expansum*, the Main Cause of Postharvest Spoilage and Patulin Production in Apples

Contamination of apple products with the highly toxic and mutagenic mold toxin, patulin, is an ongoing problem. Reducing the levels of patulin relies mainly on inhibiting or retarding the growth of the patulin-producing mold, *Penicillium expansum*, on apples after harvest. The use of chemical pesticides often fails to control the mold because of developed resistance to these chemicals. The goal of this research is to isolate and evaluate non-pathogenic yeasts from apples and other plant materials that have high inhibitory effects against *P. expansum*. Such strains can be applied to apples in storage, greatly limiting mold growth and patulin production, therefore, resulting in apples and apple products with acceptable patulin levels.

Thermal Resistance of *Coxiella burnetii* in Dairy Products with Differing Levels of Solids and Fat Contents

This project continues previous FDA research on measuring the destruction of *Coxiella burnetii*, a thermal resistant vegetative pathogen, during milk pasteurization. Pasteurization requirements were established in the 1950s using techniques which are not as accurate as modern methods. The goal of this research is to develop a method to accurately measure the number of live *Coxiella* in milk in order to determine the effective conditions for pasteurization. Skim and whole milk will be heated at specific time and temperature combinations, and the numbers of

live *Coxiella* will be determined for comparison to the older method. Through this work, traditional and novel processes may be evaluated for destruction of the pathogen in various milk products.

Evaluation of Enzyme-Based Time-Temperature for Validation of Thermal Sterilization of Low-Acid Foods

Thermal processing parameters are based on providing enough heat to kill specific pathogens expected to be present in each specific food. Traditionally, these parameters are determined and tested using microorganisms such as *Geobacillus sterothermophilis* (GS).Using these microorganisms are often a time consuming task. Recently, enzymes from heat-resistant microorganisms such as *Pyrococcus furiosus* (PF), which may serve as a substitute for GS, have been isolated. The goal of this research is to utilize an enzyme obtained from PF to determine thermal processing parameters for inactivating food pathogens. Substituting an enzyme for GS would reduce the time required to determine and verify new thermal processing parameters without impacting safety.

Utility of Generally Regarded as Safe (GRAS) Products to Reduce Microorganisms of Public Health Concern During Storage of Seafood

Bacteria can affect the safety of raw, processed and ready-to-eat (RTE) seafood. Several histamine-producing bacteria and *Listeria monocytogenes* can cause serious illness or death in humans, and have been associated with raw, processed, and RTE seafood. The presence of these bacteria in seafood products can result from temperature abuse and cross-contamination in food processing environments. The goal of this research is to evaluate the effectiveness of antibacterial products that are safe for human consumption on the reduction of bacterial populations that affect human health. Antibacterial products that are safe and effective could be used by seafood industry to reduce the risk of consumer illness from raw, processed, and RTE seafood.

Decontamination of Dry Ingredients

There have been outbreaks of illness associated with consumption of powdered infant formula, raw almonds, and dry spices contaminated with pathogens. As a result, there is a growing need for methods of decontaminating these dry foods. Traditional thermal processing approaches to decontaminate these foods can cause adverse effects on product quality. Therefore, alternative processes for decontaminating dry materials are desirable. The goal of this research is to evaluate the effectiveness of decontaminating dry ingredients during processing using novel emerging technologies. If successful, these technologies could be used to reduce microbial pathogen levels in dry ingredients to improve their safety.

The Analysis of Microwave Cooking Instructions for Not-Ready-To-Eat Food

Not-Ready-to-Eat (NRTE) meals have been the source of foodborne illness outbreaks due to inadequate instructions for preparation in a microwave oven. With the variability in performance of ovens used by consumers, it is difficult to determine if such instructions are truly universally

adequate. Experimentation with each different existing oven is impractical. As an alternative approach, the goal of this research is to use mathematical modeling to simulate microwave heating, allowing any heating condition to be easily examined simply by changing model parameters like oven size, magnetron power, *etc*. The results would indicate where such instructions would be the most sensitive to failure and identify those NRTE foods that are at risk of not being heated properly.

Temperature Fluctuation and Pathogen Survival on Fresh Produce During Transit

Despite numerous campaigns, U.S. consumption of fresh produce remains below recommended levels. Possibly due to an increasing number of highly publicized food safety incidents, there has been no significant increase in consumption in the U.S. Assurance of food safety is absolutely critical for consumers to increase their consumption of fresh product. While proper transportation and distribution are critical, there is not a great deal of consistent, science-based information available to the industry. The goal of this research is to determine if poor temperature management practices documented by FDA lead to enhanced survival and pathogen growth, and to determine the risk of cross-contamination by measuring transfer coefficients for pathogens under conditions that exist during transportation, repacking and distribution using tomato fruit as the model system. The research proposed is significant because it will provide critical, science-based information that can be used to ascertain actual food safety risks involved in transporting fresh produce.

Enteric Viruses

Effects of Temperature on Viral Survival in Contaminated Fruits and Vegetables

Viruses are responsible for most of the foodborne illnesses in the U.S. annually. Fruits and vegetables are the main commodities associated with foodborne viral diseases. Grown in a natural environment, fruits and vegetables may be contaminated in fields or packing houses via irrigation water or hand contact by infected workers. The goal of this research is to determine ways to reduce viruses during storage, shipping and other processes in which temperature control is involved. The results of this study would provide FDA with information to predict and prevent the risks associated with virus-contaminated foods and would be useful to the food industry for improving produce safety before products reach consumers.

Detection of Microbial, Chemical, and Radiological Hazards in Food, Feed, and Dietary Supplements

Chemical Contaminants – Targeted

Development and Validation of a High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry Method for Determining Toxic Species of Arsenic in Fruit Juices

The presence of the toxic element arsenic in food is a public health issue. The different chemical forms (species) of arsenic exhibit a range of toxicities. The goal of this research is to develop a method for analyzing these species and generating the data needed to evaluate the actual health risk posed by arsenic in food, specifically in pear and other fruit juices. If successful, this method will allow FDA to detect the different arsenic species in fruit juices and generate data for the evaluation of the health risk.

Development and Validation of an Ion Chromatography-Inductively Coupled Plasma-Mass Spectrometry Method for Arsenic Speciation in Chicken Feed and Tissue

Arsenic-containing feed additives are given to poultry to control intestinal parasites. While most of the arsenic is eventually excreted, it is unclear whether any toxic metabolites (inorganic arsenic) remain in edible chicken tissue, which would pose an exposure risk to humans. The goal of this research is to evaluate existing methods for extracting and detecting inorganic arsenic in edible chicken tissue. Selected methods would be optimized and used to evaluate the arsenic exposure risks posed by use of arsenic-containing poultry feed additives.

Detection of Methyl Mercury in Seafood by Direct Analysis in Real Time (DART) Mass Spectrometry

Regulatory analysis of seafood for mercury contamination currently involves a lengthy process involving extraction and analysis by Inductively-Coupled-Plasma/Mass Spectrometry (ICP-MS). The goal of this research is to develop an alternative method using Direct Analysis in Real Time (DART) technology. This will allow a much reduced analytical timeframe in order to increase laboratory efficiency and maximize laboratory resources.

Analysis of Food Samples and Dietary Supplements for Mercury (Hg)

Mercury is a highly toxic element. Some dietary supplements, consumed on a daily basis, may contain mercury at levels that are unhealthy for high-risk groups such as expectant mothers and children. In the analysis of foods, mercury detection requires microwave digestion that can cause extreme safety concerns and is labor-intensive. The goal of this research is to develop a method to analyze dietary supplements for mercury using an inexpensive, rapid, and accurate technique. The primary cost savings from this approach results from elimination of labor-intensive sample preparation prior to analysis. If successful, the method would be employed to analyze and screen more food samples for mercury in less time than current methods.

Sample Preparation and Chromatography Methods for Single Residue Pesticide Analysis

Existing methods for detecting multiple pesticide residues in regulatory samples do not always recover all pesticide compounds present in a sample. This is due to numerous factors, including incompatible sample preparations or the chromatographic techniques employed. The goal of this research is to develop additional methods to identify pesticide residues, individually or by category, that are known contaminants in regulatory food samples, but are not recovered by multi-residue analytical methods. This will provide improved surveillance capability to enhance regulatory action.

Development and Validation of a Modified QuEChERS and Gas Chromatography- and Liquid Chromatography-Tandem Mass Spectrometry Methods for Analysis of Selected Pesticide Residues in Fatty Food Products

The current method employed to identify pesticides in high fat products is time consuming, solvent intensive, and screens for a limited number of pesticides. The goals of this research are to develop and validate modified QuEChERS and gas chromatography- and liquid chromatography-tandem mass spectrometry methods to significantly shorten the analysis time, decrease solvent use and screen for more pesticides. If successful this method(s) will increase laboratory productivity and expand the scope of pesticides screened thereby better ensuring public safety in a more cost effective way.

A Rapid Extraction Method for Multi-Residue Pesticide Analysis in Fresh Food Products using Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Tandem Mass Spectrometry.

Classic multi-pesticide residue analytical methods are expensive and labor intensive. The goal of this research is to address the analytical limitations of present methods by developing an environmentally friendly, less toxic, rapid multi-residue extraction procedure. FDA will validate the method using gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry instrument platforms. The method will improve upon existing procedures by providing a faster and simpler analytical method for screening for pesticide residues in agricultural produce.

High-Throughput Determination of Over 200 Pesticides Commonly Found in Regulatory Samples by Gas Chromatography/Tandem Mass Spectrometry

The global food supply-chain presents a continual challenge to the FDA in its mission to monitor the nation's food supply for chemical contaminants (pesticides & industrial chemicals). Current procedures for monitoring pesticides and chemicals require improvements in efficiency. The goal of this research is to investigate technologies, such as gas chromatography/tandem mass spectrometry, and will evaluate these methods for their ability to offer a reduction in analytical timeframes and required resources that will allow for increased surveillance and expedited sample throughput.

Qualitative Determination of Over 600 Pesticides and Chemical Contaminants Using Gas Chromatography/Mass Spectrometry

Although there are over 1,000 U.S. Environmental Protection Agency (EPA) registered pesticides, crop protection requires the development of new chemicals to address pesticide resistance. For many food exporters to the U.S., regulation of the safe use of pesticides is lacking. Thus, the FDA pesticide monitoring program must be dynamic in its vigilance. The goal of this research is to utilize food surveillance data to expand the list of compounds that have been detected in food products domestically and internationally. Additional pesticides will be added which have had EPA tolerances established recently. Efforts will be also be made to simplify data review to facilitate significant time savings and allow for increased surveillance of the food supply.

Multiresidue Analysis of Pesticides and Other Chemical Contaminants in Foods

FDA enforces tolerances for all pesticides that may be present in a wide variety of foods to ensure that the nation's food supply is safe. Because there are thousands of pesticides and food types, however, developing methods to identify, quantify, and confirm pesticides presents many challenges. This research involves improvement of current procedures for pesticide analysis and establishes validated methods to be used for regulatory monitoring and surveillance programs. If successful, this work should help ensure safer food products, such as fresh produce, dried botanical dietary supplements, and other FDA-regulated food products.

Method Extension of Poison Screening to Additional Toxic Chemicals and Food Commodities

The goal of this research is to extend and validate current methods to detect additional poisons in foods as well as to identify and improve specific detection issues. Pesticides, rodenticides, drugs of abuse, and plant toxins such as yellow oleander have been evaluated and added to the initial set of highly toxic and available poisons targeted by current methods. A variety of analytical platforms and sample treatments are being evaluated in order to improve the detection of a specific toxic chemical, sodium fluoroacetate. Modifications have been made to the current methods for fluoroacetate screening, which improve sensitivity and reduce interferences.

Methodologies for Determining Volatile Organic Compounds in Food by Using Vacuum Distillation and Vacuum Extraction Sampling Followed by Gas Chromatography/Mass Spectrometry

Volatile Organic Compounds (VOCs) occur in foods as natural components, contaminants from processing and handling, and/or contaminants from food packaging materials. Some VOCs are carcinogens, genotoxins, or neurotoxins, and their occurrence in food may impact public health. Analytical methods that reliably determine targeted VOCs in various complex foods and food packaging are needed to assess human exposure to VOCs. The goals of the research are to evaluate, develop, and validate gas chromatography/mass spectrometry methods that determine targeted VOCs in food and food packaging. If successful, the validated methods would be used to conduct food surveys and enforce current regulations. The survey data would be used by FDA to determine the impact of the targeted VOCs on public health.

Determination of Cyclic Siloxanes in Polydimethylsiloxane (PDMS) Materials

A number of toxicity studies suggested that cyclic siloxanes pose potential safety concerns. The goal of this research is to develop analytical methods for determining the concentrations of cyclic siloxanes in milk, infant formula and silicone nipples. This will help FDA in assessing whether cyclic siloxanes can migrate into milk or infant formula via direct food contact and estimate the corresponding human exposure.

Developing a Method for the Liquid Chromatography/Mass Spectrometry Detection of 3-MCPD and Glycidyl Esters in Processed Edible Oils

The processing of crude edible oils is important in removing odors, pesticides, and other contaminants, as well as giving oils a longer shelf life. During this process 3-monochloro-1,2-propanediol (3-MCPD) esters and glycidyl esters are formed which are potential carcinogens. These compounds are difficult to detect and quantitate using conventional analytical methods. The goal of this research is to develop new sample preparation and liquid chromatography/mass spectrometry detection methods. If successful, the new methods would identify and measure these contaminants and assist FDA in encouraging the development of processing techniques that minimize their formation.

Development and Validation of a Screening Method for the Presence of Polycyclic Hydrocarbons in Select Seafood using Liquid Chromatography-Fluorescence Detection

The goal of this research was to develop a screening method for the analysis of polycyclic aromatic hydrocarbons (PAHs). The screening method, based on liquid chromatography with fluorescence detection, cut the analysis time from 5- 7 days down to 24 hrs. The method's detection limit was established at a concentration of 5 ng/g, making it effective for screening purposes. This procedure is applicable to screen a variety of seafood matrices including oysters, shrimp, finfish, and crab for the presence of PAHs due to oil contamination. The method was successfully developed, validated, and published as a lab information bulletin (LIB) in 2010 and used in the reopening of the Gulf State fisheries after the Deepwater Horizon oil spill.

Development and Validation of a Method for Formaldehyde Determination in Chicken Jerky

Recently, consumer complaints have been received reporting that chicken jerky treats have caused illness or death in dogs. Formaldehyde, which is a toxic, hazardous substance has not been ruled out as the causative agent. Formaldehyde is not approved for use in foods but may be improperly added for its preservative and bleaching effects. The goal of this research is to develop and validate an analytical method to detect formaldehyde in jerky treat products as a means of determining the possibility of formaldehyde presence in the animal food supply. This method will allow FDA to investigate a potential cause of illness or death in dogs eating chicken jerky treats.

Qualitative Screening of Toxic Glycols in Glycerin-Based Products by Direct Analysis in Real Time with Orbitrap Mass Spectrometry (DART-MS)

Multiple public health threats related to the adulteration of glycerin-containing products with toxic glycols led FDA to require testing of these products. The goal of this research is to compliment the current approach by adding a qualitative screen by DART-MS, which can be performed without sample pretreatment and provides data acquisition in seconds. If successful, this method will add the capability to rapidly process samples, the great majority of which will be negative. Positively-identified samples can then be confirmed using the official method, while negative samples would not require this, which saves time and resources. In addition, this method would increase the FDA's readiness in the event of another public health emergency related to this issue.

Liquid Chromatography/Mass Spectrometry Determination of Biogenic Amines in Seafood and Matrix Extension

A quantitative method has been developed to identify numerous biogenic amines in canned and frozen tuna that are indicative of product degradation. These compounds include agmatine, cadaverine, histamine, phenyethylamine, putrescine, tryptamine, tyramine, and urocanic acid. The goal of this research is to extend this method to the analysis of biogenic amines in non-tuna food matrices that are known to be associated with scombroid poisoning, including canned and frozen mackerel and frozen mahi-mahi. Salmon and Thai fish sauce will also be tested. This effort will serve to extend the range of food products that can be monitored for these product degradation indicators.

Analysis of Polyaromatic Hydrocarbons (PAH) in Seafood Using Gas Chromatography/Mass Spectrometry

In the event of an oil spill, current regulations require that seafood samples be analyzed for polyaromatic hydrocarbons (PAH) contamination before the fisheries can be reopened. Because of low PAH concentrations (>100 ppb) and the complex seafood tissue matrix, pre-concentration and cleanup are necessary prior to introduction onto analytical instrumentation. The goal of this research is to develop, optimize and validate an automated solid phase microextraction (SPME)-gas chromatography/mass spectrometry method for the determination of PAHs and PAH homologs in seafood. If successful, this technique has the potential to reduce analysis time and decrease analysis costs, and could provide FDA with a tool for rapid response to future oil spills.

Polyaromatic Hydrocarbon Analyses by Stir Bar Sorptive Extraction and Thermal Desorption Injection on Gas Chromatography/Mass Spectrometry - A Collaborative Study

The current analytical response to an oil spill, such as the Deepwater Horizon, includes the application of a complex method of analysis for polyaromatic hydrocarbons (PAHs). The complexity of this method limits the analytical output capability to about two results per week per analyst. The goal of this research is to develop a new technology that provides a rapid, simple and inexpensive method for extraction of PAHs, which will allow for the analysis of up to

five samples in two days per analyst. If successful, this will increase productivity, decrease turnaround time and decrease needed resources during emergency response situations.

Determination of Perfluorinated Carboxylates and Sulfonates in Fish

Wide scale industrial use of perfluorochemicals (PFCs) has led to their global distribution in the environment. The high amounts of PFCs detected in river water and soil samples indicate that fish may be a large source of human dietary exposure. Previous studies show that PFC concentrations in fish vary greatly depending on geographical location, fish type, and which part of the fish is tested. The goal of this research is to develop an analytical method for the determination of PFCs in fish. If successful, this method could be used to survey fish for determining the variability of PFCs concentration and human exposure.

Screening of Phthalates by Direct Analysis in Real-Time (DART)/Orbitrap Mass Spectrometry

A recent food safety issue involves the contamination of a broad range of food and nutraceutical products from Taiwan with industrial plasticizers. The goal of this research is to develop an analytical method to rapidly qualitatively analyze these compounds in a wide variety of suspected food and nutraceutical matrices. The method will utilize direct analysis in real-time (DART) coupled to an orbitrap mass spectrometer, which will allow a very rapid qualitative screen. This will greatly increase FDA's analytical capability to respond to this type of food emergency.

Development and Validation of Isotope Methods for Distinguishing Between Naturally Occurring and Synthetic Phthalates in Food

Contamination of the food supply with suspected endocrine disrupters, such as phthalates, is of great concern, particularly for young children and woman of childbearing age. Faced with the prospect of regulating phthalates in food, which may or may not be derived from natural processes, it would be beneficial for FDA to have a method to accurately determine the natural and industrially derived phthalate ester contributions in food matrices. The goal of this research was to develop a method for separating and determining phthalate analytes (specifically diethylhexyl phthalate) from fatty foods (cheese). This result of this research demonstrated that the method could be used to extract and quantify phthalate analytes in foods and help determine human exposure to phthalates from food.

Development and Inter-Laboratory Validation of a Modified QuEChERS (quick, easy, cheap, effective, rugged, safe) process and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Method for Analysis of >200 Pesticides in Fatty Food Products

The QuEChERS process and LC-MS/MS methods have been applied to the development of improved multi-residue methods for pesticide analysis. A 2009 multi-lab collaborative validation was based on analyses of 180 pesticide residues in high moisture products. The purpose of this research is to perform a method modification and matrix extension using the QuEChERS approach. This new method using LC-MS/MS detection will be validated for 250

pesticide residues in high and low fat food products (specifically, milk, salmon, shrimp, olive oil, almonds and avocado). The development of this method will broaden FDA laboratory capability for analysis of pesticide residues in food products.

Isolation and Concentration of Crystals from Kidney Tissues

The two major melamine adulteration events (U.S. pet food in 2007 and China infant formula in 2008) demonstrated the need for rapid methods to test large numbers of food samples. Melamine-cyanurate crystals can form in the kidneys of animals that eat contaminated food. FDA is evaluating several ways of isolating and enriching these crystals from the kidney using various solvents and centrifugation techniques and working with other food safety laboratories (Food Emergency Response Network and the USDA's Food Safety Inspection Service) to improve existing testing methods in various food matrices. This project will (1) increase FDA's capability to rapidly analyze large numbers of samples for presence of melamine and cyanuric acid at or above the level of concern; (2) facilitate interaction with state laboratories for timely exchange of information that can be crucial in the event of an outbreak; (3) help the Agency gain insight into the potential for residue accumulation with varying levels of melamine or cyanuric acid feed contamination; (4) help the Agency conduct risk assessments to maintain animal and food safety; and (5) help the Agency to evaluate selected commercially available kits in analysis of melamine to improve preparedness in the event of feed or food contamination.

Development of a Liquid Chromatography-Mass Spectrometry Method for Phorbol Esters in Jerky Treat Products

Recently, FDA found out that some pet food manufacturers may have substituted non-food grade glycerin for the more expensive food grade product in certain edible products for pets. One of the byproducts of biodiesel production is glycerin. The Jatropha plant is a major source of oil for biodiesel fuels. Non-food grade glycerin made from Jatropha plant may contain toxic compounds, namely phorbol esters. Phorbol esters or phorbol ester breakdown products could be toxic contaminants in non-food grade glycerin. Use of this glycerin could result in these toxic compounds being present in an animal feed. Additionally, some of the breakdown products could prove to be a marker for the unapproved use of non-food grade glycerin in products. The goal of this research is to develop methods to detect toxic phorbol esters. If successful, the method could support rapid detection of these contaminants and allow FDA to take regulatory action against the use of glycerin that may be unsafe for use in animal feed.

Development of a Liquid Scintillation Counting Method for Determination of Gross Alpha and Beta Radioactivity in Foods

To effectively detect and respond to radioactive food contamination, the FDA must maintain adequate radioanalytical capability and surge capacity for screening alpha/beta radioactivity in foods. At present the limitations in our regulatory capabilities stem from the laborious and time-consuming procedures needed to detect alpha/beta radioactivity at regulatory levels. This limits the agency's ability to promptly obtain sufficient radiological data for effective decision-making and response. We will develop and validate a rapid and robust solid-phase extraction, liquid

scintillation counting technique to expedite the FDA's ability to respond to food safety threats within the first 24-hours after a nuclear incident.

Radium Measurements in Foods Utilizing a Sequential Precipitation Method

Radium isotopes are long-lived radionuclides with a long chain of radioactive alpha, beta and gamma-emitting progeny. Radium occurs naturally in soil and rocks and may concentrate in nature due to its physical, chemical, and biological properties. Radium is chemically and biologically similar to calcium in that it concentrates in both milk and bone making it both more likely to be found in a child/infant diet and a source of life long exposure after consumption. This project seeks to test the efficacy of the water method for identification of Radium-228 in food matrices including milk, dairy infant formula, and juice matrices. A robust assay method will allow the FDA to routinely analyze samples for radium contamination.

Method Development Plan for Radium Measurement in Apple Juice

Radium-226 is a long-lived, alpha-emitting radionuclide with a long chain of radioactive alpha, beta and gamma-emitting progeny that occurs naturally in soil and rocks and may concentrate in nature due to its physical, chemical and biological properties. Radium is chemically and biologically similar to calcium in that it concentrates in both milk and bone making it more likely to be found in an infant diet and a source of life long exposure after consumption. The goal of this research is to develop and validate an analytical method for the identification of Ra-226 in apple juice which will enhance surveillance capabilities for the presence of this contaminant in liquid products.

Method Validation of Food Matrices for Tritium Analysis

FDA will validate a method for the analysis of tritium, a radionuclide of health concern, to extend the applicability of the method to more varieties of foods. Water containing tritium may be inadvertently released into the local environment around nuclear reactors due to a nuclear power plant incident, potentially turning up in groundwater and soil. The U.S. Environmental Protection Agency sets limits to tritium levels in drinking water and the FDA monitors tritium content of food grown in the vicinity of nuclear reactors. The goal of this project is to validate a rapid method for analyzing tritium in various foods. This method will also enhance our ability to process large sample numbers that may result from a nuclear power plant emergency.

<u>Chemical Contaminants – Non-targeted</u>

Physical Chemistry Models Study for Sample Preparation

Food sample preparations for chemical analysis, such as multi-pesticide residue analysis, employ various methods to clean up the sample extracts without compromising the target analytes. The goal of this research is to employ physical chemistry models to analyze the different procedural steps chosen for sample preparation. This will provide a tool for method development, for predicting the limitation and performance of a method, for optimizing experimental design, and

for troubleshooting. The results will ultimately provide a systematic approach to process improvement and analytical quality control.

Using Comprehensive 2-D Gas Chromatography-Time-of-Flight Mass Spectrometry for the Analyses of Polychlorinated Biphenyls and Polybrominated Diphenylesters

Exposure to persistent organic pollutants has been linked to an increased incidence of cancer, neurological disorders and other long-term health concerns. Additional concerns exist with infant exposure, both pre-natal and via breast milk. Exposure to these pollutants is generally obtained through dietary intake of fats or lipids. The goal of this research is to analyze multiple families of these compounds using 2-D gas chromatography coupled with a time-of-flight mass spectrometer during a single analytical procedure. This method is currently being used in response to the European Union requirement to quantify polychlorinated biphenyl levels in raw milk and egg samples.

Radiological Exercise 2011

The goal of this research was to extend a liquid scintillation method for screening select alpha and beta radionuclides in a wide variety of foods. A total of 120 foods from food categories of beverage, dairy, meat, vegetation, grains, and composite meal were included in this collaborative study. The foods were selected by taking into account the contamination pathways, typical compositions, customary consumptions and analytical complexity. The study provided useful data to evaluate the method performance, a set of reference values for extraction yields associated with different food groups and assessed the competency of the participating laboratories. This method is now available for Agency use in the event of a radiological event that may contaminate the food supply.

Targeted and Non-targeted Mass Spectrometry Techniques for Pesticides and Persistent Organic Pollutants in Foods and Dietary Supplements

There are more than 2,000 pesticides in use worldwide on foods and current FDA methodology detects residues from only fraction of these chemicals. The goal of this research is to develop advanced mass spectrometry techniques and computerized mass spectral libraries in establishing a new paradigm for the detection of pesticides residues and persistent organic pollutants (POPs) and other contaminants. The procedures developed will help in the eventual improvement of unknown detection and identification of food safety relevant chemicals by FDA. The ultimate goal of these studies is to enable FDA to be more proactive in detecting pesticides, POPs and previously unrecognized chemical contaminants in foods before increased numbers of Americans are exposed to these compounds.

Advancement Poison Screen Data Interpretation by Development of Standardized Mass Spectral Libraries

The goal of this research is to develop a searchable spectral library to provide consistency in the ability of laboratories with varying expertise to screen for and identify unknown compounds. A set of tuning and operating parameters to provide uniform results for all models of the ion trap

liquid chromatography/mass spectrometry instruments used in the poison screen and currently deployed has been defined. Individual standard solutions of more than 100 compounds have been prepared. Each participating laboratory analyzes individual standards using the defined conditions to obtain the mass spectral data. Spectral data is double-checked for accuracy and consistency and compiled into a library available to food analysis.

Developing Targeted and Non-targeted Data Analysis Tools for Mass Spectral and Spectroscopic Analyses

Methods to screen for new or unexpected contaminants in foods are required to protect the public from the use of adulterants in foods. Although powerful spectroscopic tools for contaminant identification exist, software tools for the rapid analysis of data are needed. The goal of this research is to develop software to reduce data processing time of liquid chromatography/mass spectrometry and spectroscopic data. If successful, the software developed would identify components that appear to be contaminants, determine whether these components are on existing target lists, and provide putative identifications of unknowns. These tools could reduce discovery time, allowing FDA to focus on identification and the eventual remediation of the contaminants in food.

Improvement and Optimization of Anticoincidence Instrumental Neutron Activation Analysis of Foods for Toxic and Nutritional Elements

FDA has ongoing needs for new methods to accurately measure toxic and nutritional element levels in foods and related products such as dietary supplements. The goal of this research is to optimize instrumental neutron activation analysis for the simultaneous determination of low levels of important elements (including arsenic, mercury, cadmium, selenium, and antimony) in FDA regulated consumer products with the aim of lowering detection limits for these elements. This work has potential applications for emerging areas of public health interest, including silver (from nanoparticles), uranium and thorium (radioactive elements) in food, and product origin determination.

Development and Validation of Analytical Methods for Chemical Food Safety Hazards

Due to the increasing amounts of imported produce and botanically-derived ingredients on the market, there is a need for simple methods for rapidly detecting chemical hazards, such as pesticides, in order to reduce levels of these hazards in food. The goals of this research are to evaluate and validate a method for detecting a variety of pesticides in botanical ingredients and then use it to study the effects of washing treatments on pesticide removal from different produce and from other botanical materials. Information gathered in this study would help regulators and the food industry identify better methods for detecting pesticide residues in botanical materials, and would result in ways to reduce levels of these chemical contaminants in food.

Development of a Field Capable Device Designed to Reduce Environmental Sampling Analysis from Five Days to Hours

Foods and devices are often contaminated during handling with pathogens that can sicken or kill. Environmental screening programs are implemented with the intent of reducing contamination during handling. Environmental screening analyses currently require at least five days to complete. Nanotechnologies are emerging which could allow this analysis to be completed in minutes. The goal of this research is to evaluate a new nanotechnology, oxidative Chemical Vapor Deposition (oCVD), which could allow environmental screening to be conducted in minutes rather than days. If successful, this technology would result in a cost-effective tool that could be employed widely by FDA and industry alike, reducing or eliminating contamination and disease.

Methods for Authentication of Skim Milk Powder and Fruit Juice Food Ingredients

Chemical contaminants can be introduced into foods through accidental or intentional means. Of those contaminants added intentionally, some are added as economic adulterants, while others are added for malicious intent. Economically motivated adulteration (EMA) presents a persistent and significant challenge in the market place. Skim milk powder and fruit juice are frequent targets for EMA. The goal of this research is to evaluate several methods for detecting chemical adulterants in skim milk powder and fruit juices. Results from this research would enable the food industry and regulators to establish reliable methods for authentication of foods and ingredients and for detecting the presence of chemical adulterants.

Development, Evaluation, and Single Laboratory Validation of In-line Turbulent Flow Extraction-Tandem Mass Spectrometry (TF-MS/MS) Methods for Food Adulterants

Adulteration of foods for economic purposes can expose the public to significant health risks, as was the case with melamine adulteration of high protein foods. Analysis of adulterants can be slow and labor intensive, with low sample throughput. The goal of this research is to develop, evaluate, and validate an in-line sample preparation and analysis technology based on turbulent flow chromatography/tandem mass spectrometry to reduce sample preparation and analysis times from hours to minutes. Model adulterant systems such as melamine and cyanuric acid in infant formula would be used to optimize this approach. If successful, the resultant methodology could allow FDA to analyze a larger number of samples more quickly when confronted with situations where foods are adulterated.

Developing Ambient Ionization-Mass Spectrometry to Rapidly Screen Food for Adulteration, and Packaging & Cosmetics for Quality/Compliance

Food and food packaging can be chemically contaminated in many ways, but which chemicals and which products are not known in advance. Contaminant identification can take days or weeks even when a problem is known to exist. The goal of this research is to develop ambient ionization-mass spectrometry methods to rapidly identify chemical contaminants and screen food and packaging for unknown contaminants. These techniques will give FDA the capability of detecting and identifying unexpected chemical contamination faster, with less effort. This

identification should allow quicker control of contaminated products limiting consumer exposure to chemical contaminants. It will also allow more targeted enforcement, and more relevant health advice.

Toxins

Development and Validation of Improved Microchip Surface Chemistries and Detection Techniques for More Sensitive Surface Plasmon Resonance Detection of Small (Toxins) and Large (Bacteria/Viruses) Analytes

A FDA extramural cooperative research grant was awarded to the University of Washington to develop novel affinity biosensor technology for rapid, sensitive, specific multi-analyte detection of foodborne toxins and pathogens. Collaboration between the Department of Chemical Engineering at the University of Washington (UW) and the Institute of Radio Engineering and Electronics (IREE) at the Academy of Sciences of the Czech Republic in Prague has resulted in the development of a multi-channel surface plasmon resonance (SPR) instrument with unique detection capabilities. Collaboration between FDA, UW, and IREE is aimed at acquiring, improving and evaluating advanced biosensor technology capable of rapid, sensitive and accurate multi-analyte detection in foods. The immediate benefit of this project is an improved detection capability using SPR biosensor technology for detecting marine biotoxins and pathogens. Results will enhance food safety and food security research and regulatory activities at FDA.

Surface Plasmon Resonance Biosensors for Antibody Screening and Quantitative Analysis of Foodborne Toxins

Paralytic shellfish poisoning (PSP) arises from consumption of toxin contaminated seafood. The ability to determine sample toxicity is a challenge due to the large number of PSP toxins, which are produced by an alga, *Alexandrium fundyense*. The goal of this research is to develop a surface plasmon resonance (SPR) assay for improved detection of the PSP toxins. FDA will also investigate new biological reagents that could better detect sample toxicity from the SPR assay measurements. If successful, this real-time technique could improve upon current tests with respect to reliability, efficiency, and sensitivity and better protect the public from consuming potentially toxic shellfish.

Biomarker Identification and Method Development for Neurotoxic Shellfish Poisoning Toxins in Clams

Neurotoxic shellfish poisoning (NSP) of seafood consumers is caused by eating shellfish that have concentrated toxins (brevetoxins) from the red tide algae *Karenia brevis*. Red tides occur periodically in the Gulf of Mexico, and along the southeastern coast of the U.S. Prevention of NSP is currently managed by monitoring coastal waters for red tides and testing shellfish by mouse bioassay. The goal of this research is to develop and validate a faster and more accurate method to test clams (*Mercenaria* spp.) to replace the mouse bioassay. The improved method

could be used by FDA, States, and the seafood industry to prevent the harvest and distribution of NSP toxin contaminated shellfish.

Single Laboratory Validation of an *In Vitro* Cell Cytotoxicity Assay and Confirmatory Liquid Chromatography-Mass Spectrometry (LC-MS) Method for the Determination of Ciguatoxins in Finfish

Ciguatera fish poisoning (CFP) of seafood consumers is caused by eating tropical and subtropical fish that have concentrated highly potent neurotoxins (ciguatoxins: CTXs) from microscopic algae (*Gambierdiscus* spp.). It is estimated that more than 100,000 people world-wide are affected every year by CFP. Currently available methods to test fish for CTXs are not validated. The goal of this research is to optimize and validate *in vitro* cell assay and LC-MS methods. If successful, these methods could be used in the development of testing programs by FDA and States, and support efforts to prevent toxic fish from entering commerce.

Development and Refinement of Methods for Emerging Marine Toxins

Marine toxins can accumulate in seafood and pose a risk to U.S. consumers. They are regulated through appropriate monitoring and management programs. Only a select few of the numerous described marine toxins have established FDA guidance levels, including diarrheic shellfish poisoning toxin (DSP). However, there are no established methods for analysis of DSP. In other cases, emerging toxins such as palytoxin have no guidance levels yet, in part because there are no reliable methods for analysis. The goal of this research is to develop methods for screening and quantification of DSP toxins and palytoxin. If successful, these methods would support FDA's monitoring and management programs for DSP, and support development of guidance on palytoxin.

Screening and Confirmatory Methods for Cyanobacteria and Their Toxins in Blue-Green Algal Supplements

Blue-green algal (BGA) supplements are marketed for elevation of mood, increased energy, and to help with attention deficit disorder. While these products are made directly from cyanobacterial blooms in freshwater lakes, there is the possibility of product contamination from a co-occurring species known to produce a group of liver toxins and potential tumor promoters. There are no validated methods to determine the presence of such toxins in BGA supplements. The goal of this research is to develop and validate methods to rapidly screen BGA products for toxin-producing algae and the toxins themselves, and develop methods for toxin quantification. If successful, these methods could be used to ensure that BGA supplements are safe and free of these toxins.

Improved Detection Methods for Domoic Acid

Domoic acid (DA) is an algal-derived toxin that accumulates in shellfish. Amnesic shellfish poisoning is the human illness that may manifest as a result of dining on seafood contaminated with high levels of DA. Shellfish harvesting is banned in areas where toxin levels exceed 20 ppm and improved detection methods could help to prevent contaminated seafood from entering

into interstate commerce. The goals of this research are to evaluate, develop, and validate new sample preparation and extraction methods, screening methods, and liquid chromatography-based confirmatory analysis methodologies for DA. If successful, this work could improve FDA's rapid screening capabilities and provide faster, more reliable methods for regulatory enforcement.

Development and Evaluation of Methods for Mycotoxins in Foods and Dietary Supplement Raw Ingredients

Molds may occur on agricultural products used to produce foods or dietary supplements. These molds may produce toxic substances known collectively as mycotoxins. Many of these mycotoxins are toxic to humans. FDA lacks information on the occurrence of specific mycotoxins (aflatoxins, zearalenone and deoxynivalenol) in consumer products where they may be present. The goal of this research is to develop and validate analytical methods for these mycotoxins to accurately measure their levels in products commonly consumed (flour, corn meal, pasta, ginger, ginseng, brown rice, oat flour, and soybeans). If successful, FDA would be able to monitor the food supply for the occurrence of these mycotoxins and develop a regulatory strategy for protecting consumers.

Rapid Test Kits for Histamine in Fish: Investigation and Validation

To reduce scombroid poisoning due to mishandling of seafood in the marketplace, FDA laboratories test for histamine levels in susceptible fish. Since the currently accepted official method for histamine testing is based on a time-consuming procedure, the goal of this research is to investigate three commercial rapid test kits and determine the best one for regulatory use. Two of these kits, one an enzyme-linked immunoassay (ELISA) and another an enzymatic assay, will be investigated further in an inter-laboratory study.

Development of Liquid Chromatography/Mass Spectrometry (LC/MS) Methods for Detection of Mycotoxins in Animal Feed and Feed Ingredients, Including Distillers Grains

In the U.S., contamination of crops by mycotoxins occurs widely due to growth of molds in farming and storage. These mycotoxins pose significant threats to animal health when domestic animals eat contaminated feed. Subsequently, if humans consume animal-derived products, they may become sick. The goal of this research is to develop a more efficient analytical method to determine the quantity and confirm the identity of multiple mycotoxins in feed, especially dry distiller's grains (DDGs). The DDGs are a byproduct of ethanol production. The large increase in fuel ethanol production has resulted in a large increase in the use of DDGs for animal feed. Data gaps exist concerning the presence of mycotoxins in DDGs associated with the use of myxotoxin-contaminated grains in ethanol production. The LC/MS method developed will enhance the ability of FDA and other U.S. agencies to monitor and mitigate the health risk brought by mycotoxins in animal and human food chains.

Evaluation of *Jatropha* species Toxic Compounds as a Potential Food Safety Risk From Glycerin Produced During Biodiesel Production - Developing Detection Methods

Jatropha curcas is a drought-hardy shrub being developed as a source of vegetable oil for biodiesel production. However, most Jatropha plants worldwide are toxic, causing severe gastroenteritis and disrupting liver and kidney functions. Glycerin can be produced from oil of the Jatropha plant. If glycerin made from Jatropha oil is not detoxified, it will contain toxic chemicals. Foods or drugs made with such glycerin could pose a serious risk to both humans and animals. FDA needs robust methods to detect Jatropha toxins in foods or drugs to deter use of any ingredients which may contain these harmful compounds. The goal of this research is to develop chemical and biological assays for toxins from the Jatropha plant. If successful, these methods will help to mitigate this emerging food safety threat.

Drug Residues and Hormones

Incursion of Residues for Bridging of Chemical and Microbiological Detection Methods

The official microbial methods to detect antibiotics are outdated. They often require equipment that is no longer available or use chemicals that are unsafe. New chemical methods will replace microbiological methods after their equivalence to the older microbiological methods has been established. Residue-incurred tissues are an essential part of validation of the new chemical methods. The goal of this research is to provide tissues with incurred drugs to the method development chemists.

Incursion of Drug Residues in Milk

The use of veterinary drugs in food animals must be carefully monitored. Drug usage that differs from the label instructions affects food safety. Monitoring of drug residues in milk from dairy cattle requires accurate analytical methods. Methods for the detection of drug residues in milk must be validated. To this end, the intentional incursion of drugs in milk is required. The goal of this research is to provide the incurred milk samples necessary for method validation. These methods will be used to detect drug residues in bovine milk. FDA scientists require incurred bovine milk samples to validate residue detection methods. These methods are used for post-approval monitoring of compounds that are not approved for use in lactating dairy cattle. If successful, the methods will be used in the milk survey conducted by during 2012.

Incursion of Drug Residues in Food Animal Tissues

FDA often receives requests, both internally and from external stakeholders such as the Veterinary Laboratory Response Network, for food animal tissues that contain drugs or other compounds. The goal of this research is to provide the incurred food animal tissues required for analytical method development and validation and respond to the many incursion requests. Developed methods will be used for post-approval monitoring of drug use in food animals.

Residue Incursion in Fish

The U.S. imports most of the seafood it consumes, and much of that seafood comes from fish farming. A large portion of domestic production is also from fish farming. The FDA needs to monitor for illegal use of chemicals such as sedatives, antibiotics and hormones. The goal of this research is to provide incurred residues in edible fish tissues. These tissues will be used by FDA scientists for development of methods for detecting illegal drugs and chemical contaminants in seafood.

Evaluation of Rapid Screening and Confirmatory Methods for Residues of Chloramphenicol and Nitrofuran Drugs in Aquaculture Products

Aquaculture is an important source of seafood worldwide. Drugs are often used to treat diseases that occur in farm-raised fish and shellfish. Regulations for the use of drugs in food animals vary between countries. To comply with U.S. Federal and state regulations, domestic and imported seafood products must not contain unapproved drug residues. The misuse of approved drugs or illegal use of unapproved drugs can lead to unsafe residues in aquaculture products. The goal of this research is to develop and validate rapid screening and confirmatory testing methods to monitor drug residues in aquaculture products. If successful the methods could be used by FDA and the seafood industry to prevent drug-tainted aquaculture products from entering U.S. commerce.

Improvement of a Liquid Chromatography/Tandem Mass Spectrometry Method for Determination of Total Nitrofuran Metabolites in Seafood

Nitrofurans are broad spectrum antibiotics used in feed additives for food-producing animals. The FDA limits the quantity of these residues to 1 part per billion because of their toxicity and mutagenicity. Analysis of nitrofurans is challenging because they rapidly hydrolyze in the tissues, forming muscle-bound metabolites. The goal of this research is to make improvements to the existing analytical method to properly monitor this hydrolysis and derivatization step by using an internal standard and matrix standards. FDA will then validate the method for shrimp and catfish and monitor the assay performance. If successful, the protocol will be submitted for publication to make the improved method globally.

Development of a Liquid Chromatography-Tandem Mass Spectrometry Method for the Detection of Nitrofuran Metabolites in Catfish

The use of nitrofurans in aquacultured or 'farmed' seafood is well documented, with catfish being the number one farmed species. With the increase in the amount of farmed seafood being produced and the decrease in the wild species being caught, the consumption of farmed seafood is expected to increase. Therefore, rapid and sensitive methods for the detection and quantification of nitrofurans in various seafood species, including catfish, are required to protect the American food supply. Current methods for the detection of nitrofurans in catfish are labor intensive and time consuming. The goal of this research is to develop a new method that will decrease sample preparation time by approximately 33% thereby greatly increasing sample throughput.

Direct On-Line HPLC Analysis of Multiple Antibiotics in Fish Using Restricted Access Materials Technology

Restricted Access Materials technology is a new tool for use with High Pressure Liquid Chromatography (HPLC) that allows for multi-analyte screening of tissues with very little sample preparation. The use of this technology enhances surveillance of the American food supply by more efficient utilization of both the FDA's workforce and its analytical instrumentation. Development of this technology will provide FDA analysts with the ability to analyze more samples in less time using instrumentation that is readily available in analytical labs (i.e. HPLC) while reserving the use of highly sophisticated instrumentation (i.e. LC-MSMS) for those samples with a higher probability of being violative.

Identification of Stilbene Residues in Fish by Liquid Chromatography/Tandem Mass Spectrometry

Stilbenes are synthetic estrogen compounds formerly used in human medicine for reproductive conditions and for livestock growth production and treatment. Diethylstilbestrol (DES) is an endocrine disruptor that causes cancer and birth defects in humans. DES and other stilbenes are banned for food animal production in the U.S. and E.U. DES has been investigated for both growth promotion and for reversing gender in farmed seafood. The goals of this research are to develop an analytical method to detect residues of stilbenes in aquaculture fish and to determine the possibility of harmful stilbene residues present in the food supply. Validated methodology will help the FDA establish a monitoring program to test for residues of these compounds in seafood products.

Determining Isoeugenol Concentrations in Edible Fish Fillet Tissue

Anesthetics are used in the aquaculture industry to reduce the stress levels of fish during handling, transport, or harvesting. One such product is AQUI-S in which the active ingredient is isoeugenol. The residual levels of this anesthetic in fish may adversely affect human and animal food safety. The goal of this research is to validate the accuracy, precision and sensitivity of a quantitative method for the detection of isoeugenol in fish and will provide the FDA with a means to prevent potential adverse effects resulting from antibiotic contamination of human and animal food products.

Simultaneous Screening and Confirmation of Multiple Classes of Drug Residues in Fish by Liquid Chromatography-Ion Trap Mass Spectrometry

Veterinary drugs are widely used for the treatment and prevention of disease in fish. But uncontrolled use of these drugs can leave residues in edible tissues, which can lead to health problems for consumers. The goal of this research is to develop a multiresidue liquid chromatography-ion trap mass spectrometry method to effectively monitor the drug residues in seafood as a means of ensuring the safety of fish products, whether produced domestically or imported. If successful, this method will be utilized for simultaneous screening and confirmation of multiple classes of drug residues in fish.

Bridging of a Quantitative and Confirmatory LC-MS/MS Method to the Microbiological Method Used to Set the Tolerance of Oxytetracycline, Tetracycline, and Chlortetracycline in Bovine Kidney

Tetracyclines are widely used antibiotic veterinary drugs for treating and preventing infectious diseases in many food-producing animals such as cattle. However, if used improperly, these drug residues can remain in the food products and can cause health problems in consumers. The current regulatory method for detecting unsafe tetracycline drug residues was developed in the 1960's and is now obsolete. A new and more efficient method has been developed. The goal of this bridging research is to establish a correlation between the new and old methods. If bridged successfully, the new method will replace the obsolete regulatory method in FDA surveillance and compliance activities.

Development and Validation of a Quantitative and Confirmatory Liquid Chromatography/Tandem Mass Spectrometry Method for Bridging to the Regulatory High Performance Liquid Chromatography/Ultraviolet Method Used to Determine Ceftiofur in Bovine Kidney

Ceftiofur is an antibiotic veterinary drug widely used to treat infectious diseases in many food-producing animals such as cattle. However, if used improperly, drug residues can remain in the food products and can cause health problems in consumers. The current regulatory high performance liquid chromatography with ultraviolet detection method for determining unsafe levels of ceftiofur residue has become obsolete. The goal of this research is to develop and validate a more efficient method based on liquid chromatography/tandem mass spectrometry. Once validated, the new method will be bridged to the existing regulatory method and implemented in FDA surveillance and compliance activities.

Development of a Quantitative Multiresidue/Multiclass Procedure for Determining Drug Residues in Shrimp

Shrimp is one of the most frequently consumed seafood in the U.S. A large portion of the product on the market is imported and comes from aquaculture. Producers use veterinary drugs to prevent or treat disease. Some of the drugs used are approved for use in other animals but not in shrimp and other drugs used are not acceptable for use in food animals anywhere in the world. This can lead to unsafe drug residues in shrimp meat, which poses a potential health risk to consumers. FDA has a program to monitor aquaculture products, but the number of samples tested is often limited due to resource constraints. The goal of this research is to develop a new, efficient method which can monitor many animal drugs in shrimp meat at the same time. If successful, the method will be used by FDA to monitor the presence of veterinary drugs in shrimp on the market, and to take action to prevent shrimp meat containing high levels of drug residues from being marketed. Additionally, this method may be used as a template for prospective drug sponsors to develop the information needed to demonstrate the human food safety of their products.

Animal Feeds and Ingredients: Development of Mass Spectrometric Methods for Drugs and Contaminants Using Targeted and Non-targeted techniques, and Differential Analysis

Contaminants in feed can result accidentally or through adulteration. Current methods for testing of feed are based on targeted analysis, looking for specific compounds or groups of compounds. The goal of this research is to develop methods to analyze drugs and other contaminants in feed based on non-targeted analysis. The development of non-targeted methods has become more widespread with the rapid development of analytical instrumentation and increases in computing power. If successful, the method would support rapid detection of contaminants and help FDA to ensure feed safety.

Method Development for the Determination of Drugs in Fish Feeds

Bacterial kidney disease (BKD) is widespread among salmon populations in the U.S. and this is a problem for salmon farming. An effective drug is known to control BKD; however, this drug is not approved by the FDA to be used in salmon hatcheries. Congress has authorized FDA to provide publicly available information that can be used by a company to obtain FDA approval for a product in niche markets, such as aquaculture. These markets are known as "minor use minor species." One of the requirements for approval is to provide an analytical method that can verify the amount of drug in the medicated feed. The goal of this research is to develop such a method for this drug in salmon feed. If successfully developed, the method could support drug approval process and allow the drug to be used for salmon to lessen mortality.

Determination of Quinolone Residues in Fish by Laser Diode Thermal Desorption Mass Spectrometry

Seafood testing for the presence of unapproved antibiotic drug residues presents many analytical challenges. Fish is a complex matrix and extracting and separating residues from the matrix for analysis is often a lengthy process. The goal of this research is to develop a rapid method to detect quinolone antibiotic residues in edible fish muscle based on the direct sample introduction technique of laser diode thermal desorption mass spectrometry (LDTD-MS/MS). LDTD allows sample analysis in 30 seconds or less per sample. We will explore the use of LDTD for regulatory residue screening and residue confirmation of identity. The development of rapid analysis techniques will provide the FDA with tools to increase sample testing capacity.

Screening for Non-targeted Veterinary Drug Residues in Milk and Aquacultured Species Using High Resolution Mass Spectrometry

In order to protect the food supply from chemical contaminants, the FDA needs to monitor for chemical residues that are not on any predetermined list. The goal of this research is to develop broad screening methods for veterinary drug residues in food using high resolution mass spectrometry. High resolution mass spectrometers provide accurate mass measurements that facilitate the identification of unknown compounds. If successful, FDA will utilize this technology to greatly expand the number of drug residues that can be detected in foods such as milk and farmed fish and publish data that will help other laboratories more readily detect and identify these chemical contaminants.

Incurred Residues of Hormones in Bovine Tissues

The use of hormones in food-producing animals has been grounds for disputes between E.U .and U.S. for many years. In addition, hormones might be used illegally in animal meat production, causing residues to occur in the meat products. Because of these issues FDA has a tremendous need for specific and accurate assays for multiple hormone residues. The goal of this research is to provide beef tissues containing these target hormones required for detection method development and validation.

Development of a Multiresidue Method for the Determination of Hormones in Bovine and in Fish Muscle Tissues

Hormones are used in animals to enhance weight gain and feed efficiency. While several hormones are approved for use, there is a concern that amounts which exceed the label directions are being used. Additionally there is a concern that some producers may be using hormones that should not be used in food animals. These illegal uses could cause harmful residues to be present in meat and are a public health concern. The goal of this research is to develop methods to measure hormone residue levels in meat. When developed, these methods will help regulatory agencies ensure the safety of animal derived foods.

Allergens and Gluten

Development and Validation of Multiplex (Bio-Plex) Immuno-based Analytical Method for Determination of Allergenic Food Components or Allergens

Over 11 million individuals in the U.S. are susceptible to allergens in foods. Many rely on a strict avoidance diet to prevent serious reactions. Despite the Food Allergen Labeling and Consumer Protection Act (FALCPA), mislabeling still occurs due to the inadvertent presence of allergens. There are eight major allergenic food groups (egg, milk, soybeans, wheat, peanuts, tree nuts, fish, and shellfish) identified by FALCPA. Currently available commercial allergen test kits can only test for a single allergen at a time. The goal of this research is to develop a method that will enable the detection of multiple allergenic food groups simultaneously. If successful, this method would enhance food safety by helping the FDA's enforcement of the food allergen labeling regulation.

Detection of Food Allergens Using Commercial Test Kits

The lack of a cure for food allergies means that over 11 million food allergic consumers must rely on the accuracy of food labels and the enforcement of the Food Allergy Labeling Consumer Protection Act (FALPCA) of 2004 to avoid consuming food allergens. To confirm the accuracy of the labels and compliance with FALPCA, the FDA uses commercial immunodiagnostic enzyme-linked immunosorbent assay (ELISA) test kits as part of the methodology employed in the analysis of food samples. The research being undertaken will enable the application of test kits and the development of the necessary methodology to detect the presence of food allergens and help assure the accuracy of allergen labeling.

Rapid Methods for the Detection of Food Allergens and Toxins

The current method used by FDA for detecting food allergens and proteinaceous toxins is based on selective binding of a protein to a capture agent, which is slow and unreliable. The goal of this research is to develop a method that accelerates the binding reaction (resulting in equilibrium) which will maximize the response generated, which increases sensitivity and reduces variability (increases reliability). If successful, this work will provide faster, more cost effective methods for detecting food allergens and toxins and help FDA in meeting its mandate to protect the consumer from undeclared components.

Field-deployable, Rapid Detection of Food Allergens Using Hand-Held Diagnostic Devices

Over 11 million Americans suffer from food allergies with one-third allergic to multiple foods. It is therefore common for samples suspected of causing an allergic reaction due to the presence of an undeclared food allergen to require extensive testing for multiple allergenic foods. This is a time-consuming and expensive process that delays FDA's response. Lateral flow devices (dipsticks) enable the analysis of food samples for the presence of multiple food allergens in a short period of time with minimal labor and cost. The goal of this research is to determine the utility of these devices that can also serve as a field–deployable tool for the screening of food samples for the presence of food allergens.

Application of Enzyme-linked Immunosorbent Assay (ELISA) Test Kits for the Detection of Food Allergens to the Analysis of Gum Arabic and Foods Containing Gum Arabic

The FDA relies on the use of ELISAs to detect undeclared peanuts in food. However, the reliability of ELISAs to detect peanut in the presence of gum arabic was brought into question when a food manufacturer observed problems when attempting to analyze pure gum arabic. Though no problems were observed with finished food products and the methods employed by the FDA functioned properly, the inability of ELISAs to analyze gum arabic raised performance questions. T he goal of this research is to determine the cause of the analytical problems and identify an indicator to determine when ELISAs may not function reliably. If successful, the results would increase the utility of ELISA methods to test ingredients and help assure the accuracy of food labels.

Mass Spectrometric Analysis of Food Allergens

Food allergy and celiac disease affect 3% and 1% of the U.S., respectively. Those affected depend on accurate food labeling. Current food allergen/gluten analysis methods are limited in terms of specificity, reproducibility and food processing effects. The goal of this research is to develop a mass spectrometric method that will provide greater specificity, more accurate quantitation and a more flexible, multi-allergen/gluten capability with an increased scope of applicability. The fates of allergens/gluten in food processing will also be determined, thereby allowing for the identification of more realistic analysis targets. The results of this work could enable more reliable food allergen/gluten labeling and enforcement.

Development of an Immuno-based Multiplex Analytical Method for the Simultaneous Detection and Quantification of Egg, Milk, and Wheat Allergenic Food Groups and Gluten

The Food Allergen Labeling and Consumer Protection Act (FALCPA) and proposed gluten-free labeling regulations require food labels to declare the presence of eight major allergenic food groups and gluten. Despite these regulations, mislabeling and the inadvertent presence of allergens and gluten still occur. This places individuals at risk of severe reactions. Current methods of analysis are limited to a single allergen or gluten test at a time. The goal of this research is to develop a method to test for egg, milk, wheat, soybeans, and gluten simultaneously. The new method will detect and quantify the presence of allergens and gluten to aid FDA in the enforcement of labeling regulations.

DNA Biomarkers for Detection of Crustacean Shellfish in Foods

Allergy to crustacean shellfish (shrimp, crab, and lobster) affects 2% of American adults and is on the rise. Allergic people can react to minute quantities of allergen which get into a food product during processing or handling. There is no effective treatment, so people rely on avoidance of offending foods in part through labeling. One way to detect an allergenic food is to detect its DNA. The goal of this research is to develop and validate a method based on polymerase chain reaction (PCR) to detect the DNA of shrimp, crab, and lobster in several food types. If successful, these methods could help FDA detect trace amounts of the allergens in food products and help ensure accurate labeling, thereby allowing consumers to avoid allergenic foods.

<u>Dietary Supplements</u>

Analytical Method Development for Isolation and Characterization of Major Marker Compounds of Botanical Dietary Supplements

The lack of analytical methods for the characterization of major marker compounds in botanical dietary supplement ingredients can lead to problems of misidentification, mislabeling, adulteration and toxicity related to the intended ingredient or one substituted for it. The goals of this research are to develop methods for isolating and identifying sufficient quantities of marker compounds (i.e. pyrrolizidine alkaloids) found in authenticated plant materials (i.e. *Symphytum* species and *Momordica charantia*) and then use the markers as standards in quantitating these components in dietary supplements containing these botanicals. If successful, this work will improve FDA's understanding of the composition of dietary supplements containing the botanicals of interest and help ensure the safety of these supplements.

Electron Spin Resonance Spectroscopy (ESR) Studies of Factors Affecting the Antioxidant-Prooxidant Activities of Dietary Supplements

Increasingly, health claims are being made for nutritional antioxidants and dietary supplements. However, the function of antioxidants in biological systems is not well understood. The goal of this research is to use electron spin resonance (ESR) spectroscopy to systematically examine the antioxidant/pro-oxidant activity of selected dietary supplements under different physiologically

relevant conditions (e.g. variations in partial pressure of oxygen in tissues, changing concentrations of metal ions, and interactions with other antioxidants). FDA will use the results in defining limitations on antioxidant claims for dietary supplements and for framing questions that can be addressed in additional animal or clinical studies on antioxidant dietary supplements.

Development of a LC-MS Method for the Analysis of Vitamin K and Related Compounds in Infant and Adult Nutritional Products

The current method employed for the analysis of vitamin K1 in infant and other nutritional products utilizes the FDA-specified liquid chromatography-fluorescence (LC-FLD) protocol. The goal of this research is to develop a more efficient and robust method with LC-mass spectrometry (LC-MS). The newer method will expedite sample analysis, which will help to ensure accurate labeling of infant formulas and other medical and nutritional foods, including multi-component dietary supplements.

Isolation and Identification of Compounds in the Botanical *Acacia Rigidula*

Acacia rigidula (AR) is a plant without a history of cultivation or use by humans. AR is increasingly found in dietary supplements advertised for weight loss. The scientific literature on this plant consists of a single manuscript which claims that the plant contains primarily amines. Some of these amines can have adverse health effects. The lack of reliable analytical data of marker compounds found in AR can lead to problems of misidentification, mislabeling, adulteration and toxicity related to the intended ingredient. The goal of this research is to analyze AR-containing products, using liquid chromatography-mass spectrometry, to establish a reliable list of amines. If successful, this work would provide information on the composition of AR-containing products useful in assessing the risk for adverse health effects.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) Method Validation for Total Diet Study Program

The methods used for elemental analysis in the FDA Total Diet Study are time consuming, costly and use outdated technology. The program currently uses five analytical techniques and four mineralization procedures. The goal of this research is to consolidate the methods into one analytical technique (ICPMS) and one mineralization procedure (microwave assisted nitric acid digestion). The consolidated method will use current technology and if successful, result in a considerable time and money savings over current methods for sample preparation, analysis, and data processing.

Analytical Method Development for Isolation and Characterization of Major Marker Compounds in Botanical Dietary Supplements Containing *Liriosma ovata*

Liriosma ovata (LO) is a plant native to the Brazilian Amazon and found in dietary supplements advertised to improve sexual functions. The lack of analytical methods for the determination of marker compounds found in LO can lead to problems of misidentification, mislabeling and adulteration related to the intended ingredient. The goals of this research are to develop methods to isolate and identify sufficient quantities of marker compounds found in LO, using preparative

liquid chromatography, nuclear magnetic resonance and mass spectrometry and to quantitate these components in dietary supplements containing LO. If successful, this work would provide information on the composition of LO-containing products.

Determination and Quantization of Terazosin, Doxazosin, Prazosin and Finasteride in Dietary Supplements by Ultra High Pressure Liquid Chromatography/Tandem Mass Spectroscopy

Many consumers use dietary supplements for self-care, mainly to improve their health. Lately, a number of supplements have appeared on the market that target patients having prostate problems. Some of these products actually contain active pharmaceutical ingredients (API) such as terazosin, doxazosin, prazosin and finasteride, which are used to treat symptoms of enlarged prostate (benign prostatic hyperplasia). The goal of this research is to develop a method to identify API adulterants in dietary supplements by use of Ultra High Pressure Liquid Chromatography/Tandem Mass Spectroscopy. This project will provide FDA with technology to identify, characterize, and quantitate illegal API-adulterated dietary supplements that can put the consumer's health at risk.

Screening of Dietary Supplements for *Bacillus* Contamination Using Chromogenic Agar

Dietary supplements and food products contaminated with enterotoxigenic strains of *B. cereus* bacteria can cause human illness that may be underreported due to the short duration of symptoms; however, there have been reports of fatal outcomes linked to this foodborne disease. The goal of this research is to evaluate newer media formulations that reportedly simplify detection, quantification, and identification *B. cereus* bacteria. Molecular methods will also be evaluated to determine if enterotoxigenic strains can be differentiated from negative strains. Rapid identification of food contamination is important to protect the consumer from food related illnesses and quickly identify contaminated food products. If successful, the developed method would be validated and submitted for inclusion into the FDA Bacteriological Analytical Manual and be available for regulatory use.

Fungal and Aflatoxin Contamination of Milk Thistle Dietary Supplements

Milk thistle supplements have been found containing molds that produce toxic and carcinogenic substances. The goals of this research are to conduct a survey of milk thistle dietary supplements to determine if toxigenic molds are present and to develop a method for determining levels of toxin in these commodities. If successful, FDA could use the method to test commercially available products for toxin contamination. Determining the types and levels of fungal contaminants would help FDA establish procedures to reduce such contaminants or remove contaminated product from the market.

Science-based Authentication of Botanical Dietary Supplements

The widespread usage of botanical dietary supplements in the U.S. markets dictates that a thorough analysis of the plants that make up this market be evaluated for their inherent safety. The goal of this research is to evaluate botanical dietary supplements in commerce in order to provide an overall fingerprint profile for each authenticated plant and potential adulterant. A

botanical fingerprint can include but is not limited to chemical/analytical identification, metabolomic evaluation, macro/microscopic analysis, and genetic profiling. Initial investigations for this work involve the isolation and identification of compounds of interest that are contained within the botanicals studied. Building a full understanding of what constituents the make-up of authentic botanicals provides a basis for identifying potential adulterants and additives that can be introduced into these products. The knowledge of what is phytochemically expected within these materials can also provide FDA with information on potential health risks that might be associated with botanical products.

National Center for Natural Products Research: University of Mississippi (UM-NCNPR) Cooperative Agreement on Botanical Dietary Supplements—Science Base for Authentication and Analysis

The primary focus of the UM-NCNPR/FDA collaborative agreement is to develop and disseminate botanical natural product research with an emphasis on public safety according to the needs of the FDA. The cooperative research, education, and outreach programs developed by UM-NCNPR will address scientific issues related to the safety of botanical dietary supplements (BDS) and botanical ingredients and will complement the diverse activities of both the public and private sectors. This collaborative research serves FDA by providing authentic botanical reference materials, rare chemical standards, and the development of unique laboratory approaches to the assessment of new botanical dietary ingredient's safety. Since these standards form the basis of analytical methods, toxicological studies and regulatory enforcement strategies, a reliable source of these materials is essential to the Agency's mission. During the 2011-2012 funding period, a number of compounds were isolated from *Labisia pumila*, *Commiphora wightii*, *Tripterygium wilfordii*, *Terminalia* species, *Casearia sylvestris*, *Centella asiatica*, *Centella erecta*, *Drynaria fortunei*, and *Pfaffia paniculata*, *Casearia sylvestris, Scutellaria lateriflora, Curcuma longa, Matricaria recutita L., Anthemis nobilis, Rhodiola spp., Hoodia gordonii, Actaea racemosa, Lonicera Japonica Thunb., Caulophyllum thalictroides, and Sutherlandia frutescens*. These compounds could be used as standards for the development of analytical methods, toxicological studies, and regulatory enforcement strategies. Utilizing the compounds isolated over the span of this project it was possible to develop or modify various authentication methods, as well as metabolomic profiling for common botanicals including *Rhodiola* species, *Podophyllum peltatum* L., *Tripterygium wilfordii* Hook. f., *Hoodia gordonii*, *Ginkgo biloba*, *Morus spp., Lepidium meyenii (Maca), Rhodiola spp., Lonicera Japonica Thunb., Lancea tibetica, Curcuma spp., Scutellaria lateriflora, Pausinystalia johimbe* (yohimbe*), and Sutherlandia frutescens.*

Filth and Other Animal Material

The Development and Manufacturing of PCR Primers for the Detection of Animal Material

FDA regulates all animal feed and feed ingredients used for food-producing animals in the U.S. The main strategy for preventing prevent bovine spongiform encephalopathy (BSE, also known as mad cow disease) is to prohibit most mammalian proteins from being used in ruminant feed. The FDA has developed a PCR-based test to detect material that came from ruminants (cattle, sheep and goat) in meal and feed. The FDA supplies the key parts of this test, e.g. PCR primers,

to laboratories that test animal feed. Currently, FDA is supplying these test components to 20 federal and state laboratories.

Updating Filth and Extraneous Materials Methods for the 21st Century

Strengthening food inspection and analysis is important in detecting food adulterants that may render these products injurious to health. Current methodologies for the detection of filth and extraneous materials in food, particularly for the detection of insects and/or insect fragments, and mold fragments are cumbersome and out-of-date. The goal of this research is to evaluate the feasibility of modern technologies, such as molecular techniques, for detecting filth and extraneous materials in foods. If successfully identified, new methods or technologies could provide rapid, accurate, reliable, and validated methods that can be used by FDA in meeting its regulatory mission to ensure the safety of the U.S. food supply.

Development of a Novel PCR-Based Rapid Method to Detect Common Pests Responsible for Spreading Foodborne Pathogens: A Public Health Priority

The FDA's filth program focuses on contaminants of food products resulting from insect or animal pests, called the 'Dirty 22' species. At present pest contaminants recovered from food facilities are identified by conventional microscopic methods. However, sometimes features needed for identification are missing or decomposed to the extent that a trained analyst can identify a specimen to the family level, but not to the required species level. The goal of this research is to develop novel DNA-based molecular methods that will be able to differentiate the four different groups of 'Dirty 22' species to the desired species level.

Developing a Rapid Molecular Assay Specific to the Detection of 22 of the Most Common Pests Contributing to the Spread of Foodborne Pathogens

FDA is one of several public health authorities to recognize rodents, flies, and cockroaches as contributing factors to the spread of foodborne pathogens. FDA laboratories have identified the 22 most common pests that contribute to the spread of disease from food. The methods for detecting these pests, called the “Dirty 22,” in food are difficult and time-consuming. The goal of this research was to develop a rapid molecular assay specific to the detection of the “Dirty 22”. A unique region of DNA was used to identify each of the 22 common pests (“The Dirty 22”). By sequencing this DNA region, FDA obtained information for each pest that can serve as unique DNA barcodes for rapid identification. Tests that are quicker to perform and more sensitive will enhance food safety and decrease food costs to the consumer.

Validation Studies – Chemical

Validation of a Method for Quantification of Arsenic Species in Rice and Rice Products

Some samples of rice have been shown to contain relatively high levels of arsenic. Since the different chemical forms (species) of arsenic exhibit a range of toxicities, analytical methods that can differentiate these species are needed for generating data to evaluate the actual health risk. FDA has developed a method for determination of arsenic species in rice. The goal of this research is to validate the new method to ensure that it is reliable, accurate, and applicable to all the rice-based products to which it might be applied. If successful, this method will allow FDA will be able to detect the different arsenic species in rice and rice products and generate data for the evaluation of the health risk.

Multi-Laboratory Method Validation for the Determination of Total Arsenic and Inorganic Arsenic in Fruit Juice and Rice

The goal of his research was to validate methods capable of detecting and accurately quantifying *total arsenic* and *inorganic arsenic* in fruit juice at concentrations of 5 parts-per-billion or less. The validation data include more than a hundred fruit juice samples analyzed for arsenic and inorganic arsenic. The data could also be used for risk assessment studies. FDA and cCAP labs have used these methods to monitor imported juices. Validation data for the recently developed method to determine inorganic arsenic in rice and rice products from cCAP labs will be compiled with data from other participating FDA laboratories resulting in a multi-laboratory validated method that can be used for monitoring and regulatory actions.

Multi-Laboratory Validation Study on High Resolution Accurate Mass Analysis Coupled with an FDA Standardized Extraction Procedure for Detection of Pesticides and Other Toxic Chemicals

The goal of this study was to combine high resolution mass accuracy with a standardized extraction procedure to provide a simple screen for 250 pesticides in three different produce composites in a single analysis. The use of high resolution mass analysis will allow large, complex compound screens to be performed in relatively short periods of time and with better standardization of performance between laboratories. This method could allow routine screening of 1000 or more compounds by any laboratory equipped with the appropriate mass spectrometer and software. Results of the multi-laboratory validation show a high percentage of the pesticides detected well below the levels of concern.

Validation of Surface Plasmon Resonance Biosensors for Quantitative Analysis of Paralytic Shellfish Toxins

Paralytic shellfish toxins (PSTs) in contaminated seafood can cause numbness and respiratory paralysis in humans. FDA has developed a surface plasmon resonance (SPR) biosensor that can rapidly and sensitively detect the PSTs in shellfish matrices. The goals of this research are to transfer the developed PST method to an instrument platform that can be easily used in monitoring laboratories and to validate the method according to the National Shellfish Sanitation Program guidelines. If successful, this validated method could allow for real-time detection of

seafood toxins in monitoring and regulatory laboratories, providing better protection for consumers from PSTs, while also reducing unnecessary closures of non-contaminated harvesting areas.

Validation of an Inter-Agency Phthalate Esters Liquid Chromatography Tandem Mass Spectrometry Method

In 2011, the FDA was on alert for unusually high levels of phthalate esters (PE) in various imported food products. In response, FDA laboratories developed a method based on liquid chromatography-tandem mass spectrometry to monitor the PE's in various products. This method, with modifications for some matrices, provides the advantages of a simple extraction method and high sensitivity and reliability. The goal of this research is to survey a large number of suspect samples to determine if the occurrence of the adulteration is limited to certain foods and manufacturers. The results will be published in the FDA Laboratory Information Bulletin to make the method publicly available for use by regulatory and private analytical laboratories.

Validation of a Liquid Chromatography/Tandem Mass Spectrometry Method to Detect Select Phthalate Esters in Foods

Adulteration of Taiwanese food products with plasticizers is currently a global concern. FDA has initiated an urgent effort to screen foods coming from Taiwan for the presence of elevated levels of phthalate plasticizers. Although phthalates are not permitted as food additives, they can leach into food at low levels from plastic caps and containers. The goal of this research is to develop a quantitative analytical method to detect phthalates in specific foods. FDA will collaborate with the Canadian Food Inspection Agency on this method development effort. If successful, the method will be used to screen specific categories of imported foods from Taiwan to prevent violative phthalate-contaminated products from entering the market.

International Method Validation of Nitrofuran Liquid Chromatography/Tandem Mass Spectrometry Approved by AOAC

Nitrofurans are broad spectrum antibiotics which have been banned both in the U.S. and E.U. Despite this, they have been detected in imported food products such as seafood and meat. Various methods for detecting antibiotics are currently in use. The goal of this research is to determine a consensus method for antibiotic detection and coordinate an international multi-lab validation. This will provide a uniform analytical method that will minimize the ambiguity of analytical results and help both importing and exporting countries better control the occurrence of banned nitrofurans in trade food products. The initial matrices will be seafood products, with the potential to expand to eggs, honey, milk, and game meat.

Method Validation of Milk/Infant Formula Matrix for Radium-226 Detection

Radium-226 is a long-lived, alpha-emitting radionuclide with a long chain of radioactive alpha, beta and gamma-emitting progeny that occurs naturally in soil and rocks and may concentrate in nature due to its physical, chemical and biological properties. Radium is chemically and biologically similar to calcium in that it concentrates in both milk and bone making it both more

likely to be found in a child/infant diet and a source of life long exposure after consumption. This study seeks to validate the Ra-226 analytical method for milk, dairy infant formula and soy infant formula in order for FDA to have the capability to routinely analyze these high risk matrices.

Nanomaterials

Determination of Organic and Inorganic Chemicals Released from Polymer-Clay Nanocomposite Food Packaging

Polymer-clay nanocomposites (PCNs), plastics in which nanoscale clay particles are dispersed, have had commercial success in food packaging. However, when physically abused or exposed to high temperature, there is concern that nanoparticles can be released from PCNs. The goals of this research are to fabricate PCNs, submerge them in substances which mimic the food properties, and exposing them to conditions relevant to intended use as a means of evaluating potential migration. This will be accomplished by analyzing the food-like substances for clay residuals to determine if clay particles have migrated from packaging to food. Results from this research could lead to increased confidence in the use of nano-engineered packaging materials for food markets.

Study of Nanoparticles Migration from Food Contact Nanomaterials: Characterization and Quantification of Silver Nanoparticles in Food Simulants

The greatest risk of consumer exposure to nanoparticles from packaged food using nanomaterials is due to potential migration into food or drinks. Nanoparticle migration data are not available despite the fact that a number of nanomaterials are already available for use. Silver nanoparticles are a high priority because of their broad range of potential applications. Silver nanoparticles have been used in several food contact materials and coating materials as anti-microbial agents, and also in culinary items. The goal of this research is to evaluate and quantify silver nanoparticle migration from polyethylene into food simulants. Results from this work will help FDA refine current risk assessments as they relate to nanosilver migration from food contact materials.

Understanding Migration of Nanoparticles in Polymer Films Using Semiconductor Nanocrystals (Quantum Dots)

Polymer nanocomposites (PNCs), polymers with nanoscale particles dispersed throughout, exhibit properties that make them usable for food packaging applications. However, when physically abused or exposed to high temperature, there is concern that nanoparticles can be released from PNCs. The goal of this research is to utilize fluorescent quantum dots (QDs) as models to probe whether nanoparticles dispersed in polymers will migrate from the polymers into foods. Initial steps are to fabricate PNCs containing QDs of known size and determine whether the QDs migrate into food simulants under conditions relevant to the food industry. If successful, this work would aid FDA in determining the size threshold over which nanoparticle migration and inform risk assessment.

The Development of Methods to Identify and Characterize Various Nanoparticles in Edible Tissue and Animal Feed

Because little is known about where nanoparticles travel in the bodies of food animals, studies are essential before large-scale industrial production and use are implemented. The goal of this research is to determine where nanoparticles travel in the bodies of food animals and animal by-products (eggs, milk, blood etc.). Different types of nanoparticles (metal-based, carbon-base, and lipid-based) will be evaluated in several types of food animals (pigs, chickens, sheep, etc.). The tissues and by-products will then be analyzed for the presence of the nanoparticles. Results from this study would provide the FDA with important food safety data and allow FDA to make recommendations to future sponsors who wish to utilize nanomaterial for animal therapy.

The Development of Methods to Identify and Characterize Various Nanomaterials in Aqueous Biological Matrices

Because little is known about the possible toxic health hazards of nanoparticles, studies on toxicology and food safety are essential before large-scale industrial production can begin. The goal of this research is to develop and validate *in vitro* detection methods for nanoparticles (metal-based, carbon-base, and lipid-based nanomaterial) in aqueous biological matrices (blood, milk, eggs, etc.). The methods would be used to conduct toxicity and food safety studies.

Adulteration and Misbranding

Continuing Development of DNA Barcoding for the Identification of Seafood Products

Enforcement of seafood labeling is a challenge because of the diversity of marketed species, many of which are imported. The FDA Seafood List contains acceptable market names for seafood sold in interstate commerce in the U.S. FDA has developed a DNA-based method for identifying processed fish that is now routinely used to aid in labeling enforcement. The goal of this research is to expand the method to include crustaceans (shrimp, crab, and lobster), and disseminate these methods to FDA's regulatory partners and other entities through the development of a public website. If successful, this project would significantly expand the number of products which can be identified based on DNA, further protecting public health and reducing fraud.

Fish Sample Collection, Identification, Vouchering, Storage, and Fish Gene-Sequencing

FDA has the responsibility to develop, apply, and provide the necessary technology and methods for the effective identification of fish species for the purposes of protecting the public from improperly and illegally imported fish species that may constitute a health threat. The FDA is also responsible for the having the necessary tools to detect and enforce regulations against substitution of one species for another in cases of economic adulteration (i.e. seafood fraud). The FDA has developed a method for fish species identification using a technique known as DNA barcoding that will address this problem. In order for the FDA to proceed with the use of the DNA Barcoding method an authenticated sample library with validated DNA sequences is required. The Smithsonian Institution, as a federally chartered museum and learning institution,

has exactly the facilities and expertise necessary to help the FDA develop a fish DNA database. This repository can serve as the basis for a government-wide seafood library, a process that was recommended in the 2009 U.S. Government Accountability Office report on seafood fraud.

Developing and Validating a Regulatory Vertebrate DNA Barcode Database

It is vital to be able to confirm the label claim of animal-based foods in order to determine whether there is any risk to human health from eating that food. The goal of this research is to expand the current regulatory DNA barcode database so FDA can identify more species using molecular methods (for example, DNA sequencing or polymerase chain reaction, PCR). The current DNA barcode database only contains information on fish. FDA needs to expand the current animal species DNA barcode database to include vertebrates (livestock, for example). Expanding the DNA barcode database will also help identify currently unidentifiable species. These improvements will allow FDA to confirm the label claims of specific animal-based foods. This will give FDA the ability to assign risk with a known animal species and aid the Agency's mission to protect the U.S. food supply.

Developing Regulatory Assays using DNA Barcoding

FDA needs to be able to detect mislabeled products, which occur when the labeled species is replaced by a substitute species. Species substitution can have a significant impact on human and animal food safety. The goals of this research are to develop molecular identification methods for targeted species, and to validate and transfer the methods to one or more FDA field laboratories. With these methods, FDA can rapidly confirm product label claims for species content by matching short DNA sequences (DNA barcodes) from products to the DNA sequences in a database of DNA barcodes. This study provides barcoding method development for multiple laboratories within FDA.

Development of Mini-Barcode Primers for the Identification of Fish Species in Commercial Food Products Using DNA Sequencing

DNA barcoding is currently being implemented at FDA field laboratories for the identification of fish species by DNA sequencing. However, full-length DNA barcodes cannot be reliably recovered from heavily processed fish products due to DNA degradation. The goal of this research is to develop mini-barcode primers that can be used in combination with DNA sequencing to identify fish species in heavily processed products. The results will provide FDA with a means to detect hazardous or economically fraudulent instances of misbranding in canned products.

Automation of DNA Barcoding Methods for Use in FDA Laboratories

DNA barcoding is being implemented at FDA laboratories for the identification of fish species by DNA sequencing. However, there are several steps in this procedure that could be improved with automation. Two instruments currently at FDA laboratories that have the potential to be incorporated into the DNA barcoding protocol are the MagMax 96-Express for use in DNA extraction from fish tissue and the QiAgility liquid handler for use in setting up samples for

PCR. Our goal for this project is to optimize these automation methods for potential use with DNA barcoding. The results of this project will improve the FDA's ability to rapidly detect potentially hazardous or economically fraudulent cases of seafood misbranding.

DNA Barcoding for Species Identification of Fish for FDA Regulatory Compliance

Seafood species substitution is difficult to identify in cases where the morphological characteristics used for species identification have been removed. DNA barcoding (DNAB) is a DNA sequencing method that uses short standardized markers in the genome to discriminate species. FDA published a single laboratory validated (SLV) method, and subsequent Laboratory Information Bulletin (LIB) reporting a method of DNAB suitable for regulatory use. The LIB provides a detailed protocol with updates to the SLV to suit the instrumentation and software utilized by FDA field laboratories, as well as additional verified procedures for rapid tissue extraction and PCR. Use of DNAB will provide the FDA with a precise means for identifying seafood.

Species Identification Analysis of Regulatory Samples

Species identification assists FDA in overseeing food safety and economic deception. Molecular methods (PCR and DNA sequencing) can be used to determine the identity of the animal species in a food product. These molecular methods have shown to be powerful tools for species identification and have vast potential for use in regulatory science. The goal of this research is to validate the use of these methods for analyzing human and pet food for the authenticity truthfulness of label claims (species identification), with the possibility of legal action if violations are found. If successful, this work would help FDA in the detection of species substitution and identifying and controlling specific species related hazards to reduce economic fraud.

Evaluation and Peer Verification of Methods for the Identification of Genetic Engineered (GE) Material

Genetic Engineered (GE) animals may come from laboratory animal species or species traditionally used as food. FDA needs the capability to identify GE animals to ensure that only GE animals that are approved are introduced into commerce, and that only GE animals approved for food use are found in the food supply. The goal of this research is to assist in evaluating and peer-verifying a sponsor's molecular methods to detect GE material. These methods may be used to distinguish GE animals from non-GE animals. These methods may also be used to distinguish between GE animals that are approved to be introduced into commerce from those that have not been approved. Results of this research contributed to the first GE animal approval to produce a drug product for use in humans.

A Method to Determine 12 Sweeteners in Foods Using Ultra Performance Liquid Chromatography/Tandem Mass Spectrometry

To ensure food products are in compliance with U.S. regulations regarding allowable sweeteners, the FDA needs to develop a method for analysis of these compounds in foods. The list of

allowable sweeteners varies from country to country and the current method for testing is outdated and does not encompass some of the sweeteners which have recently been introduced into the market place. These non-nutritive sweeteners are added in relatively small quantities often in combination with other sweeteners to reduce after-tastes. The goal of this research is to develop a modern, rapid and reliable ultra-performance liquid chromatography/tandem mass spectrometry method for analysis of sweeteners. If successful, this would allow FDA to detect adulterated or misbranded foods for both domestic and imported products.

Microbial Pathogens

Salmonella

Assay Development for Molecular Subtyping of *Salmonella* using the PlexID Biosensor System

FDA collaborates with industry stakeholders developing technologies for food pathogen screening and detection. Rapid screening of environmental samples is important for food safety because the approach quickly identifies a target pathogen from a multitude of background organisms. The PlexID Biosensor System combines mass spectrometer technology with PCR technology to provide a detailed DNA fingerprint of foodborne pathogenic microbes. The goal of this research is to develop a PlexID method to subspeciate and serotype *Salmonella* from food samples directly. If successful, this method could be validated and used by FDA with many different types of samples as a high-throughput method for analysis.

Isolation and Identification of *Salmonella enterica* from Naturally Contaminated Cilantro

Detection of *Salmonella enterica*, one main cause of gastroenteritis, is a high priority for food safety. There are over 2500 *Salmonella* serovars and the conventional serotyping method takes five to ten days to complete. The goal of this research is to develop a multiplex PCR assay capable of detecting 30 of the most common serotypes of *S. enterica* subsp. Enteritidis from pre-enrichment and selective enrichment broth from produce. This research will be useful in developing faster detection and characterization of *Salmonella* during outbreaks and thus improve the safety of the food supply.

Detection of *Salmonella* in Animal Feed by qPCR

Animal feeds that are contaminated with *Salmonella* bacteria can make animals sick and may lead to illness in humans. It is important that animal feeds be tested and kept free of *Salmonella* contamination. Rapid methods are needed for testing a wide variety of animal feed. The goal of this research is to evaluate a qPCR method for detection of *Salmonella* in animal feed. If successful, the method will be used by the Agency for routine testing of many animal feeds.

Development of a Real-Time PCR Rapid Method for Detection of *Salmonella* Enteritidis in Environmental Swabs

In the year 2010, the FDA was involved in the investigation of a multi-state outbreak of *Salmonella* Enteritidis infection that was associated with shell eggs and led to the inspection of

numerous farms and poultry houses. As evidenced by the focus of these efforts, the FDA recognizes the potential economic and public health burden posed by *S.* Enteritidis and the need for a rapid response. The goal of this research is to develop a real-time PCR screen for *S.* Enteritidis that will deliver negative and "cannot rule out" results in a very short time frame allowing FDA to allocate more resources to potentially positive samples.

Validation of a Real-Time PCR Rapid Method for Detection of *Salmonella* in Environmental Swab Samples

Investigation of food firms is a critical part of FDA's regulatory mandate to protect public health. Environmental swab sampling is now considered to be an important tool to evaluate the sanitation conditions of manufacturing processes. In one week, approximately 200-600 environmental swabs are collected and analyzed individually for *Salmonella* by any single FDA laboratory, dramatically increasing its workload. The goal of this research is to validate the Real-Time PCR method using the ABI 7500 FAST system. If successful, this rapid screening method will provide a faster, less laborious process of analyzing high throughput swab samples.

Comparison of Three Different Polymerase Chain Reaction Technologies for the Detection of *Salmonella* in Produce

Molecular methods that amplify DNA sequences from target bacteria offer a rapid, sensitive, and specific way to detect *Salmonella* contamination. A fast and accurate molecular detection method is needed to pre-screen produce samples for *Salmonella* while reducing time and labor compared to conventional culture methods. This method would also eliminate further microbiological testing of negative samples. The goal of this research is to evaluate the efficiency of these methods for detecting *Salmonella* in produce before recommending a particular protocol for widespread us. Once evaluated, the best detection method can then be implemented in FDA surveillance programs and also be used to improve outbreak investigations.

Development and Validation of Conventional and Real Time PCR Assays for the Detection of *Salmonella* in Foods

The current FDA Bacteriological Analytical Manual method takes a minimum of four days to detect *Salmonella* in foods. Integration of molecular screening technology into the isolation and detection process may hold the potential for reducing this time significantly. The goal of this research is to develop a multi-target real-time PCR assay that will effectively detect *Salmonella* in overnight enrichments of produce in less than a half a day thus reducing the time necessary to detect *Salmonella* in foods from four days to one. This project promotes public health by providing a rapid screening tool that can be used to survey and subsequently stop contaminated foods from entering the food supply and by removing those foods previously implicated in outbreaks.

Evaluation of the Pathatrix System for the Isolation of *Salmonella* Species

Due to an increase in outbreaks of foodborne illness associated with produce, the FDA has intensified its efforts to rapidly identify pathogens responsible for produce contamination. To

assist in this effort, it is essential to develop methods that reduce the amount of time needed for detection and identification of pathogens. The goal of this research is to validate a method using Pathatrix Auto, an immunomagnetic separation system, coupled with real-time PCR. Since the presence of *Salmonella* as a contaminant of tomatoes has been previously confirmed, this commodity will be the initial matrix of choice for this research. If successful, this will aid FDA in reducing the time required to detect and confirm the presence of *Salmonella* species in contaminated products.

Development of a Method for the Detection and Isolation of *Salmonella* from Internally and Externally Contaminated Tomatoes

In the past decade, at least 12 different *Salmonella* outbreaks in the U.S. have implicated tomatoes. Since most tomatoes are consumed raw in salads, sandwiches and other foods, it is important to ensure that tomatoes remain pathogen free through the farm-to-table continuum. *Salmonella* can internalize within the fruit, so the sample preparation methods used prior to testing are important. The goal of this study is to examine the effectiveness of whole soaking, quartering, stomaching, and blending in the recovery of *Salmonella* from tomatoes. This could provide the FDA with a more sensitive and effective method to identify *Salmonella* contaminated tomatoes before they enter into the food supply.

Detection of *Salmonella* in Animal Feed and Pet Food via Loop-mediated Isothermal Amplification

Animal feed and pet food contaminated with *Salmonella* raises concerns in animal health and human food safety. Therefore, it is important that FDA laboratories have access to rapid and reliable testing methodologies. Recently, a rapid, accurate, yet simple and cost-effective molecular testing method using loop-mediated isothermal amplification (LAMP) was developed by FDA for *Salmonella* detection. The goal of this research is to further develop and validate LAMP to detect low levels of *Salmonella* in various animal feed and pet food samples. Rapid, accurate, and robust detection of this important human pathogen will provide FDA feed testing laboratories a tool to better identify potential contamination problems. Timely intervention can ensure the safety of these products.

Method Development and Validation Program for High-Throughput Molecular Biological Technologies using the BioPlex Instrument

FDA is evaluating the Bio-Rad BioPlex instrument as high through-put molecular biological technology. The CDC *Salmonella* molecular serotyping (SMS) method, which is faster than conventional serological methods, was selected for implementation on the Bio-Plex. FDA has worked with CDC to acquire reagents, provide workshops, and implement the SMS method in seven FDA laboratories. Implementation of the SMS method will provide FDA with a rapid method to identify *Salmonella* serotypes during foodborne outbreaks.

Development of Methods for the Detection and Isolation of *Salmonella* from Various Spices

Many spices contain substances that are inhibitory or toxic to microorganisms; however, microorganisms, such as *Salmonella*, can remain viable, but dormant, in spices and thus lead to salmonellosis outbreaks. The spices' inhibitory effect on microorganisms poses a challenge for FDA since it also prevents the growth of pathogens on or in the media. The goals of this research are to evaluate the current Bacteriological Analytical Manual *Salmonella* culture method and several alternative methods for detection in spices and to perform inclusivity/exclusivity tests. An improved spice method will protect the public health by enabling FDA to detect contaminated spices before and/or after entering the food supply.

Accelerated *Salmonella* Procedure for Detection and Isolation of *Salmonella* spp. from Produce Samples using two methods: Neogen Reveal and SDI RapidChek .

Fresh produce is increasingly found to be involved in foodborne disease outbreaks. Rapid and reliable methods for detecting bacterial pathogens in produce samples would allow for more testing of food samples. The goal of this research was to evaluate the utility of an accelerated plating procedure and rapid screening technique using two different lateral flow devices (Neogen Reveal and SDI RapidCheck) for *Salmonella* detection. The research resulted in the inclusion of the following method in the COMPACT: (1) RapidCheck® Device for *Salmonella* Detection using a modified BAM procedure (2) Neogen Reveal Devices for Detection of *Salmonella* using BAM Enrichment as a modified procedure.

E. coli O157:H7 and STEC

Improved High-Throughput qPCR Screening and Sample Analysis through Laboratory Automation

Foodborne illness outbreaks involving fresh produce are a major public health concern. Testing strategies to detect produce that are potentially contaminated with pathogenic *E. coli* can be difficult and time-consuming. This project will investigate the use of semi-automated instruments to improve sensitivity and testing capacity for produce samples. It is anticipated that by improving detection and recovery, as well as testing more samples in a shorter amount of time, a greater number of samples can be tested for harmful pathogens, thus ensuring safer produce for consumers.

Luminex Micro-bead Based Suspension Array for Five Shiga Toxin Producing *Escherichia coli* Genes Identification

There are about 300 Shiga toxin producing *E. coli* (STEC) serotypes, of which 100 have been associated with significant disease in humans and have caused numerous foodborne outbreaks. An approach for quick confirmation of STEC isolates, especially non-O157 serotypes, is to test for the presence of virulence genes. The goals of this research are to develop an assay detecting five STEC-specific virulence genes using the PCR-Luminex platform, which can simultaneously test for multiple targets, and to evaluate the assay's specificity and sensitivity. The rapid

detection of STEC virulent genes in bacterial isolates would allow illness-causing STEC in foods to be identified faster and enable prompt removal of these products from the food supply.

Validation of a Method for Rapid O Serogroup Identification of the Eleven Most Clinically Relevant Shiga Toxin-Producing *Escherichia coli*

To ensure the validity of its analytical results, the FDA requires that new laboratory techniques undergo rigorous testing before being included in its official methods. FDA has developed a new PCR based suspension array technique, using a Luminex microbead-based suspension, which can identify the most virulent and most common disease-causing Shiga toxin-producing *E. coli* strains. The goal of this research is to conduct a multi-laboratory validation (MLV) to test the accuracy, ease of use, and reproducibility of this new method. If successful, the MLV study will enable FDA to include the technique in its official methods and protect public health from these potentially life-threatening bacteria.

A PCR-Bioplex Assay for the Detection and Identification of Shiga toxin-producing *Escherichia coli* Serotypes O26, O45, O91, O103, O111, O113, O121, O128, and O145

Identification and serotyping of Shiga toxin-producing *Escherichia coli* (STEC) by classical methods is time consuming, labor intensive and often gives ambiguous results. The goal of this research is to evaluate a Luminex microbead-based suspension array for identification of the O serogroup of the ten most clinically relevant STECs: O26, O45, O91, O103, O111, O113, O121, O128, O145, and O157. The use of PCR followed by Luminex xMAP technology will enable the FDA to detect multiple analytes in a single multiplex reaction with high throughput capabilities to prevent consumption of STEC-contaminated foods and to minimize the impact of foodborne outbreaks.

Validation of a Real-Time PCR Assay for Rapid and Simultaneous Detection of Enterohemorrhagic *E. coli* (EHEC) of O157:H7 and Other Serotypes

E. coli O157:H7 (EHEC) is a major foodborne pathogen associated with the consumption of fresh produce. The current FDA-approved qPCR screening method for STEC/O157:H7 *E. coli* can lead to false negative results in the presence of a high background of *E. coli* and miss those EHEC expressing non-O157 serotypes. The goal of this research is to develop a strategy to identify target DNA sequences unique to EHEC of O157:H7 as well as target DNA sequences common to major non-O157 serotypes as a first step to developing a new screening method to cover the EHEC of both O157 and major non-O157 serotypes.

Investigation into New qPCR Assay Design and Development for Rapid Detection of Foodborne Pathogens: Shiga-Toxin Producing *Escherichia coli* (STEC)

Significant foodborne disease is caused by *Escherichia coli* O157:H7 and other shiga-toxin producing *E. coli* (STEC). The goal of this research is to develop multi-target DNA assays (PCR) to detect these organisms based on serotype and virulence markers. FDA will use a tiered approach that maximizes our ability to rapidly configure and deploy various combinations of multi-target assays for new and emerging situations. Methods will be optimized and validated

for use on multiple PCR instrument platforms. The availability of validated rapid methods for these pathogens will provide FDA with needed tools to monitor the food supply, remove contaminated product before it reaches the consumer, and respond to outbreak situations.

Characterization and Detection of Established and Putative Shiga-toxin Producing *Escherichia coli* Virulence Factors

Shiga-toxin producing *E. coli* (STEC) can cause life threatening infections in people. While *E. coli* O157:H7 has been well studied, pathogenesis of non-O157 STECs is not as well understood, yet these pathogens increasingly cause illnesses via contaminated food. The goals of this research are to evaluate non-O157 STEC strains isolated from humans, animals, and food to determine the virulence factors that may be causing human illness and to develop assays to detect these genes in a variety of STEC strains. Improved detection methods would enable FDA to monitor and test regulated food and reduce outbreaks of human illness caused by STECs.

Comparison of Screening Methods for Recovery of Enterohemorrhagic *E. coli* from Leafy Greens

Many outbreaks of *E. coli* O157:H7 have involved the consumption of leafy greens. Current FDA methods for detecting illness-causing *E. coli* on spinach and lettuce require a rinse step to remove the bacteria from the surfaces for testing. If pathogens are present in low levels and/or are strongly attached to the produce surfaces, rinsing may be inadequate and result in false negative results. The goal of this study is to evaluate the effects of soaking the leafy greens prior to testing as an alternative treatment. Improved sample preparation methods could reduce false negative rates and enhance FDA's ability to identify *E. coli* O157:H7 contaminated produce in the food supply.

Comparison of Agars for the Detection of Shiga toxin-producing *Escherichia coli* in Produce

More than 100 Shiga toxin-producing *E. coli* (STEC) serotypes have been linked to disease in humans. Distinguishing non-pathogenic from pathogenic STEC colonies on culture media is critical for detection and isolation, but the existing media are inadequate for this purpose. The goal of this research is to evaluate agars currently used by the FDA and compare them with commercially-available media, and/or media reported in the scientific literature for their effectiveness in identifying pathogenic STEC. A more efficient agar medium would reduce the time and labor currently required for the detection and isolation STEC strains from foods.

Development and Validation of Nanosensors for Detecting and Subtyping Foodborne Pathogens

Traditional methods for the detection of *E coli* O157:H7 involve labor-intensive, time-consuming tests and trained personnel. Therefore, there is a need for portable, rapid, simple and sensitive identification techniques. The goal of this research was to develop an alternative method, using a field-effect transistor (FET) biosensor for detecting foodborne pathogens, as proof of concept. The capture of *E. coli* O157:H7 DNA by a specific oligonucleotide probe coated onto the transistor array was compared to capture of a different *E. coli* strain that is non-complementary to the probe. Targeted *E. coli* O157 DNA concentrations as low as 1 pg/μL were

detected. The ability of this method to detect low levels of crude DNA suggests its potential for time and labor savings compared to traditional methods.

Listeria

Investigation into New qPCR Assay Design and Development for Rapid Detection of Foodborne Pathogens: *Listeria*

Foodborne disease attributed to *Listeria monocytogenes* is an important public health concern. The presence of other *Listeria* species in a sample can signal the potential presence of pathogenic *L. monocytogenes*. A multi-target DNA test (PCR) to simultaneously detect *L. monocytogenes* and other *Listeria* species has previously been validated to confirm isolate identification. The goal of this research is to add quality control features and extend the use of this assay to additional high throughput PCR instrument platforms for screening food enrichments. When validated these methods will provide FDA with rapid, sensitive approaches to monitor the food supply, remove contaminated product before it reaches the consumer, and respond to outbreak situations.

Matrix Extension Validation of AOAC Methods for *Listeria monocytogenes* in Pet Treats/Food

Currently there is no procedure in place in the FDA laboratories for testing pet treats/foods for *Listeria monocytogenes*. The goal of this research is matrix extension validation of AOAC methods currently used by the FDA laboratories to test other products. If successful, this will satisfy the need for testing pet treats/foods for *Listeria monocytogenes* in the current FDA Pet treats/Foods *Salmonella* Domestic Assignment.

Accelerated *Listeria* Procedure for Detection and Isolation of *Listeria* from Food and Utilization of Neogen Reveal and SDI RapidChek in *Listeria* Detection

The goal of this research was to evaluate a streamlined Bacteriological Analytical Manual (BAM) procedure for detection and isolation of *Listeria monocytogenes* in high risk foods (Queso Fresco, guacamole, Asadero Cheese, Brie Cheese, coleslaw and smoked salmon). The performance of two enrichment procedures, two VIDAS procedures (VIDAS LIS and VIDAS LMO2) and two additional screening tests (Neogen Reveal and SDIX RapidChek) for rapid detection of *Listeria monocytogenes* were also examined. The research resulted in the inclusion of the following method in the COMPACT: (1) RapidCheck® Device for *Listeria* spp. Detection using BAM or AOAC Enrichment (2) Neogen Reveal Devices for Detection of *Listeria* spp. using BAM or AOAC Enrichment.

Shigella

Improving the Pre-Enrichment of *Shigella* Species

The recovery rate of viable, detectable *Shigella* from food matrices using the current FDA method is low. This diminishes the effectiveness of surveillance efforts for this pathogen. Contributing factors to low recovery rates include the low numbers of *Shigella* on contaminated surfaces, naturally occurring microflora that compete for nutrients, and strong adhesion of *Shigella* to the food surfaces due to biofilm formation. The goal of this research is to develop an enrichment media system for recovery of viable *Shigella* that promotes the degradation of biofilms, thereby releasing viable bacteria, while selectively optimizing *Shigella* growth. If successful, this method will allow the FDA to more effectively identify *Shigella* in regulatory food samples.

Modification of *Shigella* Isolation and Detection Methods

Shigellosis can be caused by as few as ten *Shigella* cells or by approximately one million enteroinvasive *Escherichia coli* (EIEC) cells. The goal if this research is to evaluate a new molecular PCR assay designed to detect both pathogens and compare the efficacy of DNA extraction methods for different produce samples. In addition, FDA will develop a molecular fingerprinting assay that can replace the use of antisera for differentiating EIEC and multiple *Shigella* species. If successful, this work would provide FDA with faster and more sensitive protocols that are also more suitable for the high-throughput screening of *Shigella* and EIEC pathogens in contaminated foods.

Reassessment of the Current FDA Bacteriological Analytical Manual (BAM) Method for the Detection and Isolation of *Shigella* spp. in Foods

In instances of food contamination, *Shigella* cells are often present in low numbers and in a stressed physiological state, thereby requiring special enrichment (resuscitation) procedures to enable successful detection by bacterial growth. Current methods described in the BAM and in the FDA domestic and import produce assignment may be either too selective or not selective enough, which can result in false-negative results. The goal of this research is to examine the effects of sample preparation (rinsing versus soaking) and various bacterial growth conditions (temperature, pH, pressure, oxygen content) on the recovery of *Shigella* from produce. These modifications of existing methods could improve FDA's ability to identify produce contaminated with *Shigella*.

Validation of a Real-Time PCR Assay for Rapid and Simultaneous Detection of *Shigella* Genus and Species

Since 2008, *Shigella* has been listed as the third most common food-borne illness in the United States. There are four *Shigella* species with different demographic distributions throughout the world. The current real-time Polymerase Chain Reaction (PCR) screening method targets the ipaH gene present in all *Shigella* and closely related enteroinvasive *E. coli* (EIEC) species. The goal of this research is to develop molecular identifiers for individual *Shigella* species as well as

identifiers common to all species. FDA will also develop new screening methods that can detect all *Shigella* species and differentiate them from EIEC as well as attempt to further modify the method to accommodate the demographic prevalence of each individual *Shigella* species around the world.

Optimization of a Real-Time PCR Assay for Detection of *Shigella* spp. for Use with the BAX Instrument

The open availability of reagents and protocols that are employed in FDA analytical assays provides an incentive for other analytical laboratories, in the U.S. and around the world, to adopt FDA methods. As such, FDA and DuPont Qualicon will perform collaborative research focused on the development of a highly specific and rapid detection method for *Shigella* species, the third most common pathogen causing food-borne illness in the U.S. This effort combines FDA food safety expertise with DuPont BAX technology in an effort to develop new *Shigella* detection technologies that will be commercially available to public health labs and the food industry.

Isolation and Detection of *Shigella* from Produce Samples by Capillary Electrophoresis/Laser-Induced Fluorescence

Since 2008, the CDC has listed *Shigella* as the third most reported disease-causing foodborne bacterial pathogen. Unfortunately, conventional culture methods often fail to identify *Shigella* when it is overwhelmed by ubiquitous, non-detrimental bacteria during analytical enrichment procedures. The goal of this research is to validate a new enrichment procedure using a 30 minute acid shock to promote *Shigella* growth over competitors. This modified enrichment will be followed by microbial capture and concentration by capillary electrophoresis with detection by laser-induced fluorescence. If successful, this new method of isolating and identifying *Shigella* will provide the FDA with an enhanced means of detecting *Shigella* tainted produce and reduce the risk of illness.

Clostridium

Validation of an Enzyme-linked Immunosorbent (ELISA) Method for Detection of Botulinum Neurotoxin in Foods

Clostridium botulinum toxin is extremely potent and responsible for the deadly neuroparalytic disease botulism. The bacteria and their toxin are usually associated with improperly processed or stored food commodities. The purified toxin could also be added to foods in an act of deliberate food contamination. The current standard method for detecting this toxin involves a mouse survival assay. The goal of this research is to validate a mouse-free ELISA-based rapid detection system to be used by the Laboratory Response Network and the Food Emergency Response Network. In a food contamination event involving botulinum toxin, this method could reduce numbers of human illnesses by allowing for a quicker response.

Development of a Cell Based Assay for the Detection of Botulinum Neurotoxins

The reliable detection of Botulinum toxin in foods is an important food safety and national security issue. Current lab-based methods are sensitive and robust. However, they are limited to detecting the physical presence of the toxin and fail to detect the potency of the toxin in a food sample. T he goal of this research is to develop a rapid screening method for foods that will help confirm the presence of functional toxin. This will enhance FDA's ability to protect public health and minimize human illness that could be associated with such a contamination event.

Development of Novel Antigens for Detection of a Botulinum Neurotoxin in Food Samples

Botulinum neurotoxin causes deadly neuroparalytic diseases in humans. Human botulism is caused by oral exposure of a toxin produced primarily by *Clostridium botulinum* from serotypes A, B, E, or F. The goal of this research is to develop unique reagents for the further development of a rapid screening system for food samples. If successful, this would help FDA to confirm the presence of toxin in the event of *C. botulinum* contamination in industrial and food processing environments, thereby enhancing FDA's ability to protect the public health and minimize human illness from the severity of botulism.

Development of a Rapid Non-Culture Based Method for Distinguishing Viable and Non-Viable Bacterial Endospores

The detection of *Clostridium botulinum* presents a unique health risk to the analytical microbiologist who must often grow the bacterium in order to detect it in foods. Growth of this organism can lead to the production of dangerous levels of neurotoxin. PCR based methods allow for detection of this organism without the need for sub-culture. A limitation of the PCR method is the inability to distinguish between viable and non-viable organisms. The goal of this research is to evaluate the feasibility of utilizing psoralen to limit the PCR signal to viable clostridial spores only. This will allow for the rapid detection and determination of viability without the need for culture-based enrichment.

Clostridium Botulinum Toxin Bioassay – Determination of Human Health Hazard in Regulatory Food Samples

Clostridium botulinum toxin is extremely potent and responsible for the deadly neuroparalytic disease botulism. The bacteria and their toxin are usually associated with improperly processed or stored food commodities. The goal of this research is to develop a mouse bioassay to detect *C. botulinum* toxins in food or other sources that may affect human health. If successful, this work would provide another means to test the potency of the toxin present in food and thus the risk to public health.

Staphylococcus

Detection of Thermally-Treated Staphylococcal enterotoxin B in Milk Using a Biological Indicator Assay

Staphylococcal enterotoxin B (SEB) is known to induce food poisoning in humans regardless even if it has been exposed to elevated temperatures used in cooking or pasteurization. Unfortunately, commercially available SEB detection assays cannot recognize the toxin in food once it has been heated. The goals of this research are to identify SEB-induced biomarkers associated with certain immune responses *in vivo* and to use these molecular indicators for detecting heated SEB in milk or yogurt. If successful, this research can lead to assays for detection of SEB in various heated food matrices, which would otherwise go undetected and lead to continued morbidity and mortality due to this toxin.

Utilization of Microsphere Protein-Protein Interaction in the Identification of Staphylococcal Enterotoxins

Staphylococcal food poisoning results from ingestion of pre-formed enterotoxins in foods and is one of the leading causes of foodborne illnesses worldwide. The goal of this research is to evaluate a new technology that uses specific proteins and antibodies bound to unique microspheres to detect staphylococcal enterotoxins. The technology will enable FDA to detect multiple toxin targets simultaneously and directly in foods, thereby simplifying testing procedures and decreasing detection time.

Polymerase Chain Reaction (PCR) Identification of Enterotoxigenic Staphylococci

Approximately 242,000 cases of staphylococcal enterotoxins food poisoning are reported nationwide annually. Analysis of staphylococcal isolates from suspect food products requires the use of specific antibodies to identify the five traditional staphylococcal enterotoxins; however, new staphylococcal enterotoxins that are undetectable by the current detection methods have been identified. The goals of this research are to develop a molecular method to detect staphylococcal enterotoxins gene targets from food matrices and to characterize the toxigenic potential of bacteria isolated from foods. The new molecular method will be useful for detecting suspect outbreaks of staphylococcal enterotoxins that are negative by conventional antibody detection methods, thereby improving staphylococcal enterotoxin surveillance and control.

Validation of Enzyme-Linked Immunosorbent Assays for Staphylococcal Enterotoxins in Culture Supernatant

Identification of staphylococcal enterotoxins and enterotoxigenic isolates from food is important in FDA's regulatory mission. The goal of this research is to validate commercially available test kits, designed originally for food products, for use in testing bacterial isolates. A panel of 50 enterotoxigenic *Staphylococcus aureus* and 30 staphylococcal isolates are being used to evaluate the specificity and sensitivity of selected commercially-available antibody kits in comparison to a molecular method. Once validated, these rapid test kits could be used to identify enterotoxigenic strains.

Other Bacterial Pathogens and Detection of Multiple Pathogens

Method Development for the Isolation, Detection, and Identification of *Brucella* spp. from Soft Cheeses

Brucellosis, caused by *Brucella* spp., can be contracted through the consumption of dairy products made from the milk of infected animals. Of concern are cheeses, particularly soft, Latin American-style cheeses made from raw milk, brought into the U.S. through informal channels from Mexico, where *Brucella* is endemic. Currently there are no adequately validated methods for detection of *Brucella* in cheeses. The goals of this research are to develop assays to distinguish *Brucella* from other bacteria found in cheese and to identify different *Brucella* species. A more efficient *Brucella* detection method will enable the FDA to insure the safety of the dairy products entering the U.S. and facilitate investigation of *Brucella* outbreaks and illnesses.

Chromogenic Agar and Polymerase Chain Reaction (PCR) Identification of Enterotoxigenic *Bacillus cereus*

Foodborne diarrheal illness caused by enterotoxigenic strains of *Bacillus cereus* may be underreported due to the short duration and mildness of symptoms; however, there have been reports of deaths linked to these bacteria in food. The goals of this research are to evaluate a new chromogenic growth medium that simplifies the detection, enumeration, and identification of *B. cereus* from foods, as well as to compare a molecular method specific for the toxin gene to conventional methods to determine if enterotoxigenic strains can be differentiated from non-toxigenic strain. If successful, the simplified method will shorten the testing time for *B. cereus* in foods.

A Comparison Study of Two Secondary Enrichments for the Detection and Recovery of *Cronobacter* spp. from Infant Products

Cronobacter species, particularly *Cronobacter sakazakii*, pose a critical medical concern to premature neonates and infants. As a foodborne bacterial pathogen, these microbes have been associated with powdered infant formula (PIF) as well as other dried foods, e.g. cereals. Although the FDA has a revised method to isolate and detect these pathogens in PIF, there remain challenges as they may be present in low numbers and in a stressed-physiological condition. In addition, other difficulties to recover *Cronobacter* species can be attributed to the high levels of background microbiota. The goal of this research is to compare two secondary enrichments as a means of improving recovery of our target pathogen in infant products.

Toward a Rapid and Reliable Pathogen Detection System in Produce

This collaborative project between FDA and the University of Maryland is funded by the Center for Produce Safety at the University of California, Davis. *Salmonella* and *E. coli* in produce pose a significant threat to public health and the produce industry. The goal of this research is to use a novel molecular-based testing method called loop-mediated isothermal amplification (LAMP)

for the detection of low levels of these pathogens in various produce items using conditions mimicking real-world contamination. Rapid, accurate, simple, and robust detection of important human pathogens in produce will provide the produce industry with better information that can then be used to help prevent microbial contamination.

Development of a Multiplex Real-time PCR Method for Simultaneous Detection of *Salmonella* spp., *Escherichia coli* O157:H7 and *Listeria monocytogenes* in Soft Cheese and Spinach

The presence of bacterial pathogens in food creates a public health burden and strains the economy due to the loss of work hours caused by foodborne illness. Hence, it is important to rapidly detect these foodborne bacterial pathogens in suspected food samples and thereby more rapidly eliminate contaminated food from distribution to the public. The goal of this research is to develop a method to simultaneously detect three important foodborne pathogens in soft cheese and spinach: *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes*. Once validated, this method could be used by FDA as a rapid analytical tool for ensuring industry compliance and during outbreak investigations.

Validation of a Multiplex Real-Time PCR Method to Detect *Salmonella*, *Shigella*, and *E. coli* O157:H7 in Foods

Monitoring and protecting our nation's food supply using rapid, efficient, and effective laboratory methods is a pressing issue for FDA. The goal of this research is to develop a multiplex real-time PCR system that simultaneously detects the most prevalent enteric pathogens, *Salmonella*, *Shigella*, and *E. coli* 0157:H7, in one test. This system will apply to all food types, reducing the reporting time, labor and cost of analysis in FDA laboratories. It will also increase the throughput numbers of import and domestic samples that are analyzed by the FDA, effectively expanding the agency's food surveillance capability.

Validation of *Salmonella* and *Shigella* Real-Time PCR SmartCycler II Methods on ABI 7500 Platforms

The food pathogens, *Salmonella* and *Shigella*, cause thousands of foodborne illnesses every year. Currently, the qPCR method employed to screen for these pathogens utilizes an outdated PCR Smart Cycler platform. The goal of this research is to update this method by adapting the protocol to the ABI 7500 FAST PCR thermocycler platform. This compatible protocol will provide a solid basis for high throughput sample analysis and will allow qPCR methods to be more widely used throughout FDA and other Federal agencies, academic research laboratories, State Public Health laboratories and the private sector.

Testing the Foodborne Bacterial Pathogen Assay Plate on the Plex-ID

To protect the public from foodborne bacteria and viruses, the FDA is developing rapid techniques to detect and identify these pathogens. The goal of this research is to identify foodborne bacteria and viruses that commonly cause illness using a novel combination of existing rapid technologies, including the polymerase chain reaction (PCR) coupled with mass spectrometry (Plex-ID). An assay for bacteria will be developed, and tested using known and

unknown bacterial samples. FDA will also develop an assay for norovirus using the same technology. These assays will allow for the rapid detection of foodborne bacteria and viruses during foodborne outbreaks.

Development of Rapid and Universal Detection Methods for Proteinaceous Toxins in Food Using Nanoflow Liquid Chromatography-Mass Spectrometry

Certain foodborne bacterial pathogens, such as *Staphylococcus aureus* and *Clostridium botulinum*, secrete protein toxins. Consumption of food contaminated with these bacteria may result in infectious or toxic foodborne illnesses, often associated with nausea, vomiting, diarrhea, and fever. The goal of this research is to evaluate new methods of bacterial sample preparation and toxin extraction that can be combined with liquid chromatography-mass spectrometry (LC/MS) detection. Implementation of these methods would enable the FDA to unambiguously detect and quantify the presence of protein toxins at the molecular level, without the use of costly antibodies, and provide for more rapid and reliable analysis to protect public health.

Top-Down Mass Spectrometry for the Rapid Identification of Bacterial Proteins

Protection of public health requires faster and more effective methods for identification of bacteria involved in foodborne contamination. Proteins produced by bacteria may act as markers to identify specific strains or identify virulence factors. The goal of this research is to develop an intact protein chromatography and top-down tandem mass spectrometry method, for rapid identification of bacterial proteins. The method allows the same approach and instrument to be used regardless of the bacterial target, thus simplifying development. If successful, this approach could identify protein markers unique to bacteria that cause a public health risk and produce targets for the development of additional rapid and specific methods for bacterial identification.

Isolation and Detection of Foodborne Pathogens by Novel Chromogenic Media

Detection and isolation of foodborne pathogens from foods is a challenge for FDA, with the lack of effective media and enrichment broths as an important limiting factor. The goal of this research is to compare selective chromogenic media and new broths to those media currently used in FDA's Bacterial Analytical Manual for detection and isolation of *Salmonella*, *E. coli* O157 and *Listeria monocytogenes* from FDA regulated foods. Better and more effective media and enrichment broths can greatly enhance the sensitivity of detection methods and could reduce the time and labor required for pathogen detection and isolation, thereby improving FDA's response during outbreaks.

Detection of DNA Microarrays on Spotted Glass Slides by Infrared Imaging

The rapid identification of pathogenic bacteria involved in foodborne illness is important for public health protection. The goal of this research is to develop a novel infrared (IR) chemical imaging procedure for the detection of DNA microarrays, which are emerging tools for the rapid identification of pathogenic bacteria. In addition to the identification of pathogens, the IR DNA microarray methodology will be extended to the measurement of DNA for rapid fish species identification. The development of such an approach could be used by FDA to rapidly identify

mislabeled and potentially hazardous fish, as well as reduce economic fraud, thereby protecting the public health.

Free-Living Amoebae as Tools to Recover *Salmonella*, *Shigella*, and *E. coli* O157:H7 from Produce

Current bacteriological testing methods for enteric foodborne pathogens in fresh produce require lengthy enrichment steps to increase the number of pathogens to facilitate their detection. However, these enrichments are often not specific enough to allow only for the growth of the pathogenic microorganisms of interest, which complicates subsequent testing. The goal of this research is to evaluate the ability of enteric pathogens to survive and grow inside certain amoebas, and its potential use as a specific enrichment step to enhance the detection of pathogens from different produce types. More effective enrichment steps would improve FDA's ability to identify produce contaminated with low counts of pathogens, thereby helping to ensure the American public of a safe food supply.

Enteric Viruses

Application of Microarray Technology to the Detection and Identification of Foodborne Viruses

Millions of foodborne illnesses per year in the U.S. are caused by enteric viruses transmitted through food, water, or food handlers. Low levels of virus particles in food present unique challenges to their extraction, detection, and identification. The goal of this research is to evaluate the discriminatory power of a multi-virus DNA microarray as a single, rapid alternative to current methodologies that require multiple steps to provide equivalent results. The detection and identification of virus contaminants are essential for developing and implementing effective responses to disease outbreaks, for developing a surveillance strategy for virus detection, and for preventing the sale of contaminated products.

Development of Cell Culture Methods for Foodborne Viruses

Each year millions of foodborne illnesses caused by enteric viruses transmitted by food and water are reported in the U.S. Current molecular-based detection methodologies cannot readily distinguish infectious from non-infectious particles, and important enteric viruses such as hepatitis A and norovirus lack reliable culture methods. Determining whether a commodity contains infectious virus contaminants will require developing virus culture methods. The goals of this research are to expand the knowledge base of replication characteristics of these viruses and to develop improved detection methods. Providing rapid methods to detect infectious virus will help prevent human exposure and illness and reduce the economic impact of these outbreaks.

Detection of Hepatitis A Virus and Human Noroviruses in Representative Produce Items

Reducing the overall incidence of foodborne viruses in the food chain is a critical mission of the FDA. There is a need to develop and validate sample extraction and preparation methods for the most epidemiologically important viruses in high risk food commodities. The goals of this research are to develop capture methods for hepatitis A virus and human norovirus from complex

food matrixes such as produce and ready-to-eat items and develop rapid molecular detection methods. If successful, this research will further enhance the FDA's capabilities for timely response during inspections, surveillance, and disease outbreak management, and for preventing the sale of foodborne virus contaminated products.

Sample Preparation for Detection of Viruses on Fresh Produce

Fresh fruits and vegetables have been a source of large outbreaks of illnesses caused by viruses. Viruses are usually not present in high numbers in fresh produce, and methods for their detection are difficult, lengthy, and expensive, thus prompting the need for more efficient detection methods that are accurate, easy and quicker. The goal of this research is to evaluate various ways to recover and detect viruses in fresh vegetables, using different chemical and physical techniques. The results from this research should reveal the best ways to recover these viruses from foods for the quickest and most sensitive detection and enable FDA to monitor and test regulated food and reduce outbreaks of human illness caused by these viruses.

Improving Detection of Hepatitis A Virus in Foods

Hepatitis A virus (HAV) is environmentally stable and readily transmitted via the fecal-oral route. Foods often implicated in HAV outbreaks include shellfish, fresh produce, and ready-to-eat products. Lack of a suitable methodology for detecting HAV contamination has limited the FDA's ability to prevent or intervene in HAV outbreaks. This research is aimed at establishing a rapid and sensitive method for detecting HAV in foods that could increase the FDA's ability to be more pro-active in public health protection directed against foodborne virus infections.

Real-Time, Label-Free Detection of Foodborne Viruses Using Surface Plasmon Resonance Biosensors

With increased seafood consumption in recent years, there has been a rise in shellfish associated illnesses from enteric viral pathogens. Norovirus is the viral pathogen most common in seafood, primarily in molluscan shellfish. The goal of this research is to develop a rapid and sensitive new method based on surface plasmon resonance biosensors to better detect viral pathogens, including Norovirus. If successful, this work could improve FDA capabilities for protecting public health from risks of foodborne viral pathogens.

Validation of Extraction and Detection Methods for Enteric Viruses in Shellfish

Norovirus is a leading cause of illness associated with raw or undercooked shellfish consumption in the U.S. This human virus is excreted in high numbers by infected individuals and occasionally contaminates shellfish in coastal growing waters. Current methods for recovery and identification of norovirus from shellfish are labor intensive and unreliable. The goal of this research is to develop and validate a rapid, sensitive, and accurate method to test shellfish for these viruses. If successful this method could be used by FDA and State public health laboratories for outbreak investigations and regulatory monitoring to reduce the risk of consumer illness.

Development of a Bioplex Method for the Simultaneous Detection of Multiple Viruses

There are a number of viruses that can cause illness in humans through consumption of contaminated foods. The goal of this research is to develop a method capable of both detecting and identifying multiple foodborne viruses in a large number of food samples simultaneously. The development of this high-throughput assay, utilizing the BioPlex instrument, will help enhance FDA's food safety surveillance initiatives, trace-back analyses, and its response to disease outbreaks of viral food-borne illness.

Development of a Single-Tube Nested Real-Time PCR Assay for Foodborne Virus Detection

Because of the lack of sensitive detection methods, foodborne viruses, such as Hepatitis A Virus (HAV), are rarely identified through direct detection in food. To address this issue, we will develop a method that should greatly improve sensitivity, reliability, and turnaround time for foodborne virus detection. With this method, all reactions following sample preparation, including the Polymerase Chain Reaction (PCR) DNA amplification procedures, will be performed in a single tube. This novel method, employing a single tube device, will be more sensitive than other PCR methods, reduce the risk of carry-over contamination and improve food risk assessment critical for public health.

Development of an Infectivity Assay to Detect Human Norovirus from Contaminated Food

One of the problems facing FDA in cases of foodborne norovirus is the lack of technical methodologies to confirm the presence of infectious virus in contaminated food. Molecular methodologies cannot assure integrity or inactivation of the virus particle. Thus, FDA actions would require overcoming the lack of an available cell line or animal model capable of sustaining norovirus infection. The goal of this research is to combine *in vitro* cell culture and molecular assays to determine the presence of infectious particles, as well as quantification of virus load.

Protozoan Pathogens

Genomic Analysis of *Cyclospora cayetanensis* and *Cryptosporidium* spp: Methods Development for Organism Detection, Viability and Molecular Epidemiological Applications

Illness caused *by Cyclospora cayetanensis*, *Cryptosporidium hominis*, and *Cryptosporidium parvum* has been associated with contaminated fresh produce and water sources. Pathogen detection using molecular-based approaches has replaced conventional microscopic techniques. PCR is an example of a sensitive molecular-based approach that can quickly detect these pathogenic foodborne and waterborne microorganisms by providing faster diagnostic times plus enhanced sensitivity and specificity. The goals of this research are to increase our understanding of these parasitic pathogens and to develop improved detection methods for use in epidemiological studies and outbreak investigations.

Evaluation and Development of Molecular Diagnostic Techniques for Rapid Detection and Differentiation of *Cyclospora* Transmission in Fresh Produce and Other Food Products

Cyclospora cayetanensis is a protozoan parasite that infects the gastrointestinal track and causes acute diarrheal disease in both immunocompromised and immunocompetent humans. The emergence of human cyclosporiasis disease caused by *C. cayetanensis* has led to several foodborne outbreaks in U.S. and Canada associated with imported produce, predominantly raspberries. The goal of this research is to develop a Polymerase Chain Reaction (PCR) DNA amplification protocol based on the sequence of the 70 kDa heat shock protein gene for the rapid detection of *C. cayetanensis*. The method will be tested and validated by characterizing human *C. cayetanensis* isolates from three endemic regions, namely Nepal, Mexico, and Peru.

Validation Studies - Microbial

Validation of the ABI MicroSeq 500 DNA Sequencing Method for Bacterial Foodborne Pathogen Identification

Genotypic identification is becoming the new standard for bacterial identification. The genotypic approach uses DNA sequencing of the 16S rDNA gene and comparison with gene sequences of known bacterial species to identifying unknown bacteria to the species or subspecies level. The MicroSeq 500 rDNA Bacterial Identification System is based on sequencing the first 527 base pairs of the 16S rRNA gene. The goal of this research is to optimize MicroSeq 500 System (MS500) for use in a regulatory setting, and validate its use for confirmatory identification of food pathogens.

Single-Laboratory Validation (SLV) and Multi-Laboratory (MLV) of the *Escherichia coli* O157:H7 RIMS (Recirculating Immumomagnetic Separation) Method

Fresh leafy produce has been associated with multiple outbreaks of *E. coli* O157:H7 infections. A recently developed method incorporates a 5 hour enrichment followed by a large sample volume immunomagnetic separation (IMS) step to facilitate recovery and concentrate *E. coli* O157:H7 from leafy produce for the detection and isolation of this pathogen. Screening results are available in 12-36 hours and full confirmation within 4 days. The goal of this research is to provide higher level validation of this method with representative leafy produce matrices consistent with FDA validation guidelines. This will provide FDA with another tool to rapidly screen leafy produce to detect contaminated product before it reaches the consumer.

Single Laboratory Validation (SLV) Study on qPCR Detection of *E. coli* O157:H7 from 24 hour Food Enrichments

The use of real-time PCR as a tool for detecting foodborne pathogens has shown great promise in the efforts to safeguard our food supply against acts of bioterrorism and outbreaks of disease. The current need for improved microbiological assays requires transitioning screening methods to high-throughput platforms. The goal of this research was to develop and validate a real-time PCR method targeting Shiga-toxin producing *Escherichia coli* (STEC) and the *E. coli* O157:H7 serotype on the ABI7500 Fast. Based on the results from this work, a multi-laboratory validation

study was conducted by FERN cooperative agreement laboratories and the method was accepted as a FERN method in FY2012.

Multi-Laboratory Validation Study on qPCR Detection of *E. coli* O157:H7 from 24 h Food Enrichments

The goal of this research was to validate a real-time PCR method developed at the Applied Technology Center targeting Shiga-toxin producing *Escherichia coli* (STEC) and the *E. coli* O157:H7 serotype on the ABI7500 Fast in 14 laboratories with 5 food matrices. In summary, the ABI7500 Fast *E. coli* O157/STEC qPCR method was equivalent or slightly improved to the reference method in this multi-laboratory validation study. The assay showed improved platform versatility in accommodating greater sample throughput (96-well plate format), rugged internal control performance in high background food matrices and an additional qPCR tool to screen food enrichments for the potential presence of *E. coli* O157/STEC in samples. The method was accepted as a FERN method in FY2012.

Multi-Lab Validation for High-Throughput DNA Extraction and qPCR of *E. coli* O157:H7 and *Salmonella* from Food Enrichments

It is not uncommon for a large number of food samples to be tested with only a very small proportion containing a potential human pathogen. Thus, it may be necessary for a very large number of samples to be tested in order to identify those that potentially pose a threat to consumers. The goals of this research are to develop rapid testing strategies, to improve testing sensitivity, and to focus on those strategies that can be automated and test a large number of samples. Upon identifying and optimizing instruments that meet these needs, FDA will conduct tests in multiple laboratories to ensure successful implementation in various laboratory settings. These methods will help FDA to meet increasing testing demands, keeping consumers safe from contaminated products.

Multi-Laboratory Validation of a qPCR Method for Detecting *Salmonella* in Foods

The goal of this research was to validate the conversion of the real-time PCR *Salmonella* detection method originally designed for the Cepheid SmartCycler II® to the Applied Biosystems ABI 7500 FAST platform in 16 FERN laboratories with four food types: chili powder, soft cheese, fish and tomatoes. Statistical analysis of the data showed there was no significant difference (P-value ≥ 0.05) between the methods for these two qPCR platforms with the exception of chili powder which performed better with the ABI 7500 Fast. The results of this research provide a basis for using the 24-hr qPCR ABI 7500 FAST system screening method and incorporating high throughput samples analysis to detect *Salmonella* in foods.

Multi-laboratory Evaluation of MicroSEQ® *Salmonella* spp. Detection Kit in Comparison with an FDA Rapid Screening qPCR Method

The goal of this research was to conduct a multi-laboratory evaluation to compare the sensitivity and specificity of the MicroSEQ® *Salmonella spp.* detection kit to those performance parameters previously validated for the FDA qPCR rapid screening method for *Salmonella* in foods. Four

food types (chili powder, soft cheese, fish and tomatoes) were evaluated by 12 FERN labs. The study indicated that there was no significant difference statistically between the MicroSEQ® *Salmonella* spp. detection kit method and the corresponding reference method. The consistent results support the utility of the MicroSEQ® *Salmonella spp.* kit as an alternative method for detecting *Salmonella* in food.

Single Laboratory Validation of Existing Chromogenic Agars for *Salmonella* Serovars

Chromogenic (color-based) bacterial growth media speeds up the detection process for foodborne bacteria. These agars are specific, easier to interpret, and faster for detection of *Salmonella* from foods. The goal of this research is to evaluate this plating technology with several hundred different *Salmonella* strains, representing the diversity of this bacterium, using commercially available chromogenic agars. Successful agars would be compared directly with FDA's current method to develop optimal plating schemes for this pathogen in produce and other high-risk foods. More accurate methods for the identification of *Salmonella* would enable FDA to improve emergency response and compliance efforts when testing the food supply.

Single Laboratory Validation (SLV) Study for qPCR Detection of *Listeria* spp. and *L. monocytogenes*

The goal of this research was to develop and validate a comprehensive, rapid and sensitive method for detecting and isolating *Listeria* spp. and *L. monocytogenes* from food matrices using the ABI7500 Fast qPCR platform. Two assays were evaluated 15 participating laboratories. These assays offer improved detection, turn-around time and greater sample throughput (96-well plate format). Based on this study, a multi-laboratory validation study was done by FERN cooperative agreement laboratories result in the acceptance of the method as an official FERN method in August 2012.

Multi-Laboratory Validation Study for qPCR Detection of *Listeria* spp. and *L. monocytogenes*

The goal of this research was to develop and validate a comprehensive, rapid and sensitive method for detecting and isolating *Listeria* spp. and *L. monocytogenes* from food matrices using the ABI7500 Fast qPCR platform. Two assays were evaluated by 15 participating laboratories. In summary, both assays were equivalent or slightly improved to the reference method in this multi-laboratory validation study. These assays offer improved detection, turn-around time and greater sample throughput (96-well plate format). The method was accepted as a FERN method in August 2012.

Single Laboratory Validation of the BAX qPCR *Vibrio* Assay for Identification of *Vibrio* Isolates

The goal of this research is to validate real-time PCR assays for detection and quantification of *Vibrio* spp. in seafood. The first phase in this process is to demonstrate specificity of these assays for the intended target organisms using pure cultures. The BAX *Vibrio* qPCR assay for use in identification of *V. cholerae, V. parahaemolyticus* and *V. vulnificus* isolates has been evaluated in collaboration with FDA's Gulf Coast Seafood Laboratory. This validation offers an

additional reference method for identification of these pathogenic *Vibrio* spp. including the next validation phase for detection of these organisms in seafood enrichments.

Validation of Real-time PCR Assays for Detection, Identification, and Characterization of *Vibrio* spp. in Seafood

Modern molecular methods, like Polymerase Chain Reaction (PCR) are powerful tools that can be used to quickly detect microorganisms in food. Several real-time PCR assays have been developed for detection of *Vibrio* spp. including *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus*. These methods have increased FDA's knowledge about these bacteria, although they have not been fully validated. The goal of this research is to validate real-time PCR methods for the detection of *Vibrio* spp. using FDA's validation guidelines for microbiology. If successful, these methods would be submitted for inclusion in FDA's Bacteriological Analytical Manual for use in regulatory applications, surveillance and outbreak response.

Multi-Laboratory Validation of BAX *Vibrio* Assay for Identification of *Vibrio* spp. Isolates

The goal of this research was to conduct a multi-laboratory validation study for the BAX® Vibrio Assay which is a multiplex real-time PCR that includes proprietary targets and reagents for simultaneous identification of total *Vibrio cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Six laboratories participated in this study. Statistical analysis of data showed that the sensitivity and the selectivity met the validation requirements (>95%). This validation provides laboratories an additional method for identification of these pathogenic *Vibrio* spp.

Molecular Characterization of Foodborne Pathogens as It Relates to Research on the Mechanism of Disease and/or Epidemiology of Foodborne Disease

Salmonella

Characterization of Genotypic and Phenotypic Traits Possessed by Salmonellae and the Predominant Microorganisms which are Associated with Tomatoes and Tomato-growing Environments of the DelMarVa Area.

Not much is known about how salmonellae, a cause of human gastroenteritis, contaminate produce such as tomatoes. Understanding the ecology of salmonellae on the farm and the means by which it is transferred to tomatoes is vital for the design of preventive strategies to reduce contamination events. The goals of this research are to collect tomato plant and environmental samples from tomato farms, for a year and to isolate and characterize salmonellae and associated predominant microorganisms from those farms. This work should help the FDA to assess the risk of environmental factors on tomato-borne salmonellosis. The information from this research will also be useful in developing improved detection and characterization methods for salmonellae in foods.

Whole-genome Sequence Analysis of *Salmonella enterica* Serovars Commonly Associated with Foodborne Outbreaks from Fresh-cut Produce and Poultry

Traceback of a foodborne outbreak to its source is important in understanding where a pathogen came from and how it entered the food supply. FDA is currently using whole-genome sequencing as a high-resolution strain typing method to resolve outbreaks arising from *Salmonella* contamination. The goal of this research is to apply the method to *Salmonella* associated with recent outbreaks in produce, eggs, and spices to examine potential of the technology to pinpoint contamination sources and delimit the scope of outbreaks. If successful, the technology could be used by FDA to identify pathogen reservoirs and points to specific geographic regions that harbor unique pathogenic strains. This would help FDA in traceback efforts during foodborne outbreaks.

Genetic Differentiation among the Five Most Common *Salmonella* Enteritidis Phage Types in the U.S.

In foodborne outbreaks, genetic typing methods are often used to determine if the bacterial pathogen from a patient matches that found in a suspected food. Phage typing is one method that has historically been used to help determine this match. *Salmonella* Enteritidis is a bacterium often associated with outbreaks from poultry and eggs. The goals of this research are to screen DNA-sequence information about phage types of *S.* Enteritidis and to develop a rapid molecular typing scheme for the most common phage types. If successful, this work could result in a fast and accurate phage typing method for *S.* Enteritidis isolated from patients and food samples and could aid industry compliance to FDA's 2009 rule for prevention of *S.* Enteritidis in shell eggs.

Utilization of Molecular Methods for *Salmonella* Serotyping

The standard Kauffman-White scheme, used by the FDA to serologically classify *Salmonella,* is tedious and lengthy. Two newly developed molecular methods can each rapidly and efficiently serotype approximately 100 common, though different, *Salmonella* serovars in less than a day from pure culture. The goal of this research is to evaluate and validate these methods. If successful, this could reduce time for serotyping from a week or more to a single day, thus providing FDA with critical information during outbreak investigations.

Developing a Molecular Serotyping Scheme for *Salmonella* Serovars Relevant to Animal Feed

Among over 2,500 *Salmonella* strains identified to date, eight are of major animal health concern. Rapid methods for these specific *Salmonella* strains are therefore needed for feedmill facilities to identify potential contamination problems. Recently, CDC developed a standard protocol for the molecular serotyping of *Salmonella* using PCR-Luminex assays. However, not all of the eight critical strains were included. The goal of this research is to develop a molecular serotyping scheme for all of these feed-related *Salmonella* strains using the PCR-Luminex technology and to validate it using FDA's strain collection. This development will facilitate better compliance with preventive control guidelines at feedmill facilities and protect both animal and human health.

Multi-laboratory Evaluation of DiversiLab System for Characterization of *Salmonella* Outbreak Isolates

The DiversiLab system is a rep-PCR based DNA fingerprinting system that can provide results in <5 hours from a pure culture of bacteria. The goals of this research were to conduct a multi-laboratory collaboration with 15 laboratories to: 1) examine the performance of the DiversiLab in data reproducibility and overall efficiency, and 2) optimize a semi-automated DNA extraction procedure using the Roche MagNA Pure Compact (MPC) along with the DiversiLab. Both manual DNA extraction and MPC method generated highly reproducible and consistent rep-PCR DNA fingerprinting data. This study suggests that DiversiLab has the potential to serve as an additional tool for molecular typing and for tracking the sources of contamination in foodborne outbreaks.

Plasma and Pulsed Light Inactivation of *Salmonella* on Almonds and Black Pepper

There have been outbreaks of illness associated with consumption of powdered infant formula, raw almonds and dry spices contaminated with pathogens resulting in a need for methods for decontaminating these dry foods. Traditional thermal processing approaches to decontaminate these foods can cause adverse effects on product quality. Therefore, alternative processes for decontaminating dry materials are desirable. The goal of this research is to evaluate the effectiveness of decontaminating dry ingredients during processing using novel emerging technologies, such as cold plasma and pulsed light. I f successful, these technologies could be used to reduce microbial pathogens in dry ingredients to improve their safety.

Thermal Resistance of *Salmonella* as Influenced by Matrix Water Activity

Recently, large outbreaks of gastrointestinal illness involving the presence of *Salmonella* in low moisture foods have indicated a need to re-evaluate the behavior of foodborne pathogens in these types of products. Simple systems that can be used to rapidly determine if an isolate possess unusual heat tolerance that could be useful for solving food safety related questions that involve outbreaks originating from low moisture foods. The goal of this research is to develop simple model food systems that will allow for an estimation of the heat resistance of *Salmonella* in complex low moisture food matrices that are difficult to study under laboratory conditions. If successful, the resistance data gathered in these studies would fill knowledge gaps about the survival of *Salmonella* in heat processed foods and help to establish prevention strategies to minimize *Salmonella* survival in low moisture foods.

Evaluation of Microbial Communities in Sediment Basins and Related Conservation Practices and their Impact on Nationwide Food Safety

Sediment basins on irrigated agricultural sites are an important conservation practice to capture and detain sediment laden runoff and allow sufficient time for the sediments to settle out. The goal of this research in collaboration with the USDA Natural Resources Conservation Service was to evaluate sediment basins of differing design and in differing ecological regions nationwide to determine which designs allow for maximal reduction of bacteria in surface water. While the use of sediment basins for the recovery of irrigation water has been proven to be effective in reducing sediment loads into waterways, the results indicated that these surface waters may contain pathogenic bacteria, specifically *Salmonella*. This research demonstrates that re-using these waters for irrigation of ready-to-eat produce may pose a human health risk, which should be addressed in pre-harvest good agriculture practices.

Characterization and Co-management of Potential Food Safety Risks from Wildlife in Riparian and Wetland Habitats near Fresh Produce Fields

The goal of this research was to examine the impact of water quality factors and management practices on the occurrence of pathogens in cold-blooded vertebrates and waterbodies in and adjacent to fresh produce production areas. A total of 1,444 samples were tested during the 2011 growing season; *Salmonella* was cultured from amphibian and reptile samples and non-irrigation waterbodies, but *Salmonella* was not found in pre-irrigation reservoirs used for storage of well water. All samples were negative for *E. coli* O157:H7 except a single tailwater pond sample. The results underscore the importance of pre-season and pre-harvest environmental assessment-related produce safety practices, in particular those addressing animal intrusions and irrigation water quality.

Salmonella Desiccation Resistance and Survival in Extremely Low Water Activity Foods

Salmonella has caused numerous outbreaks of illness linked to consumption of dry foods, such as peanut butter, chocolate, cereal, and grains. Most other pathogens cannot live in dry foods due to the lack of water, but *Salmonella* can live for extended periods of time. The goal of this research is to evaluate the mechanisms by which *Salmonella* survives conditions of low humidity

and water content, as well as some processing and sanitation treatments. The knowledge gained from this research would provide FDA with basic understanding of the physiology of *Salmonella* in dry foods and help in developing more effective interventions to minimize *Salmonella* contamination in dry foods.

Effect of Sanitizers on *Salmonella* on Shell Eggs

Eggs continue to be a major source for *Salmonella* infections. The conditions of the poultry houses may contribute to the contamination of the eggs with *Salmonella*. More information is needed to understand how *Salmonella* survives on eggs and in the poultry house environment, and to improve methods for decontamination of eggs. The goal of this research is to obtain data on the survival of *Salmonella* on the eggshell surface and on the transfer of *Salmonella* into the interior of the eggs, after cleaning with commercial egg sanitizers. The results of this research would help industry to develop better decontamination methods, on eggs and in the poultry house environment.

E. coli O157:H7 and STEC

Molecular Characterization and Virulence Determination of Shiga-toxigenic and Enterohemorrhagic *Escherichia. coli* Strains Isolated from Foods and Produce

Escherichia coli O157:H7 remains the primary *E. coli* pathogen that causes foodborne outbreaks worldwide. Atypical O157:H7 strains are causing a portion of these illnesses, yet these variants are not detected by existing assays. Additionally, other shiga toxin producing *E. coli* (STEC) have also emerged as important pathogens. Aside from the six serogroups recently focused on by the USDA for meats, there are many others that have been found in foods and these variants may also be pathogenic. The goals of this research are to characterize atypical O157:H7 as well as non-O157 STEC strains isolated from foods to determine their virulence potentials and to develop specific detection assays. If successful, this work would enable FDA to identify the presence of these pathogens in foods.

Validation and Optimization of Multi-Locus Variable-Number Tandem Repeats Analysis for Shiga Toxin-Producing *Escherichia coli* O157

Multi-Locus Variable-Number Tandem Repeat Analysis (MLVA), a PCR based technique used to target areas of the bacterial genome that evolve rapidly, is highly reproducible and discriminatory of closely related strains of *E. coli* O157. According to the CDC, current Pulse Field Gel Electrophoresis (PFGE) and MLVA identification results are well correlated. Both results are complementary and the evaluation of MLVA and PFGE in outbreaks enhances investigators' ability to differentiate outbreak and non-outbreak cases. The goal of this research is to optimize and validate MLVA of Shiga toxin-producing *E. Coli* O157 using the Applied Biosystems Genetic Analyzer 3500xL platform. If successful, this method will improve FDA's ability to respond quickly and correctly to foodborne outbreaks caused by this pathogen.

Fate of *Escherichia coli* O157:H7 in Field-inoculated Lettuce and Cilantro

The goal of this research was to evaluate factors that impact the fate of *E. coli* O157:H7 and *Salmonella* in field-inoculated lettuce and cilantro plants by testing the influence of inoculation method and humidity levels on the post-inoculation survival. High humidity levels and speed of desiccation consistently supported greater post-inoculation survival of *E. coli* O157:H7 in a laboratory environment. Inoculation of field lettuce or cilantro in the evening when humidity levels begin to increase also supported greater initial survival. Both the laboratory and field data showed, however, that bacterial survival on cilantro plants was different than that of lettuce; and survival of *Salmonella* was significantly better than for *E. coli* O157:H7. The results suggest that difference in the leafy green commodity may impact bacterial survival, which may lead to the development of different agricultural practices based on commodity.

Listeria

Genomic and Transcriptomic Analysis of *Listeria monocytogenes* Strains Involved in Invasive and Gastroenteritis Listeriosis Outbreaks

Listeriosis is one of the major foodborne diseases caused by a bacterium, *Listeria monocytogenes*. Listeriosis can be divided into two groups based on the invasiveness of the disease. Invasive listeriosis occurs in the susceptible groups such as pregnant women, neonates and immuno-compromised adults whereas gastroenteritis is developed in otherwise healthy populations. Efforts have been made to differentiate between these two types of bacterial strains that cause different types of diseases. The goal of this research is to utilize several molecular techniques to identify and differentiate between the *L. monocytogenes* strains that can cause gastroenteritis or invasive listeriosis. T he information gained from this research would help in understanding the mechanism(s) underlying different types of listeriosis.

Understanding Survival Strategies by *Listeria monocytogenes* and *Salmonella enterica* in RTE (Ready-To-Eat) Foods

Listeria monocytogenes is a bacterial pathogen that can cause an invasive life-threatening disease, listeriosis. *L. monocytogenes* in ready-to-eat foods is a significant public health concern. It is known that biofilms of *Listeria* facilitate resistance to sanitizers and other antimicrobial agents, but little is known about the physiological processes involved in *L. monocytogenes* growth on surfaces. The goal of this research is to conduct transposon mutagenesis studies to identify *L. monocytogenes* determinants that contribute to biofilm formation. If successful, this study would provide new insights into the genetic elements involved in biofilm formation by *L. monocytogenes* and advance efforts to understand biofilm development on food preparation surfaces.

Evaluation of *Listeria monocytogenes* Recovery from Food Matrices Co-Contaminated with Other Species of *Listeria*

The sensitivity of many currently available pathogen detection technologies depends on the population density of the target bacteria at the time of testing. This is why a period of enrichment growth is first employed. However, a complete understanding of the effect of other

non-harmful bacteria on the growth of the target bacterium during enrichment is lacking. The goal of this research is to evaluate the ability of current enrichment methods to amplify pathogenic *L. monocytogenes* in matrices that also harbor non-pathogenic *Listeria*. Results from this research could have a positive impact on human health by determining the frequency at which pathogenic *L. monocytogenes* could be missed when using molecular detection methods following conventional culture-based enrichment.

Vibrio

Molecular Characterization and Virulence Assessment of *Vibrio parahaemolyticus* and *V. vulnificus* Isolates from Shellfish and Clinical Samples

Naturally occurring marine bacteria, *Vibrio vulnificus* and *V. parahaemolyticus,* are leading causes of death and illness associated with eating raw oysters in the U.S. These bacteria are often found in coastal waters where oysters and other seafood are harvested. However, not all strains of *V. vulnificus* and *V. parahaemolyticus* cause death or illness. The goal of this research is to compare the DNA of strains that caused illnesses and deaths to those which did not. The comparisons will help identify the genes necessary for the bacteria to cause illness. The results would inform the development of more accurate testing methods and monitoring programs to reduce the risk of illness for seafood consumers.

Clostridium

Characterization of *Clostridium botulinum* Strains using the Molecular Subtyping Method, Pulsed-Field Gel Electrophoresis (PFGE)

Ingestion of foods containing *Clostridium botulinum* and its poisonous toxin, botulinum neurotoxin, results in a severely paralytic disease called botulism. The rapid onset and severity of botulism necessitate the prompt isolation and characterization of the organism and its toxins from foods implicated in an outbreak. The goal of this research is to utilize genetic tools to characterize a library of *C. botulinum* foodborne isolates to generate a genetic 'fingerprint' for each strain. These data will be used to populate CDC's PulseNet, a worldwide database that aids epidemiologists in determining whether a botulism outbreak is occurring, and facilitate the identification of the suspected source of the outbreak.

Other Pathogens

Whole Genome Analyses of Enteric Pathogens Using Optical Mapping for the Identification of Outbreak Markers

Bacteria can adapt to new environments by acquiring new traits, often by insertion and substitution of genetic material. In a foodborne outbreak of illnesses, the rapid detection and characterization of a bacterial agent serves as a means to track the source of the outbreak and to determine if the pathogen is similar or different to previous outbreak strains. The goal of this research is to evaluate changes in chromosomes using optical mapping, which can distinguish

very closely related bacteria. By quickly identifying details of an outbreak strain, the source can be pinpointed more quickly, thus shortening the spread and impact of the foodborne outbreak.

Whole Genome Sequencing of Animal and Zoonotic Pathogens

FDA has the responsibility to develop methods for the effective screening, detection, and identification of pathogens in foods derived from animals and to ensure the safety and effectiveness of drugs used in veterinary medicine. Many of the bacterial pathogens important in veterinary medicine and food production are poorly understood due to the lack of bacterial genetic information available to the scientific community. Such information would be useful in the development of vaccines and tests that can be used to detect and monitor infectious diseases. The goal of this research is to provide the complete genetic sequences of important animal pathogens. If successful, the results of this research would provide FDA with information on animal diseases and could lead to the development of tests that can be used to improve animal health and detect pathogens in food derived from animals. The availability of whole genome sequences (complete DNA sequences) for pathogens of veterinary importance would be beneficial in designing in-depth studies that can address animal health, drug effectiveness, and inform regulatory decision-making.

Microbial Metagenomics of High Risk Produce Commodities in Diverse Biogeographic Regions

Increased microbial risks are associated with specific crops and specific geographical growing regions, but a commodity knowledge gap remains. Understanding the structure and kinds of natural microorganisms associated with many produce types will serve as a valuable indicator of the geographic source of contaminated produce. The goal of this research is to utilize culture independent metagenomic snapshots of the microbiology associated with high-risk produce commodities to determine a role for this technology in identification of the origins of contaminated fruits and vegetables. Application of metagenomic technology may contribute to improved specificity for detection and pinpointing of vectors, and the minimization of microbial risks associated with production.

Fingerprinting of Foodborne Pathogens by Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) is a technique for bacterial subtyping (fingerprinting). This study will improve FDA's database of PFGE subtyping data for significant foodborne pathogens and will contribute to the national PulseNet laboratory network. The PulseNet website allows many laboratories to share information bacterial subtyping data to help rapidly determine possible sources of many foodborne infections and other infectious diseases. Data from this project is submitted to PulseNet so that public health professionals have access to the most recent and comprehensive data available for use in investigating foodborne disease outbreaks.

Development of a Custom DNA Reference Library to Improve the Accuracy of the ABI MicroSeq 500 DNA Sequencing Method for Identification of Bacteria Isolated from Foods

Foods have the potential to be contaminated with numerous bacterial pathogens. Current biochemical methods to identify bacteria in food to the species level are time consuming and subjective. The goal of this research is to show that DNA sequencing using the ABI MicroSeq500 instrument is a rapid and precise method to identify bacterial species. To optimize this approach, a DNA reference library of food-associated bacterial species is required to improve the accuracy of the method. FDA will also develop a custom DNA sequence library from bacterial isolates of confirmed identity, including pathogens and non-pathogens, isolated from foods and food-production environments, which would provide an accurate means for identifying bacterial pathogens in foods.

Metagenomic Microarray Development for Gene Discovery of Enteric Pathogens and Community Analysis of Gastrointestinal Commensals

The human form serves as a habitat for microbes that stably (commensal) or transiently (pathogens) colonize various regions of the body. This is significant because these microbes collectively outnumber our own human cells by approximately 10 to 1. There is increasing scientific interest to characterize these communities and determine their role in health and disease. The task will involve sophisticated techniques that are accurate and precise enough to determine changes in microbial diversity of enteric pathogens and gut commensals under disease conditions or upon challenge with ingestibles. The goal of this research is to develop these tools using bacterial DNA sequences that are arrayed for hybridization. If successful, this work would provide a useful tool with the capacity to monitor the metabolic and bacterial flux upon challenge with pathogen outbreak strains.

Metabolic Profiling of Foodborne Pathogenic Enteric Bacteria

E. coli, *Salmonella*, and *Shigella* are common foodborne pathogens in the U.S. Early detection of these pathogens in foods will help to halt foodborne outbreaks. This task is challenging as the pathogens adapt to new environments in foods by acquiring new genetic traits, potentially resulting in altered phenotypic traits. The goal of this research is to evaluate those phenotypes related to metabolism. The altered metabolic trait can be detected by phenotypic microarray technology and can be used as a metabolic marker. These studies on enteric pathogens will not only lead to a better understanding how they evolve to adapt and survive in foods by altering their metabolism, but will also promote the use of metabolic markers for identification and tracking.

Investigating the Global Genomic Diversity and Evolution of Enteric Pathogens via the Development and Utilization of Next-Gen Microarray and Sequencing Technologies

Rapid response to foodborne outbreaks requires the development of molecular methods that can both identify strains that cause outbreaks of disease and discriminate strains that contaminate food sources in order to track the causative strain. The goal of this research is to develop rapid and high throughput methods, using DNA sequencing and microarray technology, that will allow

the detection, identification, and tracking of pathogenic strains of *E. coli*, *Shigella*, and *Salmonella*. DNA sequence, bioinformatic, and cladistic analyses can then be used to differentiate natural isolates of each pathogenic group. If successful, this will result in faster and more precise methods to speed response time to foodborne disease outbreaks.

Novel Molecular Typing Methods for Analyzing Shiga Toxin Producing *E. coli* (STEC) and *Salmonella*

The goal of this research was to evaluate DNA sequence-based genetic structures of several of the most common *Salmonella* strains and *E. coli* (STEC) strains associated with human illness. These data can facilitate the design of specific diagnostic tests for effective detection, identification, and differentiation of foodborne outbreak strains of *Salmonella* and *E. coli*. Next-generation sequencing (NGS) of microbiologic isolates was used in the molecular tracking of these pathogens. NGS data decoupled one *Salmonella* isolate from other isolates despite its perfect genetic identity to the other isolates based on the conventional epidemiologic traceback method, pulsed-field gel electrophoresis (PFGE). This research using NGS clearly demonstrates the utility of this technique in revealing subtle genotypic differences essential to the traceback of bacterial pathogens as they emerge in the food supply.

Molecular Evolution of Enteric Pathogens

FDA's food safety research involves methods that can both track strains of bacteria involved in foodborne outbreaks and identify strains that have been used to deliberately contaminate food sources. The goal of this research is to develop tools to detect, identify, and trace strains of *E. coli*, *Shigella*, and *Salmonella* using DNA sequences. Bioinformatic and cladistic analyses will be conducted to capture the diversity of these enteric pathogens and identify novel DNA signatures that will differentiate natural isolates of each pathogenic group. If successful, this research will have a positive impact on consumer health by improving the ability to quickly identify strains related to an outbreak and trace back to the source of the contaminating pathogen.

Characterization of Phenotypic and Genotypic traits of *Cronobacter* spp. for a Better Understanding of Virulence Mechanisms and Inclusion into the Pathogen Annotated Tracking Resource Network System

Cronobacter spp. cause life-threatening foodborne illness in neonates and elderly individuals. The goals of this research are to 1) isolate and characterize virulence factors and enterotoxin homologs expressed by *Cronobacter* spp.; 2) investigate the role of plasmids in the pathogenesis of *Cronobacter* infection; 3) design and implement a molecular serotyping scheme; and 4) develop a rat neonatal meningitis model for the study of *Cronobacter* pathogenesis. The information from this research will be useful in developing improved detection methods for these organisms in foods and thus improve the safety of the food supply and reduce illness.

Antimicrobial Resistance/Susceptibility of Foodborne Microorganisms

Sequence Analysis of Multi-Drug Resistant (MDR) Plasmids and Whole Genome of Foodborne Bacteria Pathogens

Multi-drug resistant (MDR) *Salmonella enterica* strains are a significant public health risk but there is little data on how the bacteria are spread or why they persist. Also, it is not known why certain serotypes/clones of *Salmonella* are frequently associated with human infections and antibiotic resistance. Many studies have shown that bacterial plasmids (mobile genetic elements) play an important role in bacterial infections and that the genes for antibiotic resistance are commonly found on plasmids. FDA is studying selected strains of multi-drug resistant *Salmonella* species. The goal of this research is to use analyses of the entire genomic and plasmid DNA sequences of these strains to study relationships between specific genes and the ability of the bacteria to cause severe disease and maintain genes that cause MDR. This study will increase FDA's understanding of how genes work together to increase the severity of *Salmonella* infections. Determining how antibiotic resistance genes work and transfer would help identify ways to limit the spread of antibiotic resistance.

Characterization of Plasmid Associated Antimicrobial Resistance in *Salmonella enterica* Serovars Associated with Poultry and Human Infections

Bacteria have developed resistance to many antimicrobials commonly used for treatment of human and animal infections. Many infections are resistant to multiple antibiotics, which reduces the treatment options in both human and animals. Understanding the mechanisms of antimicrobial resistance development helps to control the emergence and spread of antibiotic resistance, and protects the potency of the currently available antimicrobials. The goal of this research is to conduct DNA sequencing studies on novel genes associated with antimicrobial resistance and virulence that may contribute to the persistence and transmission of *Salmonella* in poultry and eggs. Selective pressure assays could provide valuable information that may be used to carry out risk assessments of the contribution of the veterinary use of different antimicrobials on the spread of multidrug resistance among zoonotic bacterial pathogens.

Developing a High Density Microarray Platform to Study Molecular Mechanisms of Antimicrobial Resistance in Foodborne Pathogens

Antimicrobials are used for the control, prevention and treatment of infectious bacterial diseases as well as for improving growth and feed efficiency in animals. The undesired consequence of antimicrobial use is the development of resistance. To monitor the emergence of antimicrobial resistance as a consequence of use and to help protect the public from antimicrobial resistant foodborne pathogens, FDA has developed a comprehensive antimicrobial resistance gene detection and expression tool, called a DNA microarray. This DNA microarray encompasses all the currently-known antimicrobial resistance genes, mobile genetic elements by which resistance genes spread, and whole genome information from *Campylobacter*, *Enterococcus*, *E. coli*, *Salmonella* and methicillin-resistant *Staphylococcus aureus*. The goal of this research is to evaluate the DNA microarray for rapid strain discrimination, and detection of expression of antimicrobial resistance genes in foodborne pathogens. If successful, this would improve FDA's

science-based decision-making process to safeguard the public from antimicrobial resistant foodborne pathogens.

Changes in Antimicrobial Susceptibility of Bacteria in Feces and Water of Tilapia Treated with Oxytetracycline Feed

Bacteria that are resistant to antibiotics are problems for fish farming, hazards to the fish being grown in aquaculture systems, and possible threats to the environment through wastewater discharge. The risk of various bacteria becoming resistant to drug treatment can be evaluated by studying a bacterium representative of aquaculture systems. A suitable bacterium, *Bacillus cereus*, has been identified to monitor resistance to oxytetracycline, an antibiotic used by veterinarians to treat fish diseases. The goal of this research is to evaluate the antibiotic resistance of *B. cereus* isolated from tilapia feces and water before, during and after treatment with oxytetracycline. This information will help FDA scientists understand how fish antibiotics affect drug susceptibility among bacteria in the surrounding environment.

Clinical Laboratory Standards Institute Antimicrobial Susceptibility Testing Methods for Gliding Aquatic Bacteria

Gliding aquatic bacteria are major disease concerns on fish farms. However, there are no standard tests to determine if antibiotics that might be used to control disease outbreaks can kill these bacteria. Developing standard methods to determine if these bacteria are sensitive to antibiotics will help guide effective treatment options. The goal of this research is to develop and validate susceptibility tests to determine the effectiveness of specific antibiotics to kill these gliding bacteria. If successful, the methods will be added to existing Clinical Laboratory Standards Institute guidelines for antimicrobial susceptibility testing of aquatic bacteria.

Development of Interpretive Criteria for Florfenicol to Use for Antimicrobial Susceptibility Testing of *Flavobacterium columnare* Isolated from Channel Catfish, *Ictalurus punctatus.*

The FDA recently approved the drug Aquaflor® (active ingredient: florfenicol) to treat a bacterial disease in channel catfish. Methods have been developed to test how the bacterium *Flavobacterium columnare* responds to this drug. The goal of this research is to test how well the methods predict treatment success in fish. This will allow fish health professionals to better understand when the drug should be used. The drug should only be used when preliminary tests predict that drug treatment will be effective in killing the bacteria. Otherwise there is a risk of contributing to development of drug-resistant bacteria.

Detection of Antimicrobial Resistant Genes by DNA Microarray

Bacteria have developed resistance to many antimicrobials commonly used for treatment of human and animal infections. Many infections are resistant to multiple antibiotics, which reduces the treatment options in both human and animals. Understanding the mechanisms of antimicrobial resistance development helps to control the emergence and spread of antibiotic resistance, and protects the potency of the currently available antimicrobials. The goal of this research is to develop in-house technology (low-density resistance microarray) to quickly and

comprehensively screen bacteria for the presence of antimicrobial resistance genes, and also to maintain the ability to add new resistance genes to the platform rapidly. If successful, this method would provide a more rapid and informative assay for the characterization and identification of resistant microbial pathogens present in the food and animal feed supply and facilitate FDA's ability to make timely science-based decisions to protect the public from antimicrobial resistant foodborne pathogens.

Genotypic and phenotypic analysis of historical foodborne pathogens

The emergence of antimicrobial resistance as a consequence of use is a major public health concern. Antibiotics are vitally important for treating human bacterial infections. Antimicrobial drugs also are approved for animals, where such use may select for resistant bacteria that are transmitted through the food supply. FDA approves antimicrobials for use in food animals and fosters the judicious use of medically important antibiotics in food animals to minimize the development of drug resistance. The goal of this research is to provide information on antimicrobial resistance development over time, thereby helping FDA to understand the evolution of multidrug resistance at the genetic level, and assists in addressing the public health impact of antimicrobial use in food-producing animals.

Antimicrobial Resistance in *Campylobacter* spp. Isolated from Animals

Campylobacter species are a type of bacteria that are common in the intestines of poultry and can cause foodborne illness in humans. Although fluoroquinolone antibiotics are no longer used in poultry, *Campylobacter* species frequently possess specific antibiotic resistance (AR) genes that make them resistant to these antibiotics. The goals of this research are to: (1) determine the mechanisms of AR in historical isolates to see if there is a pattern of acquisition of AR that correlates with the use of antibiotics in animals, (2) determine whether compounds that are used in poultry feed can cause the AR genes to develop, (3) determine whether special molecular pumps help AR to develop and be retained, and (4) determine whether the bacteria with the AR genes grow better or remain in the infected animal longer than the same bacteria without the AR genes. The results of this research will help FDA by providing a sound scientific basis for making regulatory decisions on the safe use of antibiotics in food producing animals.

Rapid and comprehensive detection of antimicrobial resistance in bacterial pathogens

This project seeks to develop and test a standardized, microarray-based system to rapidly detect antibiotic resistance genes in a broad range of bacteria (not just foodborne pathogens) so that physicians, veterinarians, and other public health care officials can quickly implement antibiotic treatments that are likely to be successful. The method is needed in the event of a bacterial epidemic in animals or humans. The microarray being developed will detect all known antimicrobial resistance genes and will use a standardized, widely used technology so that laboratories all over the country can use this method easily. The overall goal is to enable public health officials to quickly decide what antibacterial therapy is likely to be successful during naturally-occurring epidemics or terrorism-related events. Once developed and fully validated, the microarray chips can be used for detecting antibiotic resistance genes in any bacterium

isolated from animals or food. This will allow more rapid detection of antimicrobial resistance when foodborne disease outbreaks occur.

Characterization of Antimicrobial Resistance Mechanisms of Human and Animal Bacterial Pathogens

Better understanding of antimicrobial resistance mechanisms is crucial to combat, control, and prevent the development and spread of antimicrobial resistance in bacteria. Better understanding of resistance mechanisms also helps public health officials to protect the effectiveness of currently available antibiotics. The goals of this research are to identify antimicrobial-resistant pathogens, characterize genes associated with antimicrobial resistance, and determine how resistance genes are transferred among human and animal bacterial pathogens. Detection of antimicrobial resistance genes in bacteria and information about how those genes are transferred among different bacterial species is critical for making regulatory decisions regarding the use of antibiotics in food production animals. This study directly supports FDA Center for Veterinary Medicine's Guidance for Industry # 152 (Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern).

Genotyping of Methicillin-resistant *Staphylococcus aureus* Isolated from Retail Meat Products

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified in hospital and community-acquired human bacterial infections world-wide and also has been found in animals and in environmental sources. Because this microorganism has been isolated from food animals, it is possible that retail meats may be contaminated with MRSA, which could then be transmitted to people. FDA is using the sample collection procedures already used for the National Antimicrobial Resistance Monitoring System (NARMS) to determine how common MRSA is in retail meat. The goal of this research is to conduct detailed molecular characterizations of the NARMS isolates as well as MRSA isolates from other sources in order to determine whether isolates from retail meat are similar to those from humans, animals, and the environment. The detailed characteristics of MRSA provided by this study will allow for comparisons of retail meat isolates with those obtained from various sources, such as animals, humans, and environmental sources. Such comparison would aid FDA in determining how food products may be exposed to MRSA.

Microarray Analysis for the Detection of Targeted Gene Expression Changes Resulting from Exposure of *Clostridium perfringens* to Fluoroquinolones

C. perfringens, as a cause of food poisoning, is on the list of bacterial pathogens of concern to the public, USDA and FDA. Comparing toxins, metabolites, and enzymes from different fluoroquinolone-resistant mutant strains with the wild-type parent strains in *C. perfringens* shows changes in these bacterial products whose activities affect human health. Use of microarrays an efficient way to detect the genotypic changes underlying the phenotypic changes observed, not only between parent and mutant strains, but also among other strains. Understanding the effects of antimicrobial agents on pathogens, particularly the mutational response of pathogens to specific antibiotic exposure, is of concern to the FDA. The goal of this research is to enhance

FDA's understanding of the effects of antimicrobial agents on anaerobic bacteria in an area where basic research is lacking.

Designed Epidemiologic Studies of Foodborne Illness/Associated Organisms

Ecology of East Coast *Salmonellae* Associated with Tomato-Production Environments

Fourteen percent of all foodborne outbreaks reported in North America can be attributed to the consumption of raw or minimally-processed fruits and vegetables. Large numbers of cases of salmonellosis have been associated with the consumption of tomatoes grown along the east coast of the U.S. Many of these tomatoes were found to be contaminated with *Salmonella* Newport. The association of *S.* Newport with tomatoes is ecologically complex. The goal of this research is to evaluate potential natural environmental reservoirs, as well as possible mechanisms of tomato plant contamination, through extensive sampling of the tomato farm environment. Results from this research would aid FDA in understanding the endemic nature of *S.* Newport in an important produce production ecosystem and provide insights into possible intervention strategies in the farm-to-fork continuum.

Evaluation of the 2011 *Salmonella* Agona Outbreak Associated with Papaya

When FDA's investigatory response to a foodborne illness incident involving a regulated product has ended, an assessment is conducted to identify weaknesses, strengths, and areas for improvement. Between January 1 and July 22, 2011, FDA, CDC and state authorities responded to a *Salmonella* Agona outbreak associated with papayas from Mexico in which 106 human cases of illness from 24 states were identified. Ten patients were hospitalized and no deaths were reported. In April 2011, CDC detected an early signal for produce, specifically mangoes, papayas and/or melons and identified similar demographic and exposure characteristics among case-patients. FDA issued an Import Bulletin in May 2011 to increase sampling of the suspected produce items entering the U.S. and the Texas Rapid Response Team (RRT) initiated a traceback of Texas case-patients who consumed the suspected produce items. The increased sampling identified two violative import samples that matched the outbreak strain. The violative samples and the Texas RRT traceback converged to a common papaya grower/packer in Chiapas, Mexico. The firm then recalled the implicated papaya. Sixteen percent of Mexican papaya samples collected at the border tested positive for multiple serotypes of *Salmonella* and in August 2011, a country-wide Import Alert for papaya from Mexico was issued. The lessons learned from such incidents are used to make improvements to Agency response, policy and planning, and food safety to prevent future incidents.

Epidemiologic Analysis and Risk Management Practices for Reducing *E. coli* in Irrigation Source Water Supplies and Distribution Systems

The goal of this research was to conduct a comprehensive epidemiological analysis of irrigation water monitoring data currently being collected by the leafy greens produce industry in order to characterize the occurrence of generic *E. coli,* which has been used as an indicator of microbial produce contamination. Two datasets (2007-2010) were compiled that together represent a large number of produce growers from throughout California and from various sources of irrigation water across all seasons of the year. The results showed that the rate of exceedances of generic *E. coli* was uncommon for most locations and sources of irrigation water used for leafy greens during this time period at the locations evaluated. This finding will provide useful information to FDA when developing guidance. In addition, this supports development of a risk-based

approach to using generic *E. coli* to monitor irrigation water quality and suggests that testing could be reduced at locations with persistent high water quality and increased at locations that have a history of exceedances.

Prevalence, Distribution, and Diversity of *Vibrio cholerae* in Mobile Bay, Alabama

The naturally occurring bacteria, *Vibrio cholerae* (non-O1), are a leading cause of seafood related bacterial illness in the U.S. These bacteria can be found in coastal waters where oysters and other seafood are harvested. However, little is known about *V. cholerae* in seafood and in the environment. The goal of this research is to evaluate the distribution, prevalence, and abundance of *V. cholerae* in Mobile Bay and relate these findings to environmental conditions. This information will be useful for FDA in developing tools to predict the occurrence of *V. cholerae* in seafood.

Integration of a Multifunctional, Curated, Searchable, Strain-Tracking (PATRN) System for Surveillance and Outbreak Analyses

The purpose of this research project is to develop an enhanced standardized microbial characterization data repository and software system called the Pathogen Annotated Tracking Resource Network (PATRN) system. The PATRN system shall capture and integrate a group of dynamic, up-to-date set of phenotypic and genotypic traits that define a pathogen. This would result in an expanded, transparent and rapid epidemiological tool which will help manage the research and surveillance efforts of FDA's global response during a foodborne outbreak investigation. The PATRN system approach shall unify historical information, and cutting-edge knowledge with surveillance information, both domestic and global, and shall tie research activities of a global "expertise pool" of partners together with FDA's current research, surveillance and outbreak investigation activities all under a single computer-managed and informational focal point.

Feasibility Assessment for Studying the Long-Term Effects of Foodborne Illness

Foodborne pathogens can cause serious acute and/or long term health outcomes in some patients. However, the incidence, severity and burden of long term health outcomes associated with foodborne disease are not well understood and there are few guidelines for long-term medical care. The goal of this research project is to summarize current knowledge, identify data gaps and stakeholder information needs and assess potential frameworks for systematically studying and tracking long term health outcomes and estimating the chronic burden of foodborne disease. The results of this project will be used to help determine which research model(s) is/are most appropriate, given the dynamics of current research capabilities, for systematically studying the long term health outcomes of foodborne disease in the U.S.

Risk Assessment, modeling, management, and communication

Chemical Contaminants

Pharmacokinetics: Physiologically-based Pharmacokinetic (PBPK) (Exposure) and Pharmacodynamic (PBPD) (Dose-Response) Modeling for food-related compounds in Quantitative Risk Assessment.

The PBPK computer model is a powerful tool for making safety extrapolations from animals to humans. Pharmacokinetics and PBPK modeling are a critical part of chemical safety assessment. The goal of this research is to develop various human PBPK models (adult and children) for the following uses: to provide assessment of target tissue doses, including brain; to evaluate low-dose and long-term effects of chemicals and toxins; to extrapolate from rats to humans to determine target tissue doses that correlate with adverse effects; and to assess increased risks due to the effects of age, gender and disease. Results from this work would provide FDA with information for conducting rigorous quantitative risk assessment of food contaminants.

Dietary Concentrations of Residual Styrene and Styrene Oligomers from Various Types of Polystyrene Products Currently Authorized for Food Contact Applications

In response to the recent National Toxicology Program report on the carcinogenicity and other toxicology effects of styrene, the FDA is updating its safety review of this compound. The goal of this research is to collect and determine the concentrations of residual styrene and its oligomers in foods from polystyrene containing products currently authorized for food contact applications. The results would be used by FDA to update dietary concentrations and contribute to exposure assessments of styrene and styrene oligomers.

Migration of Pressure-Sensitive Adhesive Components to Food

The transfer of adhesive components from pressure-sensitive food labels across thin plastic films covering foods, such as meat and poultry products, is not well understood. The goals of this research are to characterize adhesives used in food packaging, identify chemical species that may transfer to plastic films, and evaluate the potential for these species to enter the food. The results of this work would help clarify potential risks associated with using pressure-sensitive labels on thin plastic films in contact with food such as meat, poultry, fish, fruits and vegetables.

Development of a Risk Assessment Framework for Folate Metabolism and the Identification of Applicable Risk Assessment Models

Folic acid fortification has reduced the incidence of neural tube defects (NTDs) among infants in the U.S. However, the incidence among infants of Mexican American women of childbearing age remains higher compared to other subpopulations, possibly due to the lack of fortification of staple foods such as corn masa flour. Conversely, there is a concern that high folic acid fortification may mask B_{12} deficiency, resulting in cognitive impairment. The goal of this research was to establish a risk-benefit analysis methodology to quantify the beneficial verses harmful effect of folic acid fortification. The results showed that no more than 140 µg/100 g of

corn masa flour may prevent NTDs, while preventing excessive intake in the subpopulation. This research provides information for regulatory decisions regarding folic acid fortification and presents a new methodology that can be applied to the field of nutrition and risk assessment.

Toxins

Investigation of Paralytic Shellfish Poisoning in New England Waters

Waters off the New England coast harbor extensive molluscan shellfish resources, large portions of which are frequently contaminated with paralytic shellfish poisoning (PSP) toxins produced by an alga, *Alexandrium fundyense*. Harvesting closures are implemented when toxins exceed a safe limit. The goal of this research is to evaluate the relationship between *Alexandrium* surface blooms and shellfish toxicity in Federal waters currently closed to shellfishing, in collaboration with other researchers from federal agencies, academia, States, and private industry, to ensure that closures are made in the presence of risks, while avoiding unnecessary closures. Results from this work would inform FDA regulatory decisions and guidance allowing for improved monitoring and management of PSP risks.

Amnesic Shellfish Poisoning: Assessment of Seafood Safety in the Northern Gulf of Mexico

Domoic acid is an algal toxin that can cause amnesic shellfish poisoning (ASP) in seafood consumers. Symptoms of ASP include gastrointestinal upset, fatigue, disorientation, seizures, permanent loss of short-term memory, and in severe cases, death. Toxic blooms of domoic acid-producing algae occur world-wide, and appear to be increasing in frequency. The goal of this research is to evaluate the distribution and environmental conditions promoting the growth and toxicity of domoic acid-producing algae in the Northern Gulf of Mexico. This information would be useful to FDA and State public health authorities in developing models to predict the occurrence of toxic blooms and prevent the harvest of domoic acid-contaminated seafood.

Drug Residues and Hormones

Risk Assessment of Drug Residues in Milk and Milk Products

Since the time that requirements for testing dairy products for drug residues were established, dairy cattle have been given an increasing variety of antibiotics and other drugs. The goal of this research is to develop a risk assessment to determine which drugs are of greatest risk to consumers if the drugs enter the milk supply and to provide options to the current dairy sampling and testing requirements that could reduce or eliminate the risks. The results of this work would help FDA address concerns about the potential for harmful residues of these drugs in dairy foods.

Allergens and Gluten

Food Allergen and Gluten Research

For allergic consumers, avoidance of the allergenic food is the only method of disease prevention. Despite regulations under the Food Allergen Labeling and Consumer Protection Act (FALCPA) mandating the labeling of eight major food allergens, undetected allergens are responsible for many adverse reactions and recalls. Allowable threshold levels have not been established for the purposes of allergen labeling or detection, nor for the prevention of an allergic reaction. The goal of this research is to determine the mechanisms involved in sensitization, which will help to define regulatory threshold levels using models that mimic host allergic response. These results would be useful for FALCPA enforcement, for establishing limits of detection for method development, and ultimately for protection of the allergic consumer.

Impact of Processing on Food Allergens

The success of allergen control programs depends on an accurate assessment of the allergenic potential of proteins of interest. Most foods are cooked or processed prior to consumption, therefore it is important to know how food processing can affect the allergenicity and detectability of allergens. The goals of this research are to evaluate the impact of thermal processing on the biochemical and immunological properties of food allergens, characterize the underlying structural changes and evaluate the adequacy of commercial ELISA test kits for detection of allergenic residues in thermally processed foods. If successful, this work will help FDA in determining if thermal stability can be used as a criterion to assess the allergenic potential of novel proteins or bioengineered foods.

Filth and Other Animal Material

Quantify Potential Health Risks Associated with Insect Adulterants in Food

There is a need for improved science-based information and data assessment to support for regulatory actions concerning filth and extraneous materials in foods. The goal of this research is to evaluate two important health risks introduced by insects: the spread of foodborne pathogens by flies and the allergenicity of food-associated insects and mites. This will be accomplished by collecting insect samples for pathogen detection to provide data on the distribution of fly populations, bacterial load and frequency of foodborne pathogens carried by flies from urban areas. In addition, the allergenicity of insects and mites found in food will be studied. If warranted, changing the status of mites and insects from incidental to pests of regulatory concern may be recommended.

Microbial Pathogens

Salmonella

Enhancing the Safety of Nuts and Nut Products

Nut and nut products have been implicated as the contamination vehicle in previous foodborne disease outbreaks. The goal of this research was to conduct two separate risk assessment to complement ongoing field and laboratory studies on the safety of nuts and nut products. In addition to these ongoing studies, the ability of *Salmonella* to survive in dry environments was also evaluated. The aims of the risk assessment were to: 1) assess the risk of salmonellosis associated with almond and pistachio consumption in North America, with current treatments in effect; 2) determine the resilience of the current production system to increases in prevalence or concentration of *Salmonella* on almonds and pistachios; 3) assess the impact of treating less than 100% of the crop; and 4) investigate conditions that could explain the number of cases associated with the 2001 almond outbreak. The ability of *Salmonella* to survive during desiccation was shown to be influenced by strain and environmental conditions. This work enhances food safety and hazard analysis efforts at FDA for nuts and nut products linked to outbreaks and recalls.

E. coli O157:H7 and STEC

Enhancing the Quantitative Predictive Risk Assessment Model (QPRAM) for Improved Produce Food Safety

The goal of this multi-year field research in the Salinas Valley, California is to generate real-world data regarding *E. coli* O157:H7 contamination of lettuce prior to harvest due to fecal pathogen transfer during foliar irrigation. Results from the first year study suggest that a 5-day interval between final irrigation and harvest combined with removal of the outer lower leaves from the head during harvest and due diligence to remove scat prior to final irrigation, would substantially reduce the risk of microbial contamination for lettuce. These initial results assisted FDA in the development of transfer coefficients–rates of pathogen transfer from soil to crops–to help inform the Produce Risk model, a quantitative risk assessment model under development.

Listeria

Risk Assessment of the Effects of Retail Practices on Public Health Impact of Foodborne *Listeria monocytogenes* in Ready-to-Eat Food

The bacterium *Listeria monocytogenes* can result in listeriosis, a leading cause of death and stillbirth among foodborne illnesses. Ready-to-eat (RTE) foods, such as those sold in delicatessens, are among the highest-risk products associated with this bacterium, which tends to cross contaminate in the deli and is extremely hard to eliminate. The goal of this research is to develop a computer model that simulates patterns of cross contamination in the deli, based on studies of real-world processes for food preparation and serving in this setting. The model would identify points of risk and predict effectiveness of various prevention options in order to help

FDA risk managers develop new approaches for control of *Listeria* in the deli and guidance for retailers and consumers.

Expert Services for a Dose Response Risk Assessment for Pregnancy-Related Listeriosis

Recently, pregnant gerbils have been proposed as an alternate animal model for human listeriosis during pregnancy. Researchers have shown that gerbils have two key receptors similar to humans while guinea pigs only have one receptor. Therefore it is anticipated that a pregnant gerbil model would be physiologically closer to human than guinea pigs for *Listeria* infection. FDA scientist will work with scientist from the University of Georgia because they have the advantage of having worked with and published data from both pregnant guinea pigs and non-human primates. The goal of this project will be to determine the dose response of stillbirths in pregnant gerbils and to compare this with concurrently exposed pregnant guinea pigs. This research will generate state of the art, scientifically relevant data to understand human listeriosis and to incorporate this knowledge in FDA's risk assessment and policy development with the goal of protecting the public, particularly the most vulnerable populations, from this important foodborne disease.

Enteric Viruses

Joint U.S./Canada Public Health Risk Assessment of Norovirus in Bivalve Molluscan Shellfish

Norovirus causes millions of cases of illness in the U.S. and thousands in Canada each year. Contaminated bivalve molluscan shellfish (*e.g.*, oysters, clams, and mussels) are a foodborne source of the virus and outbreak investigations suggest that contamination usually takes place during growth and harvest, likely from human waste in typical growing areas. The goals of this research are to conduct a risk assessment, in collaboration with Canadian health authorities, to estimate the risk of contracting norovirus from eating shellfish and to evaluate the impact of various risk-management strategies. The results would help the two countries assess current prevention policies and guidelines for this product and identify new mitigation strategies.

Investigation of Norovirus Cross-Contamination during Foodservice Procedures Used in the Preparation of Fresh Produce

Norovirus infection is the leading cause of foodborne viral disease in the U.S. A major transmission mode for norovirus infection is through hand contact by infected humans. Ready-to-eat (RTE) foods (*e.g*,. salads) which involve human hands to prepare before serving are at high risk for transmitting norovirus. The goal of this research is to determine the viral cross-contamination rates among produce items, cutting utensils, and human hands during RTE food preparation procedures. The results of the research will help to identify the critical control points that could reduce or prevent disease through education and communication of our findings to the food retail, food service industries, and the public.

General Microbial Pathogens

Host Susceptibility to Foodborne Pathogens Evaluated Using Immunological Biomarkers

When assessing the impact of foodborne illness, certain groups of individuals are more susceptible to disease (infants, pregnant women, the elderly, those that are compromised pharmacologically, nutritionally, or by underlying disease). Susceptible groups represent 20% of the U.S. population and will increase in the coming years. Disease severity, treatment difficulties, and incidence of chronic sequellae are also increased in these patients, causing much personal and economic hardship. The goal of this research is to identify immune biomarkers of exposure and susceptibility, which can be used to predict the risk of infection following foodborne pathogen exposure.

Evaluation of Risk Management Options to Reduce Microbial Hazards in Sprouts through Quantitative Risk Assessment

Recent research has resulted in new mitigation options to minimize microbial contamination of sprouts. Questions remain as to which mitigation technologies should be implemented, and at what stage of the supply chain, to achieve the greatest reduction in microbial hazards and maximize protection of public health. The goal of this research is to develop a series of mathematic models to assess the risk associated with microbial contamination in sprouts and the extent of risk reduction that can be achieved through applications of a combination of mitigation steps and microbial testing programs. These models would be used to prioritize intervention strategies and provide the FDA and sprout industry with a science-based approach to optimize risk management programs.

Geospatial Risk Assessment Model of Environmental Contamination of Produce by Enteric Pathogens

Under an inter-agency grant from FDA, scientists at the National Aeronautics and Space Administration (NASA), Goddard Space Flight Center will create a geo-referenced environmental database and develop a prototype geospatial predictive risk assessment model to: study, understand, map, model, and predict the confluence of environmental factors (such as topography, contaminated ground and surface water, human actions, proximity of livestock to produce fields, wildlife activity, irrigation practices, and climate) that lead to enteric pathogen contamination of produce and leafy greens. Development of a geospatial, environmental and climatic database and model, will enable FDA to understand, characterize and predict the likelihood, amount, time, and location of environmental contamination of produce by *E. coli* and norovirus (Enteric pathogens), in the continental U.S. Upon development and validation, the database and model could be used to predict the potential areas of possible produce contamination, and evaluate possible field application, response, and risk management actions.

Survey of the Microbiology of Animal Feed and Feed Commodities

FDA conducts an animal feed survey program that routinely screens farm animal feeds and pet foods. The feeds are tested for the presence of certain bacteria and antimicrobial resistance

profiles of public health concern. The survey test results provide useful information to FDA on the risks associated with animal feeds and pet foods. The program also allows FDA to monitor the quality of feeds provided to animals in the U.S. Data collected from this program and the trends observed over time allow a long-term assessment of the role that animal feeds play in the introduction of pathogens into the food chain and human environment. The data also allows assessment of the potential for feed to disseminate resistance to bacteria in the livestock and pet animal population and provides the opportunity to isolate specific bacteria from feed and feed commodities, for use in other research studies at FDA.

Scientific Assessment and Evaluation of Food Safety Risks Throughout the Food Supply System from Farm-to-Fork

The purpose of this work is to assist FDA in the evaluation, analysis, and development of scientific assessments of current and emerging information and technology in selected areas of food safety. The models and methods that are developed will provide the means with which to analyze the significance of various hazards (bacterial, viral, protozoa and chemical) in a variety of food products along the farm-to-fork continuum. This will facilitate the prioritization of food-related threats to public health, identification of future research priorities and consideration of alternative approaches for the mitigation of food safety hazards. The effort will support the Agency in its mission to assure the safety of the U.S. food supply and protect public health.

Surveys on Consumer Knowledge, Perception, and Beliefs About Current Foodborne Illness Outbreaks for Food Recalls

Part of FDA's is to develop messages that can be disseminated to consumers during major food-related emergency events such as a foodborne illness outbreak or a food recall. The goal of this research project to determine consumer knowledge, perceptions, beliefs, and self-reported behaviors during a current major foodborne illness outbreak or food recall and help them understand FDA's messaging regarding taking appropriate action to minimize the risk. The results will be used to help the Agency adjust its communication strategies and messages for foodborne illness outbreaks or food recalls, when needed.

Safety Assessments of Foodborne Hazards, Including Toxicological Studies

Chemical Contaminants

Effect of Maternal Exposure to Silver on Thymic Function in F1 Generation Offspring

An evaluation of the available data on the use of silver mixtures as antimicrobial agents in food contact polymers by FDA revealed that *in utero* exposure to silver could adversely affect the developing immune system. A review of the available literature suggests that insufficient data were available to identify a dose at which this effect did not occur. The goal of this research is to identify the dose at which adverse effects from silver ion exposure will not occur and provide FDA with the information needed to perform a safety assessment for silver exposure from food contact uses.

An *In Vitro* Model System for Screening Food-Related Neurotoxic Compounds

Pesticides, heavy metals, seafood toxins, and plant alkaloids found in foods, cosmetics, and dietary supplements may exhibit negative effects on the brain and nervous system. The goals of this research are to use rat and human nerve cells grown in culture to rapidly identify potential neurotoxic compounds in botanicals and dietary supplements and to understand their toxic mechanisms. The data obtained from this work will serve as a simplified model of how substances affect the nervous system, and therefore may quickly identify neurotoxic compounds and reduce the amount of animal testing.

Incursion of arsenic metabolites in livers of untreated broilers and broilers after treatment with roxarsone

In a previous study, chickens were dosed with feed containing the arsenic-based animal drug roxarsone. The livers were tested for different chemical forms of arsenic. The goal of this research is to generate liver samples which may contain incurred levels of roxarsone and other forms of arsenic derived from roxarsone. Results of this research would be used to determine the stability of the residues during the storage period and to refine the treatment and tissue handling parameters during a subsequent tissue depletion study.

Testing for Concentration, Homogeneity, and Stability of Arsenic Species in Medicated Feed

Previous FDA research determined that arsenic residues could be detected in the edible tissues of poultry given feed containing roxarsone. The purpose of this research is to answer some of the questions raised by that previous study. These issues include things such as stability of roxarsone in feed over time. The study will also determine if the drug has been uniformly mixed in the feed. Other questions that will be examined include determining the amount of organic and inorganic forms of arsenic in the feed samples and in the pure drug. The data from this study would be utilized in developing further studies addressing speciation of arsenic residues in chicken liver.

Speciation of Arsenic Residues in Chicken Liver, Roxarsone Medicated Feed, and Type A Medicated Article

FDA will repeat its poultry 2009-2011 roxarsone study. In that study, feed containing roxarsone was given to chickens. The treated birds had higher levels of inorganic arsenic in their livers than control birds. The study is being repeated to verify these results. Additionally, several questions raised by the first study, such as distribution and stability of roxarsone in the medicated feed and stability of residues in frozen chicken liver, will be addressed. This study provides the method development and chemical analysis (speciation) for all other related arsenic studies described, and it will provide FDA with additional data to determine whether the use of roxarsone results in carcinogenic inorganic arsenic being present in the chicken tissues. The results would support FDA's effort to determine if the approval to use roxarsone in medicated animal feed should be withdrawn.

Triazine Depletion and Crystal Formation in Fish

During 2007 the triazine chemicals melamine and cyanuric acid were found in pet feeds, livestock feeds and fish feeds. FDA developed methods to detect these compounds in food animal tissues, and to diagnose harmful melamine-cyanurate crystal formation in kidneys of animals that had consumed contaminated feeds. The goal of this research is to conduct a depletion study evaluating residues of melamine and cyanuric acid in muscle, kidney and serum of fish after dosing with each chemical and with the combination of the two. A second goal is to understand the time course of crystal formation in kidneys: how long do the crystals reside in kidneys, does the sequence of ingesting the chemicals affect crystal formation, and what are the No Observable Adverse Effect Levels for single and continuous dosing. Preliminary data from this study was used by the FDA risk assessors during the 2008 Interim Safety and Risk Assessment of Melamine and its Analogues in Food for Humans. The data have also been used by the World Health Organization during the 2008 and subsequent melamine risk assessments. This information is important for FDA to determine the risk of injury from these chemicals and to be able to limit their presence in foods.

Teratology/Reproduction Toxicity Study of Melamine in Rats

In recent years, melamine was inappropriately added to pet food ingredients and milk. The purpose was to fake higher protein levels. As a result, many pets and Chinese infants developed urinary tract and kidney problems. There is a need for data to understand how melamine may affect mothers and younger children. The goal of this research is to evaluate how melamine affects pregnant and nursing rats, their fetuses, and newborns. Results from these studies would help the FDA develop improved risk assessments.

Effect of Maternal Melamine Exposure on the Developing Fetus and the Postnatal Animal

As a consequence of the wide spread contamination of food products with melamine the possibility exists that pregnant women could consume melamine tainted products and that melamine could cross the placenta adversely affecting the developing fetus or could accumulate in breast milk adversely affecting the renal function in the nursing new born. The goal of this

research is to conduct animal studies to understand the association between melamine exposure and acute kidney injury, including stone/crystal formation, in sensitive subgroups including the fetus and neonate. T his research will provide additional data to identify the mechanisms of toxic actions and interactions of melamine in the developing fetus and neonate and help FDA develop improved risk assessments.

NCTR-collaborative rat study: Assessment of the Nephrotoxic Effect of a Combined Exposure to Melamine and Cyanuric Acid

The intentional adulteration of food ingredients with melamine and its related chemicals (triazines), including cyanuric acid, caused kidney failure and death of a large number of cats and dogs in the U.S. in 2007 and illness in infants in China in 2008. The goal of this research is to determine what concentrations of triazines will cause the formation of crystals and injure the kidney. This information is important for determining the risk of injury from these chemicals and limiting their presence in foods.

Melamine and cyanuric acid newborn rat study (E02189) CVM-NCTR collaborative study

During 2008, adulteration of milk in China caused devastating effects on kidneys of babies. The goal of this research is to determine if newborn rats are more susceptible than adult rats to the toxic effects induced by co-exposure to melamine and cyanuric acid. Understanding differences in susceptibly to triazines between adults and infants is key to performing risk assessments for these compounds. Results from this study would help address data gaps identified by FDA during the melamine risk assessment related to the susceptibility of infants to exposure to melamine and cyanuric acid.

Impact of Melamine on Human Intestinal Microbiota and Evaluation of Enzymatic Capacity for Cyanuric Acid Formation

Regulatory bodies such as the FDA and international agencies such as the World Health Organization (WHO) are developing recommendations on what toxicological information is necessary for evaluating the risks of melamine exposure. WHO recommends a tolerable daily intake (TDI) of 0.2 milligrams per kilogram of body weight for melamine and 1.5 milligrams per kilogram of body weight for cyanuric acid. Exposure to both melamine and cyanuric acid may confer a higher risk, and there are unknowns about long-term renal and other risks. The current limit set by the FDA for melamine in food is 2.5 parts per million, calculated on the basis of ingestion by a person weighing 60 kg. This goal of this research is to evaluate the impact of melamine on human intestinal microbiota and determine the enzymatic capacity for cyanuric acid formation. Result from this work would provide FDA and other regulatory agencies with data to enhance the toxicological evaluation of safety of melamine residues in food.

The incursion and evaluation of cyanuric acid residues in the milk of lactating dairy cattle

The intentional addition of melamine and cyanuric acid to animal feed and human food can cause kidney failure and death. These effects show the need to know how animals metabolize and excrete these chemicals. FDA is concerned about biuret, an approved, non-protein nitrogen

supplement. The supplement may contain cyanuric acid as a by-product of the manufacturing process. The goal of this research is to measure the amount of cyanuric acid in milk from cows fed cyanuric acid to simulate amounts that could be present in biuret. The results of this research would provide the data needed by FDA for making regulatory decisions concerning the use of biuret as a non-protein nitrogen supplement in dairy cattle rations.

CD-1 Mouse Diet Pilot Study: Evaluation of Various Diets on Various Endpoints Critical to Evaluation of Bisphenol A and other Endocrine Active Agents in Mice

Bisphenol A (BPA) is an industrial chemical used to make a hard, clear plastic known as polycarbonate, which has been used in many consumer products, including reusable water bottles. FDA has been and continues to pursue a set of studies on the exposure to dietary BPA and the safety of low doses of BPA, including assessment of the novel endpoints where concerns have been raised. The goal of this research is to determine an appropriate diet for assessing the effect of endocrine active compounds, including BPA, on reproductive and metabolic parameters in CD-1 mice. Chemical and biological assays will be conducted on the diets to determine the occurrence and variability of several component activities that could alter the *in vivo* response of the animal to such endocrine active compounds. The data from the study will provide guidance for the selection of conditions for evaluating hormonally active compounds in CD-1 mice.

Physiologically-based Pharmacokinetic (PBPK) Models for Bisphenol A

Bisphenol A (BPA) is an industrial chemical used to make a hard, clear plastic known as polycarbonate, which has been used in many consumer products, including reusable water bottles. FDA has been and continues to pursue a set of studies on the exposure to dietary BPA and the safety of low doses of BPA, including assessment of the novel endpoints where concerns have been raised. The goal of this research is to development of a suite of dose response computational tools that would be capable of simulating BPA and its glucuronide metabolite (BPA-G) kinetics in laboratory animals used in toxicity testing and in the extrapolation of laboratory animal findings to humans. The human BPA models (child, adult, pregnant mom, and fetus) would predict the probable internal exposures to the aglycone PBA using BPA-G biomonitoring data (e.g. urine and blood). Results from this research would help FDA in science-based regulatory decision-making regarding the use of BPA.

Di(2-ethylexl)phthalate (DEHP) and Bisphenol A (BPA) Exposure in Pediatric Patients

Bisphenol A (BPA) is an industrial chemical used to make a hard, clear plastic known as polycarbonate, which has been used in many consumer products, including reusable water bottles. FDA has been and continues to pursue a set of studies on the exposure to dietary BPA and the safety of low doses of BPA, including assessment of the novel endpoints where concerns have been raised. The goal of this research is to provide estimates of concentrations of active BPA aglycone in potential target tissues of developing fetuses and children for BPA due to exposures from food and medical devices so the Agency can make science-based decisions on risk.

Evaluation of the Impact of Polycyclic Aromatic Hydrocarbon-Contaminated Seafood Residues in Edible Tissues on the Human Intestinal Microbiota

FDA, NOAA, and State agencies monitor petrochemical contamination levels in seafood. Polycyclic aromatic hydrocarbons (PAHs) are a component of crude oil and are regulated as carcinogens and are known to have harmful health impacts on humans. Exposure of ingested PAH residues in the human gastrointestinal tract may actively interfere with the host's physiology. The goal of this research is to evaluate of the impact of PAH contamination in seafood on the human gastrointestinal (GI) tract. The research approaches developed from these studies would help elucidate the intestinal fate and toxicity profiles of PAHs and reveal the ecophysiological changes between GI tract microbial communities and their relationships with the GI tract with respect to PAH exposure. Results from this research would provide the FDA and other regulatory agencies with data to enhance the toxicological evaluation of safety of PAH residues in food.

Toxins

Discovery and Development of Proteomic and Transcriptomic Biomarkers of Ciguatera Fish Poisoning

Ciguatera fish poisoning (CFP) of seafood consumers is caused by eating tropical and subtropical fish that have concentrated highly potent neurotoxins (ciguatoxins: CTXs) from microscopic algae (*Gambierdiscus* spp.). It is estimated that more than 100,000 people world-wide are affected every year by CFP. No tests exist to diagnose and confirm CFP in humans. The goal of this research is to identify biomarkers of CTX exposure in clinical samples to enable diagnosis of CFP in patients. Results from this research may also describe pathologic pathways and effects of CTXs and inform medical treatment to improve clinical management of CFP.

Drug Residues and Hormones

Metabolism and Residue Depletion of Albendazole in Yellow Perch

Very few drugs are approved for use in farmed fish because the market for these products is small, and information on drug metabolism and depletion of unapproved drugs in farmed fish is limited. The goal of this research is to continue previous work at FDA where the metabolism of albendazole in a variety of farmed finfish species was studied. In the new study, yellow perch will be treated with albendazole and tissues collected at various times post-dose and the muscle tissue will be homogenized and assayed. The results of this work would help to ensure that farmed fish in the marketplace are safe for human consumption.

Disposition of ^{3}H-Ivermectin in Hybrid Striped Bass, Largemouth Bass, and Yellow Perch

Very few drugs are approved for use in farmed fish because the market for these products is small, and information on drug metabolism and depletion of unapproved drugs in farmed fish is limited. The goal of this research is to continue previous work at FDA in determining a marker residue of ivermectin in various farmed fish species. In the new study, fish will be treated with

ivermectin and tissue samples collected and analyzed for potential marker residue(s). The results of this work would help FDA ensure farmed fish in the marketplace are safe for human consumption.

Proof of Concept Study for Identification of Metabolites of the Large Molecule Drug Improvest

Typically there are few human food safety concerns with the use of large molecule protein-based drugs, because they are digested by the body just like food. However, to improve the effectiveness of some of these drugs, manufacturers are chemically modifying the protein. Potentially-toxic compounds could be released when the body digests these modified proteins. The goal of this research is to develop laboratory models in which enzymes extracted from the stomach break down these molecules, which will allow analysis of the potentially generated compounds. This would help FDA understand the implications of any residues from large molecule protein-based drugs which might occur in edible swine tissues and aid in developing regulations for the safety of veterinary large molecular drugs.

Impact of Antimicrobial Residues on the Human Gastrointestinal Tract Microbiota

Because of incomplete absorption, ingestion of residues of antimicrobial agents present in edible products may cause adverse ecological effects on the human gastrointestinal (GI) microbiota. Perturbations of the GI microbiota by residues of antimicrobial agents may lead to reduced colonization resistance of the indigenous microbiota with subsequent overgrowth of resistant bacteria arising from strains already present in the GI tract or establishment of exogenous resistance pathogenic bacterial. To elucidate answers to these questions, data from *in vitro* and *in vivo* models have been used by the FDA and other regulatory bodies to evaluate the effect of residue levels of antimicrobial agents on the human intestinal microbiota. The goal of this research project is to develop methodology to enhance the toxicological evolution of the safety of veterinary antimicrobial residues in food that may impact the human intestinal microbiota.

Allergens and Gluten

Studies of Gluten-mediated Cell Signaling and Immune Modulation in Cultured Intestinal Epithelial Cells - Impact on Women's Health and Celiac Disease

Celiac disease, an intestinal inflammatory disorder, is caused by gluten from wheat, rye and barley. Currently, avoiding foods containing gluten is the only choice for those afflicted with celiac. Hence, proper food labeling is essential so that gluten-free labeled foods will not induce harmful immunologic responses. FDA proposed the term gluten-free for foods not containing 20 ppm or more gluten based on the currently available quantitation methods, which show considerable variability because of gluten complexity. The goal of this research is to use an intestinal epithelial cell line to identify biomarkers of gluten-mediated immune response. Information obtained from this work will further help to define gluten-free standards to protect patients with celiac disease.

Toxicological Studies Related to Dietary Supplements

Development of High-Throughput, *In Vitro* Screens for Toxicity: A Screen for Disrupters of Vitamin A Signaling

Because of the large number of new chemicals introduced into foods, supplements, and cosmetics each year, it is no longer possible to insure the safety of these chemicals through animal testing alone due to the high cost of animal studies and societal pressures to limit the use of test animals. The National Research Council has proposed the development of rapid, cell-culture tests as a solution to this problem. The goal of this research is to develop high-throughput *in vitro* screens for toxicity. Specifically, FDA is working to develop a test for chemicals that interfere with the action of vitamin A in fetal mouse cells because any chemical that interferes with the action of this vitamin in the fetus can cause serious birth defects.

Development and Validation of *In Vitro* Hepatotoxicity Assay(s) for Dietary Supplemental Materials

The increased use of dietary supplements has led to concerns about the possible health risk associated with their use. The goal of this collaborative research between FDA and University of Maryland scientists was to develop high throughput *in vitro* hepatotoxicity assay(s) to rapidly evaluate potential liver toxicity of dietary supplement materials. Sixteen phenolics were tested in both human and rat hepatoma cells using eight toxicity endpoints. From these results, the phenolics were grouped based on toxicity to the cell lines. Five herbal extracts were prepared and then tested in the same manner. The results support the use of this *in vitro* multi-endpoint screening model for identifying potential liver toxicity of herbal dietary supplements that are rich in phenolics.

Development of *In Vitro* Assays to Assess the Toxicity of Components in Dietary Supplements and Identify Biomarkers of Chemically-Induced Kidney Damage

Translating animal toxicity studies into accurate, rapid and cost-effective human risk assessments has led to the development of alternative methods to identify chemicals that may cause adverse health effects. *In vitro* assays with human kidney cells are being used to aid the FDA in identifying components of dietary supplements and other food-related compounds that may produce kidney damage. The goal of this research is to determine the mechanism(s) that contribute to renal toxicity and evaluate the role of the immune response in chemical-induced kidney damage. This work can provide valuable information that complements animal studies, while enhancing consumer safety by identifying food components or supplements suspected of causing kidney damage.

In Vitro Models of Fatty Liver Disease and Gender-specific Metabolizing Enzyme Activities in Cultured Human Liver Cells

Fatty liver, a common chronic liver disease, and sex-related differences in liver metabolism are poorly characterized but potentially important factors in the susceptibility to liver toxicity from chemicals in FDA-regulated products. The goal of this research is to develop models, using

human liver cells grown in culture, to evaluate the effects of dietary fat and sex-specific hormones on the metabolism and liver toxicity of herbal dietary supplements. This work will assist the FDA in determining whether adjustment of the safety profiles of some regulated products is needed for specific groups of people, such as women or people who consume a high fat diet, who may be at elevated risk for chemical-induced liver injury.

Biomarkers of Liver Toxicity Studied with Liquid Chromatography/Tandem Mass Spectrometry-based Global Metabolomics and Cultured Human Hepatocytes

Liver toxicity is a frequent finding in the FDA's Adverse Event Reports for dietary supplements. In order to assess the hazards to public safety from these reports, FDA is using human liver cells treated directly with extracts of dietary supplements. Cell growth medium is then screened, using mass spectrometry, for metabolites that are indicators of liver toxicity. The goal of this research is to develop and validate a method for rapid hazard assessment of dietary supplements suspected of causing liver toxicity. If successful, FDA can use the data from this method either directly for regulatory action or as a basis for targeting whole animal testing to further define hazards prior to taking regulatory action.

Development of an *In Vitro* Assay for Identification and Characterization of Liver Toxicants from Botanical Dietary Supplements

FDA has a need for additional toxicity data related to the chemical components found in botanical dietary supplements for more informed risk assessments. Toxicity to the liver from dietary supplement constituents is often evaluated using animal models. The goal of this research is to utilize metabolically active human liver cells in assessing the potential toxicity of botanical dietary supplements. This work will aid FDA in elucidating the cellular mechanisms involved in the toxicity and identify specific chemical components responsible for the toxicity. These studies are expected to provide more rapid and reliable data for understanding hepatotoxicity due to botanical dietary supplements and thus could help reduce the risk of injury due to their consumption.

Utilization of a Metagenomic Microarray (FDA-GutProbe) for Analysis of Probiotic, Supplement, and Food Additive impacts on Gastrointestinal Microbiome

The gastrointestinal tract of humans is inhabited by a large number of mostly beneficial microbes that aid in digestion of the diet. This community remains largely understudied in terms of health and disease. We have FDA has developed a DNA microarray (FDAGutProbe) to examine the effects of food additives on gut microbial content. FDAGutProbe will be utilized to determine the microbial content of fecal samples following the addition of various food additives to the diet. These studies fill a gap in FDA's ability to survey the effect of probiotic supplements used by the public, and confirm accurate labeling. The can also help to provide new insights into food additive safety as it relates to the effects on the gastrointestinal microbiome.

Potential Toxicity of Nanomaterials

Use of Electron Spin Resonance Spectroscopy to Characterize the Interactions between Nanoscale Materials and Model Biological Systems

Nanoscale materials are purported to have unique biological activities and may emerge as commercially important ingredients/components of FDA-regulated products. The goal of this research is to use electron spin resonance spectroscopy (ESR) to examine fundamental interactions between model biological systems and nanoscale materials. Work will focus on the way in which nanoscale materials affect reactive oxygen species and their subsequent interaction with components of biological systems. These studies will give FDA insight into the bioavailability and biological activity of nanoscale materials potentially found in foods and cosmetics.

Use of Electron Spin Resonance Spectroscopy (ESR) and Biomarkers of Oxidative Damage to Assess the Safety of Nanoscale Materials Used in Foods and Cosmetics

Nanomaterials are reported to be used in a wide range of products, including foods and cosmetics. The physical and chemical properties that make nanoscale materials uniquely useful may also contribute to toxicity, possibly through oxidative damage due to generation of free radicals and other reactive oxygen species (ROS). There are few methods to study potential nanomaterial-induced reactions. The goal of this research is to develop an Electron Spin Resonance (ESR) spectroscopy method to study the formation of free radicals and other ROS by nanomaterials. If successful, the ESR-based methods developed could be used by FDA as tools for rapidly screening potential nanoscale materials for use in foods and cosmetics, and provide insights into mechanisms of potential toxicity of nanomaterials.

Evaluation of the Applicability of *In Vivo* Micronucleus Assays for Assessing Genotoxicity of Engineered Nanomaterials

Nanotechnology holds great promise for the development of new treatments and diagnostics. Current methods of evaluation of genotoxicity of the FDA-regulated products were developed for molecular forms of materials, and may not be applicable to nanomaterials because materials may behave differently at the nanoscale. The goal of this research is to evaluate the applicability of *in vivo* micronucleus assays for assessing the genotoxicity of engineered nanomaterials. Results from this research could fill data gaps and help FDA to better understand the prediction of current tests for genotoxicity of nanomaterials.

Caenorhabditis elegans as an Alternative Model to Evaluate Nanomaterial Toxicity

Nanomaterials can be used in FDA-regulated products, such as cosmetics or foods, and it is important to better understand the long-term effects of nanomaterial exposure. Nanomaterial size, charge, and coating can alter function and toxicity profiles. FDA's previous work indicates that *C. elegans* can be used as a higher-throughput and cost-effective alternative animal model to assess nanomaterial toxicity. We will use the assays developed to evaluate the effect of various nanoparticle characteristics for the toxicity in *C. elegans*. Data acquired in this study will assist

in the development of risk assessments for specific nanomaterials and novel cost-effective assays for the detection of nanomaterial toxicity.

Genotoxicity and Cytotoxicity of Nanoparticles *In Vitro*

Nanomaterials are reported to be used in cosmetics and foods. We will investigate the potential toxicity of nanoparticles on *in vitro* human skin cells using markers such as cell morphology, cell uptake, cell viability, and DNA damage. The nanoparticles will be characterized in various solutions and media by size, size distribution, shape, surface charge, UV absorption, agglomeration, and aggregation. The results will be used to help predict the potential toxicity of nanomaterial ingredients when used in cosmetics and food contact substances.

Assessment of Iron Oxide Nanoparticle-induced Neurotoxicity in Cell Cultures and Whole Animal Models

Nanomaterials such as nanotubes, nanowires, fullerene derivatives (buckyballs) and quantum dots have received widespread attention as new types of analytical tools for biotechnology and in the life sciences. These nanomaterials have been shown to produce toxicity via oxidative stress. The goal of this research is to generate new information that would have a direct impact on the field of neuroscience, especially in the nanosciences and nanotoxicology of these materials. The results could provide a better understanding of the potential toxicity of these nanomaterials in dietary supplements or cosmetics.

Microbial Pathogens

In vitro Toxicogenomics and Molecular Mechanisms of Host-Pathogen Interactions in the Liver

Liver toxicity of foodborne agents including microbial pathogens is a food safety concern. The goal of this research is to utilize *in vitro* liver models for high-throughput screening of potential hepatotoxins, including microbial pathogens that occur naturally or that are added deliberately to foods and dietary supplements. These *in vitro* liver models can reduce animal use and associated costs. They can also provide more accurate data related to the interaction between low level pathogen exposure and liver toxicity from chemicals found in foods and dietary supplements.

Caenorhabditis elegans as Host Organism to Study Host Innate Immune Responses against Bacterial Infection and to Study Bacterial Virulence Factors of *Vibrio cholerae* and Other Foodborne Pathogens

Foodborne pathogens cause severe outbreaks responsible illness and the possible loss of lives. Understanding disease and dissemination mechanisms used by these pathogens is important to help prevent the outbreaks and to treat the affected individuals. The goal of this research is to use *Caenorhabditis elegans*, a genetically tractable simple organism, as an alternative to traditional vertebrate models. This model will be used to study virulence and host-defense mechanisms of foodborne bacterial pathogens, biothreat agents, and bacterial toxins. Besides being much less expensive than the standard animal models, the rapidity of the assay would make it useful during large scale foodborne outbreaks.

Alternative Toxicological Assays

Development of Methods for Evaluating DNA Damage using Single Cell Gel Electrophoresis (Comet Assay) in Rodents

The goal of this research is to evaluate the *in vivo* alkaline comet assay for use in preclinical hazard identification and the genotoxicity testing of food ingredients and other chemical agents such as nanoparticles for risk assessment and regulatory purposes. The comet assay has proved more popular in European and Japanese laboratories than in the U.S. As a result there is less experience with the assay in U.S. laboratories and among FDA personnel. With successful establishment and validation of the assay, the ability of FDA to evaluate the quality of the European Centre for the Validation of Alternative Methods (ECVAM) and Japanese Center for the Validation of Alternative Methods (JaCVAM) validation studies or evaluation of regulatory data submitted to the agency would be enhanced and the expertise within the agency can be developed to evaluate the results from comet assay that may be used for genotoxicity of FDA-regulated products, including food ingredients.

Phosphatidylinositol Glycan Complementation Group A (Pig-a) Mutagenesis; An International Validation Study Comparing Pig-a Mutation in Rats with Other Biomarkers of Genetic Toxicity

The FDA uses genotoxicity data to assess the safety of many of the products that it regulates, including human and veterinary drugs, food additives, food contact substances, and medical devices. At present there is no practical method for assessing the ability of these regulated products to induce gene mutation in humans or laboratory animals. In most instances, *in vivo* genotoxicity assessments are limited to assays that measure chromosome breakage or indicator assays like the Comet assay. The Pig-a assay may fill this void by providing a rapid, cost effective method for measuring gene mutation in rodents (and humans). The goal of this research is to conduct a validation study as the first step in developing a regulatory assay based on measuring mutation in the rat Pig-a gene. Results from this work would provide information for designing *in vivo* assays for assessing the safety of the various products regulated by the Agency.

Quantum mechanical and Nuclear Magnetic Resonance (NMR) Spectral Approaches for the Rapid Prediction of Estrogen Activity of FDA Regulated Chemicals

The U.S. EPA has recently required toxicity testing for endocrine disrupting compounds in the environment and FDA may need to perform similar tests for food additives and cosmetics. The goal of this research is to design advanced models for prediction of estrogen activity using quantum mechanical and nuclear magnetic resonance spectral approaches. If successful, these models could have improved performance compared to traditional structure activity relationship approaches for evaluating the estrogenicity of environmental contaminants having diverse chemical structures. The information on activity would be added to the FDA's Endocrine Disruptor Knowledge Base and could be used by FDA, U.S. EPA and other regulatory agencies for improved risk assessment.

Economic Analysis

The Costs to Farms of Controlling Human Health Risks in the Products They Produce

Because of the human health risk associated with fresh food and farm products used as ingredients in processed food, FDA is experiencing ever increasing pressure to regulate food commodities while they are still located on farms. This is highlighted by the recent outbreaks in leafy greens and tomatoes, and the ongoing problem of *Salmonella* Enteritidis in shell eggs. One way of reducing that risk is through government mandate and guidance of on-farm controls and certain practices. Most recently, FDA has worked on a regulation designed to improve on-farm handling practices associated with shell (table) eggs to prevent contamination with Salmonella. Developing a versatile model to assess the impacts of food safety regulations on farms will improve the FDA estimates of burden to industry associated with farm-related regulatory efforts. Such a model will improve the efficiency and decision quality when FDA is faced with the need to set policy.

FSMA Section 110(g) Report
Appendix C [ARS Food Safety Research List[15] for FY 2011 and FY 2012][16]

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[15] The primary source of research project information was the USDA's *Agricultural Research Service, National Program 108: Food Safety Summary and Completion of Project Reports, 2012*.

[16] *Disclaimer: Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its approval, endorsement, recommendation, or favoring by the United States Government or any department, agency, office, or branch thereof.*

Prevention, Intervention, and Control of Foodborne Hazards

Chemical Contaminants – Heavy Metals

Working with U.S. Producers to Protect Sales of Grains with Normal Levels of Cadmium

A European Union panel recommended lowering of the Potentially Tolerable Weekly Intake of cadmium be lowered from 7.0 to 2.5 micrograms per kg body weight peer week, and then proposed lower levels of cadmium for important U.S. export crops. The Codex Alimentarius (World Health Organization) program did not support lowering the cadmium intake recommendation (previously 7 micrograms cadmium per kg body weight per week), and slightly lowered it in changing to a monthly intake recommendation in order to stress the long term nature of dietary cadmium risk to humans (Potentially Tolerable Monthly Intake to be 25 micrograms per kg body weight per month). Previous research at Beltsville, MD, showed that the low iron, zinc and calcium levels of rice grain promote cadmium absorption by animals compared to animals fed adequate levels of these nutrients, but the EU committee did not take these important findings into account. Research findings such as these were brought to the attention of Foreign Agricultural Service and commodity group scientists who participate in international negotiations regarding food cadmium limits so they could use available scientific information to support the U.S. farm community. The EU Commission group dealing with the proposed lower limits for Cd in grains has decided not to adopt such regulations at this time, but have not formally rejected the proposal. Additional feeding trials would help settle these questions.

Cultivar Variation of Cadmium Accumulation in U.S. Soybean and Durum Wheat

Grain cadmium can be a limit for marketing of crops. Studies were begun or continued at Beltsville, MD, to assess cultivar variation in cadmium accumulation in grain of durum wheat and soybean. Breeding lower cadmium accumulating cultivars is one approach to protect markets for U.S. crops, but this requires knowledge of genetic variation and genotype-by-environment variation in crop cadmium. Cooperative studies were undertaken with plant breeders in Montana, Arizona and California to assess durum wheat genetic variation, and in Iowa and North Dakota to assess both genetic variation and soil series effects on cadmium accumulation in soybean. Crops grown during FY11 were analyzed, and the crops grown again during FY12 and will be analyzed. Both genotype and location affected grain Cd in soybean.

Flooding Rice Soils Causes Transformation of the Chemical Species of Zinc and Cadmium Present

Previous studies at Beltsville, MD, reported on the chemical forms of Cd present in contaminated paddy soils at varied redox status. Additional studies examined the effect of soil redox on the forms of Zn, and the extractability of soil Zn in the highly contaminated paddy soil from Mae Sot, Thailand. The soils were alkaline because the farmers had added limestone to reduce yield reduction due to the massive Zn and Cd contamination of the soil. Extended X-Ray Absorption Fine Structure Spectrometry was conducted to characterize the forms of zinc present and changes during flooding and drainage. Flooding this soil caused no apparent change in the forms of Zn

present, nor did draining the flooded soil to allow it to become aerobic. Interestingly, the two major forms of Zn present, the zinc-aluminum layered double hydroxide (hydrotalcite like), and Zn phyllosilicate are capable of holding Zn in forms which have little phytoavailability. The previous work showed that Cd and Zn were in different positions on solid surfaces in the flooded soil helping to explain how Zn and Cd in rice soils have such different phytoavailability.

Testing Use of Zinc Fertilizer and Ground Rubber to Reduce Cadmium in Durum Wheat in Arizona

Durum wheat grain produced on AriZona soils rich in chloride may contain cadmium at levels which might affect marketing in the European Union. High soil chloride has been shown to interact with low soil zinc (Zn) availability to promote cadmium accumulation, such that application of zinc fertilizers may reduce grain cadmium to protect markets. Scientists at Beltsville, MD, designed a field test in cooperation with scientists at Arizona State University to evaluate the effect of zinc sulfate and ground rubber application on cadmium in leaves and grain of two durum wheat cultivars with different cadmium accumulation potential. The plots were established in advance of planting to allow reactions of the amendments in the soils. All soils, plant tissues and irrigation waters were analyzed for trace elements and nutritional status. Wheat yield was not affected in these soils which were thus not zinc-deficient. The analyses showed that there was no reduction in grain Cd in response to the added Zn, in contrast with some soils which had true Zn deficiency without the high chloride in irrigation water. Other treatments such as foliar Zn spray during grain filling will be examined.

Toxins

Edible Food Compounds Inactivate Virulent Bacterial and Plant Toxins

Stx is the primary virulence factor of Shiga-toxin producing Escherichia coli (STEC), so decreasing active Stx in contaminated food is a strategy for minimizing illness. Stx virulence is based on the 3-D conformation of the protein. In collaborative studies at Albany, CA, we discovered that (a) orally ingested Stx damages kidney, spleen, and thymus tissues in mice; (b) freshly prepared juice from Red Delicious apples inhibited the biological activity of Stx; (c) 4-hydroxytyrosol from olives inactivated *Staphylococcus aureus* and its enterotoxin; (d) olive powder inhibited multiple foodborne pathogens; and (e) reconstituted milk inhibited activity of ricin toxin (isolated from castor beans), a toxin similar in activity to Stx. These findings suggest that safe food-compatible compounds can inactivate toxins.

Repression of Mycotoxin Genes in *Aspergillus flavus*

The biocontrol yeast, Pichia anomala WRL-076 has been demonstrated by scientists at Albany, CA, to repress the expression of aflatoxin and cyclopiazonic acid biosynthetic genes from *Aspergillus flavus* by quantitative reverse transcriptase PCR (qRT PCR). Genes demonstrated to be repressed in aflatoxin biosynthesis are: aflR (coding for transcription activator), aflJ (coding for transcription enhancer), omtB (coding for 0-methyltranferase B) and pksA (coding for polyketide synthase). Genes demonstrated to be repressed in cyclopianzonic (CPA) acid biosynthesis are: pks-npls1 (coding for polyketide synthase and nonribosomal peptide synthase),

hydA (coding for CPA amidohydrolase and ctfR1 (coding for CPA C6-type transcription factor). The molecular data validated that the patented P. anomala is a suitable biocontrol agent for reducing both aflatoxin and cyclopiazonic acid in plant food products.

New Maize Inbred (Pure Breeding Line) Lines Demonstrate Resistance to Aflatoxin Contamination in Field Trials

Of the six inbreeds previously developed and released by the International Institute of Tropical Agriculture and ARS scientist at New Orleans, LA, aflatoxin-resistance maize breeding program, five were tested in 2011 as single-cross hybrids (combination of different varieties) in field trials held in MS, TX (College Station and Lubbock), and LA. Aflatoxin resistance was demonstrated in at least two of the five on a consistent basis and at levels comparable to other well-known resistant lines. Promising yield numbers were also attained by two of the lines compared to other resistant lines and to two commercial hybrids (mixed between two types of parents). The development of new resistant corn lines in good agronomic backgrounds (suitable for desired environmental conditions) offers the possibility of enhancing resistance of commercial lines through marker-assisted breeding. New linkages of resistance genes may have also been facilitated through the breeding of these lines.

Ochratoxin and Fumonisin Production in Conventional versus Organic Orchards

Black *Aspergillus* isolates have been collected and identified from vineyard samples of raisins by scientists from Albany, CA. These isolates have been screened for ochratoxin and fumonisin production. Initial analysis showed no significant differences in population diversity of black *Aspergillus* species between conventional and organic vineyards. This result supports the hypothesis that organic farming practices do not lead to greater risk of ochratoxin or fumonisin contamination.

Identification of Toxins in *Stenocarpella* Rotted Corn

Stenocarpella maydis is a fungal pathogen that causes a dry-rot of maize ears and can contaminate grain with neurotoxins that are harmful to livestock. ARS scientists at Peoria, IL, collaborated with a scientist at the University of Iowa, Iowa City, IA, to perform chemical investigations of *Stenocarpella* rotted ears from a field outbreak in Illinois. This research resulted in the isolation of diplodiatoxin and a second set of toxins, chaetoglobosins, as major components. Toxin levels increased ~ 100 fold in grain exposed to a period of post-harvest moist incubation. Contamination of corn ears and stalks with chaetoglobosins could explain reported toxicological effects in livestock grazing harvested fields infested with *Stenocarpella*. Specific knowledge of the toxins produced by Stenocarpella in corn cultivation is an essential first step in interpreting the mechanism of action by which these toxins affect livestock, poultry or humans, and is critical to the development of practical methods for estimating the levels of these toxins in corn and in assessing potential risk.

Identification of a Fungal Protein Used by *Fusarium* to Cause Disease in Corn

Fungi known as *Fusarium* can cause devastating damage to U.S. agriculture through crop loss and by contaminating the food and feed supply with harmful toxins. Plants produce proteins called chitinases that act as a natural defense against fungi. ARS scientists at Peoria, IL, previously identified specific plant chitinases that are inactivated by fungal proteins. In the current research, project scientists have isolated and identified one of the fungal proteins responsible for this inactivation. The protein was identified in *Fusarium* verticillioides, an important fungus that causes corn ear rot and produces toxins that cause adverse health effects. This research has identified a target that can be exploited by chemists, breeders, and genetic engineers to improve disease resistance and reduce mycotoxin contamination of economically important crops.

Discovery of Genetic Mechanisms that Control Toxin Production in the Fungus *Fusarium*

The fungus *Fusarium* verticillioides is frequently associated with corn and is of concern to food and feed safety because it produces toxic metabolites that are common contaminants of corn. ARS scientists at Peoria, IL, have identified multiple gene clusters (i.e. groups of genes located next to one another) in *Fusarium* verticillioides that are responsible for the production of toxins, such as the carcinogenic fumonisins and the mutagenic fusarins. The scientists have also demonstrated that a gene, LAE1, is required to activate the gene clusters and thereby induce production of the corresponding toxins in F. verticillioides. The identification of these toxin biosynthetic genes and the discovery of mechanisms used by the fungus to control toxin production provide scientists with information needed to develop novel strategies aimed at suppressing toxin production and improving the quality and safety of food and feed derived from cereals.

Genetic Control of Toxins in Emerging Fungal Pathogens of Cereal Crops

Fusarium head blight is a devastating disease of cereal crops. It is a concern for food and feed safety because the fungus causing the disease also contaminates the grain with a mycotoxin, deoxynivalenol (DON). In the U.S., most of the *Fusarium* strains that cause this disease make one type of DON. New *Fusarium* strains that produce a different form of DON, and higher amounts of the toxin, have been spreading across North America. Scientists in ARS at Peoria, IL, used modern molecular and genetic techniques to design mutant strains that differ only in the single gene that determines which form of DON is made. Greenhouse tests found that the form of DON produced was not correlated with the amount of toxin made in infected grain and that other factors need to be considered. This information is needed by food safety scientists and plant breeders to develop strategies to limit toxin contamination of wheat, and to maintain the quality and safety of food and feed derived from cereals.

Genetic Basis of Fumonisin Production

Genetic analysis of fumonisin-nonproducing *Aspergillus Niger* and *Aspergillus awamori* strains showed that in *A. awamori* strains, a larger section of the fumonisin bysynthetic gene cluster is deleted. This deletion in the gene cluster resulted in a loss of fumonisin production. In A. Niger,

fumonisin-nonproducing strains contain the entire biosynthetic gene cluster, indicating that the nonproduction is due to other genetic factors. This research from Peoria, IL, increases the fundamental understanding of the genetic basis of fumonisin production in these species.

Alkaline Processing of Maize Unequivocally Reduces Fumonisin Toxicity in Maize-based Foods

Detoxification of fumonisin-contaminated, whole kernel corn by nixtamalization was for the first time demonstrated using a well-characterized rat feeding bioassay. The study conducted at Athens, GA, combined cooking and rinsing (three times) under conditions that are relevant to households and industry. Unlike earlier *in vitro* experiments, the in vivo approach unequivocally demonstrated the absence of any significant toxicity that could be attributed to unknown fumonisin by-products remaining in the cooked corn.

Characterizing Antibodies that Offer Protection against BoNT Intoxication

Botulinum neurotoxins (BoNTs) are highly dangerous molecules responsible for botulism food poisoning. These toxins are rapidly absorbed in small amounts that are very difficult to detect. ARS scientists in Albany, CA, developed monoclonal antibodies (mAbs) for the sensitive detection of BoNT serotype B, one of four serotypes that cause human disease. Some of these mAbs were shown to confer strong protection to BoNT/B. Protection by antibody was correlated with the antibody potentiated depletion of BoNT/B toxin in blood of intoxicated mice. Knowledge of the impact of antibodies on the toxicokinetics will be valuable for advancing food safety and defense.

Discovery of Fungal Metabolites that Inhibit Botulism Neurotoxin

Fungi that colonize and kill other fungi are potential sources of novel antifungal agents and other compounds useful to agriculture or medicine. A scientist at Peoria, IL, in collaboration with a University of Iowa scientist in Iowa City, IA, have isolated several antifungal compounds which scientists in the Division of Integrated Toxicology, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD, have identified as inhibitors of a neurotoxin causing a life-threatening illness known as botulism. Improperly preserved home-processed foods such as green beans, beets, and corn are a common source of botulism food poisoning. These are the first fungal metabolites reported to inhibit the botulism neurotoxin and can serve as lead compounds for further chemical modifications to improve potency and other pharmacological parameters.

Development of a New Material to Absorb Ochratoxin A from Liquids such as Wine

Ochratoxin A (OTA) is a toxin produced by certain fungi that contaminate and reduce the value of agricultural commodities. In an effort to reduce exposure to OTA, ARS scientists at Peoria, IL, investigated the ability of a nanoporous carbohydrate-polymer material to reduce levels of OTA in water and wine. The interaction between OTA and the material was investigated using experimental and computational techniques. It was discovered that the material is capable of reducing OTA levels in water and wine, through a complex binding mechanism. In addition, these results provide important information on the application of this class of polymers to aid in

the detection of toxins and will assist in more broad uses of cyclodextrin polymer materials. This research provided a new type of material and strategies to selectively reduce exposure to ochratoxin A.

Drugs and Hormones

Natural Antimicrobials to Replace Antibiotics in Swine Diets

The use of antibiotics in animal production is a controversial issue due to the concern of transmission of antibiotic resistance genes. Young swine are often fed dietary antibiotics to improve health, reduce pathogen load, and enhance performance. Few natural alternatives have been identified to replace these compounds if producers are required to eliminate antibiotic use. ARS scientists at Clay Center, NE, determined that a commercial product containing lysozyme (naturally found in eggs) could replace dietary antibiotics. The impact of this research, particularly for industry, is that the use of lysozyme in diets of young piglets could maintain a safe food supply and reduce the use of prophylactic antibiotics that are typically used for swine production.

Microbial Pathogens

Salmonella

Finding the 'Danger Zone' on a Jalapeño Pepper

Consumption of *Salmonella*-contaminated jalapeño peppers has been implicated in foodborne illness outbreaks. Working with artificially inoculated peppers, ARS researchers at Wyndmoor, PA, recovered > 90% of the introduced *Salmonella* from the stem/calyx and recovered only a small proportion from fleshy pods. *Salmonella* grew by 3 logs on peppers after incubation at 20C (68F) for 48 hr and could survive for at least 8 weeks on peppers stored at 4C (40F). Immersion of inoculated peppers in 200 ppm of sodium hypochlorite, acidified sodium chlorite, or peroxyacetic acid for 10 min could reduce the number of *Salmonella* on stem/calyx by 96.8-98.0% and on flesh by 99.2-99.6%. By clearly identifying where pathogens reside on peppers, processors will be better able to provide safer produce to consumers.

A Simple Treatment to Eliminate *Salmonella* from Tomatoes

Numerous *Salmonella* outbreaks have been associated with fresh tomatoes. ARS researchers at Wyndmoor, PA, dipped artificially contaminated green tomatoes in hot water for 3.5 minutes. This treatment at 70C (160F) reduced *Salmonella* by up to 99.9999% without visible injury or changes to maturation. When combined with refrigerated storage, the treated tomatoes remained free of *Salmonella*. This chemical-free process of surface pasteurization holds promise for use in the fresh produce industry, and can enhance the microbiological safety of tomatoes.

Integrated Approach to Enhance Microbial Safety of Tomato Fruits

Fresh tomatoes have been implicated in recent outbreaks of foodborne diseases. ARS researchers at Wyndmoor, PA integrated ultraviolet C (UV-C) light with low dose gamma radiation to control human pathogens on tomato fruits. Results indicated greater than 99.999% of *E. coli* O157:H7 and *Salmonella* enterica strains inoculated onto tomato fruits was inactivated by a combined treatment of low dose UV-C and gamma radiation. Furthermore, this treatment significantly reduced the population of native microbes during 3 weeks of storage at 10C, without causing substantial quality changes in tomatoes. This approach may be used by the produce industry to decontaminate fresh tomatoes.

Salmonella Contamination Occurs in the Stem Scars of Tomatoes

The goal of this work was to determine antimicrobial treatments effective at inactivating *Salmonella* on tomato stem scars with or without vacuum perfusion. ARS researchers at Wyndmoor, PA tested sixty-three antimicrobial combinations against *Salmonella* on tomato stem scars. Twenty-four of the antimicrobial washes inactivated more than 3.0 log CFU/g (99.9%). Even more *Salmonella*, 4.8 log (99.998%), were reduced by seven of the washes (viz., 40% ethanol, sulfuric acid, in addition to five organic acid combinations). Vacuum perfusion + 200 ppm chlorine increased inactivation by 0.7 log (80%) over chlorine alone. Results from this study provide tomato processers with sanitization options effective at inactivating *Salmonella* from the stem scars of tomatoes.

In-package Pasteurization for Fresh and Fresh-cut Produce

Fresh fruits and vegetables are usually consumed by consumers directly without a cooking step to kill foodborne pathogens. Any pathogenic contamination in fresh fruits and vegetables can potentially cause severe illnesses or even deaths. ARS researchers at Wyndmoor, PA, developed an in-package chlorine dioxide releasing film that can kill pathogens on packaged foods. Results indicated antimicrobial films in a food container can inactivate 99.9% of *Salmonella* inoculated onto fresh grape tomatoes and some other types of produce. The in-package pasteurization provides a single, simple, inexpensive technique to enhance the microbial safety and extend the shelf-life of packaged fresh and fresh-cut produce.

Antimicrobial Activity of Plant-derived Compounds Against *Salmonella* on Organic Leafy Greens

Organic foods are produced without the use of any chemicals, and hence, natural plant compounds may be good alternatives to chemicals. The antimicrobial effect of apple, hibiscus, olive, and lemongrass extract was evaluated against *Salmonella* on organic romaine and iceberg lettuce, and on spinach. Research conducted at Albany, CA, showed that olive extract exhibited significant antimicrobial effect, resulting in up to 3 log CFU/g *Salmonella* reduction followed by lemongrass and apple extract. The antimicrobial effect of hibiscus extract was marginal. The antimicrobial effect of these extracts increased with exposure/contact time. Plant extracts were more effective in reducing *Salmonella* on romaine and iceberg lettuce than on spinach. This study demonstrates potential use of natural plant extract to reduce *Salmonella* on organic leafy greens.

Salmonella on Chicken Parts

The number of *Salmonella* on chicken wings, chicken breast fillets, chicken thighs and chicken drumsticks was investigated by an ARS researcher at Princess Anne, MD. Most pieces of chicken (97%) contained no *Salmonella* and those that did have *Salmonella* only had a few cells (i.e. 1 or 3 cells). These results indicate that the chicken examined posed a very low risk of foodborne illness from *Salmonella*.

Salmonella and the Cutting Board

Sometimes consumers use the cutting board and utensils used to prepare raw chicken for cooking without first washing them to prepare other foods. This could result in the transfer of *Salmonella* from raw chicken to other foods and consumption of this pathogen by consumers. This scenario was investigated by an ARS researcher at Princess Anne, MD, and it was found that on only 1 out of 57 occasions was *Salmonella* transferred from raw chicken to cooked chicken during meal preparation when the cutting board and utensils were not washed before cutting the cooked chicken. On that one occasion where *Salmonella* was transferred only a single cell was found on the cooked chicken. These results illustrate that when the chicken company provides the consumer with chicken containing low levels of *Salmonella* that even when the consumer makes a food handling mistake, the risk of getting foodborne illness is low.

Salmonella and Cold Chicken

Although chicken meat may contain low levels of *Salmonella* when purchased, if the consumer stores the chicken meat in a refrigerator at an improper temperature for an extended period of time, the *Salmonella* could grow to high and dangerous levels. An ARS researcher at Princess Anne, MD, investigated this possibility and developed a model for predicting growth of *Salmonella* on different types of chicken meat during cold storage. The model predicts that *Salmonella* grows best on thigh meat, second best on chicken skin, and third best on breast meat. *Salmonella* survived and did not grow on chicken meat stored at temperatures from -8C (17.6F) to 10C (50F) for up to 8 days. However, at temperatures from 11C (51.8F) to 16C (60.8F), *Salmonella* grew on chicken meat with the amount of growth increasing as time of storage (up to 8 days) and temperature of storage increased. In fact, after 2 days of storage at 16C, one cell of *Salmonella* became 1,383 cells on thigh meat, 164 cells on chicken skin, and 31 cells on breast meat. The model will be incorporated into the ARS, Pathogen Modeling Program (http://portal.arserrc.gov/) where it can be used by consumer's to help them decide whether or not their chicken is safe to eat after extended time in the refrigerator.

New Insights on How *Salmonella* Interacts with Chicken Immune Cells

Food-poisoning bacteria such as *Salmonella* are significant causes of human disease; these pathogens can often be found as contaminants in poultry meat products. New approaches are needed to produce poultry that are not colonized by these harmful bacteria, given that absence of the pathogens in living birds will largely translate into pathogen-free meat products for human consumption. ARS researchers at College Station, TX, found that after exposure to S. typhimurium, a type of chicken immune cell known as a heterophil responded by up-regulating

(turning on) genes associated with regulation of cell differentiation, protein transport, macromolecule localization, and heterocycle metabolic processes. The work also established that bacteria attacked by the heterophils responded by increasing fatty acid biosynthesis, flagellar assembly, glutathione metabolism, and the Type III secretion system. This work is important because for the first time, it has been shown how *Salmonella* and the bird host interact and how these interactions ultimately result in protection versus illness/death. This work has identified specific targets to design new immune modulatory and/or antimicrobial compounds that can be utilized by the poultry industry to produce microbiologically safer poultry meat products for the consumer.

Bactericidal Effects of Chitosan in Poultry

A number of supply, production, and environmental sources can serve as *Salmonella* contamination points during food animal production. Prebiotic feed additives offer potential alternatives to antibiotic use against gut pathogens in poultry production. ARS researchers at College Station, TX, showed that incorporation of chitosan into the diet significantly reduced *Salmonella* cecal contamination in young broilers. Chitosan is a derivative of chitin, which is the structural element of the exoskeleton of insects, shrimp, etc. Chitosan is totally safe to higher animals. This discovery has important food safety implications because chitosan as a feed additive offers potential application against *Salmonella* contamination during pre-harvest poultry production. Reduced pre-harvest pathogen load will result in fewer *Salmonella* in live broilers entering the processing plant and will lessen the potential for carcass cross-contamination.

Frequency and Persistence of Fecal Shedding following Exposure of Laying Hens to Different Oral Doses of *Salmonella* Enteritidis.

ARS researchers in Athens, GA, determined that exposing laying hens to quantitatively increasing oral doses of *Salmonella* Enteritidis (ranging from 104 to 108 cells) resulted in corresponding increases in both the initial post-inoculation frequency of fecal shedding and the duration of this shedding over the course of the experiment. The results of this study indicate that higher oral exposure doses can significantly increase fecal shedding of S. Enteritidis into the laying house by infected hens. Accordingly, the probability of detecting infection by environmental testing protocols which depend on fecal shedding may be relatively low when hens in a flock are exposed to low doses of S. Enteritidis.

Thermal Death Time Model for *Salmonella* in Chicken

Adequate cooking time and temperature ensures safety against pathogens in ready-to-eat foods while minimizing quality losses. ARS researchers at Wyndmoor, PA, defined the heat treatment required to achieve a specified lethality for starvation-stressed *Salmonella* in ground chicken supplemented with natural antimicrobials, cinnamaldehyde and carvacrol. The thermal death predictive model for the pathogen, which can predict the D-values for any combinations of the factors that are within the range of those tested, was developed. Using this model for *Salmonella*, food processors can design thermal processes for the production of a safe chicken product with extended shelf life.

Antimicrobial Coatings Effectively Inactivate Foodborne Pathogens

Pathogenic contamination of food usually starts from food surfaces. The presence and potential growth of pathogens in food during storage and transportation is a safety concern. ARS researchers at Wyndmoor, PA, used antimicrobial coatings to kill pathogens on food surfaces and to prevent further cross-contaminations during transportation, storage, and store display. The coating treatments were capable of reducing *Salmonella* by more than 99.9999% on cantaloupe, tomato stem scar, and shell eggs, and no re-growth during 14 days storage at 10C. Results from this study will provide food processors with viable options for designing antimicrobial coatings to improve the microbiological safety and quality of produce.

Evaluation of Washing Aid in Inactivation of *Salmonella* in Biofilms

The effect of the washing aid, T-128, on inactivation of *Salmonella* enterica serovars Thompson or Newport, or Pseudomonas fluorescens populations in biofilms on stainless steel was evaluated under conditions of increasing organic matter loads by researchers at Beltsville, MD. For both *Salmonella* and Pseudomonas, the sanitizing effect of free chlorine (1.0-5.0 mg per L) was enhanced significantly when combined with T-128. Application of T-128 decreased the free chlorine depletion rate caused by increasing organic matter in wash waters, and significantly augmented inactivation of bacteria in biofilms compared to treatments without T-128. Results show T-128 can aid in sanitizing stainless steel contact surfaces during fresh-cut produce processing.

Existing Method for Pasteurizing Shell Eggs Significantly Affects Quality

Currently, hot water immersion is the only commercial method used to pasteurize eggs and it may damage egg functional properties. In the initial phase of this research, shell eggs were inoculated with *Salmonella* and immersed in hot water at various temperatures for various times. ARS researchers at Wyndmoor, PA, determined that *Salmonella* was reduced by 4.5 log (99.997%) following treatment at 56.7 deg C for 60 min. The hot water pasteurized eggs were then refrigerated for 4 weeks to determine the fate of the surviving *Salmonella*. The *Salmonella* neither grew nor died during the storage. In the final phase of the research, the quality of the eggs was evaluated in collaboration with ARS scientists at Athens, GA. Compared to fresh (unpasteurized) eggs, the hot water immersion eggs had significantly greater shell dynamic stiffness, albumen height, and Haugh unit score and significantly lower yolk index. There was no significant difference in shell strength or vitelline membrane elasticity. This research established that current pasteurization methods affect egg quality parameters and that an alternate pasteurization method is required.

Seafood, When Adjusted for Per Capita Consumption, is Associated with Foodborne Illness more than Meat, Poultry, or Produce

In research conducted by ARS researchers at Wyndmoor, PA, catfish fillets were rinsed with near-neutral electrolyzed water (anaolyte) having a pH of 6.0-6.5 and an oxidation reduction potential greater than 700 mV. Catfish fillets which were inoculated with *Salmonella* were treated with anolyte with a residual chlorine level of 300 ppm for 3 minutes. The treatment

reduced *Salmonella* levels by 90%. In addition, *Salmonella* levels did not increase when the catfish fillets were held for 8 days at refrigeration (4C) temperature. The treatment with anolyte had no negative effect on catfish fillet quality. This method can be used by seafood processors to provide safer fish fillets to consumers.

E. coli O157:H7 and STEC

Inactivation of *Escherichia coli* O157:H7 (ECOH) and Non-O157:H7 Shiga Toxin-producing *E. coli* (STEC)

Escherichia coli O157:H7 (ECOH) and non-O157:H7 Shiga toxin producing *E. coli* (STEC) are the cause of many outbreaks of illnesses and deaths. Infections are generally foodborne with ground beef a major conduit. ARS researchers at Wyndmoor, PA, evaluated the fate of *E. coli* O157:H7 and non-O157 strains in both flattened and wafers of ground beef in a heated water bath and commercial grills. Studies showed that regardless of the level of fat or type of heat/grill used, cooking ground beef patties to an internal temperature of ~71.1C was effective for destroying the pathogens. These data were transferred to the USDA-Food Safety and Inspection Service for their use in consumer related advice for safe food handling.

Vaccine Development for *Escherichia coli* O157:H7

Escherichia coli O157:H7 can cause life-threatening foodborne illnesses. Beef cattle are a major asymptomatic carrier of the pathogen, and development of a vaccine for cattle to eliminate the pathogen is a major goal for government and industry. *E. coli* O157:H7 colonize the terminal portion of the large intestine in cattle by "sticking" to a specific type of tissue. Specific bacterial proteins are required for adherence and studies have implicated the protein (intimin) responsible for adherence. However, ARS researchers at Ames, IA, have now determined that *E. coli* O157:H7 lacking the intimin protein use additional proteins for adherence. This finding is significant in the context of developing efficacious vaccines for blocking adherence of the bacteria. Therefore, better vaccines would be those that would include not only the intimin protein but other proteins to reduce adherence. ARS will redirect its vaccine development studies to address this critically important observation.

Fate of *Escherichia coli* O157:H7 (ECOH) in Mechanically Tenderized Prime Rib

The process of mechanical and/or chemical tenderization transfers surface-residing cells of pathogens into the deeper tissues of the meat wherein they may be more resistant to subsequent thermal challenge. Similar to previous work with thermal inactivation of ECOH compared with non-O157:H7 Shiga toxin-producing *E. coli* (STEC) in steaks prepared from blade/chemical tenderized subprimals following cooking on commercial grills, ARS researchers at Wyndmoor, PA, evaluated the effect of the commercial practice of low-temperature, long-time heating when preparing prime rib roast for elimination of ECOH from mechanically tenderized beef prime rib. These results demonstrated that to meet the required 5.0-log reduction of ECOH to produce a product that was both safe and of high quality, it would be necessary to sear, cook to internal temperatures of 48.9C, 60.0C, or 71.1C, and then hold previously inoculated and tenderized prime rib roasts in a warming oven at 60.0C for 8 h. These data support regulators in the

development of science-based cooking guidelines and assist restaurants and/or food service establishments to enhance the safety of non-intact prime rib served at the point of consumption.

Role of Curli and Cellulose Expression in Adherence of Shiga-toxigenic *E. coli* to Spinach Leaves

Cellular appendages, such as curli fibers, and cellulose, a constituent of extracellular matrix, have been suggested to be involved in *E. coli* attachment and persistence in fresh produce. ARS researchers in Beltsville, MD, documented that curli-expressing *E. coli* O157:H7 strains developed stronger association with spinach leaf surfaces, whereas curli-deficient mutants attached to spinach at significantly ($P < 0.01$) lower numbers. Attachment of cellulose-impaired mutants to spinach leaves was not significantly different from that of curliated strains. The relative attachment strength of *E. coli* O157:H7 to spinach increased with incubation time for the curli-expressing strains. Scanning confocal microscopy (LSCM) analysis of inoculated leaves revealed that curli-expressing *E. coli* O157:H7 were surrounded by extracellular structures strongly immunostained with anti-curli antibodies. Production of cellulose was not required to develop strong attachment to spinach leaf. These results indicate that curli fibers are essential for strong attachment of *E. coli* O157:H7 to spinach whereas cellulose is dispensable.

Chlorine and Washing Aid to Reduce Pathogens in Biofilms on Cantaloupe Rind

The efficacy of chlorinated water (CW) solutions, with or without the washing aid, T-128, on inactivation of natural microbial flora and enteric pathogen biofilms on cantaloupe rinds was evaluated by scientists at Beltsville, MD. With free chlorine (FC) at 500-2000 mg per L, the sanitizing effect on the natural microflora and on the inoculated strains were enhanced significantly by 1.0-2.0 log CFU per square cm when combined with T-128 in the washing solutions. An additional significant reduction of 0.7-1.0 log CFU per square cm for *Salmonella poona* and *E. coli* O157:H7 was observed in CW at 500-1000 mg per L containing T-128 when rinds were brush-scrubbed during the washing. These results indicate that T-128 can aid in reducing pathogen viability in biofilms on cantaloupe rinds, and thereby can aid in reducing food safety risks associated with fresh cantaloupes.

Functional Metagenomics Assay for Alternatives to Antibiotics

ARS researchers in Ames, IA developed and successfully tested a high throughput, robotic-based assay to identify ‘toxic’ genes whose products either inhibit *Escherichia coli* growth or disrupt *E. coli* bacteria. This ‘functional metagenomic’ assay screens all DNAs in subfractions of environmental samples in order to find genes toxic for foodborne pathogens, such as *E. coli*. The researchers plan to add to their collection of inhibitory genes while starting to identify the cloned toxic genes. This research and products benefit animal producers, animal health and food safety industries, commodity groups, and pharmaceutical industries seeking alternatives to existing antibiotics.

Listeria

Modeling Heat Resistance of *Listeria monocytogenes* in Ground Beef

Adequate heat treatment destroys *L. monocytogenes* and is the most effective means to guard against the potential hazards in cooked meat products. Due to public health concerns associated with high salt levels in the diet, consumers these days are increasingly demanding processed meats with reduced salt levels. ARS researchers at Wyndmoor, PA, defined reduced heat treatment required to achieve a specified lethality for *L. monocytogenes* in beef supplemented with salt and apple polyphenols (antioxidants). The predictive model will assist processors to develop beef products with reduced salt and apple polyphenols.

Airborne Transfer of *Listeria monocytogenes* from Floor Drains during Wash

Listeria monocytogenes, a human pathogen, can be found contaminating the environment inside poultry processing plants especially the floor drains. It is unclear if or how *Listeria* can travel from a floor drain to product. The objective of this study conducted at Athens, GA, was to determine if an accidental discharge of a water hose into a contaminated floor drain could result in airborne transfer of live *Listeria* cells to other surfaces. Using a two second spray into an experimental model drain systems, we were able to detect airborne *Listeria* within the experimental rooms. *Listeria* was detected settling out of the air as far away as 4.0 m (13 ft) on the floors and even 2.4 m (8 ft) high on the walls. Poultry processors will use this information to guide sanitation standard operating procedures relative to avoiding inadvertent hose spray into floor drains. Researchers will find this information critical as they design and test intervention strategies to prevent the escape of live *Listeria* from contaminated sites during poultry plant wash down.

Predictive Model for *Listeria monocytogenes* in Ready-to-eat (RTE) Meat

Consumption of contaminated ready-to-eat meat may cause bacterial foodborne illnesses. ARS researchers at Wyndmoor, PA, developed models to describe the growth of *L. monocytogenes* on cooked ham surface-treated with lactic acid. These models will help the manufacturers to select a suitable lactic acid treatment to improve the safety of their food products.

Listeria monocytogenes in the Ready-to-eat (RTE) Foods at Retail

Significant efforts have been made to control *Listeria monocytogenes* (Lm) in foods over the past decade. Outbreaks of foodborne illness are especially associated with ready-to-eat foods such as deli-meats, soft cheeses, raw and smoked fish, and raw or partially processed vegetables. At the request of the Food and Drug Administration and the USDA-Food Safety and Inspection Service, ARS researchers at Wyndmoor, PA, undertook a study to determine the current prevalence and levels of Lm in deli-packaged versus pre-packaged RTE foods purchased at retail establishments in four FoodNet sites. The study indicated an observed Lm prevalence from 0 to 1.0 percent for seven product categories. This is the most comprehensive survey of Lm in retail RTE foods in the past decade, and provided data critical for policy decisions on further control for this pathogen, and its contribution to the public health burden. The study received the FDA Commissioners Award.

Attachment of *Listeria monocytogenes* on the Surface of Ready-to-eat (RTE) Products

Understanding the mechanisms of attachment may help to elucidate the persistence of *Listeria monocytogenes* in ready-to-eat products and thus find ways to inhibit it by detaching it from the food or not allowing attaching to the food. A gene was identified as responsible for attachment (at least in part) of *Listeria monocytogenes* in RTE foods like fresh fruits and vegetables. The binding was found to occur with cellulosic material in the fresh produce. This hypothesis was tested and validated. Thus, a system to prevent attachment to cellulosic material or to detach the organism can be designed. This can enhance the safety of fresh and fresh-cut fruits and vegetables, a category of foods responsible for a large number of foodborne outbreaks annually.

Understanding how *Listeria monocytogenes* is able to Survive Inactivation Treatments

Nisin is an antimicrobial compound, known as a bacteriocin, that can be used to control *Listeria monocytogenes* in food, and high hydrostatic pressure has also been used to inactivate *L. monocytogenes* in food. However, *Listeria* can become resistant to these inactivation treatments, and how this resistance occurs is not known. Studying a naturally-occurring strain of *L. monocytogenes* that was tolerant to high pressure treatment but showed increased sensitivity to nisin, ARS researchers at Wyndmoor, PA, provided information to show that a combination of high pressure and nisin can be applied to effectively control *L. monocytogenes* in food. This study also revealed the mechanism of this interaction, and this knowledge may contribute to the design of safe and economically feasible treatments to inactivate *L. monocytogenes* during food processing.

Control of *Listeria monocytogenes* in Ready-To-Eat (RTE) Meats

L. monocytogenes can be reintroduced onto the surface of RTE meats via post-process contamination. Thus, ARS researchers at Wyndmoor, PA, evaluated the efficacy of food grade antimicrobials, alone or in combination, to inhibit *L. monocytogenes* on the surface of uncured turkey breast at 4C. Inclusion of levulinate, alone or in combination with diacetate and propionate, as ingredients, suppressed outgrowth of *L. monocytogenes* during extended refrigerated storage. These data were the first to validate the synergistic effect of using levulinate in combination with diacetate and propionate to control *L. monocytogenes* on an RTE uncured turkey breast product. Thus, in the event of post process contamination, inclusion of this levulinate-diacetate-propionate blend as an ingredient in uncured turkey breast would preclude outgrowth of *L. monocytogenes.*

Listeria monocytogenes Is Transferred from Cantaloupe Rind Surfaces to Fresh Cut Pieces during Preparation

Recent outbreaks of foodborne listeriosis due to consumption of contaminated cantaloupes led ARS researchers at Wyndmoor, PA, to investigate the effects of holding time prior to refrigeration as well as variations in storage temperatures on survival of *L. monocytogenes* transferred to fresh-cut pieces during preparation. Holding contaminated fresh-cut melon pieces at 20C for 4 h or more prior to refrigeration (5C) increased the chances of *L. monocytogenes* proliferation. The information will help food service industry and consumers as well as fresh-cut processors in implementing HACCP plans and good manufacturing practices (GMP's).

Vibrio

Natural Antimicrobials that Can Inhibit *Vibrio (V.) vulnificus* and *V. parahaemolyticus* in Shucked Oysters

Vibrio in oysters account for many foodborne outbreaks, are responsible for economic and social hardship to fishermen and others, and also responsible for increased health care costs. This work looked at some natural antimicrobials that could be utilized to inhibit *Vibrio* spp. growth and thus prevent foodborne illnesses. Research at Dover, DE, found that citric acid, grape seed extract and lactic acid solutions at fairly high concentrations could be used to suppress growth of these pathogens. These findings can serve to develop formulations to add to oysters to minimize foodborne illnesses when consumed.

Bacterial Viruses (phages) Save Larval Shellfish

Oysters, clams and mussels begin life as free- swimming larvae which are susceptible to infection by the shellfish pathogen *Vibrio tubiashii*. This bacterium has caused major die-offs at shellfish hatcheries, particularly on the U.S. West Coast. Using Hawaiian seawater, ARS researchers at Dover, DE, discovered 15 phage viruses that kill various strains of *V. tubiashii*. A mixture of these phages protected larval oysters against high levels of *V. tubiashii*, suggesting applicability of in commercial shellfish hatcheries. The phages have been approved for licensing for commercial application.

Predatory Bacteria Naturally Suppress *Vibrios* in Seawater and Oysters

Vibrio bacteria are a significant threat to shellfish safety causing numerous illnesses, some deaths, and the closure of shellfish harvesting areas each year. ARS researchers at Dover, DE, isolated, identified, and characterized naturally-occurring bacterial predators against a variety of pathogenic *Vibrio* species. Electron microscopic analysis revealed a broad group of bacteria responsible for the decline in *Vibrios*. These predators were shown to reduce *Vibrios* in seawater and oysters and appear to be a natural control mechanism to enhance seafood safety. *Vibrio* predatory bacteria are important modulators of pathogenic *Vibrios* in seawater and oysters.

Clostridium

Swine Are Not Carriers of a Hyper-virulent, Multi-drug-resistant Strain of *Clostridium difficile*

C. difficile is a ubiquitous opportunistic bacterial pathogen that is usually associated with infections acquired by immune-compromised patients during hospital stays. Recently there have been an increasing number of "community-acquired" cases among otherwise healthy individuals with no readily explained origin of infection; however, infectious disease scientists suspect that food-producing animals such as swine may be a potential reservoir of *C. difficile*. ARS scientists at College Station, TX, demonstrated that while *C. difficile* was isolated at low frequency from non-diseased human and swine populations, none of the isolates were the hyper-virulent, multi-drug-resistant strains common to the community-acquired infections. Also, there was no

evidence of transfer of the different isolates between animals and humans. This work is important because it indicates that swine should not be implicated as carriers of the newer "community-acquired" *C. difficile* strains.

Identification and Cloning of Bacteriophage or Prophage Lytic Enzymes using Genomic Analyses

Due to an increase in reports of antibiotic resistant bacteria, there has been resurgent interest in the use of bacteriophages or their gene products to control bacterial pathogens as alternatives to currently utilized antibiotics. *Clostridium perfringens* is a Gram-positive, spore-forming anaerobic bacterium that plays a significant role in human food-borne disease as well as non-food-borne human, animal and poultry diseases. ARS researchers in Athens, GA, isolated two types of bacteriophage from poultry processing plants, those that had long non-contractile tails, members of the family *Siphoviridae*, and those with short non-contractile tails, members of the family *Podoviridae*. Several bacteriophage genes were identified that encoded amidases, lysozyme-endopeptidases, and a zinc carboxypeptidase domain not previously reported in viral genomes that can potentially digest the cell wall of *C. perfringens*. Additionally, bioinformatic analysis was utilized to identify an amidase lytic enzyme in the genome of *Listeria monocytogenes*, the leading cause of bacterial food-borne death in humans. Lytic proteins were cloned and expressed as recombinant proteins, then utilized to kill *C. perfringens* and *L. monocytogenes* in the laboratory. Future investigations will examine the ability of these phage lytic proteins to control these pathogens in the chicken gastrointestinal system during poultry production. Reducing populations of pathogens associated with poultry during production will lead to fewer pathogens entering the processing plant and reaching the subsequent consumer and will reduce the risk of human food-borne illness.

Proper Means for Cooling of Cooked Beef

Inadequate rate and extent of cooling is a major food safety problem. ARS researchers at Wyndmoor, PA, determined the germination and outgrowth of *Clostridium* perfringens spores during cooling of cooked beef products. A predictive model for growth of *C. perfringens* during cooling of cooked beef products based on the product composition factors was developed. Also, growth of the pathogen was quantified in ten commercially prepared acidified beef, pork, and poultry products. The growth data/predictive model on the safe cooling rate of meat will provide the food industry means to assure that cooked products remain pathogen-free.

Campylobacter

Naturally Occurring *Campylobacter* in a Broiler Rinsate Can Survive in Cold Storage

While *Campylobacter* are thought to be fragile, ARS scientists in Athens, GA, demonstrated that *Campylobacter* survives after long-term frozen storage (20 months) at 4°C or - 23°C in rinsate alone. Therefore frozen raw foods that are naturally contaminated with *Campylobacter* may still pose a potential health hazard even after 1-2 years in a freezer.

Optimization of Chicken Challenge Trials

Dose-response and transmission parameters were monitored for *Campylobacter* challenge in the chicken. Transmission between co-housed animals was determined to 1) reduce the apparent value of the infectious dose (ID); 2) increase the variability between replicates; and 3) produce a distinctive "all-or-nothing" response. Individual housing was determined to be the most efficient experimental design for ascertaining dose-response relationships and estimates of transmission for *C. jejuni*. The findings of ARS researchers in Athens, GA, suggest that the age-dependence of transmissibility between hosts, rather than their susceptibility to colonization, is the mechanism behind the 'lag-phase' reported in commercial broiler flocks, which are typically *Campylobacter* free the first 14-21 days of life. The individual housing model was employed to determine the ID of distinct *C. jejuni* isolates previously determined to differentially invade a eukaryotic cell line (Caco-2). Preliminary data revealed that highly invasive isolates were observed to be strong colonizers relative to the less invasive isolates. Further characterization of isolates with different cell invasiveness potentials will allow for the delineation of colonization factors that will subsequently facilitate the development of interventions.

Use of Specific Compounds during Poultry Processing Increases the Survival of *Campylobacter*

Campylobacter species are responsible for a large number of cases of food-borne illness annually in the developed world; however, these pathogens do not survive well in food processing and storage environments. It is therefore important to understand what factors contribute to the ability of *Campylobacter* to survive in sufficient numbers to cause such a large amount of human illnesses. ARS researchers at Wyndmoor, PA, previously identified a food safety risk factor in the use of compounds known as polyphosphates during poultry processing for the primary purpose of enhancing moisture retention in the poultry product. Use of these polyphosphates increased the survival of *Campylobacter* species during normal poultry processing and storage conditions. It was determined that the certain types of polyphosphates more than others were responsible for changing the acidity level during processing of poultry. This research identified specific polyphosphates that could be used during poultry processing that are less of a food safety risk since they do not enhance *Campylobacter* survival.

Other Bacterial Pathogens

Enhanced Functional Properties of Tannic Acid after Thermal Hydrolysis

Tannic acid has been proven to possess antioxidant and antimicrobial properties thus being a powerful bioactive component. However, there could be potential for enhancing its functional properties by manipulation of the compound per se or in food or other matrices. Thermal hydrolysis studies conducted at Stoneville, MS, showed that at high pressures of fresh tannic acid resulted in shorter chain but more powerful components produced: gallic acid and pyrogallol. This process could be utilized to enhance natural antimicrobial properties of tannic-acid containing foods or to enhance functional properties of tannic acid to be used as additives in food and feed.

Temporal Study of Pathogen ecology in Poultry Processing

ARS scientists in Athens, GA, demonstrated changes in the bacterial quality of scalder tank water during commercial processing of poultry resulted in the recovery of higher levels food safety-related pathogens from the water within those tanks. Chiller tank water quality did not change as significantly, however, and no pathogens were recovered from chiller water. Results of this study will provide data to researchers and commercial processors on the importance of understanding the role of microbial ecology of processing water in controlling poultry food safety-related pathogens in the processing operations.

Airborne Pathogen Transport during Cattle Hide Removal

For a long time, it has been speculated that bacteria present in airborne droplets released during hide removal were a source of beef carcass contamination but no data had been collected to confirm or refute this idea. ARS scientists in Clay Center, NE, demonstrated the contribution of liquid splatter during hide removal as a means of transferring bacterial pathogens to carcasses. Recognition of the contamination risk posed by pathogen-containing airborne droplets should lead to the implementation of strategies to protect adjacent carcasses.

Transmission of *Salmonella* and *Campylobacter* among Caged and Cage-free Laying Hens

ARS scientists in Athens, GA, conducted a series of experiments to evaluate the potential for the transmission of the foodborne pathogens, *Salmonella* and *Campylobacter*, among table egg producing hens in different housing systems. Among *Salmonella*-challenged hens, the percentage of cecum and reproductive tract samples positive for *Salmonella* were similar, and infection from the cloaca likely resulted in contamination of the reproductive tract. The horizontal transmission of *Campylobacter* among non-challenged hens was significantly greater on shavings than in cages. The litter in the shavings pen most likely contributed to the survival of the *Campylobacter* that was shed through the feces of challenged hens. Findings of this study will assist commercial poultry growers in determining best growing methods to reduce the spread of human foodborne pathogens among laying hens.

Scalding Operation Protocols for Hard and Soft Scalding of Broilers

Electroporation-spray studies conducted at Athens, GA, showed that spraying with levulinic acid (3%) and SDS (2%), and spraying with bromine (500mg/L) failed to significantly lower carcass skin *Salmonella* and *Campylobacter* from challenged breast skin samples compared to treatment with chlorine (20 ppm). Chemicals that have been approved for direct food use are continuously evaluated for potential use in the commercial processing plant to reduce and/or eliminate *Salmonella* and *Campylobacter*.

Safer, Cleaner Cantaloupes in 45 Seconds

Cantaloupes have been implicated in some of the deadliest foodborne illness outbreaks due to contamination with *Salmonella* and *Listeria monocytogenes*. A surface pasteurization process developed by ARS researchers at Wyndmoor, PA, is now being tested commercially for whole cantaloupe. A very brief, direct-from-field treatment in 70C (160F) hot water reduces total plate count by at least 99.9% and total coliforms to non-detectable levels. This 45 second process

dovetails in existing commercial cantaloupe processing lines. Collaboration between ERRC and the industry partner is underway to evaluate the effect of this commercial process on microbial quality, shelf-life, and sensory quality of fresh and fresh-cut cantaloupes.

Cold Plasma Inactivates Pathogens on Almonds

Contamination of raw nuts, including almonds, is a food safety concern. ARS researchers at Wyndmoor, PA, used rapid cold plasma treatments, 10 or 20 seconds, to inactivate both *Salmonella* and *Escherichia coli* O157:H7 from dry almonds. Inoculated almonds were treated with at 2, 4 or 6 cm from the cold plasma emitter. The greatest effect observed was a 95.4% reduction of *E. coli* O157:H7. The interaction of treatment time with distance from plasma emitter head was complex, and isolate-dependent. In general, air was more effective cold plasma feed gas than nitrogen. This treatment is waterless, contact-free and uses no antimicrobial chemicals. Short pulses of atmospheric pressure cold plasma therefore show promise as a nonthermal treatment for tree nuts, a technological advance which will have applications in improving the safety of this commodity for producers and consumers.

Effects of Housing on Egg Microbiology

While the layer hen production environment is known to contribute to the microbial quality of shell eggs, it is more important to consider any residual effects after processing has been completed. Studies were conducted at Athens, GA. Eggs from traditional cages, aviary, enriched cages, and eggs from the floor of aviary housing were sampled before and after processing (washed as required for retail shell eggs). None of the washed eggs was contaminated with *Salmonella* or *Campylobacter* and counts for aerobic microorganisms and Enterobacteriaceae were very low. This indicates that the commercial process is sufficient for decreasing shell contamination of eggs, regardless of where they are collected. Including floor eggs for shell egg producers that use an aviary style of housing can add to profits. This data shows that there is not a significant effect to shell egg safety based on housing.

Novel Probiotics Target Human Food Safety Pathogens and Improve Poultry Health

Campylobacter and *Salmonella* are the most commonly reported bacterial pathogens causing foodborne infections in the U.S. Epidemiological evidence has implicated poultry products as a significant source of these pathogens. A novel probiotic method was developed by ARS at Fayetteville, AR, capable of inhibiting growth of specific enteric pathogens. These probiotic cultures (composed of non-pathogenic "healthy" bacteria) target *Salmonella* and *Campylobacter* in the gastrointestinal system of poultry. This discovery was licensed to an Arkansas-based start-up company in cooperation with the University Arkansas. The commercial product (FloraMax-B11) is marketed in 16 countries with approximately 300 million birds dosed per/year.

Effect of power of hydrogen (pH) of alkaline salts of fatty acids on the inhibition of bacteria associated with poultry processing

ARS scientists in Athens, GA, conducted experiments using the agar diffusion assay to examine the effect of pH on the ability of alkaline salts of three fatty acids (FA) to inhibit growth of

bacteria associated with poultry processing. FA solutions were prepared by dissolving fatty acids in potassium hydroxide (KOH), and citric acid was added to the mixtures to adjust the pH. Results indicated that reducing the pH of some of the fatty acid mixtures caused decreases in the size of zones of inhibition of some of the bacteria. Findings demonstrated that the pH of solutions of alkaline salts of FA may play an important role in the antibacterial activity of these surfactants towards bacteria associated with poultry processing. Research will be used in the development of novel, non-chlorine based sanitizers that can be used to reduce microbial contamination during poultry processing.

Use of Hypobromous Acid as an Antimicrobial Intervention

Beef processing companies continually seek more effective and/or less expensive antimicrobial interventions to prevent carcass contamination by bacterial pathogens. ARS scientists in Clay Center, NE, demonstrated that application of hypobromous acid to cattle hides significantly reduced the prevalence of *E. coli* O157:H7 and *S.* enterica on hides. These results demonstrate that adoption of a hypobromous acid hide wash by beef processors would be effective in reducing carcass contamination.

Source Tracking in Surface and Groundwater using Multiple Indicators

Bacterial contamination caused by discharge of human and animal waste is a serious challenge to maintaining and preserving the quality of water resources. ARS scientists in Athens, GA, in collaboration with Environment Canada and the Universiti Teknologi MARA, applied a multi-indicator approach to identify sources of fecal contamination in Fraser Valley, a province of British Columbia, Canada. Poultry waste generated from the industry in this area is used as fertilizer and spread onto the fields thus creating a potential source of surface and groundwater pollution. Sterol analysis, Enterococcus Bacterial Source Tracking, and chemometric analysis were used as pollution source identifiers to determine if fecal bacteria in the environment originated from humans, livestock, or wildlife. Fecal contamination was detected in 100% of surface water and 15% of groundwater sites tested. Contribution from the poultry industry to surface water pollution was detected at nine sampling locations. Human fecal pollution was also detected at four surface water and one groundwater location. An ability to ascribe sources when confronted with a complex pollution situation is essential for planning management actions and implementing best management practices. Researchers can use this information to further efforts to protect and preserve surface and ground water quality from the impacts of human and agricultural activities.

Microwave Cooking Impact on Pathogen Inactivation on Catfish Fillets

When adjusted for per capita consumption, seafood is associated with food borne illness more than beef, poultry, or produce. Microwave cooking is used extensively at consumer, wholesale and retail levels, which can include the cooking of seafood. However, microwave cooking can be uneven, resulting in cold spots, and allow the survival of foodborne pathogens. ARS researchers at Wyndmoor, PA, designed a microwave oven (1250 watts) which used automatic feedback (80-90C/ 2 min) and a specially formulated phosphate solution to enhance even cooking of catfish fillets. The Food and Drug Administration recommendation for a 5 log

(99.999%) reduction of foodborne pathogens including *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7 were attained, without damage to the quality of the fish fillets normally attributed to microwave cooking. These results will help the microwave and food service industries provide safer microwave cooked seafood products to consumers.

Inactivation of *Francisella tularensis* Utah-112 in Fish and Fish Exudates Using Ultraviolet Light

When adjusted for per capita consumption, seafood is associated with food borne illness more than beef, poultry, or produce. In this study, ARS researchers at Wyndmoor, PA, used ultraviolet light (UV-C, 254 nm) to inactivate the avirulent foodborne pathogen surrogate *F. tularensis* Utah-112 on catfish and tilapia fillets and their exudates inoculated onto food contact surfaces. When Utah-112 was suspended in catfish and tilapia exudates and placed on stainless steel and plastic food contact surfaces, which were then passed through a commercial UV-C conveyor, 0.5 J/cm2 inactivated >99.99% of the microorganism. UV-C (1 J/cm2) inactivated 90 and 150% of Utah-112 inoculated on catfish and tilapia fillets, respectively. UV-C had no negative impact on fish fillet quality. This study demonstrates the effectiveness of UV-C for decontamination of fish and food contact surfaces using actual commercial equipment. This will assist seafood processors provide safer products to consumers.

Fungi

Improving the Efficacy of Commercial Fungicides with Natural Products

Crop (agricultural) and human (medicine) fungal diseases are very difficult. Oftentimes the fungi are resistant to available antifungal agents and the antifungal agents are toxic to the environment or to the patient. ARS scientists at Albany, California, in collaboration with scientists at VNIIF, Russia, and IHMT, Portugal, are looking for new methods to improve agricultural fungicides and chemotherapeutic antifungal drugs, respectively. A number of safe, natural phenolic compounds were identified that synergize the activity of fungicides against a number of phytopathogenic fungi of barley and wheat (Russia) and antifungal drugs against human yeast pathogens, such as *Candida* sp. and *Cryptococcus neoformans* (Portugal). Both of these discoveries may have significant impact on improving wheat and barley production and for the chemotherapy of patients suffering from these diseases.

The Almond-based Host Plant Volatile Blend Reported Last Year is Undergoing a Second Year of Intensive Field-trapping Studies

Moreover, based on previous testing results and electroantennographic studies conducted at Albany, CA, additional blends are being formulated and field tested for attractive behavior toward the navel orangeworm, the major insect pest to California tree nuts. The additional blends are based on host plant volatile emissions from both almonds and pistachios and various treatments of both (i.e., fungal contamination, damage). Results from the previous year attributed to the funding of a specialty crop block grant from the California Department of Food and Agriculture. The two-year award helps fund field trapping studies of the blend and additional formulations and comparison of efficacy to the current navel orangeworm monitoring

standard, almond meal. Results thus far for the 2012 growing season confirm the blend's ability to significantly attract more adult navel orangeworm moths than almond meal.

Improving the Efficacy of Commercial Fungicides with Natural Products

Human fungal diseases are very difficult to treat and the fungi causing these diseases are often resistant to the antifungal drugs currently available. ARS scientists at Albany, CA, are looking for new methods to improve antifungal drugs. ARS scientists in collaboration with Russian colleagues discovered that the culture filtrates of a fungus used in Russia as a biological control agent contains compounds that are effective chemosensitizing agents for the control of wheat and barley pathogens. Combining this filtrate with either azole or strobilurin type fungicides greatly improves the efficacy of the fungicides and prolongs their antifungal activity.

Inoculation of Aspergilli and Other Orchard Fungi

Scientists at Albany, CA, have performed intensive in vitro inoculation experiments of aspergilli and other common orchard fungi on almonds using almond fatty acids and proteins as the fungal media. Once completed, scientists will compare the emissions of various fungi relative to the volatile emissions of the orchard fungal bouquets to best determine the unique volatile emission pattern and/or emission ratios. A second objective is to determine which actual fungus is responsible for the unique volatile(s). Later this year, these results will be transferred to almond and pistachio media to determine the fungal candidate for inoculation.

Crops Influence Aflatoxin Contamination by Altering Population Structures of Crop Associated Fungi

Aflatoxins are potent cancer causing toxins that frequently contaminate several important crops including corn, peanut and cottonseed. The ability of fungal populations associated with crops to produce aflatoxins varies widely but it is unknown what causes particular aflatoxin producers to dominate in specific areas. A team of scientists working in the ARS laboratory at the University of Arizona in Tucson used sophisticated genetic analyses to reveal that crops exert selection on which aflatoxin producing and closely related fungi dominate. Crops do this by influencing the success of certain fungi during competition. This is the first empirical demonstration that specific genetic groups of aflatoxin-producing fungi receive advantage from specific crops and will provide bases for designing optimal crop rotations and for selection of elite biological control agents for aflatoxin reduction in diverse cropping systems.

Novel Synthetic Peptides Demonstrate Significant Antifungal Activity Against Mycotoxigenic Fungi including *Aspergillus (A.) flavus*

Each year millions of dollars are lost to crops contaminated with toxic and carcinogenic aflatoxins produced by *A. flavus*. ARS scientists at New Orleans, LA, have tested a number of novel antifungal peptides for control of mycotoxigenic fungal pathogens. Peptides were provided by collaborators at Tuskegee University and AgroMed LLC. The ß-sheet (form of secondary structure of protein) peptides D4E1, AGM181 and AGM182, and the a-helical (form of structure of protein) peptides AGM184 and RCJ-1, demonstrated potent in vitro inhibitory

activity compared to natural peptides against *A. flavus*, *Fusarium verticillioides*, and *Verticillium dahliae* and showed little or no hemolytic (destructive to red blood cells) activity. Development of transgenic crops expressing genes encoding these antifungal peptides will provide an effective means of controlling aflatoxin contamination in susceptible crops such as corn, cotton, peanut, and tree nuts thus benefitting both producers and consumers.

Mating between Non-aflatoxigenic and Aflatoxigenic *Aspergillus (A.) flavus*

Non-aflatoxigenic strains of *A. flavus* are being used to reduce contamination of cotton seed, corn and peanut by aflatoxin-producing fungi. The success of this program depends on genetic recombination being a rare or non-existent event following field application of the biocontrol strain. An ARS scientist at New Orleans, LA, found that, under optimized laboratory conditions, non-aflatoxigenic strains of *A. flavus* that are currently being used for biocontrol are capable of mating with aflatoxin–producing strains after only two months of incubation. The new strains arising from the mating produce conidial chains that are either entirely fluorescent, entirely non-fluorescent, or a mixture of fluorescent and non-fluorescent spores. These results clearly demonstrate that mating between soil populations and the biocontrol population is able to occur at least under these *in vitro* conditions.

Hydrolase Production by Non-aflatoxigenic and Aflatoxigenic *Aspergillus flavus*

Biocontrol depends on the ability of the fungus to reach and penetrate the seed of the susceptible crop. An ARS scientist at New Orleans, LA, in preliminary studies has found that among the hydrolytic enzymes necessary for seed invasion, esterase activity (an enzyme activity that breaks down specific organic compounds into acid and alcohol) is significantly reduced in the non-aflatoxigenic *Aspergillus flavus* biocontrol strain, Afla-Guard, compared to that of other strains considered for biocontrol and the aflatoxigenic strain. This result suggests that esterase activity is probably not critical for fungal colonization of plants susceptible to aflatoxin contamination.

Parasites

Prevention of Food Safety and Food Quality Problems Associated with Larval Nematodes in Commercial Catfish

The most prevalent population of turtles in commercial catfish ponds are the red-eared sliders and that they are frequently heavily infected with two nematodes, *Serpinema sp.* and *Camallanus sp*. which can also infect fish. Both *Serpinema* and *Camallanus* have been sequenced and work is underway to develop molecular probes to screen for their presence. This work conducted at Stoneville, MS, will provide for a rapid screening tool for these nematodes and thus reduce fish losses at the farm.

Demonstrated a Protective Effect in Domestic Pigs Against Infectious *Trichinella* Genotypes by Prior Exposure to Sylvatic Genotypes of *Trichinella* that are Carried by Feral Pigs

To determine if a primary infection with North American sylvatic genotypes of *Trichinella* (to which domestic pigs are resistant) could protect pigs against a challenge infection with *T.*

spiralis (to which domestic pigs are susceptible), pigs were infected with *T. nativa*, *T. pseudospiralis*, and *T. murrelli*, and then challenged with *T. spiralis*. Pigs that were exposed to encapsulated genotypes (*T. nativa*, and *T. murrelli*) were protected against a challenge infection with *T. spiralis*, while an un-encapsulated genotype (*T. pseudospiralis*) provided little protection. Antibody isotype and cytokine gene expression analysis of blood and intestinal tissues from protected (*T. nativa*, and *T. murrelli*) versus unprotected animals (*T. pseudospiralis*) determined that anti-inflammatory, or TH-2 immune, effectors were heightened in early infection in the protected pigs. These data from studies at Beltsville, MD, demonstrated that pigs can be rendered resistant to *T. spiralis* infection by immunization.

The U.S. State Department funded Biosecurity Engagement Program on *Trichinella* spiralis was expanded in the Republic of the Philippines.

Beltsville, MD, researchers in collaboration with the Bureau of Animal Industry, Philippine Animal Health Center (PAHC), Republic of the Philippines, a validated *Trichinella* testing facility was established at PAHC, Quezon City, Luzon and a nationwide prevalence study for *Trichinella spiralis* in market hogs was conducted. The sample storage capacity of PAHC was expanded for establishment of a swine serum bank for prevalence studies on other swine pathogens of interest. This project provides for improved disease surveillance capacity for a trading partner which in turn reduces risks to U.S. consumers from food-borne pathogens and to U.S. agriculture from importation of foreign animal diseases.

Detection of Microbial, Chemical and Radiological Hazards in Food, Feed, and Dietary Supplements

Chemical Contaminants

A Simple and Inexpensive Test for Bioaccessible Garden Soil Lead

With the large increase in interest in gardening and agriculture in urban areas, the high levels of lead from historic paint and automotive exhaust emissions may comprise risk to children who could ingest garden soils either at the garden or when soil is carried to the home on tools and clothes of gardeners. Some crops may accumulate enough Pb to comprise risk (lettuce, carrot, etc.). Because common garden soil amendments can induce the formation of chemical forms of lead which have low bioavailability to humans who ingest soil, it is important that advice about urban gardens be based on bioavailable/bioaccessible lead rather than total lead. The soil test presently approved by U.S.-Environmental Protection Agency to estimate soil bioaccessible lead has been shown to over-estimate lead bioavailability in soils treated with phosphate or compost, and is prohibitively expensive for most gardeners. Thus a simplified test calibrated using soil samples from the Joplin field test of using amendments to reduce soil lead bioavailability. The phosphate treated soil had 69% reduction in soil lead bioavailability to humans fed the test soils. The U.S. Environmental Protection Agency (US-EPA) test with 0.4 M glycine-hydrochloride was modified for ease of operations and calibrated against the Joplin soils. Additional evaluation of the method was conducted at Beltsville, MD, showing that the rate of shaking was a more critical factor than initially believed. The new method, conducted at pH 2.5 rather than the EPA method at pH 1.5 has nearly the same reduction in Pb bioaccessibility as observed in the human feeding test so estimated bioavailability can be measured from the correlation of bioavailability and bioaccessibility from the Joplin soils.

Development of an Efficient Method for Analysis of Novel Flame Retardants and Other Persistent Organic Pollutants in Catfish

Novel flame retardants are emerging food contaminants of interest as due to their toxicology, persistence in the environment, and bioaccumulation/biomagnification in the food chain. Catfish have become of particular interest to USDA-FSIS due to recent changes in legislation. ARS researchers at Wyndmoor, PA, have developed and evaluated a highly sensitive and selective, laboratory-based, multiclass, multiresidue method for analysis of flame retardants and other persistent organic pollutants, including pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and polybrominated diphenyl ethers (PBDEs). The new method uses efficient sample preparation coupled to low-pressure gas chromatography-tandem mass spectrometry for improved analysis of over 60 contaminants simultaneously. The generated data on occurrence of these contaminants in foods may advance the understanding of the potential risk posed by these emerging chemicals and aid in future risk assessment and regulations.

Confirmation of Triphenylmethane Dye Residues in Retail Catfish Nuggets

Samples of retail catfish nuggets collected from domestically raised catfish in New Jersey, Pennsylvania, New York and Delaware had tested positive for dyes such as malachite green,

gentian violet, brilliant blue, and their metabolites using an enzyme-linked immunosorbent assay (ELISA). These test results needed to be confirmed using an alternative protocol. In response, ARS researchers at Wyndmoor, PA, examined these samples using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), and confirmed ultratrace levels of gentian violet in nugget samples, including one sample with levels exceeding the action level set by the FDA. This study also found that the contamination may be caused by the ink used on packaging materials. The methodology developed in this study can be used by regulatory agencies for verification and confirmation of dye residues in foods.

Toxins

Rapid, Portable Test for Botulinum Neurotoxins

Produced by the common soil bacterium *Clostridium* botulinum, botulinum neurotoxins (BoNTs) are potent toxins that can cause the severe foodborne disease, botulism, and could be used as a biological threat agent. ARS scientists in Albany, CA, developed a rapid, sensitive diagnostic test for BoNTs that could be used by minimally trained personnel in the event of a foodborne outbreak or a bioterrorist threat. The simple lateral flow device, similar in design, use, and time as a pregnancy test, can detect and distinguishes between BoNT/A and B, two of the four serotypes that are known to intoxicate humans and together account for >80 percent of naturally occurring botulism. This rapid diagnostic method which has been validated and now transferred to regulatory and other biosecurity/military agency's is a valuable tool in the areas of food safety and homeland security.

Detection of Botulinum Toxin Contamination of Catfish

Clostridium Botulinum Type E is associated with seafood including aquaculture products. It has been discovered that this organism is beginning to affect fish at the farm level since it produces a powerful toxin, Neurotoxin E. It is very difficult to separate the toxin from a complex matrix like fish. Preliminary results have shown progress in eliminating enzymes responsible for eliminating the toxin and thus make the detection method more sensitive and accurate. The development of this procedure at Stoneville, MS, will aid the catfish and other aquaculture/seafood industries tremendously as a diagnostics tool to react and treat prior to total loss.

A Rapid, Reusable, Sensor for Detecting the Mycotoxin Deoxynivalenol in Wheat

Deoxynivalenol (DON) is a toxin produced by certain species of fungi that can infest wheat, barley, and corn. It results in substantial losses to the quality and value of grain worldwide. Due to the toxicity of DON and the desire to protect human and animal health, the U.S. conducts extensive monitoring of commodities both for domestic use and export. ARS scientist at Peoria, IL, investigated ways to improve upon a biosensor that they developed last year uses a novel analytical technology, biolayer interferometry (BLI). ARS scientists discovered that the signal from the sensor could be substantially increased through the use of an antibody labeled with colloidal gold, allowing the assays to be conducted more rapidly. The technique was successfully used to measure DON in samples of naturally contaminated wheat, with results that

agreed well, but which were obtained more quickly than with a reference method. The result is a rapid and reusable sensor that can be used by industrial, academic, or government laboratories to monitor contamination of wheat and assist in the protection of the human food supply.

Development of a Protein that Binds to the Mycotoxin Deoxynivalenol

Deoxynivalenol (DON) is a toxin produced by certain species of fungi that can infest wheat, barley, and corn. It results in substantial losses to the quality and value of grain worldwide. Due to the toxicity of DON and the desire to protect human and animal health the U.S. conducts extensive monitoring of commodities both for domestic use and export. ARS scientists at Peoria, IL, developed a new material for selectively binding a protein known as a scFv that was based upon a toxin binding material previously developed by this research team. An advantage of the material is that it can be produced in bacterial systems, which may lower production costs. The results suggest that despite the smaller size of the scFv, it retained much of the binding capacity of the original protein. This material, the details of its method of production, and the details of its composition, may be of use to scientists charged with developing the next generation of toxin detection kits.

A Structural Model to Improve Detection of Citrinin and Related Toxins

Citrinin is a toxin produced by several fungal species that frequently contaminate agricultural commodities. This mycotoxin is associated with kidney disease in livestock and humans. To improve detection of citrinin, a state-of-the-art computational study of the three dimensional structures of this toxin was performed by ARS scientists at Peoria, IL, in collaboration with scientists at Bradley University, Peoria, IL. Key features of the structure and properties of citrinin were identified. These results will aid researchers in the design of materials for citrinin detection and provide insight into the methods appropriate for studying related natural products. The results will be useful to food safety scientists seeking improved methods for detection of citrinin.

Discovery of “Masked” Metabolites of T-2 Toxin and HT-2 Toxin

T-2 toxin is produced by a variety of fungi that commonly infest grains. T-2 is acutely toxic and has been found at low levels in crops such as wheat, corn, barley, oats, and rye, and has also been found in human food and animal feeds. Fungi and plants have evolved mechanisms to reduce toxicity of small molecules such as T-2 toxin through metabolism to forms that are less hazardous or that can be excreted. ARS scientists at Peoria, IL, discovered the production of novel metabolites of T-2 toxin: the gluocoside derivatives of T-2 and HT-2. Such metabolites may be important because the modified versions of the toxins may evade the techniques normally used for toxin analysis, yet may retain the ability to be converted back into toxins following consumption by animals. This discovery provides the developers of analytical methods for toxin detection assays insights into which compounds are important enough to include in monitoring programs to assure the safety of grain products.

Drug Residues and Hormones

Development of a Rapid Screening Assay for the Antibiotic Roxarsone

Roxarsone is used in the poultry and swine industries as a feed additive to treat coccidiosis and other intestinal disorders as well as to improve feed efficiencies and weight gain. A rapid screening method was developed by ARS scientists at Fargo, ND, using antibodies against roxarsone in an immunoassay format. The immunoassay was suitable for the determination of roxarsone in chicken meat, having good sensitivity and specificity. The assay was able to detect roxarsone well below the maximum allowed food residue level. Because of its sensitivity and specificity the assay could be adapted to high throughput or on-site residue screening programs.

Optimization, Extension, Validation, and Technology Transfer of Multiclass, Multiresidue Method for Veterinary Drugs in Animal Tissues

Until now, USDA-FSIS used a 7-plate microbial growth inhibition assay to screen for antimicrobial drug residues in beef samples from slaughter establishments throughout the US. Last year, ARS researchers at Wyndmoor, PA, developed, validated, and transferred to FSIS an improved screening method using ultrahigh performance liquid chromatography – tandem mass spectrometry (UHPLC-MS/MS) that improved their screening logistics and capabilities for 60 of the most important drugs of regulatory concern. This year, the qualitative screening and identification plus quantitative method was optimized and extended to 120 drugs and validated for meat tissue. A single analyst can perform sample preparation of 60 samples with the method in an 8-hour day for a series of sequential 10 minute analyses. Implementation of the method in the USDA-FSIS National Residue Program has already found additional residues of concern missed previously. This new method serves to improve the monitoring and enforcement of veterinary drug residues, and thereby assure better animal husbandry practices, reduce environmental contamination, decrease microbial antibiotic resistance, and increase food safety.

Evaluation of a Multiclass Multiresidue Method for Analysis of Veterinary Drug Residues in Beef Kidney

Monitoring methods for veterinary drug residues in food have traditionally been designed for single drugs, or for multiple drugs belonging to the same class. Methods which allow for simultaneous monitoring of multiple drug residues from multiple classes can provide increased efficiency. In this work, ARS researchers at Wyndmoor, PA, optimized and validated in kidney, a multiclass multiresidue method using liquid chromatography-tandem mass spectrometry for use in the monitoring process. Control kidney samples were fortified at 4 different levels with a mixture of 120 veterinary drugs. After extraction of these samples, recoveries, precision and lowest calibration levels for each drug were reported. The method was judged to be successful for a majority of the drugs tested, and serves as an efficient and useful option for monitoring veterinary drug residues in food.

Microbial Pathogens

Salmonella

Correctly Detecting *Salmonella* in Foodborne Outbreaks

Salmonella species remain one of the leading pathogens causing outbreaks of illness. Unfortunately, the serotype implicated in actually causing any outbreak (clinical disease) is often difficult to determine since there may be many contaminating strains. During outbreak investigations it is critical to isolate the relevant strain from food and/or environmental sources. ARS researchers in Albany, CA, determined that some *Salmonella* strains were more likely to be isolated than others. Current selective enrichment media shows a bias for *Salmonella* enterica strains while strains of serogroup B, which include serovars Typhimurium, Saint-Paul, and Schwarzengrund were less likely to emerge as dominant strains. This work provides critical information to public health agencies at the Federal and State level, as well as to the industry, stressing that during investigations, multiple enrichment protocols should be used to ensure isolation of target strains.

Intragenic Sequence Ribotyping (ISR) for Serotyping *Salmonella* enterica Helps International Trade Partners Assess the Safety of Food

International trade partners have difficulty obtaining serotype for the major food borne pathogen *Salmonella* enterica at reasonable cost. ARS researchers at Athens, GA, compared ISR to a certified DNA microarray method, and found ISR it to give comparable results at a fraction of the cost. The impact was that USDA was able to provide serotype information to major trade partners in Columbia, Brazil, Peru and Argentina, as well as to multi-national agriculture companies. The ISR method facilitates exchange of information about *Salmonella* serotypes across international boundaries, which protects the American consumer by enhancing the safety of the food supply.

Biosensor Technology Robustly Detects Strains of *Salmonella* enterica that Vary in Outer Membrane Components

Preventing pathogens from spreading in the air, in water and in food supplies is facilitated by rapid methods of detection, and new approaches are needed to improve detection. A company that had developed an antibody-based biosensor approach needed more information on its ability to detect *Salmonella* enterica, which is known to have a cell surface that can vary greatly as targets for antibodies. USDA researchers in Athens, GA, provided a set of 26 strains and mutants that would stringently test the ability of the biosensor technology to detect *Salmonella* enterica with variable cell surfaces. One strain was found that could trick the technology, but does not occur in nature. The lower limit of detection for the technology was somewhat strain dependent, but it still compared favorably with other methods such as the polymerase chain reaction. This research facilitates accurate application of a promising technology. The company sponsored presentation of the research at the International Association of Food Protection Annual Meeting.

Classification and Structural Analysis of Live and Dead *Salmonella* Cells Using Fourier Transform Infrared (FT-IR) Spectroscopy and Principle Component Analysis (PCA).

ARS scientists in Athens, GA, conducted experiments on the rapid detection, identification and differentiation of different bacteria. In recent years, research on the development of rapid methods for identifying and characterizing bacteria has increased. In these experiments, the Fourier Transform Infrared spectroscopy (FT-IR) method was used to detect and differentiate *Salmonella*. Live and dead cells of *Salmonella* Typhimurium and *Salmonella* Enteritidis were used in this study. Live cells of both *Salmonella* Typhimurium and *Salmonella* Enteritidis were classified with 100% accuracy and the technology was able to differentiate between live and dead cells. This technology may be used for the rapid detection and identification of *Salmonella*.

Development of a *Salmonella* enterica Identification Assay Based on DNA Structure of the Bacteria

ARS scientists in Athens, GA, conducted laboratory experiments to develop genetic primers and probes that specifically target portions of *Salmonella* DNA. The newly developed assay will allow scientists to accurately distinguish between any combinations of the DNA from strains of *Salmonella* enterica. Research is being expanded to test environmental *Salmonella* enterica isolates. Results of this assay will provide researchers with a rapid serotyping tool for major poultry food safety-related pathogenic *Salmonella* enterica strains, and a method to understand the survival dynamics of these strains in environments along the entire poultry production spectrum.

Rapid Recovery, Concentration, and Detection of *Salmonella* from Chicken Extracts

Despite a recent decline in some bacterial foodborne illnesses, foodborne infections caused by the most common strains of *Salmonella* have not declined in 15 years. New methods to recover and concentrate foodborne pathogens from complex food matrices would greatly facilitate the rapid detection of *Salmonella* and other foodborne pathogens leading to a reduction in the distribution of contaminated foods and the prevention of outbreaks of foodborne illness. ARS-funded researchers at Purdue University's Center for Food Safety Engineering in West Lafayette, Indiana identified ultra and micro-filtration methods for the rapid concentration and recovery of *Salmonella* from chicken. The concentration method has been used in conjunction with microbiological plating technique for detection of *Salmonella* in about 24 hours or with DNA-based detection methods (real-time PCR) that can be completed in about 7 hours. The combined approach of concentrating the microorganisms and then testing the concentrated samples for the presence of food pathogens using PCR based techniques will contribute to food safety by increasing identification specificity of the target organism.

E. coli O157:H7 and STEC

Genomic Markers for Identifying Specific Pathogenic *Escherichia coli* Strains

The USDA-Food Safety and Inspection Service recently declared *Escherichia coli* strains O26, 045, O103, O111, O121, and O145 adulterants in beef trim and recently started regulatory screening for these pathogens. The current method for detecting these specific (serotypes) takes several days because there is not a specific genomic marker for each serotype. ARS scientists at Clay Center, NE, identified strain specific DNA markers for each serotype by comparing portions of the DNA. The DNA markers were licensed to a company that makes diagnostic kits for foodborne pathogens and are being used as part of a commercially available assay. This assay will be useful for industry and government researchers.

Development of Rapid and Inexpensive Multiplex Assay for STEC

The recent STEC outbreaks have emphasized the importance of developing rapid methods for genotyping virulent bacterial strains. An inexpensive colorimetric DNA microarray-based method was developed at Albany, CA, for simultaneous identification of multiple virulence genes in *E. coli* O157 and non-O157. This facilitated detection and characterization of STEC isolates recovered from multiple animal sources and environmental samples in agricultural regions in California. The efficiency of this method for STEC has led to collaboration in a major project on human noroviruses, which is the leading cause of human gastroenteritis worldwide.

Rapid Top-down Proteomic Identification of Shiga Toxin Variants

STEC are increasingly linked to severe outbreaks of foodborne illness in the US (spinach in 2006) and elsewhere (e.g. fenugreek seeds in 2011 in Germany and France). Toxicity of Stx varies by differences in primary amino acid sequences. Researchers at Albany, CA, developed a rapid mass spectrometry-based top-down proteomic method to identify sequence-specific Stx variants. The method is simple and fast and can distinguish between highly similar sequence variants. A Stx variant can be identified within 1 to 2 hours after bacterial culturing using this technique and has potential as a rapid identification tool during an outbreak of foodborne illness as well as a powerful research tool.

New Culture Method for Detection of Shiga Toxin-producing *E. coli*

Shiga toxin (Stx)-producing *E. coli* (STEC) are responsible for an estimated 265,000 infections each year in the US, including some fatalities. In order to detect the bacterium as well as the main toxins produced by STEC, scientists with ARS in Albany, CA, developed a new method based on a highly selective medium that promotes the production of Stx by cultured STEC and the killing of certain protozoa when they consume the resulting Stx-laden *E. coli* as a food source. With further development, this test would minimize the time needed for analysis, while enabling recovery of STEC from contaminated foods. An industrial cooperator has commercialized the improved medium that is expected to enhance the capabilities of industry and government to assure food safety.

Novel Growth Medium for Detection of *E. coli* Pathogens Based on Color

Some strains of *E. coli* produce Shiga toxins (STEC) and are clinically significant foodborne pathogens. The Centers for Disease Control (CDC) reported that the top six non-0157 STEC

strains accounted for 71% of the human cases in the U.S. Unlike the most notorious *E. coli* strain (O157:H7), however, these foodborne pathogens have no unique characteristics to readily distinguish them from other *E. coli*. ARS scientists in Clay Center, NE, developed a novel agar medium that indicates different bacterial types by producing different colors. The medium allowed successful detection and isolation of the naturally occurring top six non-O157 STEC present in bovine feces. This is the first bacterial agar medium validated for isolation of all of the top-6 non-O157 STEC and should significantly improve their detection.

Differentiating Non-O157 STEC Serogroups in Pure Culture and from Ground Beef on Agar Media

A hyperspectral imaging technique was developed at Athens, GA, to detect and differentiate the Shiga Toxin-Producing *Escherichia coli* (STEC) serogroups other than *E. coli* O157 on Rainbow agar plates. These non-O157 STEC (O26, O111, O45, O121, O103, and O145) are known as the "big six". A number of different classification techniques were developed to distinguish these *E. coli* colonies on spread plates of pure and mixed cultures. The classification models were tested on inoculated ground beef to measure the performance of the imaging technique. The average sensitivity and specificity was 95% and 92%, respectively. Test results obtained from these studies showed the potential of the imaging technique for rapid screening of STEC positive colonies to ensure that the correct colonies were selected for further confirmatory techniques.

Latex Agglutination Tests (LATs) for Six Pathogenic Non-O157 *Escherichia coli*.

Certain Shiga toxin-producing *Escherichia coli* (STEC) serogroups, including *E. coli* O26, O45, O103, O111, O121, and O145 cause a similar illness in humans as *E. coli* O157:H7. The USDA Food Safety and Inspection Service (FSIS) recently declared these STECs as adulterants in beef. At the request of the FSIS, ARS researchers at Wyndmoor, PA, developed and validated a rapid and simple testing method (LAT) for confirming presumptive positive non-O157 STECs to better protect the food supply and consumers. The reagents and test protocols were transferred to FSIS for validation, and the (LAT) agglutination method has now been incorporated into the FSIS Microbiology Laboratory Guidebook (MLG) Chapter 5B.02. Adoption of this method contributed to the implementation of the USDA 'zero tolerance policy' for the six non-O157 STECs in June, 2012. The scientists who developed the LAT technology received the USDA Secretary's Award.

A Method to Detect Harmful Bacteria in Beef

In addition to *E. coli* O157:H7 certain similar types of harmful *E. coli* have also been declared as adulterants (zero tolerance) in beef by the USDA Food Safety and Inspection Service (FSIS). Since methods to detect these pathogens in beef that can be used for regulatory testing were needed, ARS researchers at Wyndmoor, PA, worked with the FSIS to develop a method that is currently being used by in FSIS laboratories to test for these important pathogens in beef samples collected from federally inspected establishments and retail stores. Use of these methods will ensure that beef contaminated with these harmful *E. coli* types will not be sold to the consumers, thus preventing serious illness and deaths caused by these pathogens.

A Rapid Screening Method for Detection of Harmful *E. coli* in Food

Rapid and simple screening methods that can be used by the food industry for detection of types of *E. coli* that were recently declared as adulterants in beef by the Food Safety and Inspection Service (FSIS) are needed. The food industry cannot hold perishable foods for long periods; therefore, the availability of rapid methods for detection of contamination with these pathogens is critical. ARS researchers at Wyndmoor, PA, worked with a company to develop a rapid, simple, and reliable method that allows screening of beef for these pathogens with fewer hands-on steps. This method was developed into a commercially available assay kit that will be particularly useful for the food industry in the U.S. and throughout the world, since the FSIS also requires that beef that is imported into the U.S. be tested for these pathogens.

Staphylococcus

Food Test Based on a Biomarker of *Staphylococcus aureus* Intoxication

Staphylococcus aureus is a major bacterial pathogen which causes various clinical infections, including food poisoning. Several immunological assays have been developed for *Staphylococcus* enterotoxin A (SEA), which is the common cause of *Staphylococcus* food poisoning, but these assays cannot distinguish between active toxin and inactive toxin, which can bind antibody, but shows no toxicity. ARS scientists in Albany, CA, demonstrated that SEA induces cells of the immune system to produce a protein called tumor necrosis factor (TNF). This observation was the basis for a new, sensitive assay for measuring the presence of active toxin in foods contaminated with SEA.

Campylobacter

Development and Optimization of a Rapid and Specific Antibody Biosensor for the Detection of *Campylobacter* spp. from Environmental Samples

ARS researchers in Athens, GA, through a funded cooperative agreement, supplied a panel of target *Campylobacter* antigens for the development of proprietary, commercial polyclonal and monoclonal antibodies that detect diverse *C. jejuni* isolates as well as a variety of *Campylobacter* species. Efficacy screening of over 500 polyclonal antibodies were conducted and subsequently resulted in the incorporation of six monoclonal antibodies into the novel commercial biosensor. The development of rapid and sensitive detection technologies, and the subsequent correlation to molecular and cultural analysis, facilitates regulatory needs of FSIS.

Development and Optimization of Fluorescence *In situ* Hybridization (FISH) Technique

In an effort to improve *in situ* microbial ecology investigations of *C. jejuni* in poultry, ARS scientists in Athens, GA, designed and optimized probes for fluorescence *in situ* hybridization (FISH) detection. The newly designed FISH probes not only successfully targeted *Campylobacter* at the genus level, but were also able to discriminate among four closely related

isolates on the basis of a single base pair mismatch when used in combination with an unlabeled competitor probe. Additionally, protocols were optimized to perform FISH directly with chicken cecal samples. The optimization of FISH as a cultivation-independent tool for microbial ecology investigations will facilitate future investigations of the interaction of *C. jejuni* with the gastrointestinal tract of chickens.

Next-generation DNA Sequence Analysis Validation of a Widely Cited *Campylobacter* Specific Polymerase Chain Reaction Assay

ARS scientists in Athens, GA, tested, using next-generation DNA sequence analysis, the specificity of a widely cited *Campylobacter* spp. specific polymerase chain reaction (PCR) assay. Additionally, researchers described a method for a direct cell suspension PCR to facilitate sample screening. Pyrosequencing results showed the previously developed assay to be extremely (>99%) sensitive. Additionally, two newly designed broad range bacterial primer sets, that have wide applicability as internal amplification controls, were developed.

Comparative Media Investigation for the Recovery of *Campylobacter* spp.

A significant contributor to the current knowledge gap on the epidemiology of *Campylobacter* spp. in poultry is the use of several highly selective, cultivation media with sub-optimal performance. In an effort to determine if the use of varied media and recovery conditions introduces a bias on the recovery of *Campylobacter* subtypes during epidemiologic investigations, ARS researchers in Athens, GA, sampled three integrated broiler flocks (production, processing, and final product). Samples were cultured for *Campylobacter* using four different media coupled with four different atmosphere/temperature combinations. Quantitative cultural analyses demonstrated that recovery of *Campylobacter* was similar among the four media tested, independent of recovery conditions. Subtype analyses of isolates revealed a bias in subtype recovery relative to sample type, media, incubation temperature, and atmosphere. Next-generation DNA sequence analyses revealed that *Campylobacter* represented a small proportion (<0.04%) of nucleotide sequences present in feces. Furthermore, next-generation sequencing revealed that more non-*Campylobacter* sequences were present on plates after incubation at 42C relative to 37C. Further characterization of distinct niche-dependent subtypes will facilitate the development of specifically targeted interventions at each integrated stage to reduce and eliminate *Campylobacter* from poultry.

Aerobic Growth of *Campylobacter* in Media Supplemented with A-ketoglutaric, Lactic, and/or Fumaric Acids

ARS scientists in Athens, GA, conducted a series of experiments to determine if cultures of *Campylobacter* bacteria can be grown under aerobic conditions. *Campylobacter* is a major human, foodborne pathogens, and presently microbiologists must grow these bacteria in closed jars under microaerobic conditions. Producing microaerophilic conditions requires the use of expensive equipment and technical training. Recent experiments have indicated that growth of *Campylobacter* is stimulated in media supplemented with selected organic acids; therefore, experiments were conducted to determine if *Campylobacter* that are provided some of these acids can also grow under aerobic conditions. Findings indicated that aerobic growth of all

Campylobacter isolates was produced in media containing a mixture of organic acids. This study may lead to the development of media that will simplify and reduce the cost of growing cultures of a major human foodborne bacterial pathogen.

Other Bacterial Pathogens and Detection of Multiple Pathogens

Evaluation of TEMPO Technology for Rapid Automation of the Most Probable Number (MPN) Technique

The TEMPO instrument was developed to automate the most-probable-number (MPN) technique and reduce the effort required to estimate bacterial populations. Scientists within the ARS in Athens, GA, compared the automated MPN technique with traditional microbiological plating methods and Petrifilm methods for estimating the total viable count of aerobic microorganisms (TVC), total coliforms (TC), and *E. coli* populations (EC) on freshly processed broiler chicken carcasses (postchill whole carcass rinse samples) and cumulative drip-line samples from a commercial broiler processing facility. When samples below the limit of detection were excluded, 92.1% of the total responses were within a single log difference between the traditional plating or Petrifilm methods and the automated MPN method. These results highlighted the benefits of use of the automated TEMPO method for FSIS needs.

Characterization of Bacteria Transported through Aerosols

The role of enteric pathogen aerosolization in the contamination of produce remains unclear. ARS scientists in Albany, CA, evaluated a flow cytometry-linked culture method to monitor and characterize live bacteria from airborne particles by collecting them in liquid rather than with filters, which decreases bacterial viability. The successful detection of culturable pathogens from airborne particles with our method indicates its potential for monitoring the environmental transport of pathogens and complements current molecular-based methods to detect live enteric pathogens in aerosols transported to produce grown in proximity of dairies and feedlots. Our method has applicability in surveillance by public health, and for research agencies to improve our understanding of the epidemiology of enteric pathogens in agricultural areas where animal and crop production are geographically intertwined.

A Quick and Easy Test for Identification of Human Pathogens in Seafood and Other Products and the environment

Testing at the processing level for the presence of pathogens in the food is very tedious, time consuming and requires lots of training for the most part. Novel testing kits utilizing two-phase media with a mix of indicators, growth promoters and/or inhibitors have been developed or are being developed. These kits developed at Stoneville, MS, will allow processors and handlers of food to screen their production lots and their environments in relatively short times for the presence of pathogens and thus clear product for market in a rapid and reliable manner.

Development of improved foodborne bacteria detection methods via investigation of environmental barriers to bacterial growth and survival.

The toxicity of *Yersinia pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis* bacteria depends on the presence of a small, circular piece of DNA inside the cell which is referred to as a virulence plasmid (pYV). The pYV is unstable in all three bacteria and if it lost during growth or processing, the bacteria may not be properly detected or identified. In order to better understand pYV stability, ARS researchers at Wyndmoor, PA, developed a procedure to monitor its presence in *Yersinia* bacteria. The procedure exploits the low calcium response (Lcr) and Congo red (CR) binding by the bacteria. The Lcr-CR positive bacteria (pYV-bearing strains) were used to study the growth of and to monitor the pYV stability of virulent *Y. pestis* and *Y. pseudotuberculosis* in raw ground beef. The published finding will arm regulatory testing agencies with additional means for ensuring biosafety as well as biosecurity of foods.

Nanotechnology for Bacterial Detection

Nanobiosensors offer advantages over traditional microbiological and standard-scale biosensors for pathogen detection because of their low cost, label-free detection, and potential for massive parallelization. ARS-funded researchers in collaboration with Purdue University's Center for Food Safety Engineering in West Lafayette, IN have made significant advances toward the development of nanobiosensors for nucleic acid-based detection of pathogens. The scientists developed a method to position tiny droplets on an array of individual silicon microwave heaters, allowing precise control the temperature of droplets-in-air and subsequently perform biochemical reactions like DNA melting.

Identification of Unknown Foodborne Pathogens

Rapid detection of bacterial foodborne pathogens is necessary to prevent foodborne illness and safeguard public health. The optical light scattering sensor, BARDOT, is a noninvasive label-free detection system which allows identification of bacterial colonies in real-time. Developed by ARS-funded researchers at Purdue University's Center for Food Safety Engineering in West Lafayette, IN, BARDOT involves shining a laser light through the bacterial colony and collecting images of the light that passes through. The images collected contain descriptive characteristics of bacterial colonies, which can be used to identify bacteria by comparing the resulting light scattering image with a library of previously collected images. This method has tremendous potential for properly classifying foodborne pathogens; even emergent pathogens such as the previously unknown type of *E. coli* that recently caused a serious foodborne outbreak in Europe.

Enteric Viruses

Optimization of Method for Detection of Human Norovirus from Produce and from Sewage

Norovirus is considered the most common etiologic agent of outbreaks linked to produce, and water is one of the most important routes of transmission. However, effective methods for its concentration from produce in order to detect it and determine its infectivity are lacking. ARS

researchers in Albany, CA, have developed a rapid and sensitive method to detect human norovirus in fresh produce and sewage using a receptor-binding capture and magnetic sequestration (RBCMS) method. Compared with other commonly used approaches, the RBCMS method is more sensitive, has better concentration power, and enriches for the infectious encapsulated virus. This information is of use to public health agencies, private laboratories that perform testing for the industry, and the biotech industry that develops equipment and molecular biology kits for detection of norovirus.

Method to Differentiate Infectious from Non-infectious Human Norovirus

Simple tests to detect infective human noroviruses are needed to replace much more complex human volunteer studies, the only other alternative test for virus infectivity. ARS researchers at Dover, DE, attached swine mucin to magnetic beads to selectively bind infectious human noroviruses, but not inactivated noroviruses. Viruses inactivated by heat, ultraviolet light, and high pressure processing were unable to bind to mucin-coated magnetic beads. The binding of only infectious viruses to the beads will, for the first time, allow the detection of infectious viruses in food and environmental samples. This technology has been provisionally approved for patenting.

Parasites

Identified Giardins in the Attachment Organelle of Giardia and Developed a Fluorescence Microscopy Based Diagnostic Test for *Giardia duodenalis* in Environmental and Biological Specimens

ARS scientists in Beltsville, MD, identified the location of two unique proteins in the intestinal stage of *Giardia* parasites in the ventral disc, the organelle used for attaching the parasite to the intestine of an infected host. Because these proteins participate in controlling disc shape, essential for the parasite to attach to host cells, this finding provides a basis for developing methods of prevention and treatment of *Giardia* infections by utilizing anti-giardin antibodies, immunizations, or drugs to block attachment of the parasite to cell surfaces. Antibodies produced against these proteins labeled with a fluorescent dye were found useful for microscopic diagnosis of infection of intestinal tissue and diagnosis of the parasite in feces of infected animals and humans, as well as for the presence of the parasite in the environment (water, soil, plant contamination). A patent application is in the process of being filed with the U.S. Patent Office.

Molecular Characterization of Foodborne Pathogens as It Relates to Research on the Mechanism of Disease and/or Epidemiology of Foodborne Disease

Salmonella

Sequencing of *Salmonella* Associated with Multiple Outbreaks

In 2009, shredded lettuce at restaurants seemed the likely source of an outbreak of *Salmonella* Typhimurium. This was followed in 2010-2011 by a *Salmonella* outbreak that occurred among students and workers in laboratories. To help elucidate the exact genetic relationship of these outbreak strains, wears scientists at Albany, CA, performed genome sequencing of five outbreak-associated clinical strains that were isolated across the United States between 2009 and 2010. Comparison of these sequences with the seven completed S. Typhimurium genomes revealed that all of these strains are nearly identical to the strain of S. Typhimurium used in clinical and research labs for over two decades. These results have raised concerns about 1) the 2009 shredded lettuce outbreak source, 2) the security of laboratory strains, and 3) persistence of S. Typhimurium strains from "field to fork".

An Improved and More Rapid *Salmonella* Serotyping Method

Various supply, production, and environmental sources can serve as critical contamination points for the important foodborne pathogen *Salmonella* during food animal production. Cost-effective and rapid identification of different *Salmonella* strains (serotypes) would facilitate the recall and elimination of contaminated products from the production chain; however, traditional diagnostic protocols based on commonly used methods are slow and tedious or are not discriminating enough to enable molecular differentiation of the more than 1500 different types of *Salmonella*. ARS scientists at College Station, TX, have shown that incorporating a procedure known as Denaturing Gradient Gel Electrophoresis into the diagnostic protocol enables sufficient resolution of a highly variable gene sequence to reliably discriminate more than 50 different commonly encountered *Salmonella* types into their appropriate category. Work is now focusing on development of a digital molecular typing library that includes the most prevalent *Salmonella* serotypes isolated from human cases. This new molecular typing technology will help producers and public health officials in their food safety assurance missions by providing near real time differentiation and traceability of *Salmonella* recovered from various supply, production, environmental, and clinical sources.

Genetic Analysis of *Salmonella*-resistant and -susceptible Chickens

Food-poisoning bacteria such as *Salmonella* are significant causes of human disease; these pathogens can often be found as contaminants in poultry meat products. New approaches are needed to produce poultry that are not colonized by these harmful bacteria, given that absence of the pathogens in living birds will largely translate into pathogen-free meat products for human consumption. ARS researchers at College Station, TX, showed that certain genes in chickens that convey immune protection against *Salmonella* are more active in the *Salmonella*-resistant chicken line A than in the susceptible line B. These findings are important because they identify new targets for genetic selection of chickens to increase resistance to bacterial infections. Such

resistant birds will be much less likely to harbor microorganisms that can contaminate poultry meat products and cause food poisoning in humans.

Salmonella Habitat Conditions Overlap in Produce Soft-rot Lesions and in the Animal Intestine

The incidence of *Salmonella* contamination of retail produce has been positively correlated with the presence of soft-rot, a common post-harvest disease that affects all fruit and vegetables. ARS researchers in Albany, CA, have determined through gene expression profiling that *Salmonella* experiences in soft-rot lesions, conditions that are required also for its colonization and pathogenicity in the animal host intestine. This high adaptation of the human pathogen to degraded plant tissue has implications for safe shelf-life standards set by the produce and food industry, and means that the presence of soft-rot should be included in microbial risk assessment models for produce.

Linking Genetic Variations in Porcine Genes to Colonization and Shedding of *Salmonella* in Pigs.

To address this strategy, ARS researchers at Ames, IA and collaborators at Iowa State University searched greater than 3,000 porcine genes to identify genetic variations in pig genes that respond to *Salmonella*. DNA segments know as single nucleotide polymorphisms (SNPs) were identified in 31 pig genes whose expression is altered in response to *Salmonella*. To determine if the identified porcine SNPs were associated with tissue colonization or fecal shedding of *Salmonella*, four independent pig populations were genotyped for the SNPs. Statistical analysis revealed 13 SNPs that associated with *Salmonella* fecal shedding or tissue colonization. These genetic differences in pigs associated with *Salmonella* shedding provide insight in identifying and selecting for pigs with increased resistance to *Salmonella* colonization to improve food safety (for example, development of diagnostic tests to screen pig lines for the desired trait).

Identification of Metabolic Pathways that Allow *Salmonella* to be a Successful Pathogen

Salmonella enterica serovar Typhimurium is a food borne zoonotic pathogen that causes gastroenteritis in humans. To establish an infection, *Salmonella* must compete successfully with the host microflora in the intestine during colonization. *Salmonella* is unique in that it can utilize molecular hydrogen, an abundant molecule in the host intestine, as an energy source for growth. However, very little is known about how *Salmonella* uses hydrogen. In collaborative studies, an ARS scientist from Athens, GA, used microarray analysis to identify gene expression changes during exposure to hydrogen gas in *Salmonella*. Genes up-regulated by hydrogen included those that encoded proteins involved in the transport of amino acids and sugars. Genes involved in carbon conservation were also up-regulated and strains with deletion of two specific genes showed reduced hydrogen-dependent growth compared to the wild type. Hydrogen stimulates the expression of genes involved in nutrient and carbon acquisition and in carbon-conserving pathways, linking carbon and energy metabolism to sustain hydrogen-dependent growth. *Salmonella* strains unable to use hydrogen in this manner have significantly reduced virulence and survivability in mouse infection assays. Because hydrogen metabolism enables *Salmonella* to compete with the host flora and establish an infection, this pathway could be a target of drug or probiotic development to prevent infections with *Salmonella*. This research is of interest to

industry involved in drug and food development to combat diseases caused by food borne pathogens.

Gene Expression in *Salmonella* in Response to Acidic Conditions in the Host Digestive Tract

In the United States, *Salmonella* enterica serovar Kentucky is the most common type of *Salmonella* found in chickens while the number of *Salmonella* serovar Enteritidis isolated from chickens varies from year to year. Interestingly, while neither serovar cause disease in poultry, Enteritidis appears to result in more human disease while Kentucky only rarely causes disease in humans. The reasons for this are not well understood. In order to colonize the host, *Salmonella* must survive the low pH in animal digestive tracts. Studies of *S.* Kentucky isolated from chickens showed it responded differently to acid than other serovars. To explore this, gene expression of *S.* Kentucky was compared to *S.* Enteritidis in studies conducted at Athens, GA. These strains were tested by ten minute exposures to hydrochloric acid (pH 4.5) or to acetic acid (pH 5.5) in rich growth media. Microarray analysis indicated that more genes were turned on or off in *S.* Kentucky than in *S.* Enteritidis under these conditions. Overall, it appeared that the responses to acid by *S.* Kentucky and *S.* Enteritidis are similar, but differences exist in the scope and magnitude of the responses. These responses could explain the difference in prevalence of serovar Kentucky and Enteritidis in chickens. Further investigations could identify host responses in chickens that could be adjusted to reduce the levels of dangerous pathogens like *S.* Enteritidis in chickens. This information is useful for the poultry industry which can use this research to improve food safety by reducing the risk of *Salmonella* infection to humans via poultry.

Identification of Entire Cellular Proteins of *Salmonella* under Conditions Similar to Irrigation- and Wash-waters

The ability of *Salmonella* spp. to survive in irrigation waters enables them to enter the food chain. Vegetable wash waters and irrigation waters have been implicated in recent outbreaks of infections caused by *Salmonella* spp. In this study, ARS researchers at Beltsville, MD, analyzed the entire protein component of *Salmonella* during its growth in low osmotic media resembling irrigation waters. The study identified several cellular proteins which were essential for optimal growth of *Salmonella* in low osmotic conditions. Characterizing human pathogens grown under conditions mimicking fresh produce handling and washing practices will advance our knowledge of how enteric human pathogens enter and survive in our food chain.

<u>*E. coli* O157:H7 and STEC</u>

Studying Genes Involved in the Ability of Harmful Bacteria to Cause Illness

Harmful bacteria often carry genes on plasmids (non-chromosomal circular DNA), that are important for the disease process (virulence). ARS researchers at Wyndmoor, PA, determined the DNA sequence of a large plasmid in a pathogenic *E. coli* strain to gain a better understanding of how this important pathogen causes illness and to discover diagnostic markers for its identification. The virulence plasmid carried several of the same key virulence genes that are carried on the plasmid of a similar harmful bacterium, *E. coli* O157:H7; therefore, these genes

are expected to be important for virulence. Related studies compared all of the genes in pathogenic *E. coli* strains to identify genes important for causing illness. Knowledge of the virulence gene profiles of *E. coli* strains that cause severe disease and understanding the stepwise evolution of these pathogens provide the foundation for developing strategies for detection of highly pathogenic strains and for their control in food.

E. coli O157:H7 Forms Mixed Biofilm with Spinach-associated Microorganisms

The dynamics of mixed biofilm formation that include *E. coli* O157:H7 leading to its persistence in produce processing environments are unknown. ARS researchers in Albany, CA, have observed that *E. coli* O157:H7 produces thick biofilms on stainless steel surfaces in the presence of spinach lysates. Using physiological and metagenomic approaches, they determined that the early success of *E. coli* O157:H7 in these mixed biofilms was associated with its efficient utilization of spinach nutrients, whereas its population decline at later stages of the biofilm was due to its poor competition for macronutrients. This study provides risk assessment information for investigation of factors that may compound the occurrence of outbreaks linked to produce.

Gene Regulation in *E. coli* Biofilms

Pathogenic *E. coli* is able to form biofilms that increase the bacteria's resistance to environmental assaults. This also increases their persistence in food processing facilities. A better understanding of how these biofilm-associated bacteria respond to stresses is needed in order to reduce the incidence of foodborne illness. ARS researchers at Wyndmoor, PA, investigated the molecular mechanisms and regulation of genes related to oxidative stress in pathogenic *E. coli* biofilms. This is the first study that examined the gene expression changes in all four major peroxide resistance genes in biofilm cells, and determined the regulatory mechanisms involved that can allow a rational approach to developing targeted interventions to decrease the persistence of *E. coli* biofilms in food processing facilities.

Molecular Mechanisms Promoting Persistence and Fecal Shedding of *Escherichia coli* (*E. coli*) O157:H7 in Cattle

An understanding of the mechanisms that promote colonization and increase fecal shedding of *E. coli* O157:H7 bacteria in cattle intestines are critical for developing vaccines to reduce and/or eliminate *E. coli* O157:H7 in cattle. Reduced fecal shedding of *E. coli* O157:H7 will lower the risk of contamination of hides and carcasses, which in turn will lower the risk for contamination of meats produced from these animals. By infecting cattle with specific *E. coli* O157:H7 variants capable of secreting specific proteins that allow adherence of these bacteria to the tissues of the large intestine, ARS researchers at Ames, IA, found that these variants were shed in the feces for longer duration and at higher numbers in infected animals than those *E. coli* O157:H7 variants that were unable to secrete these proteins. The understanding of the requirement of this protein secretion mechanism and specific proteins secreted through this mechanism in colonization of cattle intestines has enabled construction and evaluation of novel heat-killed vaccines for reducing colonization and fecal shedding of *E. coli* O157:H7.

Listeria

Determination of the Genome Sequence of a Virulent Serotype of *Listeria monocytogenes*

Listeria monocytogenes is a human pathogen but some strains and other species could be non-virulent. This work determined the genomic sequence and identified 58 virulence-specific genes for a virulent strain of *Listeria monocytogenes*. This can potentially allow development of methods to distinguish pathogenic and nonpathogenic isolates. Polymerase chain reaction (PCR) assays based on eight of these virulence-specific genes were tested on a panel of *Listeria* isolates, and two of the genes showed good potential. The goal is development of a multiplex real-time PCR assay for simultaneous and rapid differentiation and quantification of high-risk and low-risk *L. monocytogenes* serovars from food, particularly aquaculture products. This assay developed at Stoneville, MS, will allow us to address important questions regarding persistence and replication of different *Listeria* serotypes in food and in processing environments.

Novel Genetic Differences between Pathogenic and Nonpathogenic Isolates

Discrimination between pathogenic and non-pathogenic *Listeria* (L.) *monocytogenes* with accurate diagnostic tools can help the industry (food and fish) better discern the safety of their products. A large number of food recalls occurs annually because of the possibility of being contaminated with *L. monocytogenes*, even though the strain may not be virulent. The identification of novel targets that could differentiate pathogenic and nonpathogenic isolates has taken on increased importance. Results show that one could discern between pathogenic and non-pathogenic strains by determining the presence or absence of certain genes. This could lead to the development of tools to identify whether a product or animal carries a pathogenic strain or whether it is safe to eat even though it may carry *Listeria monocytogenes*.

<u>*Campylobacter*</u>

Sequencing and Analysis of *Campylobacter* Genomes

Some *Campylobacter* species cause human bacterial gastroenteritis and/or disease in food animals. Although most cases of *Campylobacter* associated food-borne illness are currently attributed to *Campylobacter jejuni*, emerging *Campylobacter* species have become linked increasingly to human illness. Scientists at Albany, CA, have completed the genome sequences of 27 *Campylobacter* species, subspecies and biovars. Additionally, the genome sequences of multiple novel species, isolated from California agricultural regions, were also completed. *Campylobacter*s colonize a wide variety of mammalian and avian hosts and can be isolated from meat, water, milk and shellfish. Comparative genomics of these completed *Campylobacter* genomes will provide further insights into the genetic basis of host association, pathogenicity and survival in the environment.

Identification of Genes that Differentiate *Campylobacter jejuni* Isolated from Cattle, Chickens and Humans

Campylobacter is a leading cause of food borne illness in humans and improving our understanding of the epidemiology of this organism is essential. The objective of this study

conducted at Athens, GA, was to identify the genes that were most significant for discriminating isolates of *Campylobacter jejuni* by analyzing whole genome DNA microarrays. Statistical analyses of whole genome data from 95 geographically diverse cattle, chicken and human *C. jejuni* isolates identified a total of 142 most significant variable genes. Of this total, 125 (88%) belonged to genomic prophage and hypervariable regions. The significance of genomic prophage and hypervariable regions in determining the overall genetic diversity of *C. jejuni* is emphasized by these results. These genes should prove useful to food borne illness tracing programs, such as PulseNet, in the development of genotyping systems for *C. jejuni* as well as a means to further our understanding of the epidemiology and population genetics of this major food borne pathogen.

Validation of Sequence-based Typing for *Campylobacter jejuni/coli*

Campylobacter is the genus of bacteria that is responsible for the greatest number of human diarrheal disease. Up to now, type profiling of these organisms has relied on the use of a group of genes from the organism that were selected with the intent that they would be representative of the entire genome, an assumption that can now be tested using total genome sequences. A method was developed at Athens, GA, to simultaneously compare the variation in all of the 1029 genes we identified as being contained in a panel of genomes. The method involved measuring the differences in each of these genes for each of the 25 genomes to create a data file that was then subjected to specialized statistical analysis for determining clusters. We were able to define clusters that correlated with specific evolutionary influences, such as selective pressure for frequent changes ('mutations') or participation in cross-bacterial exchange events ('recombination' also known as 'lateral gene transfer'). These are factors that affect interpretation of population genetic data for analysis of migration, so knowing which genes have these influences will be useful in future interpretations.

<u>Other Bacterial Pathogens</u>

Identification of Pathogenic *Staphylococcus aureus* Strain by Classification of Toxin Genes

Food producers and regulatory agencies are in constant need of improved means for bacterial classification so that identification of harmful, bacterial contaminants in foods may be dealt with in a timely manner. It is of critical importance that such investigative methods be very accurate for tracing back the source for bacteria isolated from human samples (i.e., clinical isolates). In recent decades, such methods have compared genetic patterns of isolates against known bacteria. ARS researchers at Wyndmoor, PA, in conjunction with collaborators at Shanghai Jiao Tong University (China), have compared three genetic classification or typing schemes for their ability to distinguish between pathogenic strains of S. *aureus*. The gene typing schemes were PFGE (pulsed-field gel electrophoresis), MLST (multilocus sequence typing), and a custom-developed method termed "Toxin Gene Typing Method." In the analysis of over 100 S. *aureus* clinical isolates, three (of 18) known toxin genes were found to be most common and the Toxin Gene Typing Method was noted to give the best results.

Analysis of the Poultry Microbiome – Farm to Fork

ARS researchers in Athens, GA, used next-generation DNA sequence analysis to characterize the poultry microbiome throughout a 'farm-to-fork' continuum. Investigators determined that a core microbiome, including sequence types most closely related to *Clostridium*, *Campylobacter*, and *Shigella*, was present in all sample types tested. Additionally, fecal samples were determined to contain 2-4-times greater taxonomic richness relative to carcass rinse samples. Interestingly, carcasses sampled 48 hours after processing, revealed the greatest proportion of unique taxa, including Prevotella, Veillonella, Leptrotrichia. Retail products were dominated by Pseudomonas, but were also determined to contain sequence types for 27 additional genera, most of which were encountered in on-farm samples. These data represent the first next-generation DNA sequence based characterization of poultry-associated microbiomes along the farm-to-fork continuum and demonstrate the utility of next-generation DNA sequence analysis for the identification of sources of potential zoonoses.

Genetic Relatedness of Enterococci Poultry and the Environment

The potential for contamination of surface and groundwater due to poultry waste used as fertilizer on fields was investigated. ARS scientists in Athens, GA, have collaborated with Environment Canada to assess the genetic relationship of enterococci from surface and groundwater to enterococci isolated from poultry sources in Fraser Valley, a province of British Columbia, Canada. Using two molecular analysis methods, enterococci from layer litter and surface and groundwater were compared. Enterococci were isolated from all three sources, but overall grouping was independent of source by both molecular methods. Although enterococci from litter and water sources were grouped together using both methods, water isolates could not be definitively identified as originating from poultry litter. These results suggested that although poultry waste was used as fertilizer, a variety of hosts may be contributing to fecal contamination especially in aquatic environments. Researchers can use this information for source tracking environmental contamination from poultry and other potential sources of contamination and in designing strategies to reduce microbial contamination of the environment.

A New and Improved Method to Study Bacterial Proteins

To understand how harmful bacteria survive and persist within the food supply, it is necessary to be able to identify and measure the products that the bacteria produce, their proteins. This field of research is referred to as proteomics and can be studied using various techniques. However, a bacterium produces a very large number of proteins and studying them can be complicated. Thus, ARS researchers at Wyndmoor, PA, developed a novel and easy to perform proteomics method that allowed analysis of a large number of proteins produced by bacteria grown under different conditions, and the method was sensitive and reproducible. The workflow developed from this research will become an essential part of research projects that will increase the understanding of how pathogens are able to overcome environmental stresses inherent in food settings.

Determination of the Genomic Sequence of *Bacillus mojavensis* Strain RRC101

The sequence is 4.1 million base pairs in length and is most similar to sequenced strains of the type for the genus, *B. subtilis*. The impact of this work is expected to have large ramifications.

Currently, the genomic data is assisting us in generating hypotheses related to mode of inhibition of *Fusarium verticillioides* by compounds produced by the bacterium. Combined with progress on transformations of this strain, ARS will explore direct methods to test these hypotheses.

Fungi

Sequencing of All the Deoxyribonucleic Acid (DNA) of the Fungus *Aspergillus (A.) flavus* and Its Applications

Knowing the genetic makeup of this fungus is important to know how and why this fungus makes the potent carcinogen aflatoxin when it invades crops. Two different format whole genome microarrays (slides containing spots of DNA corresponding to fragments of all unique genes) have been designed recently, and used to identify critical genes involved in fungal response to various environmental factors favoring toxin production. These microarray resources have been used in large scale functional genomics studies by ARS scientists at New Orleans, LA, and our collaborators to analyze which genes are affected under varying conditions: 1) nutritional (high or low carbon source); 2) environmental (temperature); 3) developmental (veA mutant); and 4) during the fungus-corn interaction (cooperation with ARS scientists and faculty, Mississippi State University) that affect toxin production. The long-term survival of aflatoxin producing A. *flavus* strains in comparison with non-producing strains has indicated that under temperature stress (47°C), spores of toxigenic strains survived longer than non-aflatoxin producers; and there is no difference in survival under ultraviolet light for aflatoxin-producing strains vs. non-aflatoxin-producing strains. From these microarray studies, over a hundred genes were identified that may have some impact on aflatoxin production and fungal survival. The database will be accessible through a website which is expected to be housed at the ARS, Mid-South Area genomics facility, Stoneville, MS, and provide genomic information to all researchers worldwide working with this fungus. These studies carried out by ARS scientists are providing significant insights into what genes are involved in the interaction between the fungus and the crop.

Proof of Involvement of Genes in Aflatoxin Synthesis and Fungal Development Obtained

Knowing the genetic makeup of this fungus is important to know how and why this fungus makes the potent carcinogen aflatoxin when it invades crops. Using sophisticated molecular techniques, ARS scientists at New Orleans, LA, have tested specific interactions of key aflatoxin developmental regulatory factors. Further, the genetic basis for loss of aflatoxin production in toxin-deficient mutants of *Aspergillus* parasiticus (generated by physical manipulation of toxin-producing strains) has been investigated using microarrays and metabolic profiling, and specific regulatory genes causing this loss have been identified. ARS scientists at New Orleans, LA, have found that many factors are needed for complete regulation of the turning on and off of aflatoxin production. Through the use of these technologies we will rapidly assess the critical role of several genes of interest in aflatoxin formation in crops.

Cyclopiazonic Acid is Another Toxic Compound that the Fungus *Aspergillus (A.) flavus* Produces Along with Aflatoxins

In collaboration with National Peanut Research Laboratory (NPRL), ARS scientists at New Orleans, LA, have identified the gene cluster involved in cyclopiazonic acid (CPA) formation and characterized associated biosynthetic genes. The CPA gene cluster is located next to the aflatoxin gene cluster on the *A. flavus* genome. Using this information, scientists confirmed that the biopesticide Afla-Guard® (active ingredient is A. *flavus* NRRL21886), developed at NPRL and commercialized by Syngenta Crop Protection, does not contain the CPA and aflatoxin *A. flavus* gene clusters, which ensures the safe application of this genuinely nontoxic *A. flavus* strain in the field as a biocontrol agent. The colocation of the aflatoxin and CPA biosynthetic gene clusters on the fungal chromosome suggests how the fungus could make these two harmful compounds together based on one signal that allows the "turning on" of the genetic machinery within the fungus.

Deoxyribonucleic Acid (DNA) Probes (Primer Sets) Identified for Universal Screening for Genetic Variability of *Aspergillus (A.)* Group Fungi

Each year millions of dollars are lost to crops contaminated with toxic and carcinogenic aflatoxins produced by *A. flavus*. ARS scientists at New Orleans, LA, have conducted studies on the molecular characterization of the aflatoxin biosynthetic pathway from the aflatoxigenic cousin of *A. flavus*, namely toxin-producing *A. ochraceoroseus*, *A. rambelli*, as well as, nontoxigenic *A. oryzae*, were done to determine if aflatoxin production provides a competitive advantage to *A. flavus* for its ability to survive in field conditions. Strains of an *A. parasiticus* isolate with specific deletions of aflatoxin pathway genes have been compared for morphological and physiological differences due to the knocking-out of critical pathway genes. This will help us understand if the deletion of these pathway genes has an effect on the fungus in its ability to invade crops.

Enabling Rapid Identification of Toxigenic and Pathogenic *Fusarium* Species

Fusarium species rank among the most economically destructive plant pathogens and mycotoxigenic fungi, posing a constant threat to plant and animal health, and food safety. ARS scientists at Peoria IL, in collaboration with agricultural scientists in Denmark, Israel, Japan, The Netherlands, and Norway, determined that DNA sequence data could be used to accurately type agriculturally important pathogens and help predict toxin potential. In addition, the researchers identified genes responsible for the production of several toxins injurious to humans and plants in the whole genome sequence of two phytopathogenic *Fusarium* species. Lastly, the DNA sequence data have been incorporated into *Fusarium*-ID (http://isolate.*Fusarium*db.org, at the Pennsylvania State University, Philadelphia, PA, and *Fusarium* MLST (http://www.cbs.knaw.nl/*Fusarium*) at the Centraalbureau voor Schimmelcultures (CBS-KNAW) Biodiversity Center, Utrecht, The Netherlands, two web-accessible sites dedicated to promoting DNA sequence-based identifications of pathogenic and toxigenic fusaria via the Internet. The two websites promote agricultural biosecurity worldwide by facilitating global molecular surveillance of pathogenic fusaria and by enabling plant quarantine officials, plant breeders, and plant pathologists to accurately detect and identify these pathogens for the first time.

<u>Parasites</u>

Genetics of *Trichinella* Infections

Strong immune responses may strictly limit the opportunities for unrelated parasites, such as foodborne species of *Trichinella*, to mate. ARS researchers in Beltsville, MD, examined the diversity of *Trichinella* larvae in naturally infected animals. Almost without exception, they found that animals had been successfully infected by only a single pair of parasites, as judged by the fact that larvae (their progeny) were full siblings. Therefore, immunity may typically preclude establishment of subsequent infections, a finding that further substantiates vaccination as a plausible strategy for future public health interventions.

Gene Flow among Dissimilar Types of *Trichinella*

The spread of biological traits relevant to public health may be constrained by geographical, physical, and/or physiological barriers to reproduction among diverse forms of *Trichinella*. ARS biologists working in Beltsville, Maryland genotyped isolates of these food-borne parasites at loci (located in the nucleus) inherited from each parent, as well as at a locus (in the mitochondrion) inherited solely from the maternal line. Where each of two parasite lineages was known to infect wildlife, intermediate forms of the parasite (containing ancestry from both lines) were documented. In particular, one maternal lineage was found where two had been expected, substantiating the notion that introgression has resulted in the displacement, locally, of one by the other. These findings are among the first to explore the capacity of genes to flow from one lineage of *Trichinella* to another. Understanding whether traits can be shared among lineages is important, because a prevalent method to ensure the safety of meat (freezing) would be undermined if it certain traits (i.e. freeze resistance) were capable of being shared.

An Unexpectedly Widespread Chromosomal Variant in *Toxoplasma gondii*

Toxoplasma gondii, is an important and prevalent parasite that can be contracted either by eating contaminated meat or by ingesting contaminated produce or water. In spite of the fact that the parasite can undergo sexual recombination, producing myriad variants in certain locales, other strains are temporally stable and geographically widespread. The basis for persistence and dissemination of such strains was studied by ARS researchers in Beltsville, MD, in collaboration with an international team of academic scientists. They found that a particular variant of one of the parasite's chromosomes occurs in myriad, otherwise unrelated strain. This finding focuses attention on the genes encoded by this chromosome, as it may offer functional explanation for the basis of this parasite's dispersal capacity, and new means to intervene in its transmission to food animals and to people.

Antimicrobial Resistance/Susceptibility of Foodborne Microorganisms

Antibiotic Resistance in *Salmonella* Isolated from Milk in the U.S.

The degree of resistance to antimicrobial agents was determined for strains of *Salmonella* recovered from the bulk tank milk (raw milk before it leaves the farm) and in-line milk filters from U.S. dairy farms as part of two surveys conducted by USDA-Animal and Plant Health Inspection Service (APHIS) and other participants, including ARS researchers at Beltsville, MD, in 2002 and 2007. Resistance of pathogenic bacteria to antibiotics or other antimicrobials has become a growing concern. Each of 176 *Salmonella* isolates was tested for their ability to grow in the presence of 15 different antibiotics. Thirty isolates (17.0%) exhibited resistance to at least one antimicrobial agent. Twenty *Salmonella* isolates (11.4%), displayed resistance to a wide range of antibiotics. The results of this study suggest that there is a low but appreciable risk of infection by multi-drug resistant *Salmonella* from consuming non-pasteurized milk and dairy products.

Tracking the Emergence of Antimicrobial Resistant *Salmonella* Heidelberg

Surveillance conducted as part of the National Antimicrobial Resistance Monitoring System (NARMS) has shown a recent increase in extended-spectrum cephalosporin (ESC) resistance among *Salmonella* Heidelberg isolated from food animals at slaughter, retail meat, and ill humans. ARS scientists in Athens, GA, and collaborators at the Centers for Disease Control and the Food and Drug Administration demonstrated that the 2009 increase in ESC resistance among *Salmonella* Heidelberg was caused mainly by the dissemination of blaCMY on 2 types of plasmids, IncI1 and IncA/C, in a variety of genetic backgrounds and is likely not the result of clonal expansion. This data is critical for public and veterinary health officials and regulatory agencies as they investigate food borne illness outbreaks. This data is also critical for scientists studying the development of antimicrobial resistance and in the development of mitigation strategies.

A Foodborne Outbreak Associated with *Salmonella* Heidelberg

Uncomplicated gastrointestinal illness from *Salmonella* infection usually resolves within five to seven days and is not treated. If treatment is needed, particularly in the young, elderly, and immunocompromised, antimicrobial resistant (AR) *Salmonella* may compromise treatment. To track the development of AR among *Salmonella*, isolates from food at retail, ill humans, and animals at slaughter were tested for resistance to a panel of antimicrobials as part of the National Antimicrobial Resistance Monitoring System (NARMS) by FDA, CDC and USDA-ARS scientists, respectively. Further characterization using pulsed gel electrophoresis (PFGE) assisted in determining if *Salmonella* strains are related to each other. In March 2011 a multi-state outbreak of *Salmonella* serotype Heidelberg infections in humans was closely monitored. CDC and FDA collaborators demonstrated that *Salmonella* Heidelberg isolates from ill humans closely matched *Salmonella* Heidelberg isolates from ground turkey product at retail stores. Antimicrobial resistance patterns also matched between these isolates. A recall of ground turkey was initiated as a result of these findings. ARS scientists involved with NARMS in Athens, GA, in collaboration with FSIS scientists, identified isolates from turkeys at slaughter that also

closely matched the outbreak isolates. FDA, ARS and CDC, scientists also closely matched isolates originating from past routine NARMS surveillance of isolates from ground turkey at retail, turkey at slaughter and from ill human clinical isolates, respectively. This data is critical for public and veterinary health officials and regulatory agencies as they investigate food borne illness outbreaks. This data is also critical for scientists studying the development of antimicrobial resistance and in the development of mitigation strategies.

Multi-drug Resistant NTS (non-Typhoidal *Salmonella*) is a Major Food Safety Concern Worldwide

In collaboration with scientists at the Ohio State University ARS scientists in Athens, GA, characterized multi-drug resistant (MDR) *Salmonella* serovar Havana, an emerging serovar reported from multiple sources including porcine, other food animals and the environment. Polymerase chain reaction and DNA sequencing were conducted to characterize resistance gene cassettes and class 1 integrons which are genes that are known to reside either within the bacteria's chromosome or on a mobile genetic element, such as a plasmid, that can be transferred to other bacteria. An unusually large single integron was identified commonly among seven members of this serovar. This integron was found to carry gene cassettes that encode resistance to multiple classes of antimicrobials including sulphonamides, beta-lactamases, aminoglycosides and macrolides. The gene cassettes identified on the large 4kb integron include dfra16, blaPSE1, aadA2, and ereA resistance genes. Finding such a wide range of resistance against antimicrobial classes on a single integron is unique and implies the high propensity of this serovar to persist in the face of antimicrobial selective pressure and thus potentially become a major food safety concern. Additional genes that were not associated with the class 1 integrin included tetA(B), strA, strB and apha1-Iab. The findings underscore the significance of class 1 integrons in maintenance and persistence of MDR serovars of food safety significance and they are important in the development of mitigation strategies.

Antimicrobial Resistance Mechanisms of *Salmonella* enterica serovar Typhimurium

Antimicrobial resistance (AR) in food borne bacteria is a concern for both animal and human health. This is especially true when multi-drug resistance (MDR) occurs in foodborne pathogens such as *Salmonella* enterica. MDR has been found in *Salmonella* enterica serovar Typhimurium isolated from animals as part of the National Antimicrobial Resistance Monitoring System (NARMS). Some of these bacteria are resistant to five or more antimicrobials including ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (ACSSuT resistance phenotype). To investigate the genes responsible for this MDR, S. Typhimurium isolated from healthy cattle, poultry, and swine and resistant to at least ACSSuT were selected between the years 1997 to 2007 (n=33). AR and plasmid gene content of these isolates were evaluated using microarray analysis. The distribution of genes detected separated most of the isolates into two groups. Those in group A (n=15) were often a specific type called definitive phage type DT104 and were isolated mostly from swine. Isolates in group B (n=16) had many Inc A/C plasmid genes detected and were isolated mostly from cattle. Further analysis conducted at Athens, GA, demonstrated significant linkage for the associations between plasmids, phage type, and animal source for these groups. This study identified the two major genetic elements responsible for MDR in S. Typhimurium isolated from healthy food animals.

This information clearly defines the genetics behind MDR in this important pathogen and will enable the scientific community to determine the sources of these genetic elements and develop procedures to reduce their prevalence in the food chain.

Colonization of a Marker and Field Strains of *Salmonella* Enteritidis and *Salmonella* Typhimurium in Vancomycin Pretreated and Non-pretreated laying hens

ARS scientists in Athens, GA, conducted a study to evaluate the effects of pre-treating laying hens with the antibiotic vancomycin on the hen's susceptibility to colonization by *Salmonella*. The ability of *Salmonella* strains to colonize the intestinal and reproductive tracts the hens were compared. Hens were orally challenged with the *Salmonella* and housed for 72 weeks. Periodically, hens from the study were euthanized, organ samples were collected, and the presence of *Salmonella* in the organs was determined. The ability of the *Salmonella* to colonize the hens was based on the ability of the bacteria to successfully compete for nutrients and to become resistant to vancomycin. Findings of this study confirm the importance of bacterial antibiotic resistance in the colonization of poultry by human pathogenic bacteria.

Antimicrobial Resistance Mechanisms of Commensal *Escherichia coli* Isolated from Chickens

Multi-drug resistant (MDR) commensal bacteria in food animals are a potential source of MDR genes for pathogenic bacteria and could present a hazard to animal and human health. To determine the genetic cause of resistance, 32 MDR *Escherichia coli* isolates from poultry were examined (four isolates from each year 2000-2007). Microarray analysis was used to detect antimicrobial resistance (AR) genes and mobile genetic elements, such as plasmids, known to be associated with the spread of MDR. AR genes were detected consistent with the MDR phenotypes of all isolates, and a large number of IncA/C plasmid genes were detected in 27 of the isolates, indicating the likely presence of this plasmid known to carry MDR genes. Testing for 18 plasmid replicon types associated with MDR in Enterobacteriaceae detected one or more replicon types in all 32 isolates and confirmed the presence of IncA/C in the 27 isolates. Class 1 integrons were also assayed for and detected in 25 of the isolates. The class 1 integrons detected ranged in size from ~1000 to 3300bp and sequence analysis identified AR genes in those integrons. The class 1 integrons, IncA/C plasmids, and MDR-associated plasmids found in the isolates indicated the importance of these genetic elements in the accumulation and spread of AR genes in the microbial community associated with poultry. This study conducted at Athens, GA, is important for federal regulatory agencies as identifying the source of MDR elements in food animals is an important step towards developing interventions to reduce resistant bacteria which are potential hazards to human health.

Enterococci from Retail Meat and Produce

Although enterococci are considered opportunistic pathogens, they can be reservoirs of antimicrobial resistance. This is increasingly important considering food borne illnesses from meat and produce. The objective of the present study conducted at Athens, GA, was to use band-based molecular typing methods to determine if genetically related enterococci were found among different stores, food types, or years. Different enterococcal species were prevalent on fruits, vegetables, and meat from retail grocery stores. Specific species, such as Enterococcus

casseli*flavus* from fruits and vegetables, were predominant on certain food products, but were also found in lower numbers on other food items. The majority of enterococcal isolates from the retail food items were resistant primarily to bacitracin, flavomycin, and lincomycin. Resistance of enterococci to penicillin, salinomycin, and nitrofurantoin was low and none of the isolates were resistant to linezolid or vancomycin. Enterococcal isolates with identical banding patterns were identified from different stores, food types, and of different species. These data suggest that foods commonly purchased and consumed from grocery stores are a source of genetically related antimicrobial resistant enterococci that can be transferred to the human population. This research will be useful to policy makers and researchers to better understand bacterial contamination of meat and produce in order to contain and eliminate food borne outbreaks.

Analysis of Antimicrobial Resistance Genes in Environmental Soil Bacteria

Acinetobacter baumannii-calcoaceticus complex (ABC) causes wound infections in many combat casualties. An increase in ABC strains which are multi-drug resistant (MDR) has complicated treatment of these wounds in U.S. personnel participating in Operation Iraqi Freedom. In this study conducted at Athens, GA, DNA patterns from 298 ABC isolates were characterized to determine how closely they matched on a genetic level. Pulsed Field Gel Electrophoresis (PFGE) was used to classify them into 67 distinct PFGE types (PFTs). Microarray analysis of DNA from isolates detected the presence of several antimicrobial resistance genes some of which indicated resistance to the antimicrobial imipenem. Imipenem resistance (IR) in MDR isolates is of particular concern as it is one of the last drugs available to treat MDR ABC infections. DNA Southern blot analysis demonstrated that the IR specific gene was plasmid-borne or both plasmid and chromosomally located. A plasmid carrying the IR specific gene inserted into a susceptible ABC strain conferred IR to the recipient showing that the plasmid contained the resistance and could be transferred to other bacteria. The distribution of the IR-ABC with specific PFTs implied nosocomial spread in military treatment facilities. This study has determined the genes causing IR and MDR in ABC infections and how it is spread in the hospital environment. It also demonstrated that resistance developed in these ubiquitous soil bacteria which could be easily transferred to other bacteria including food borne pathogens. This information is particularly useful to the U.S. military and military scientists in order to protect the U.S. military from bacterial infections in war zones.

New Antimicrobial Compounds Kill Foodborne Pathogens

The incidence of antibiotic resistant pathogens is on the rise, reducing the effectiveness of antibiotic treatment of human infections and necessitating the development of new antibiotics. ARS researchers at Wyndmoor, PA, in collaboration with scientists at PolyMedix, Inc., Radnor, PA, tested several of the company's antimicrobial peptide mimics and determined that these compounds appeared to kill the cells by causing them to break open, and so are less likely to develop resistant strains than traditional antibiotic compounds.

Designed Epidemiologic Studies of Foodborne Illness/Associated Organisms

Prevalence of *Salmonella* in Dairy Cattle

Understanding the prevalence of *Salmonella* in animals on farms is necessary to control for bacteria entering the food supply. However, these studies are expensive and less costly testing for *Salmonella* is essential. ARS scientists in Athens, GA, and -APHIS conducted a national study on United States dairy farms to determine if the same information using pooled or composite fecal samples (less costly) for culture of *Salmonella* versus testing individual fecal samples (costly) could be obtained. The USDA's National Animal Health Monitoring System Dairy 2007 study collected samples on dairy operations from 17 major dairy states. Among 116 operations sampled, 41.4% (n = 48) were positive by individual samples, 39.7% (n = 46) by pooled samples, and 49.1% (n = 57) by composite samples. Relative to individual samples to determine herd infection status, the sensitivity was 85.4% for composite fecal samples and 91.7% for pooled fecal samples. On 33.6% of operations (39/116), *Salmonella* was cultured from all 3 sample types (individual, pooled, and composite). Of these, 20 operations (51.3%) had exact *Salmonella* serotype matches in all 3 sample types. Composite fecal sampling is less costly and time consuming and provided similar results for detecting/identifying *Salmonella* in dairy herds. Therefore, it may be considered an alternative to culture of individual samples when assessing *Salmonella* status in dairy herds. These data are important for the dairy industry and other researchers as they develop and implement prevalence studies.

Prevalence of *Salmonella* in Swine

Food borne illness is typically self-limiting and does not require antibiotics; however, when antibiotics are needed, resistant bacteria may be difficult to treat. To determine the prevalence *Salmonella* and characterize their susceptibility to antimicrobials ARS (Athens, GA) and -APHIS scientists cultured swine fecal samples as part of the National Animal Health Monitoring System's Swine 2000 and 2006 studies in over 17 states. Overall, 6.2% of the samples and 34.2% of the farms were positive in 2000 while in 2006, 7.2% of the samples and 52.6% of the farms were positive. *Salmonella* serotypes Derby, Typhimurium var. 5- and Agona were the three top serotypes recovered in both study years. In both years Derby was most often resistant to streptomycin, sulfisoxazole and tetracycline. Most isolates were resistant to tetracycline, sulfisoxazole and streptomycin in both years. The percentage of isolates susceptible to all antimicrobials was 38.1% in 2000 and 20.4% in 2006 while the percentage resistant to three or more antimicrobials was 52.8% in 2000 and 57.7% in 2006. These data indicate that the prevalence of *Salmonella* remained relatively stable between years but the total number of positive farms increased. There was no change in the types of *Salmonella* recovered between study years and antimicrobial resistance was observed mostly in drugs that are not used to treat salmonellosis. These data are important to epidemiologists, veterinarians and public health officials as they monitor the health of food animals, status of the food supply, conduct food borne outbreak investigations, and develop mitigation strategies.

Incidence of Pathogens in the Environment

Thousands of strains of STEC, including O157:H7, *Salmonella*, *Listeria monocytogenes* and *Campylobacter* species have been isolated from > 15,000 environmental samples, and characterized for serotype, genotype and virulence factors. This work was done at Albany, CA. The epidemiology and ecology of pathogens in a major food production region has stimulated multiple interactions with regulatory agencies and industry. In addition to the microbial source tracking information provided for comparison to the field data obtained, these strains provide an unprecedented resource for addressing fundamental biology objectives related to comparative genomics, analysis of foodborne pathogens from multiple sources, and gene expression profiling of related strains from different sources.

Investigation of *Listeria monocytogenes* Isolates Related to a Large Cantaloupe-borne Outbreak.

Listeria monocytogenes is a deadly human pathogen that has been associated with a variety of foods including cantaloupe in a 2011 outbreak in the U.S. Researchers at Albany, CA, gathered *L. monocytogenes* isolates from the cantaloupe outbreak, isolates from other food-related outbreaks, clinical collections, defined epidemic clones and isolates from chicken processing plants. All isolates were characterized and compared to one another using sophisticated molecular subtyping methods. It was discovered that the cantaloupe outbreak isolates matched isolates from food-related outbreaks in Canada, other worldwide clinical collections and chicken processing plants in the U.S. None of the cantaloupe isolates fit into previously described epidemic clones but two novel epidemic clones were created to include these strains. Thus, a new type of highly virulent *Listeria* has been recognized and needs to be monitored.

Dissemination of *Campylobacter* in a Georgia River

The greater part of the life cycle of *Campylobacter jejuni* is not defined – the sources of chicken infection are unknown and passage of the organism outside of the chicken to human is uncharacterized. This study conducted at Athens, GA, was designed to see if open waterways are part of the life cycle for the organism involved in human disease. Over the course of four years 560 water samples were taken from the Upper Oconee River Watershed from which 47 strains of *Campylobacter* were obtained. The strains were genetically typed in order to classify them by family lineages and this data was compared to types known to be found in chickens and humans. Of the recovered strains, some have previously been found in cattle and very few that were ever found in humans whereas most of the strains have only been associated with environmental water. This means that open water plays only a minor role if at all in the transmission of human associated *Campylobacter* and need not be a focus of intervention.

Risk Assessment, Modeling, Management, and Communication

Chemical Contaminants

Survey of Market Catfish for the Presence of Dioxins and Source Attribution

The USDA is responsible for the safety of meat, poultry, egg, and catfish products sold in the United States. Animal-based food products are the major route of exposure to dioxins for humans, and one way to reduce that exposure is to identify and remove the sources of dioxin before they enter the food chain. USDA ARS scientists in Fargo, ND, measured 17 individual dioxin compounds in catfish tissues. These data demonstrated that dioxin levels found in the catfish products were low and suggested minimal public health risks; however, they also suggested that uncontaminated mineral clays or alternatives to mineral clays in catfish feeds would minimize even further the entrance of dioxins to the food supply.

Microbial Pathogens

E. coli O157:H7 and STEC

Modeling the Growth of Shiga Toxin-producing *E. coli* in Ground Beef and Spinach Leaves

Shiga toxin-producing *E. coli* (STEC), particularly non-O157 STEC, has become a major public food safety hazard and has been declared by the USDA-FSIS as an adulterant in ground beef. ARS researchers at Wyndmoor, PA, conducted a study to investigate the growth kinetics of non-O157 STEC in ground beef and spinach leaves. This research evaluated the growth of non-O157 STEC in ground beef and spinach leaves, and developed new mathematical models to predict the bacterial growth. The results of this research can be used by regulatory agencies such as the FDA and USDA FSIS to conduct risk assessments of ground beef and spinach leaves exposed to contamination of non-O157 STEC.

Listeria

Modeling the Growth of *Listeria monocytogenes* in Fresh-cut Cantaloupe

L. monocytogenes is a deadly foodborne pathogen that causes serious illnesses in pregnant women, the elderly, and patients with compromised or suppressed immune systems. A multistate outbreak of listeriosis linked to contaminated cantaloupes from Jensen Farms, Colorado, caused 146 illnesses, including 30 deaths, in 2011. In response to this outbreak, ARS researchers at Wyndmoor, PA, conducted a study to investigate the growth kinetics of *L. monocytogenes* in fresh-cut cantaloupe, and developed mathematical models to predict the bacterial growth. The results of this research can be used by regulatory agencies such as the FDA to conduct risk assessments of fresh-cut cantaloupe exposed to contamination of *Listeria monocytogenes*.

Cronobacter

Cronobacter sakazakii in Powdered Infant Formula

Cronobacter sakazakii is a deadly foodborne pathogen found in dehydrated powdered infant formula. ARS researchers at Wyndmoor, PA, conducted a study to investigate the growth kinetics of *C. sakazakii* in reconstituted powdered infant formula (RPIF), and develop predictive models. Thermal growth studies indicated that *C. sakazakii* grows well at temperatures between10-48C. There was no significant difference between the growth rates of non-heat-treated and heat-injured cells suggesting that any *C. sakazakii* in RPIF may present a risk to infants. The results will assist industry in their production of infant formula; for regulatory agencies in conducting risk assessments of RPIF exposed to various temperature-abuse conditions and defining regulatory policy, as well as for parents and other caretakers in properly storing and preparing reconstituted powdered infant formula.

General Microbial Pathogens

Pathogens in Produce Growing Areas in the Salinas Valley, California

Several bacteria have been linked to produce associated foodborne illness outbreaks. ARS researchers in Albany, CA, in collaboration with the Food and Drug Administration and National Aeronautics and Space Administration conducted a survey of the Salinas watershed for the presence of *E. coli* O157, non-O157:H7 Shigatoxin-positive *E. coli* (STEC), *Salmonella*, *Listeria*, and *Campylobacter*. Data collected indicated substantial differences in the prevalence of the various pathogens with a definite correlation to sampling region and date. Data allowed the development of a predictive geospatial risk assessment model (PGRAM), while overall the study provided industry and public health regulatory agencies with valuable epidemiological data for development of a risk assessment for this important agricultural region of the U.S.

Predictive Microbiology for the Growth of Foodborne Pathogens on Seafood

When adjusted for per capita consumption, seafood is associated with food borne illness more than beef, poultry, or produce. Little or no models exist to predict foodborne pathogen growth in seafood. ARS researchers at Wyndmoor, PA, in cooperation with researchers at Mississippi State University, assessed the growth potential of spoilage microorganisms and foodborne pathogens on seafood including catfish fillets and yellow fin tuna. Growth curves at 5, 10, 15, 22 and 30 degrees C were completed for non-O157:H7 shigatoxin producing *Escherichia coli* inoculated onto catfish and *Salmonella* inoculated onto yellow fin tuna. This research fills a much needed gap in the field of predictive microbiology and will assist seafood processors and regulatory agencies assess risks associated with consumption of both properly refrigerated and temperature abused seafood which has become contaminated with foodborne pathogens.

Development of a Buffer Capacity Model to Predict pH Changes in Acid and Acidified Foods

The growth of spoilage bacterial may increase pH in acidified foods, resulting in unsafe products. Conversely, pH reduction in fermented foods is important for assuring safety. However, there is currently no accurate method for predicting pH changes in acid and acidified foods because of the unknown buffering compounds which affect pH changes. To address this problem, ARS researchers at Raleigh, NC, developed a buffer capacity model that can be used to

determine pH changes in acid and acidified foods as acids and bases are added for processing, or are produced by the growth of bacteria. The model showed that the complex acid buffering present in commercial vegetable fermentation brines can be predicted even in the presence of unknown buffer compounds. Research to validate the model showed that pH changes in commercial fermented vegetables can be predicted. Spoilage related pH increase in acidified foods and beverages may be similarly modeled. By determining the buffer capacity of acid and acidified foods using the model, the potential health hazards that may occur (or be prevented) due to microbial activity could be predicted during production of acid and acidified foods, improving safety.

Improved the Capability to Estimate Microbial Water Quality of Stream Water

Microbial water quality of irrigation and recreation water is known to be affected by wildlife inputs and solar radiation, which are not currently accounted for in water quality predictive models. ARS researchers in Beltsville, MD, in collaboration with Korean scientists, developed models to take into account variations in solar radiation and in wildlife contribution to surface water fecal contamination. Testing these models with the long-term microbiological water quality monitoring data has demonstrated improvement of the model accuracy. Results of this work will be useful in development of watershed management programs in which the effect of agriculture on water quality will be estimated more accurately than it is currently.

Parasites

Toxoplasma isolated from organically-raised pigs

Prevalence of *T. gondii* in organically-raised pigs in two locations (Farm 1 and Farm 2) in Michigan was investigated by researchers at Beltsville, MD. Serum and tissue samples from 33 pigs on the farm were available for *T. gondii* evaluation at slaughter. Serological testing was performed using both enzyme-linked immunosorbant assay (ELISA) and the modified agglutination test (MAT). Antibodies to *T. gondii* were detected by both ELISA and MAT in 30 of 33 animals. Hearts of all 33 pigs were bioassayed for *T. gondii*; *T. gondii* was isolated from 17 pigs including one from a seronegative (both ELISA and MAT) pig. Genetic typing of 16 of the 17 *T. gondii* isolates revealed clonal Type II from Farm 1 and clonal Type III on Farm 2. These results revealed very high prevalence of *T. gondii* in organic pigs for the first time in the U.S., indicating potentially increased health risk of consuming meat products from organically-raised swine.

Safety Assessments of Foodborne Hazards, Including Toxicological Studies

Pharmacokinetics of the Industrial Contaminant Perfluorooctane Sulfonate in Beef Cattle

Perfluorooctane sulfonate (PFOS) is a "nonstick" surfactant used in many industrial, commercial, and consumer products. Due to its extensive use, PFOS is widely found in humans, wildlife, and the environment. Cattle are exposed to PFOS while grazing in contaminated areas, but the extent to which PFOS accumulates in their meat is not known. ARS researchers at Fargo, ND, together with scientists at the USDA Food Safety and Inspection Service and North Dakota State University, conducted a metabolism study to determine the degree to which PFOS concentrates in the edible tissues of beef cattle and whether PFOS residues may be a concern for human exposure. The PFOS was not readily excreted, and nearly half of the total dose was still in the blood a month after dosing. PFOS was detected in edible tissues. This study showed that PFOS would likely accumulate in beef exposed to environmental PFOS and that consumption of contaminated beef could be a source of human exposure to PFOS.

Evidence for Fumonisin-induced Disruption of Sphingolipid Metabolism

Studies conducted in Guatemala, in collaboration with the Centro de Investigaciones en Nutricion y Salud in Guatemala (CIENSA), Creighton University, and Duke University show that fumonisin exposure, based on the levels of urinary fumonisin B1, is significantly correlated with the level of sphingoid base 1-phosphates in blood spots and the increase in the sphinganine 1-phosphate to sphingosine 1-phosphate ratio. This finding is consistent with the hypothesis that high levels of fumonisin exposure in humans can lead to disruption of sphingolipid metabolism through inhibition of ceramide synthase. This is significant because every animal disease known to be caused by fumonisin has been shown to be closely correlated with, and preceded by, evidence of disruption of sphingolipid metabolism. The findings also provide a research tool for assessing the threshold for disruption of sphingolipid metabolism in humans and for designing epidemiological studies to evaluate the potential of fumonisin exposure as a contributing factor to human disease.

Adipose Tissue Derived Cytokines Expressed During Molting

Induced molting is an important management tool for the layer producers to maximize the laying life of a flock. Cytokines have been implicated in tissue remodeling during molting but the source of the cytokines is not known. ARS researchers in Athens, GA, suggest that adipose tissue cytokines play a major role in regression of the ovary and oviduct during freed restricted induced molt. Microarray analysis and quantitative real-time polymerase chain reaction (qPCR) indicated little change in adipose tissue gene expression throughout the molt indicating that adipose tissue gene expression per se was not involved in the molting process. This research indicates that research into post transcriptional changes in proteins per se may be necessary to examine the molting process

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Appendix D [USDA/ERS Food Safety Research List for FY 2011 and FY 2012][17]

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[17] *Disclaimer: Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its approval, endorsement, recommendation, or favoring by the United States Government or any department, agency, office, or branch thereof.*

Economic Analysis

Does More Product Testing and Process Control Lead to Safer Meat and Poultry?

The USDA's Agricultural Marketing Service (AMS) is responsible for procuring safe and nutritious food for the National School Lunch Program (NSLP) and sets more stringent food safety standards for its suppliers than those required by the Food Safety and Inspection Service (FSIS). The National Academy of Sciences suggests that AMS relies too much on testing and not enough on ensuring plant-level process control. This goal of this research is to examine the food safety performance of plants processing meat and poultry destined for the NSLP compared to that not destined for this market. We classified plants into four categories: plants that begin producing for the NSLP (market entrants); plants that stop producing for the NSLP (market quitters); plants that continue producing for the NSLP (NSLP plants); plants that never produce for the NSLP (FSIS plants). These four groups enable several tests of changes in food safety performance: (1) the current food safety performance of entrants can be compared to their performance in earlier years, (2) the current performance of quitters can be compared to their performance in earlier years and (3) the performance of suppliers can be compared to their performance when they quit, and (4) the performance of current suppliers can be compared to AMS quitters. In all cases, the pool of plants that have never supplied AMS serves as a control group. Measures of performance are *Salmonella* levels and compliance in performing Hazard Analysis and Critical Control Point (HACCP) and Sanitation Standard Operating Procedure (SSOP) tasks. Preliminary results indicate that NSLP plants outperformed market quitters, but not FSIS plants. Results will shed light on the impact of NSLP food safety requirements in addition to those currently required by FSIS. It will also improve our understanding of why firms opt in and out of supplying the NSLP school food purchase program and the implications of this behavior for school lunch food safety.

Estimation of QALY Loss and Cost of Illness for 14 Major Foodborne Pathogens in the U.S.

The goal of this research is to estimate the annual cost-of-illness and Quality Adjusted Life Year (QALY) loss in the U.S. caused by 14 of the 31 major foodborne pathogens. These 14 pathogens account for 95% of illnesses and hospitalizations and 98% of deaths due to identifiable pathogens estimated by Scallan et al. These 14 pathogens cause an estimated $14.0 billion (ranging from $4.4 to $33.0) in cost of illness and loss of 61,000 QALYs per year (ranging from 19,000 to 145,000). Roughly 90 percent of this loss is caused by five pathogens: nontyphoidal *Salmonella enterica* ($3.3 billion, 17,000 QALYs), *Campylobacter* spp. ($1.7 billion, 13,300 QALYs), *Listeria monocytogenes* ($2.6 billion, 9400 QALYs), *Toxoplasma gondii* ($3 billion, 11,000 QALYs), and Norovirus ($2 billion, 5000 QALYs). A companion publication attributes losses estimated in this study to the consumption of specific categories of foods. The cost of illness and QALY loss estimates are used in regulatory impact analysis and are used in risk-based decision analysis of food safety policy.

Ranking of the Burden of 14 Foodborne Illnesses in the U.S. by Food/Pathogen Pair

Understanding the relative public health impact of major microbiological hazards across the food supply is critical for a risk-based national food safety system. This research estimates the U.S.

health burden of 14 major pathogens in 12 broad categories of food and ranks the resulting 168 pathogen/food combinations. These pathogens include *Campylobacter*, *Clostridium perfringens*, *E. coli* O157:H7, *Listeria monocytogenes*, Norovirus, *Salmonella enterica*, *Toxoplasma gondii*, and all other FoodNet pathogens. Health burden for each pathogen is measured using new estimates of the cost of illness and Quality Adjusted Life Years (QALYs) loss from acute and chronic illness and mortality Disease burden is found to be concentrated among a relatively small number of pathogen/food combinations. The top ten pairs are responsible for over $8 billion and 36,000 lost QALYs, or over 50% of the total across all pairs. Across all fourteen pathogens, poultry, pork, produce, and complex foods are responsible for nearly 60% of total cost of illness and QALY loss. Comparative ranking of the burden of foodborne illness by food/pathogen pairs is used in risk-based decision analysis of food safety policy.

Source Attribution Study for the World Health Organization Global Burden of Disease Foodborne Illness Initiative

The real impact and costs of foodborne diseases globally is unknown. The World Health Organization (WHO) is supporting an initiative to fill this gap by estimating the global burden of foodborne diseases. This initiative will provide estimates of the incidence, burden, and sources of foodborne diseases by WHO and GEMS Diet Cluster Regions. ERS is collaborating in this effort by co-leading an expert elicitation designed to attribute specific foodborne diseases to their contamination sources. The study will help improve public health and strengthen international trade of foods by improving the quality of evidence available to policy-makers and other stakeholder available to inform appropriate, evidence-informed priorities of food safety at the country level.

Exploratory Research on Use of Food Purchase Data and FoodNet Surveillance Data to Estimate the Relationship between Particular Foods and Foodborne Illnesses

Current estimates attributing foodborne illnesses in the U.S. to their food sources rely almost entirely on outbreak data. Outbreaks account for less than 10 percent of foodborne illness in the U.S. Primary microbiological research suggests that the role of specific foods in causing disease may not be the same for outbreaks as for the remaining 90 percent of foodborne illnesses. FoodNet surveillance provides the best data set in the U.S. on total foodborne illness, including both outbreak-related and sporadic illnesses. HomeScan® food purchase data provides a proxy for food consumption. While neither data set provides perfect measures of foodborne illness or food consumption, they are the best data collected on foodborne illness and food consumption in the U.S. This research effort is evaluating the feasibility of using statistical analysis of FoodNet and HomeScan® data to empirically estimate relationships between consumption of specific foods and foodborne illness in the U.S. Improved source attribution estimates will help government agencies, firms, and consumers better evaluate the likely sources of foodborne illness. This is necessary information for risk-based food safety decisions.

Cost of Chronic Foodborne Illness Caused by Shiga Toxin-producing *Escherichia coli* (STECs)

Acute foodborne illnesses can be followed by long term chronic disease. A substantial body of research has developed since 2000 identifying and measuring the rate of chronic diseases that

follow STEC infections. This study will use this research to update ERS cost-of-illness estimates. Because some of this research has been done in Europe, the project will be done in conjunction with researchers at the National Institute for Public Health and the Environment of the Netherlands, which has a leading group of researchers working on measurement of the burden of foodborne illness. This research will provide a more accurate assessment of the burden of STECs in the U.S. and Europe. This will strengthen the evidentiary basis for risk-based domestic policy decisions and in so doing; help strengthen the environment for trade in food between Europe and the U.S.

The Economic Efficiency of Sampling Size: The Case of Beef Trim

The economically optimal sample size in a food safety test balances the marginal costs and marginal benefits of increasing the sample size. The goal of this research is to provide a method for selecting the sample size when testing beef trim for *E. coli* O157:H7 which equates the averted costs of recalls and health damages from contaminated meats sold to consumers with the increased costs of testing while allowing for uncertainty about the underlying prevalence rates of contamination. Using simulations, the results show, in most cases, the optimal sample size is larger than the current sample size of 60 and, in some cases, it exceeds 120. Moreover, lots with a lower prevalence rate have a higher expected damage because contamination is more difficult to detect. Simulations indicate that these lots have a higher optimal sampling rate. This research will help inform sampling protocols used in FSIS inspections.

The Limits of Testing as You Approach Zero Tolerance: The Case of *E. coli* Testing of Beef Trim

In addition to mandatory tests conducted by FSIS, meat packers often voluntarily test beef trim, the primary component to ground beef, for pathogens including *E. coli* O157:H7. These tests are "zero tolerance" because no positive level of the pathogen is permitted, not because the test can intercept all hazardous material or can function without error. The primary purpose of testing is to monitor that the production process is controlling food safety risks in accordance with the plant's Hazard Analysis and Critical Control Points (HACCP) plan. However, testing also allows packers to divert beef trim that tests positive for the pathogen to safe, yet lower-value reconditioned uses. The goals of this research are to examine: (1) the incentives for meat packers to increase their frequency of testing lots of beef trim, (2) the market incentives to improve food safety as a result of testing, and (3) the effectiveness of testing as a filtering mechanism. This research will provide industry and policy makers with a better understanding of the economic incentives informing firm- level decisions about when to test beef and how these decisions affect food safety and efficiency of meat use.

The Mortality Burden from Foodborne Illness in the U.S.

The number of deaths is only one aspect of the mortality burden from foodborne illness because future years of life and economic productivity are both lost when individuals die prematurely. The goal of this research is to estimate the annual losses from deaths due to foodborne illness in the U.S. using three separate methods: the years of life lost (YLL) approach based on average life expectancy, the human capital (HC) approach based on lost productivity, and the value of a

statistical life (VSL) approach based on individual willingness to pay to reduce the risk of mortality adjusted for age. Data to calculate each measure were obtained from estimates of annual deaths by the Centers for Disease Control and Prevention (CDC) and tabulations of reported deaths by age from surveillance systems or death certificates, depending on the pathogen. Mortality is a major driver of estimates of the burden of almost all illnesses. This research will provide more accurate and up-to-date information on the benefits from food safety regulation.

Antibiotic Use in U.S. Livestock Production

Antibiotics are widely used in livestock production for disease treatment, but also for disease prevention and for growth promotion. With growing evidence of resistance to antibiotics used for human and for animal purposes, there is considerable scientific and public policy interest in the economics of antibiotic use. The goal of this research is to evaluate the economics of antibiotics use in U.S. hog, cattle, and poultry production. This will be accomplished by estimating the extent of use in different stages of production and production environments; the alternative inputs and production practices used in place of animal antibiotics; the effects of sub-therapeutic uses of antibiotics on production and financial outcomes at the farm level; and the likely market impacts of proposed restrictions on antibiotics use. The FDA has proposed a new rule which will likely limit the use animal antibiotics for growth promotion. Aside from federal regulations, retailers are imposing changes in production practices, with the express goal of reducing antibiotics use. This project will provide data on changes in use, and ongoing shifts in production practices to meet these goals. The project will also apply a model of the food system to estimate the likely impacts of reduced use on livestock production and prices, meat prices and consumption, and meat trade.

The Cost of Food Safety—Grower/Shipper Food Safety Costs Under the California Leafy Greens Marketing Agreement (LGMA)

Under FSMA, most produce growers will face new food safety regulations to reduce microbial risks at the farm level. There is very little information on the costs of running enhanced food safety programs. The goal of this research is to examine the annual food safety costs of grower/shippers who belong to the California Leafy Greens Marketing Agreement (LGMA), a grower-organized marketing order with food safety practice requirements. This is a demanding food safety program and will provide insight into what upping the food safety standards will cost other parts of the produce industry. Another output will be an assessment of the many challenges of measuring food safety costs. New government food safety requirements will raise the costs of food safety for many growers. An assessment of how much it costs to participate in a high-level voluntary food safety program will provide some insight into the costs of this new legislation.

Grower-Initiated Mandatory Food Safety Programs for Produce

Many produce grower organizations have adopted mandatory, or highly recommended voluntary, food safety programs. In the absence of Federal regulations on minimum food safety standards these private programs have played important roles for produce industries trying to minimize the

impact of one grower's problem on the rest of the industry. The goal of this research was to develop a theoretical model of incentives to growers to engage in voluntary collective actions. The model investigates incentives under various types of liability rules and different levels of ambiguity. This work provides an analytical framework to understand the benefits of this type of program.

Consumers' Response When Regulators are Uncertain about the Source of Foodborne Illness

In 2008, FDA attempted to trace the source of a spike in *Salmonella* infections back to its source. Evidence first pointed to tomatoes and FDA warned consumers to not eat certain types of tomatoes. When consumers continued to get sick, even when the suspect tomatoes were off the market, FDA refined its investigation, first focusing on fresh cilantro (very briefly) and then hot peppers which were the culprit. The goal of this research is to model retail demand for these products and characterize consumers' response to the evolving information. Initially consumers did make distinctions between the types of tomatoes FDA said were potentially contaminated and those that were not. However, total expenditures on all tomatoes also fell, providing some evidence that consumers did not completely escape the consequences of the hazard announcement about some types of tomatoes by making dietary substitutions among the different types. Adjustment back to pre-announcement behavior occurred over a couple of months; consumers were more responsive to the news that tomatoes were a source of potential contamination than the news that tomatoes were considered safe again. Understanding how consumers respond to warnings to avoid certain foods will help agencies and firms better manage these kinds of warnings.

Irradiation for the Mitigation of Pest Risks from Imported Fruit and Vegetables

Expanding U.S. import demand for fresh fruits and vegetables gives rise to concerns with adequately addressing risks to U.S. producers and consumers associated with invasive and non-native insect pests—or quarantine pests—that may enter the country along with fresh produce. Irradiation was approved by USDA as a post-harvest treatment in 2006 and can neutralize a range of quarantine pest problems. The goal of this research is to document the recent growth in U.S. imports of fresh produce, characterize the pest risks associated with these imports, and assess the potential applicability of irradiation technology. It will then examine the economic costs and benefits of irradiation using a case study of Indian mango exports to the U.S. Results from this study will provide data on the grown in U.S. fresh produce imports, and on pest risks associated with these imports. It will evaluate the costs and benefits of using irradiation to control pest risks on produce imports.

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China is the fastest-growing major supplier of food to the U.S., now ranking fourth as a source of U.S. food imports. The goal of this research is to investigate and monitor food safety policies, adjustments in production and marketing arrangements and changes in industry structure in China in response to food policy concerns. The effects of food safety measures on costs and competitiveness of China's agriculture will also be investigated. The project will explore the roles of information asymmetries, consumer trust, and tradeoffs between control and market

power in more concentrated market organizations. This project will report on and analyze China's explorations of new forms of market organization, subsidies, and institutional innovations that are partly driven by food safety concerns: vertical coordination, government promotion of companies and cooperatives, land tenure reforms, subsidies intended to promote farm consolidation, and emergence of "local foods" initiatives. Results from this research will provide insight into how China's international food safety standards/rules are influenced by the institutional structure of the Chinese economy and into divergence between safety of exports and products produced for China's domestic market.

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This research uses a Turkey comparison test and other statistical techniques to show how food safety technology use varies with types of third party audits, plant size, and type of firm (whether the plant is part of a single or multi-plant firm) in six meat and poultry industries. This research is linked to an earlier ERS report on technology use. Plant size and firm type are typical dimensions along which technology use is examined. Third party audits can serve as a proxy for food safety requirements demanded by major buyers, such as fast food chains and grocery stores because buyers use third party audits to ensure compliance with their standards. A better understanding of the types of food safety technologies used by meat and poultry plants and how these vary by plant size and type of firm will help regulatory agencies better target their outreach and regulatory programs.

Food Safety Technology Use in Meat and Poultry

This research is an extension of an earlier study on food safety technology use to include the poultry slaughter, processed meat and poultry, and raw meat and poultry industries. Regression analysis was used to examine the economic forces driving food safety technology use. Economic forces include regulation, a plant's own characteristics, and the demands of major buyers, exporters, and product brands. Technologies come from the ERS food safety survey. Since the nature of demand and not the type of technology is the key to this research, the age of the data is not critical. Results will provide a better understanding to regulatory agencies of the types of technologies used by meat and poultry plants.

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Previous studies suggest that the percent of Americans who consume unpasteurized (raw) milk is increasing (now roughly 5 percent of all U.S. consumers up from 1.5 percent a decade ago). FDA and other public health agencies warn consumers that unpasteurized milk poses higher risks of several foodborne diseases. There has been momentum building in many States to reduce the restrictions on the sale of unpasteurized milk. Some advocates are pressuring the Federal government to make it legal to sell unpasteurized milk across State lines. Very little is known about consumers in the U.S. who consume raw milk. The goal of this research is to provide an analysis of the demographics of raw milk consumers. Information from this study is timely as it could help target educational efforts on the consumption of unpasteurized milk and it could be helpful if new State and Federal milk regulations are considered and enacted.

Food Safety in Industrialized Countries

This project evaluates whether similarities in food safety regimes lead to greater trade among countries. Cross-country data and case studies of particular foodborne illnesses will be analyzed to determine the different degrees of success in achieving food safety. A model-based analysis will be used to determine whether similarities in food safety import regimes have an effect on trade in selected agricultural commodities. This research will focus on industrialized countries and nations that trade in animal products. These nations trade a great deal with the US, and are likely to have similar regulatory regimes. The research will address several questions: 1) Do countries with more similar food safety regimes trade more? 2) How can countries measure success in food safety? 3) What methods are countries currently using to measure success in food safety? Knowing how different countries approach food safety can provide insight into how to improve food safety, particularly if some countries have been very successful at reducing the impacts of foodborne illness. Additionally, food safety standards and differences across countries have altered trade patterns, in some cases leading to reductions in trade with developing countries.

Foregoing Subtherapeutic Antibiotics: the Impact on Broiler Grow-out Operations

Data from a national survey of broiler grow-out operations were used to analyze the use of sub-therapeutic antibiotics (STAs) in 2006. Forty-two percent of growers did not use STAs in their feed or water, and instead relied on a set of other practices, including pathogen testing, expanded sanitary protocols, altered feeding regimens, and HACCP plans, to maintain production. STA suspension has no statistically significant impact on production, given other inputs, but producers who forego STAs receive higher contract fees, suggesting that they bear higher costs to realize a given level of output. Results from this research provide data on one important element of the cost of restrictions on antibiotics use, to be use in cost-benefit analyses of proposed regulations. It also provides information on the non-medical alternatives to antibiotic use implemented on current contract operations, including alternative sanitation, ventilation, feeding, and testing regimens.

Factors Determining Milk Quality, and Implications for Production Structure under Somatic Cell Count (SCC) Standard Modification

Consumer and processor demand for high quality milk has placed increasing pressure on U.S. milk producers to achieve higher product standards. International standards for somatic cell count (SCC) are becoming more stringent, but in May 2011, the United States National Conference on Interstate Milk Shipments chose to retain the 750 thousand cells per milliliter standard. The goal of this research was to model producer and farm-level characteristics associated with SCC. Dairy Costs and Returns Report data provide information on the physical structure, input expenses, demographics, and outputs for farms in selected states. Location outside the Southeast, lower herd age, full-time farming status, use of biosecurity guidelines, good milking facilities and operations management, and application of related quality tests are all associated with lower SCC levels and thus higher-quality milk. Size of operation had little effect on SCC levels. Many of the operations that did not attain a more demanding SCC standard of

400 thousand cells per milliliter had older operators, expressed intention to exit within 10 years, were smaller size and were more likely to be located in the Southeast when compared to those meeting the tighter standard. The results suggest that the stricter scheme favors larger farms that are more committed to production and are less likely to be sole or family proprietorships. Policies intended to lower SCC count and improve milk quality are best applied to the southeast and/or should focus on lowering average herd age, using and abiding by biosecurity guidelines, and maintaining good milking facilities.

The Impacts of Food Scare Events on Brand Choice and Consumption

Food safety failures or "scares" affect food demand, prices and price margins. The objective of this research is to: (1) estimate the effect of "food scare events" on brand choice and consumption; and (2) identify the demographic profiles of consumer segments associated with different levels of changes in their preferences for national store brands as well as those who are more likely to stop consuming the adversely affected product category.

Market Effects of Alternative Food Safety Regulations for Fruits, Vegetables, and Tree Nuts

The goal of this research is to develop economic models of the fruit, vegetable and tree nut markets to assess the effects of alternative food safety regulations on relative food prices, dietary choices in the U.S., and on imports and exports of these foods. The project is focusing on mandatory recalls, traceability programs, and certification of safety practices for food moving in international trade. It considers whether the impact of regulations and food safety outcomes differ by the size of farm or firm and whether food is sold locally or shipped further distances.

FSMA Section 110(g) Report
Appendix D [USDA/ERS Food Safety Research List for FY 2011 and FY 2012][18]

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Economic Analysis

Does More Product Testing and Process Control Lead to Safer Meat and Poultry?

The USDA's Agricultural Marketing Service (AMS) is responsible for procuring safe and nutritious food for the National School Lunch Program (NSLP) and sets more stringent food safety standards for its suppliers than those required by the Food Safety and Inspection Service (FSIS). The National Academy of Sciences suggests that AMS relies too much on testing and not enough on ensuring plant-level process control. This goal of this research is to examine the food safety performance of plants processing meat and poultry destined for the NSLP compared to that not destined for this market. We classified plants into four categories: plants that begin producing for the NSLP (market entrants); plants that stop producing for the NSLP (market quitters); plants that continue producing for the NSLP (NSLP plants); plants that never produce for the NSLP (FSIS plants). These four groups enable several tests of changes in food safety performance: (1) the current food safety performance of entrants can be compared to their performance in earlier years, (2) the current performance of quitters can be compared to their performance in earlier years and (3) the performance of suppliers can be compared to their performance when they quit, and (4) the performance of current suppliers can be compared to AMS quitters. In all cases, the pool of plants that have never supplied AMS serves as a control group. Measures of performance are *Salmonella* levels and compliance in performing Hazard Analysis and Critical Control Point (HACCP) and Sanitation Standard Operating Procedure (SSOP) tasks. Preliminary results indicate that NSLP plants outperformed market quitters, but not FSIS plants. Results will shed light on the impact of NSLP food safety requirements in addition to those currently required by FSIS. It will also improve our understanding of why firms opt in and out of supplying the NSLP school food purchase program and the implications of this behavior for school lunch food safety.

Estimation of QALY Loss and Cost of Illness for 14 Major Foodborne Pathogens in the U.S.

The goal of this research is to estimate the annual cost-of-illness and Quality Adjusted Life Year (QALY) loss in the U.S. caused by 14 of the 31 major foodborne pathogens. These 14 pathogens account for 95% of illnesses and hospitalizations and 98% of deaths due to identifiable pathogens estimated by Scallan et al. These 14 pathogens cause an estimated $14.0 billion (ranging from $4.4 to $33.0) in cost of illness and loss of 61,000 QALYs per year (ranging from 19,000 to 145,000). Roughly 90 percent of this loss is caused by five pathogens: nontyphoidal *Salmonella enterica* ($3.3 billion, 17,000 QALYs), *Campylobacter* spp. ($1.7 billion, 13,300 QALYs), *Listeria monocytogenes* ($2.6 billion, 9400 QALYs), *Toxoplasma gondii* ($3 billion, 11,000 QALYs), and Norovirus ($2 billion, 5000 QALYs). A companion publication attributes losses estimated in this study to the consumption of specific categories of foods. The cost of illness and QALY loss estimates are used in regulatory impact analysis and are used in risk-based decision analysis of food safety policy.

Ranking of the Burden of 14 Foodborne Illnesses in the U.S. by Food/Pathogen Pair

Understanding the relative public health impact of major microbiological hazards across the food supply is critical for a risk-based national food safety system. This research estimates the U.S.

health burden of 14 major pathogens in 12 broad categories of food and ranks the resulting 168 pathogen/food combinations. These pathogens include *Campylobacter*, *Clostridium perfringens*, *E. coli* O157:H7, *Listeria monocytogenes*, Norovirus, *Salmonella enterica*, *Toxoplasma gondii*, and all other FoodNet pathogens. Health burden for each pathogen is measured using new estimates of the cost of illness and Quality Adjusted Life Years (QALYs) loss from acute and chronic illness and mortality Disease burden is found to be concentrated among a relatively small number of pathogen/food combinations. The top ten pairs are responsible for over $8 billion and 36,000 lost QALYs, or over 50% of the total across all pairs. Across all fourteen pathogens, poultry, pork, produce, and complex foods are responsible for nearly 60% of total cost of illness and QALY loss. Comparative ranking of the burden of foodborne illness by food/pathogen pairs is used in risk-based decision analysis of food safety policy.

Source Attribution Study for the World Health Organization Global Burden of Disease Foodborne Illness Initiative

The real impact and costs of foodborne diseases globally is unknown. The World Health Organization (WHO) is supporting an initiative to fill this gap by estimating the global burden of foodborne diseases. This initiative will provide estimates of the incidence, burden, and sources of foodborne diseases by WHO and GEMS Diet Cluster Regions. ERS is collaborating in this effort by co-leading an expert elicitation designed to attribute specific foodborne diseases to their contamination sources. The study will help improve public health and strengthen international trade of foods by improving the quality of evidence available to policy-makers and other stakeholder available to inform appropriate, evidence-informed priorities of food safety at the country level.

Exploratory Research on Use of Food Purchase Data and FoodNet Surveillance Data to Estimate the Relationship between Particular Foods and Foodborne Illnesses

Current estimates attributing foodborne illnesses in the U.S. to their food sources rely almost entirely on outbreak data. Outbreaks account for less than 10 percent of foodborne illness in the U.S. Primary microbiological research suggests that the role of specific foods in causing disease may not be the same for outbreaks as for the remaining 90 percent of foodborne illnesses. FoodNet surveillance provides the best data set in the U.S. on total foodborne illness, including both outbreak-related and sporadic illnesses. HomeScan® food purchase data provides a proxy for food consumption. While neither data set provides perfect measures of foodborne illness or food consumption, they are the best data collected on foodborne illness and food consumption in the U.S. This research effort is evaluating the feasibility of using statistical analysis of FoodNet and HomeScan® data to empirically estimate relationships between consumption of specific foods and foodborne illness in the U.S. Improved source attribution estimates will help government agencies, firms, and consumers better evaluate the likely sources of foodborne illness. This is necessary information for risk-based food safety decisions.

Cost of Chronic Foodborne Illness Caused by Shiga Toxin-producing *Escherichia coli* (STECs)

Acute foodborne illnesses can be followed by long term chronic disease. A substantial body of research has developed since 2000 identifying and measuring the rate of chronic diseases that

follow STEC infections. This study will use this research to update ERS cost-of-illness estimates. Because some of this research has been done in Europe, the project will be done in conjunction with researchers at the National Institute for Public Health and the Environment of the Netherlands, which has a leading group of researchers working on measurement of the burden of foodborne illness. This research will provide a more accurate assessment of the burden of STECs in the U.S. and Europe. This will strengthen the evidentiary basis for risk-based domestic policy decisions and in so doing; help strengthen the environment for trade in food between Europe and the U.S.

The Economic Efficiency of Sampling Size: The Case of Beef Trim

The economically optimal sample size in a food safety test balances the marginal costs and marginal benefits of increasing the sample size. The goal of this research is to provide a method for selecting the sample size when testing beef trim for *E. coli* O157:H7 which equates the averted costs of recalls and health damages from contaminated meats sold to consumers with the increased costs of testing while allowing for uncertainty about the underlying prevalence rates of contamination. Using simulations, the results show, in most cases, the optimal sample size is larger than the current sample size of 60 and, in some cases, it exceeds 120. Moreover, lots with a lower prevalence rate have a higher expected damage because contamination is more difficult to detect. Simulations indicate that these lots have a higher optimal sampling rate. This research will help inform sampling protocols used in FSIS inspections.

The Limits of Testing as You Approach Zero Tolerance: The Case of *E. coli* Testing of Beef Trim

In addition to mandatory tests conducted by FSIS, meat packers often voluntarily test beef trim, the primary component to ground beef, for pathogens including *E. coli* O157:H7. These tests are "zero tolerance" because no positive level of the pathogen is permitted, not because the test can intercept all hazardous material or can function without error. The primary purpose of testing is to monitor that the production process is controlling food safety risks in accordance with the plant's Hazard Analysis and Critical Control Points (HACCP) plan. However, testing also allows packers to divert beef trim that tests positive for the pathogen to safe, yet lower-value reconditioned uses. The goals of this research are to examine: (1) the incentives for meat packers to increase their frequency of testing lots of beef trim, (2) the market incentives to improve food safety as a result of testing, and (3) the effectiveness of testing as a filtering mechanism. This research will provide industry and policy makers with a better understanding of the economic incentives informing firm- level decisions about when to test beef and how these decisions affect food safety and efficiency of meat use.

The Mortality Burden from Foodborne Illness in the U.S.

The number of deaths is only one aspect of the mortality burden from foodborne illness because future years of life and economic productivity are both lost when individuals die prematurely. The goal of this research is to estimate the annual losses from deaths due to foodborne illness in the U.S. using three separate methods: the years of life lost (YLL) approach based on average life expectancy, the human capital (HC) approach based on lost productivity, and the value of a

statistical life (VSL) approach based on individual willingness to pay to reduce the risk of mortality adjusted for age. Data to calculate each measure were obtained from estimates of annual deaths by the Centers for Disease Control and Prevention (CDC) and tabulations of reported deaths by age from surveillance systems or death certificates, depending on the pathogen. Mortality is a major driver of estimates of the burden of almost all illnesses. This research will provide more accurate and up-to-date information on the benefits from food safety regulation.

Antibiotic Use in U.S. Livestock Production

Antibiotics are widely used in livestock production for disease treatment, but also for disease prevention and for growth promotion. With growing evidence of resistance to antibiotics used for human and for animal purposes, there is considerable scientific and public policy interest in the economics of antibiotic use. The goal of this research is to evaluate the economics of antibiotics use in U.S. hog, cattle, and poultry production. This will be accomplished by estimating the extent of use in different stages of production and production environments; the alternative inputs and production practices used in place of animal antibiotics; the effects of sub-therapeutic uses of antibiotics on production and financial outcomes at the farm level; and the likely market impacts of proposed restrictions on antibiotics use. The FDA has proposed a new rule which will likely limit the use animal antibiotics for growth promotion. Aside from federal regulations, retailers are imposing changes in production practices, with the express goal of reducing antibiotics use. This project will provide data on changes in use, and ongoing shifts in production practices to meet these goals. The project will also apply a model of the food system to estimate the likely impacts of reduced use on livestock production and prices, meat prices and consumption, and meat trade.

The Cost of Food Safety—Grower/Shipper Food Safety Costs Under the California Leafy Greens Marketing Agreement (LGMA)

Under FSMA, most produce growers will face new food safety regulations to reduce microbial risks at the farm level. There is very little information on the costs of running enhanced food safety programs. The goal of this research is to examine the annual food safety costs of grower/shippers who belong to the California Leafy Greens Marketing Agreement (LGMA), a grower-organized marketing order with food safety practice requirements. This is a demanding food safety program and will provide insight into what upping the food safety standards will cost other parts of the produce industry. Another output will be an assessment of the many challenges of measuring food safety costs. New government food safety requirements will raise the costs of food safety for many growers. An assessment of how much it costs to participate in a high-level voluntary food safety program will provide some insight into the costs of this new legislation.

Grower-Initiated Mandatory Food Safety Programs for Produce

Many produce grower organizations have adopted mandatory, or highly recommended voluntary, food safety programs. In the absence of Federal regulations on minimum food safety standards these private programs have played important roles for produce industries trying to minimize the

impact of one grower's problem on the rest of the industry. The goal of this research was to develop a theoretical model of incentives to growers to engage in voluntary collective actions. The model investigates incentives under various types of liability rules and different levels of ambiguity. This work provides an analytical framework to understand the benefits of this type of program.

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Appendix F [NIFA Food Safety Research Awards for FY 2010 and FY 2011 (Research Conducted in FY 2011 and FY 2012)][19]

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Prevention, Intervention, and Control of Foodborne Hazards

Chemical Contaminants

Improved Breeding and Variety Evaluation Methods to Reduce Acrylamide Content and Increase Quality in Processed Potato Product

Potato is the most consumed vegetable in the U.S., with production valued at $3.5 billion. This applied research will facilitate the rapid, efficient development and adoption of new potato varieties that have exceptional agronomic, processing and consumer acceptance traits. An immediate critical need will be to proactively reduce the acrylamide content of processed potato products in order to mitigate health concerns that have arisen with regard to acrylamide in food, and to remove the economic uncertainty associated with potential regulatory actions focused on acrylamide.

Dietary Flavonoids as Reactive Carbonyl Scavengers to Prevent the Formation of Advanced Glycation End Products

This research will investigate whether dietary flavonoids can trap reactive dicarbonyl species and therefore prevent the formation of advanced glycation end products (AGEs) during food processing and long-term storage under in vivo conditions. The researchers hypothesize that dietary flavonoids can trap reactive dicarbonyl compounds (e.g. MGO and GO) and thus inhibit the formation of AGEs.

Microbial Pathogens

Salmonella

Salmonella Thermal Resistance during Desiccation and Rehydration in Low Water Activity Foods

This research will use microbiological challenge studies and new molecular tools to study the stress response of *Salmonella* during desiccation and rehydration prior to and during industrial thermal processing. This work will provide important data on the heat resistance of *Salmonella* after different treatments of desiccation and rehydration, and it will provide a mechanistic understanding of the response to *Salmonella* under conditions of desiccation stress prior to heat stress that will allow the design of effective validation studies.

Contamination of Fruits, Nuts, and Vegetables by Filamentous *Salmonella*: Persistence and Virulence

This research will study the formation and persistence of Stress-Induced Filamentous *Salmonella* (SIFS) on fruits, nuts, and vegetables. Results will provide fundamental information on the formation and survival properties of SIFS that can influence persistence on fruits, nuts, and vegetables and virulence of this pathogen.

Reducing Egg-borne Outbreaks of *Salmonella* Enteritidis by Integrating Research and Extension

This applied research and extension project will investigate whether plant ingredients are effective at reducing S. Enteritidis in layers and eggs when given to birds through feed. In addition, the research will investigate whether plant ingredients are effective at killing *Salmonella* on eggs when used in an antimicrobial wash. Consumer acceptance and the overall safety of these products (eggs containing plant ingredients) will be evaluated. For the extension and outreach component of this research, the project team will develop, implement and evaluate *Salmonella* control programs for poultry farmers, egg producers and processors through extension programs, workshops, and educational materials.

Development of an Updated and Optimized Egg Quality Assurance Program to Minimize *Salmonella* Enteritidis Contamination of Shell Eggs

This project will integrate applied research and outreach to develop an updated and optimized Egg Quality Assurance Program (EQAP) aimed at reducing *Salmonella* Enteritidis (SE) contamination of shell eggs. The knowledge gained will be disseminated to poultry producers and stakeholders through extension programs, meetings, workshops, and educational materials. This study will identify critical control points, and update and optimize PEQAP for use by producers throughout the U.S. to prevent *Salmonella* contamination of shell eggs.

Solutions for the Food Safety Threat Posed by Salmonella in the Lymph Nodes of Cattle Presented for Harvest

This integrated, multi-disciplinary, and multi-state project team will conduct a series of experimental and observational studies to better understand the biology, epidemiology, and ecology of *Salmonella* in lymph nodes. These studies will be used to explore control strategies and identify those that are most effective. The researchers will use this knowledge to frame best practices that effectively mitigate the risk posed by *Salmonella* in lymph nodes.

E. coli O157:H7 and STEC

Effects of Dietary Energy Source on Colonic Microbial Ecology of Cattle

This research will reduce the pathogen load of *E. coli* 0157 in beef cattle through feed interventions. The researchers will compare the colonic microbial ecology between cattle fed corn-based or distillers' grain-based diets and link the specific changes in colonic microbial ecology with the presence and magnitude and *E. coli* 0157 shedding among naturally exposed groups of cattle.

C-DI-GMP Signaling in *E. coli* O157:H7 Biofilm Formation and Colonization of the Gastrointestinal Tract of Beef Cattle

This research will evaluate the role of c-di-GMP in *E. coli* O157:H7 biofilm formation and gut colonization. The research will impact the beef industry by providing insight into methods to

reduce or eliminate *E. coli* O157:H7 biofilm formation, as well as its colonization in the GI tract of beef cattle, improving the safety of beef.

Influence of Moisture ad Phyllosphere Microbiota on the Persistence of *E. coli* O157:H7 on Lettuce Plant

This research will determine the effects of abiotic environmental conditions (specifically moisture and temperature) and composition of the phyllosphere microbiota (biotic conditions) on the survival of virulent and avirulent (attenuated) *E. coli* O157:H7 strains on Romaine lettuce plants. This research will integrate results from field-trials with an attenuated *E. coli* O157:H7 strain with controlled growth-chamber studies using virulent isolates of the pathogen.

Reduction of *Escherichia coli* O157:H7 on Small-scale Cow/Calf Operations Using Best Management Practices

Researchers will determine educational gaps and assess production practices employed by small-scale cow/calf operations through survey-based research. They will determine the factors affecting *E. coli* O157:H7 prevalence on small-scale cow/calf operations in Louisiana and Texas. They will establish a training program on Best Management Practices (BMPs) and pre-harvest food safety for cow/calf producers across Louisiana and Texas. Lastly, they will validate the effectiveness of on-farm BMPs in reducing *E. coli* O157:H7 contamination in the cow/calf operation environments.

Reducing Seasonal increases in STEC Prevalence in Cattle to Reduce Human Exposures and Improve Public Health

This research will identify factors responsible for seasonal variation in STEC shedding using a combination of experimental and observational studies on the farm. Researchers will examine the nature of bovine supershedding and its contribution to seasonal variation in STEC shedding and evaluate practical interventions (immunization, feeds and feeding, and clear water supplies) to reduce STEC fecal shedding by cattle. This research will provide significant new information about the nature and biological basis of seasonal variation of cattle STEC colonization, which is necessary for the development of practical, cost-effective interventions to reduce the seasonally-increased human exposures and disease incidence.

Listeria

Minimizing Risk of *L. monocytogenes* Cross-Contamination of Deli RTE Foods by Developing New Sanitation Techniques and Evaluating Cost Effective Training Techniques

This applied research will evaluate the most commonly used computer-based food safety employee training programs. The project team will build an evaluation matrix that measures knowledge of the elements of the FDA's Model Food Code following participation at multiple training sites. They will then compare the commercial computer-based training to the newly developed evaluation matrix.

Vibrio

Improving Oyster Quality by Enhancing Natural Immunity

The researchers will develop simple tests for measuring antimicrobial polypeptides (AMPPs) and then determine the storage conditions that result in the highest AMPP levels. They will determine how AMPP levels affect the concentration of *Vibrio*s and other bacteria in oyster tissues and determine the susceptibility of a number of nonpathogenic versus pathogenic *Vibrio* isolates to these AMPPs. Research results will provide a consistent method for increasing AMPP levels in oyster tissue and subsequently greatly reduce *Vibrio* contamination in oyster tissues.

Campylobacter

Farm-level Practices to Reduce *Campylobacter* Prevalence in Commercial Turkeys

This applied research and extension project will identify and characterize routes and mechanisms of transmission of *Campylobacter* in turkey production, and farm-level practices associated with *Campylobacter*-negative flocks. Results will contribute to a reduction in the prevalence of *Campylobacter* in poultry, and thus towards a decrease in the incidence of human foodborne illness.

Other Bacterial Pathogens

Developing Scientifically-based Consensus Food Safety Metrics for Leafy Greens and Tomatoes

This applied research will provide scientific knowledge/data and develop analytical validation and verification tools that can be implemented on a national basis for both domestic and imported produce. These goals will be achieved by combining data from growers, packers, and processors with etiologic, intervention, and challenge studies using pathogens (e.g. enterohemorrhagic *E. coli*, S. enteric) and/or appropriate surrogate microorganisms.

The Survival of Bacterial and Viral Pathogens in Manure and Biosolids in the Southeastern United States

This research will determine inactivation/survival rates for enteric bacterial pathogens and norovirus from soils receiving manure or Class B biosolids at agronomic application rates. Researchers will provide identical comparisons of the two residual amendments across multiple southeastern U.S. soil types. The results of this study will be related to established USDA-AMS and USEPA regulations for the land application of manure and biosolids to soils intended for the growth of food and non-food crops.

Advanced Processing Technologies as Multiple Hurdles to Inactivate STEC and Viruses during Beef Processing and on Non-intact Beef Products

This research will evaluate the efficacy and feasibility for integrating several processing technologies such as EO water, LA-SDS wash, IR heating, radio frequency, UV radiation, and UV-TiO2 photocatalysis into cattle slaughter establishments and meat processing facilities as a multiple hurdle to inactivate STEC, NoV, and other pathogens on beef, non-intact beef, and RTE beef products.

Control of Foodborne Bacterial and Viral Pathogens Using Microwave Technologies

Intervention strategies are needed in the food industry to reduce food-borne illness, which continues to be a critical public health issue. The goal of this research is to bridge scientific and engineering gaps that limit commercial applications of microwave technologies for the control of bacterial and viral pathogens in packaged foods, particularly ready-to-eat foods, deli meats, and seafoods.

Enhancing the Safety of Non-thermally Processed Apple Juice by Combining Microfiltration with Ultraviolet Treatment

The main goal of this integrated, multidisciplinary, multi-institutional project will be to reduce the microbial risks associated with non-thermally processed apple juice. This will help ensure the safety of apple juice, while retaining its freshness, nutritional and sensory properties, and extending its shelf life.

Role of Surface-related Factors on Contamination and Survival of Pathogens in Fresh Produce Grown in Texas and Mexico

This research will evaluate persistence of pathogenic bacteria on the surface of produce. The project team will investigate whether these contaminants can be removed by the application of antimicrobials, and how this process is affected by product surface properties such as surface tension, available nutrients, and the presence of specific microflora. The team will also investigate whether this process is affected by seasonality and irrigation methods used in Texas and Mexico.

Reducing *Salmonella* Enteritidis and *Campylobacter jejuni* in Chickens by Dietary Supplementation of Plant-derived Antimicrobial Molecules

The overall goal of this research is to improve microbiological safety of poultry products. The research will investigate the effect of trans-cinnamaldehyde, carvacrol, thymol, and eugenol as dietary supplements on reducing colonization of S. Enteritidis and *C. jejuni* in broiler chickens as well as the effect of plant molecules on gene expression in S. Enteritidis and *C. jejuni* using DNA microarrays. The safety of these substances will be evaluated in in chickens using histopathological analyses of internal organs.

Integrating National Resource Information and Food System Signals to Identify Novel Methods for Control of Microbial Contamination in Spinach

This research will use a spinach production system to identify new and improved ways to control foodborne pathogens in produce at the pre-harvest level. The approach will allow identification of novel strategies to control foodborne pathogens in spinach at the pre-harvest level based on appreciation of the local management practices and ecological conditions.

Nanoengineered Surfaces for Controlling the Attachment of Pathogenic and Biofilm Forming Bacteria to Food Contact Surfaces

Adherence of microorganisms to surfaces and subsequent biofilm formation in food processing environments leads to increased opportunity for microbial contamination of processed foods. A key element in the fight against pathogenic microorganisms in the food industry is the use of food contact materials that are not only cleanable, but could also prevent microbial adherence and biofilm formation. The research team will evaluate the effect of nanoscale surface details on the attachment of pathogenic and biofilm forming bacteria relevant to food processing, then use this knowledge to develop nanostructured food contact surfaces with both microbial repellant and microbial killing properties.

Development of Antimicrobial Food Processing Surfaces by Nanoscale Surface Modification

This research will design and characterize self-sanitizing processing surfaces through nanoscale surface modification. By making nanoscale changes to the surface of common materials, researchers will introduce antimicrobial nanostructures, which can repeatedly recharge antimicrobial activity after rinsing in chlorine sanitizing solution. The resulting materials will exert antimicrobial activity against a broad range of food pathogens and spoilage organisms. Materials will regain antimicrobial activity during sanitization, making them practical for use in food processing environments.

Super-repellent Antimicrobial Coatings to Ensure Food Safety

This research will develop super-repellent antimicrobial nanostructured coatings for food processing facilities to eliminate a major external source of bacterial infection for food. The researchers believe these antimicrobial coatings can be applied to food processing facilities and peripheral surfaces (drains, floors, storage tanks, apparel, etc.), and will have the potential to greatly reduce bacterial attachment and biofilm formation, thus reducing foodborne pathogens.

An Evidence-based Photonovella to Encourage Safe Meat Handling and Cooking Practices Among Low Socio-economic Status African Americans

Researchers will conduct focus groups to determine access to and understanding of current nationally available food safety educational materials among African Americans in Chicago. They will identify knowledge gaps for safe handling and preparation of meat, attitudes toward this information, access to and use of meat thermometers among these consumers. Research results will guide the development of a culturally-appropriate educational photonovella that will target these knowledge gaps and will be easily accessible at clinics.

Improving the Safety and Nutritional Adequacy of the Home Food Supply of Elderly Recipients of Home Delivered Meals

Researchers will partner with the Meals on Wheels Association of America and selected Meals on Wheels agencies in five states to conduct an in-depth study of homebound, elderly individuals who receive home-delivered meals Agency employees will test a labeling system in which the food delivered to the clients will be date-labeled to alert the clients when the food is no longer safe for consumption. This applied research will identify and address the critical food safety and nutrition needs of this vulnerable population, particularly during times of emergency.

Helping Childbearing-aged Women Make Informed Decisions Regarding Seafood Consumption

This applied research will determine the effectiveness of a new web-based education program called X-Train on improving the food safety knowledge, attitudes and behavior of childbearing-aged women.

Integrated Strategies to Reduce Foodborne Illness and Food Allergic Reactions Associated with Independently Operated Restaurants

This project will use an integrated and applied approach to develop a food safety education training program for Spanish restaurant workers at independently-operated establishments. The program will include components that address risk management strategies for food allergens.
The Role of Biofilms as a Reservoir for Foodborne Pathogens in Irrigation Systems

This project will help producers and processors assess microbial water quality in irrigation systems and its impact on produce contamination by enteric pathogens. The results from this research will be used in a quantitative microbial risk assessment (QMRA) to estimate the magnitude of the risk and the probability of adverse effects of exposure to these pathogens based on their occurrence in biofilms.

Cost-effective Pathogen Reduction Strategies and Food Safety Training for Small and Very Small Meat Processors in Georgia

This project will identify suitable and cost-effective pre-slaughter spraying treatments using small home-scale and low-cost equipment commercially available to generate electrolyzed (EO) and ozonated water. Researchers will determine the efficacy of various spray-washing solutions in reducing foodborne pathogens on goats at pre-slaughter.

An Integrated Approach to Enhance the Microbial Safety of Fresh-cut Fruits and Vegetables during Processing, Packaging, and Distribution

This multidisciplinary and multi-institutional project will identify commercial slicing and dicing practices that increase the risk for cross-contamination of fresh-cut produce. The researchers will develop novel packaging strategies for minimizing pathogen growth/survival in the cold-

chain and reduce the risk of foodborne illnesses from fresh-cut produce through a series of training activities aimed at processors, retailers, foodservice workers, and regulators.

Improving Process Validation Methods for Multiple Pasteurization Technologies Applied to Low-moisture Foods

The overall goal of this project is to reduce the risk of salmonellosis associated with low-moisture foods by giving the industry sound scientific data, knowledge, and tools that are needed to ensure the effectiveness of processing interventions. The project team will develop quantitative mathematical models, develop inactivation models, and develop and assess training tools.

Development and Evaluation of Standardized, Competency-based Food Safety Education and Training Programs for the Food Industry

This project will develop, implement, and evaluate high-quality, highly-scalable, standardized education and training materials for the global food system that will improve worker knowledge, promote positive worker behaviors and reduce the incidence of food safety hazards in food products.

Enteric Viruses

Inactivation of Enteric Foodborne Viruses in High Risk Foods by Non-thermal Processing Technologies

This study will focus its efforts on the most significant foodborne enteric viruses, including human norovirus, hepatitis A virus, and rotavirus. The overall goal will be to identify effective non-thermal processing technologies and to optimize processing parameters to destroy these viruses in shellfish (oysters and clams), fresh and frozen berries (strawberries, raspberries and blueberries), berry purees, green onions, and salsa. In addition, the efficacy of these non-thermal processing technologies will be tested on pathogenic bacteria that cause large outbreaks in these high risk foods, such as *Vibrio* parahaemolyticus in shellfish and *Escherichia coli* O157:H7 and *Salmonella* in other products. The effect of the processing technologies on the quality of foods will also be evaluated. The knowledge gained will be disseminated through education curricula and outreach programs.

An Integrated Approach to Prevent and Minimize Foodborne Enteric Viruses in Vegetables and Fruits

The overall goals of this project will be to understand the attachment, internalization, and survival of foodborne viruses in vegetables and fruits, to develop novel sanitization and process technologies to inactivate the viruses, and to minimize virus contamination in fresh produce via extension, outreach and education programs using cultivable murine norovirus (NoV) and hepatitis A virus (HAV) as models to study the human enteric viruses.

Hand Hygiene Promotion: An Essential Strategy for Preventing Foodborne Illness in Elementary Schools

This integrated and applied research will reduce the incidence of foodborne infections from human norovirus in elementary schools by developing mathematical models that investigate environmental and institutional factors. These factors will include the use of alcohol-based hand rubs versus traditional hand washing. A hand hygiene campaign for elementary schools will be developed and implemented.

Detection of Microbial, Chemical, and Radiological Hazards in Food, Feed, and Dietary Supplements

Allergens and Gluten

Development of Monoclonal Antibody Based Immunoassays for Tree Nut Detection and Quantification

This research will develop monoclonal antibody (MAb)-based immunoassays to detect and quantify trace amounts of almonds and cashew nuts in various foods, as both are often implicated in tree nut-induced allergies in humans. The researchers will assess effects of various processing methods on the stability of select epitopes on targeted almond and cashew nut proteins.

Microbial Pathogens

Simultaneous Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) and *Salmonella* from Farm to Fork

This research will develop a novel, rapid, sensitive, and specific detection protocol for STEC and *Salmonella*, from pre-harvest through consumption of food products. Researchers will develop an optical microplate array that is a label-free colorimetric method that can simultaneously and visibly detect multiple pathogens (STEC and *Salmonella*) through controlling DNA hybridization conditions.

Study on Prevalence and Characterization of Shiga Toxin-Producing *E. coli* (STEC) from Cattle Farms in the Arkansas Delta Region

This research will determine the prevalence of Shiga toxin-producing *E. coli* (STEC) in cattle farms located in the Arkansas Delta region, and develop a sensitive detection assay for STEC in farm environments. Researchers will develop a novel approach for assessing STEC prevalence by focusing on only small-sized farms (30 to 40 mature cows on average) in the Arkansas Delta region, primarily managed by family members. The study will focus on mature cows, commonly used for the production of ground beef, the main food source of human STEC infection.

Rapid Detection of Foodborne Pathogenic Bacteria by Surface Enhanced Raman Spectroscopy Using Ag Nanorod Array Substrates

This research will develop a rapid detection technique for foodborne pathogenic bacteria using nanorod array surface enhanced Raman spectroscopy (SERS). Researchers will determine the SERS signatures for different pathogenic bacteria and the method to differentiate them. They will design SERS substrates for lower detection limits and differentiate different pathogenic bacteria strains and livability using SERS. They will then test the detectability of target pathogenic bacteria using a meat system. This will enable the ultimate development of portable, rapid and sensitive biosensors with on-the-spot interpretation of results for target pathogens.

Magnetoelastic Biosensors for Detection of Pathogens on Globe Fruit

This applied research will develop, demonstrate and field test inexpensive, accurate, easy to use biosensors for the detection of *Salmonella* contamination of fresh globe fruits (tomatoes, cantaloupes and watermelons). These biosensors will be used in the field to identify critical hazard points, i.e. when and where bacterial contamination enters the system.

Foodborne Pathogens Detection and Education to Support Sustainable Agriculture for Small Scale Producers

The project will develop nucleic acid-biosensor-based detection assays that will have simultaneous or multiplexed detection capacity. The researchers will integrate the specific, real time, PCR-based foodborne pathogen detection tools and antibodies with impedometric biosensor technologies and hand-held surface plasmon resonance (SPR), respectively.

Molecular Characterization of Foodborne Pathogens as It Relates to Research on the Mechanism of Disease and/or Epidemiology of Foodborne Disease

Salmonella

Defining *Salmonella* Genes Important for Colonization and Persistence in Poultry

This research will use a novel strategy to identify the genes involved in persistence that can then be targeted for vaccines or drug discovery. Identification of Typhimurium genes involved in these important biological processes is an essential stepping stone to the long term goal of developing novel strategies to eliminate cecal carriage of this organism in chickens.

Revealing the Mechanisms of Competitive Exclusion of Enteropathogens from the Intestinal Microbial Community

The researchers will perform quantitative compositional and functional metagenomic analyses of the broiler intestinal microbial community. Further, they will determine the in vivo transcriptional response of *Salmonella* to the presence of permissive or exclusive intestinal microbial communities. This information will help determine the colonization, competition and invasion behavior of *Salmonella* in the presence of a complex intestinal microbial community.

E. coli O157:H7 and STEC

Quantification of the Relationship Between Distiller's Grains Co-products and *E. coli* O157:H7 Load in Real-world, Commercial Feedlots

This research will quantitatively describe epidemiologically relevant drivers of pathogen burden in cattle populations. The researchers will identify, describe, and quantify feedlot- and pen-level variables that modify the relationship between distillers' grains and *E. coli* O157:H7 load on feedlots.

Development of a Novel Multilocus Sequence Typing (MLST) Subtyping Strategy for Tracking the Farm to Fork Transmission of *Escherichia coli* O157:H7

This research will test the effectiveness of the multilocus sequence typing (MLST) method to track and control the spread of *E. coli* O157:H7 strains within meat-processing facilities. The researchers will develop an MLST scheme for *E. coli* O157:H7 that would be useful for tracking the source of contamination during epidemiologic investigations.

Composition of the Gastrointestinal (GI) Microbiota and Predisposition to Enterohemorrhagic *Eshcherichia coli* (EHEC) Colonization as Complex Polygenic Traits in Beef Cattle

Because EHECs exist within complex microbial communities that colonize the bovine GI tract, the researchers will investigate these communities in association with high- or low- levels of EHEC shedding ("shedder" phenotype), on one hand, and the animal's genotype on the other hand. They will first identify correlations between specific features of microbial community

composition or functionality and "shedder" phenotype. They will then analyze the animals' genome for regions and candidate genes that exert influence over the patterns of microbial colonization in the gut. Aside from the insight into the biological underpinnings of EHEC shedding, this study promises to discover genes and markers associated with supershedders and thus equip animal breeders with a new tool for minimizing their number.

Host, Genetic, Microbial, and Environmental Factors Associated with Shiga Toxin-Producing *Escherichia coli* (STEC)

This research will identify bacterial genotypes and epidemiological factors important for shedding in multiple herds over time. Researchers will compare the composition, diversity and function of the microbial communities within the rectoanal junction and ruminal fluid of STEC shedders and non-shedders, determine how STEC affects the bovine immune response, identify STEC inhibitors from the ruminal fluid of non-shedders, and test control strategies aimed at decreasing shedding levels. The long term goal is to assess how bacterial, epidemiological, immunological, and host factors work together to impact shedding.

Listeria

Mechanisms of Synergistic Combinations of Growth Inhibitors for *Listeria monocytogenes* on Ready-to-eat (RTE) Seafoods

This research will identify and characterize the mechanisms of growth inhibitor combinations that result in synergistic effects on growth inhibition for *Listeria monocytogenes* in cold smoked salmon. This information will allow for development of effective, synergistic combinations of growth inhibitors for *Listeria monocytogenes*.

Campylobacter

Regulation of the CTS Type II Secretion System and Its Role in Genetic Variation of *Campylobacter jejuni*

This research will address genetic exchange and regulation, strain variation, and antibiotic resistance in the naturally competent bacterium *Campylobacter jejuni*. The research will identify factors important for the uptake and integration of extracellular DNA into the *Campylobacter* chromosome.

Characterization of Genes Involved in the Production of Pili by *Campylobacter jejuni*

Campylobacter jejuni is the leading cause of bacterial gastroenteritis in the U.S. Pili play an important role in the attachment of the bacterium to the host cell and are therefore considered important vaccine targets. This research will assess the role of the putative pili in the colonization of poultry and will contribute significant knowledge regarding virulence in *C. jejuni*, and determine whether Cj1343c would be a viable vaccine target.

Antimicrobial Resistance/Susceptibility of Foodborne Microorganisms

Effectiveness of Reduced Agricultural Antimicrobial Usage as a Food Safety Intervention

This research will quantify the impact of reduced antimicrobial use on food-borne pathogens at pre-harvest. It will assess the response of targeted fecal commensal bacterial populations to reduced antimicrobial use. Lastly, it will characterize the effect of antimicrobial use on the risk of zoonotic food-borne transmission of resistant pathogens. Results will provide data necessary for science-based public policies for antimicrobial use.

Effect of Antimicrobial Peptides on Growth and Survival of *Vibrio spp*. and Their Potential Application to Postharvest Treatment of Oysters

The long-term goal of the research is to provide a greater understanding of the feasibility of using an alternate post-harvest processing method involving application of antimicrobial peptides (AMPs) to raw oysters. The research will evaluate the susceptibility of the three primary *Vibrio* pathogens to various AMPs and determine AMP concentrations that induce growth inhibition and/or death in these bacteria.

Practical Interventions to Effectively Manage Antibiotic Resistance in Beef and Dairy Cattle Systems: A Fully Integrated Approach

The researchers' goals are to identify, evaluate, and implement practical interventions for managing antibiotic resistance in beef and dairy cattle systems. The researchers will also evaluate the potential impact of commensal bacteria on antibiotic resistance across host and environmental ecosystems.

Minimizing Antibiotic Resistance Transmission: A Dairy Farm as a Model System

This project will look at a combination of approaches necessary to reduce antibiotic resistance transmission along the food chain, including judicious use of antibiotics, targeted interventions for infection control, and communication of effective interventions to enable their broad-based implementation.

Reducing the Transmission of AMR Organisms by Wildlife within the Food Supply: A Research, Control, and Outreach Strategy

This applied research and extension project will provide an understanding of how food-producing animals acquire antibiotic resistant organisms and how this transmission can be mitigated. This knowledge will contribute to the overall control of antibiotic resistance throughout the food chain and enhance the safety of the food supply. The information will be communicated to a variety of stakeholders: the public, farmers, other scientists, and policy-makers.

An Integrated Approach to Control Methicillin-resistant *Staphylococcus aureus* and *Clostridium difficile* from Limited Resource Poultry

This integrated and applied research and extension project will determine the occurrence of Methicillin-Resistant Staphylococcus Aureas (MRSA) with acquired resistance to selected antimicrobial agents in poultry and pork products, food animals, and the farm environment. Educational materials on safe poultry management and practices for handling retail raw meats will be developed and disseminated to limited-resource poultry and pig producers and consumers.

Designed Epidemiologic Studies of Foodborne Illness/Associated Organisms

Differential Epidemiology and Ecology of Clinical and Bovine-biased Genotypes of *Escherichia coli* O157:H7

This research will determine if the management techniques used in cattle farming to reduce *E. coli* O157:H7 infection work on the strains of *E. coli* O157:H7 most involved in human illness. This work is necessary to ensure that the control measures applied to cattle are working on the strains of the bacterium that cause human illness.

Identification and Control of Microbiological Hazards in Fruits and Vegetables: A Field Epidemiological and Intervention Study in Mexico

This research will identify, quantify and prevent fecally-associated pathogen contamination in produce and, in so doing, engage the U.S. and Mexican agricultural community. This study will help to better characterize safe fruit and vegetable practices and therefore reduce the number of outbreaks related to fruits and vegetables.

Molecular Epidemiology of *Clostridium difficile* Food Contamination: Links to Human CDI

The researchers believe that humans are exposed to *Costridium difficile* (CD) via consumption of foods of animal origin. To test this hypothesis, they will obtain and characterize CD isolates from foods and from humans with CDI in Arizona. Phylogenetic relationships will be established among strains to infer source and route of transmission to humans. The researchers will use multilocus variable-number tandem-repeat analysis (MLVA) to infer temporal relationships among strains and to suggest directionality of transmission.

Risk Assessment, Modeling, Management, and Communication

Assessing Risks and Implementing Food Safety Practices: Developing an On-farm Decision Tree for Fresh Produce Growers

The primary objective of this project will be to develop and evaluate an on-farm decision tree to assist growers with risk assessment, GAPs implementation, and farm food safety plan development. This on-farm decision tree will allow growers to evaluate their own unique operations to identify microbiological, chemical, and physical hazards and generate a list of farm-specific food safety risks. The researchers will then prioritize the implementation of practices that would most effectively reduce the identified risks.

Toxins

Risk Assessment and Intervention Strategies for the Emerging Food Safety Threat of Ochratoxin in the United States

This research will provide data on the emerging Ochratoxin (OTA) issues by conducting a comprehensive national survey followed by a health risk assessment of foodborne OTA for the general public and high-risk populations. Researchers will also investigate effective strategies to reduce the exposure of the public from OTA.

Microbial Pathogens

Staphylococcus aureus: Is Raw Meat a Risk Factor for Colonization and Infection

Several recent publications have documented a high prevalence of *S. aureus* in meat products. The researchers will examine the epidemiology of *S. aureus* associated with raw meat, and determine the effect of meat contamination with *S. aureus* on human colonization. The researchers hypothesize that humans are exposed to *S. aureus* from food animals via handling of foods of animal origin.

Managing the Emerging Risk of Trichinellosis in Organic and Free Range Pork

Researchers will develop serological assays that differentiate swine chronically infected with zoonotic T. spiralis from swine only transiently exposed to T. murrelli. This importance of this work is augmented by recent findings that transient exposure to T. murrelli may render an animal immune to subsequent infection with T. spiralis. Thus, the presence of T. murrelli in the American environment may decrease the risks associated with outdoor swine husbandry. Success in developing and applying discriminatory diagnoses will enable the testing of this hypothesis.

Advancing the Understanding of the Zoonotic Risk of ExPECs and Evaluation of a Vaccine for the Control of These Contaminants in Poultry Products

This research will improve the understanding of how these bacteria are transmitted from chickens to humans by analyzing chicken food-products, including meat and eggs (vehicles) and chicken intestines (reservoir), for the presence of human-like ExPEC bacteria and evaluating the strains that cause human diseases using animal models. Moreover, the research will evaluate a safe, easy-to-use *Salmonella*-based vaccine to protect against ExPEC infections in chickens and reduce or eliminate the risk of contamination in poultry products.

A Systems Approach to Managing Microbial Threats to Greenhouse Tomatoes

This applied research will use a systems-based, trans-disciplinary approach to design Best Management Practices (BMPs) and develop tools that will improve the efficiency, profitability, safety and economic competitiveness of the greenhouse tomato industry. The systems approach will consider all phases of tomato production from seeding through propagation, production and harvest. It will identify key problems/obstacles, set priorities, develop solutions, assess the economic impact of solutions, and provide outreach to a broad community of greenhouse tomato growers.

A Comprehensive Study Investigating the Potential Health Risk of *Clostridium difficile* as a Foodborne Pathogen

This research will determine the occurrence of *C. difficile* in ground beef, pork and chicken in retail stores in CT, PA, MN and AL. The characteristics of *C. difficile* isolated from food will be compared with those of human isolates from the respective states. Researchers will determine the effect of chilling, freezing, cooking, salt, and acidity on *C. difficile*. This research will improve understanding about the mode of transmission, viability characteristics in foods, and susceptibility of *C. difficile* to the common antimicrobial steps applied in foods.

Foodborne Pathogen Persistence: From Identification of Risk Factors to Communication of Control Strategies

The overall goal of this project is to integrate applied research and outreach to augment knowledge regarding pathogen persistence. Combined field studies and molecular subtyping will be performed to identify *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7 strains that persist in the food processing plant environment to probe risk factors for persistence and phenotypes that may contribute to persistence.

Assessing Food Safety and the Persistence of Enteric Pathogens on Fresh Produce Ingredients Destined for Uncooked Sauces/Salsa

This integrated applied research and extension project will increase knowledge of the food safety risks of raw fresh herbs and fresh produce items used in uncooked or unprocessed or minimally processed sauces and condiments such as cilantro, green onions, serrano, jalapeno, and small hot peppers commonly found in Mexican, Oriental and Latino cuisines. The project will focus on understanding the distribution and prevalence of selected foodborne pathogens associated with these produces items and how current practices correlate with pathogen survival and localization.

Assessment and Reduction of Produce Food Safety Risks in the School Foodservice System

The goals of this project are to identify, assess, and reduce produce food safety risks in school foodservice systems via quantitative and qualitative research techniques and development and evaluation of a food safety training program targeting produce safety. Specifically, this project will target employees and managers in secondary school foodservice where self-service salad bars are frequently offered to middle school and high school students.

Safety Assessments of Foodborne Hazards, including Toxicological Studies

Nanomaterials

Influence of Nanoparticle Characteristics on Fate, Bioavailability, and Toxicity of Food-grade Nanoemulsions

Currently, there is only limited knowledge on the effects of nanoemulsions on the fate of the food components in the nanoemulsions. The researchers will address this lack of knowledge by systematically examining the impact of specific nanoparticle characteristics on the digestion, absorption and safety of beta-carotene encapsulated in nanoemulsions using both cell culture and animal models.

Nanoparticle Contamination of Agricultural Crop Species

Engineered nanomaterials (NM) are currently being incorporated into pesticides and fertilizers. Nanomaterial impacts on agricultural plants and potential trophic transfer is unknown. This research will characterize the toxicity, accumulation, and trophic transfer of nanomaterials by agricultural crops, including potential impacts on co-existing contaminants, and will explore potential risks to humans from exposure through food chain contamination.