Retrospective Review
Accomplishment Report for the 2006-2010 Action Plan

National Program 108 – Food Safety

Panel Assessment of Accomplishments Report

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Executive Summary:

The Review Team, a panel of scientists with expertise in the many disciplines represented in Food Safety, ARS National Program 108, met on 12-13 May 2009 to conduct an retrospective assessment of the major accomplishments of NP 108 and their impacts on food safety. A Retrospective Review Accomplishment Report for the 2006-2010 Action Plan and the Plan had been supplied before the meeting and the respective sections had been assigned to two scientists for assessment in depth. After each scientist reported on their respective Problem Statement within the specific Component and a discussion was held, an assessment was completed in relation to the Outputs listed for each Problem Statement. The complete set of Problem Statements with their respective Outputs, Review Team Rating, and Review Team Assessment are contained in this Report.

The Review Team was impressed with the strengths of many of the research activities and critical where necessary of those that did not address the stated outputs related to the Action Plan. The scientists working in NP108 are well recognized for excellence in their respective disciplines and many have published extensively. In some cases the research reported under a specific Problem Statement did not seem to be appropriate for that specific area and could be more appropriately placed in another area. In several cases it appeared that better coordination among projects working in similar areas would have benefitted from coordination and sharing of techniques and results. The arbitrary division of preharvest and postharvest may not be in the best interest of research on food safety. Coordination and a holistic approach to certain problems may be beneficial in the future. Although chemical contamination is in the title of the two sections of Component 1, only minimal efforts were directed at chemical contamination. In some cases the research appeared to not be appropriately concerned about food safety, but rather about traditional animal and crop production and how to protect the system from adverse contamination.

Individual Reviewers on the Team identified a number of areas of strengths; consequently, representative notice should be given to some examples. Methodology related to detection and validation was identified as especially strong as was intervention and pathogenicity research. Mycotoxin research is world class but appeared to be heavily directed toward crop production.

In conclusion, food safety activities within NP 108 over the past three years have been guided by the specific components of an Action Plan. This approach has been useful in creating a unified, national direction for ARS Food Safety research, and was successful in supporting achievement of numerous high-impact projects.

Introduction

Background

Food Safety, National Program 108 (NP 108) is one of 22 National Programs (NP) within USDA-Agricultural Research Service (ARS). The National Programs are organized within four broad program areas: Nutrition, Food Safety and Quality (NFSQ); Animal Production and Protection (APP); Crop Production and Protection (CPP); and Natural Resources and Sustainable Agricultural Systems (NRSAS). The Food Safety National Program is part of NFSQ. Collaborations and/or interactions do occur between the food safety researchers and some other National Programs, in particular NP 107 Human Nutrition, NP 306 Quality and Utilization of Agricultural Products, NP 103 Animal Health, NP 106 Aquaculture, and NP 206 Manure and Byproduct Utilization. The National Program structure allows ARS to link scientists in laboratories around the country to address research problems of regional, national and international interest.

The 2006-2010 NP108 Action Plan when originally developed in 2005 was under Goal 3 of the 2003-2007 ARS Strategic Plan. Research Outputs supported Objective 3.1: "To provide science-based knowledge on the safe production, storage, processing, and handling of plant and animal products and on the detection and control of toxin-producing and/or pathogenic bacteria and fungi, parasites, chemical contaminants, mycotoxins, and plant toxins so as to assist regulatory agencies and the food industry in reducing the incidence of foodborne illnesses". There were two Performance Measures: 3.1.1: Develop new on-farm preharvest systems, practices, and products to reduce pathogen and toxin contamination of animal- and plant-derived foods. Target: Develop practices and/or products that reduce preharvest contamination of major animal- and plant-derived food products; and 3.1.2: Develop and transfer to Federal agencies and the private sector systems that rapidly and accurately detect, identify, and differentiate the most critical and economically important foodborne microbial pathogens. Target: Develop practices and/or products that reduce postharvest contamination of major animal- and plant-derived food products.

Food Safety now is under Goal 4 of the current USDA-ARS Strategic Plan (February 2007): To Enhance Protection and Safety of the Nation's Agriculture and Food Supply. The Objective 4.1 of the Goal is to: "Provide the scientific knowledge to reduce the incidence of foodborne illnesses in the U.S. For the Nation to have affordable and safe food, the food system must be protected at each step from production to consumption. The production and distribution system for food in the United States encompasses a diverse, extensive, and easily accessible system that is open to the introduction of pathogens (bacteria, viruses and parasites), bacterial toxins, fungal toxins (mycotoxins), and chemical contaminants through natural processes, global commerce, and intentional means. In response to these threats, crop and livestock production systems must be protected during production, processing and preparation from pathogens, toxins, and chemicals that cause disease in humans." To ensure security of a production system, ARS conducts basic, applied and developmental research resulting in new technologies, new and improved management practices, pest management strategies, sustainable production systems, and methods of controlling potential contaminants. These ARS activities are key to providing a

safe, plentiful, diverse, and affordable supply of food, fiber, and other agricultural products. The key outcome is the reduction in foodborne illnesses associated with the consumption of meat, poultry and egg products.

In response to the new ARS Strategic Plan the Program Mission and Visions Statements were also revised. The new Mission Statement is: "NP108 provides through scientific research, the means to ensure that the food supply is safe and secure for consumers and that food and feed meet foreign and domestic regulatory requirements. Food safety research seeks 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. Since food safety and food security are global issues, our research program involves both national and international collaborations through formal and informal partnerships. Accomplishments and outcomes are utilized in national and international strategies delivering research results to regulatory agencies, commodity organizations, industry and consumers". The new Vision Statement is "To increase public health through the development of technologies which protect food from pathogens, toxins, and chemical contamination during production, processing, and preparation thus increasing the safety of the food supply."

2006-2010 Food Safety Action Plan

There are two research Components in the NP108 2006-2010 Action Plan. Component 1. Pathogens, Toxin and Chemical Contaminants: subdivided in preharvest and postharvest. Component 2. Mycotoxins and Plant Toxins.

The Action Plan is a working document, dynamic in nature, where the goals and objectives are subject to realignment and modification when necessary. Information from various sources was used to develop the Action Plan relevant to stakeholder, partners and customers, including regulatory agencies, policy makers, producers, commodity organizations, public interest groups and other scientists. The 2006-2010 Action Plan was developed based on managerial divisions of responsibility within NP 108. Dr. Robens was responsible for Components 1.1 and 2.1 and Dr. Lindsay for Component 1.2. Problem Statements, within each of the Components were identified, as were research needs, outputs, their predicted impact, and the location resources. ARS scientists at each of the laboratories participating in this and related National Programs used the Action Plan to develop Project Plans that describe the research to be conducted in each laboratory. The Project Plans provide detailed information on objectives, anticipated products or information to be generated, the approach that will be used, roles and responsibilities of ARS scientists and their cooperators, and timelines and milestones to measure progress of the research. All Project Plans were reviewed for scientific quality by an external panel of experts in the field, through the independent Office of Scientific Quality Research (OSQR). ARS scientists use input from the review panel to revise and improve their planned research. Cooperation occurs among ARS laboratories to develop the products and achieve the outcomes identified in the Action Plan. Cooperators from academia, other agencies, and industry are full partners in the research and in the outreach and technology transfer activities resulting from the research.

Report Construction and what it Reflects:

This Accomplishment Report was prepared by NP108 National Program Leaders, Dr. James Lindsay and Dr. Mary Torrence. The Report consists of major research accomplishments and their impact, in the fiscal year 2006 through 2008, with inclusion of predicted accomplishments and their impact for 2009 and 2010. Not all accomplishments during the period are listed since this would be too extensive a document. Instead Program scientists were requested to provide the Program leaders with a list of their projects, major/critical accomplishments, and their impact (2 page limit in total). These accomplishments were subsequently edited for length and clarity by Drs. Lindsay and Torrence.

The compiled list of accomplishments provided a broad picture of the research conducted in the Program. The Accomplishment Report was organized into three sections including an Introduction plus a section for each of the Components. Each Component contains a summary of the research findings followed by accomplishments organized under the Problem Areas in each Component.

The scientists were asked to address the impact of the accomplishment, noting that any assessment of an accomplishment's impact is inherently a qualitative and imprecise science. A series of criteria were identified in order to impartially conduct the evaluation, these were:

- Did the research advance the knowledge of food safety?
- Was the research innovative?
- Was there technology transfer?
- Was there regulation and policy development?
- Was there academic, industry and/or consumer relevance?

Further, it was critical that "actual impact" as described by the project was likely to be far less that the "potential impact". There is always a considerable gap between real and perceived impact; the practice of doing science and its transfer into usefulness."

Output-Assessment

Component 1.1: Pathogens, Toxins and Chemical Contaminants Preharvest

Problem Statement 1.1.1 Methodology

Outputs

- Methods to recover, identify, and quantitate pathogens and chemical residues in the tissues and matrices, including animal manure, where they are expected to be found, or are needed for regulatory purposes.
- Methods to recover and enumerate "non-culturable" pathogens from all environmental niches and to selectively enhance the growth of extremely slow growing organisms.
- Methods that are sufficiently rapid and cost effective to be useful to both producers and regulatory agencies.
- Genetic and serologic bases for new methods that will provide differentiation of bacterial clones to the extent needed for attribution of these sources.
- Highly specific serological tests with the necessary sensitivity to detect animal carriers.
- Development of microarrays to assess gene expression in various environments.

Review Team Rating:

High Impact

Review Team Assessment:

As efforts to enhance food safety have intensified in recent years, the need for rapid, accurate, cost effective and sensitive methodologies to detect and quantify foodborne hazards of biological and chemical nature has also increased. Methods are needed for laboratory as well as field use and for application to varying substrates and environments, including animal tissues, fluids and waste, produce, the environment, and foods. The methods are necessary for hazard monitoring, which is needed in studying pathogen ecology, epidemiology and control. In addition to detection and quantification, methods for pathogen typing and molecular characterization are also needed in order to address modern food safety assurance needs. Modern detection methodologies are based on molecular, genomic and proteomic approaches. The role of ARS in this arena is in developing and validating methodologies for regulatory, industrial and research applications, while in many cases methodologies are applicable to more than one of these sectors. Effective detection methodologies are useful in identifying and tracking biological pathogens and chemical hazards, developing and validating intervention technologies as well as predictive mathematical models for growth or control of pathogens, and carrying out risk assessments. Overall, availability of appropriate analytical methodologies is useful to regulatory agencies and the industry in their efforts to provide safe foods to consumers.

Accomplishments achieved are associated with most of the above listed objectives, at least for certain hazards, and substrates or target applications. Included are outputs involving use of plasmids and genetic modifications of strains in ecological studies; genetic methods for identification and characterization of serotypes such as those of shigatoxin producing *Escherichia coli*; spectral imaging or molecular approaches for differentiation of pathogenic from non-pathogenic *Campylobacter* and *Cryptosporidium*, respectively; the need for detection methodology for viable but non-culturable *Campylobacter* following exposure to dryness/temperature stresses; DNA microarray typing of *Campylobacter*; isolation of a gentamicin-resistant *Campylobacter* strain which may be useful in the study of the ecology of the pathogen; identification of common *Salmonella* serotypes by PCR; the potential use of the lux gene in *Salmonella* ecology; valuable studies on development of biofilms and an epi-fluorescence assay for enumeration of *Campyobacter jejuni* cells in biofilms; real-time PCR quantification of *Clostridium perfringens*; an ELISA method for a biomarker indicator of infection associated stress in chicken; real-time PCR methods for various pathogens of food safety and defense interest; and, method validation and application training for *Trichinella* detection in various meat products as required for their export.

In general, most of the goals listed were addressed and major advances were made in many areas. Some outcomes are very impressive, while others found immediate practical application or should be useful for further research on important food safety issues. Some of the output may be of major economic benefit, while others should lead to better or more rapid detection and/or identification of hazards or will facilitate future research on pathogen ecology or other food safety issues. Although not all output were of the same scientific significance, some are very important from a practical point of view.

No outputs were listed in association with detection of chemical hazards, toxins, or commensal bacteria. Some of the work appears to be related more to animal health than food safety issues. Some of the work deals with enhancement of sampling for microbial isolation and detection, which is useful but not directly related to detection methodology. Sampling and sample preparation could receive more attention in the future.

Additional necessary and valuable work, but not exactly associated with methodology development, is that dealing with *Trichinella* detection in horse, game and pork meat for exports, although other projects have developed improved methodologies.

For detection methods related to serotyping and subtyping of pathogenic microorganisms the current work is useful but additional focus needs to be put on detection methods for more effective identification. For example, PFGE is a very time consuming method; development of methods that are faster and yield equivalent resolution is desirable**.**

Component 1.1: Pathogens, Toxins and Chemical Contaminants Preharvest

Problem Statement 1.1.2 Epidemiology

Outputs

- Development of coordinated food animal surveillance and epidemiology programs for food animal species which recognizes early warning signs of pathogen infection.
- Estimates of the basic reproductive ratios (Ro's) for epizootic pathogens in various food animal species for epidemiologic modeling and more accurate prediction of the effectiveness of interventions.
- Epidemiological studies that elucidate chemical or pathogen presence and behavior in particularly important, suspected biological niches, including animal manure and other environmental niches.

Review Team Rating:

Low Impact

Review Team Assessment:

Research needs specified in the action plan identified the Collaboration for Animal Health ad Food Safety Epidemiology (CAHFSE) as a preferred approach. This approach was intended to address repeat testing over an extended period of time at all points of production. However, as outputs, CAHFSE results were not identified. The outputs focused on single time points of contamination. In addition, there was no focus on associating results from various points in time. Outcomes for epidemiology appeared to be more related to those for ecology (section 1.2.3) and frequently do not have direct relationships to food safety.

The reasons for why the CAHFSE approach was not followed should be articulated so that barriers can be addressed for future work that should be done on epidemiology. It is a critical need and the barriers to the development of the coordinated food-animal epidemiology should be overcome.

Component 1.1: Pathogens, Toxins and Chemical Contaminants Preharvest

Problem Statement 1.1.3 Ecology, Host Pathogen and Chemical Contaminants Relationships

Outputs

- Use of molecular methods and genomics to assess the dynamics of the microbial intestinal flora and to more thoroughly understand the dynamics of various gut environments of food producing animals in order to elucidate effective means for improved control of food pathogens in the pre-harvest stage.
- Use of such techniques as oligonucleotide fingerprinting of rDNA genes to assess species diversity and identify novel genes in order to identify opportunities for interventions to control pathogens.
- Locating sequestration and deposition sites, and metabolic pathways to determine factors affecting both holding and release of chemical residues and pathogens.
- Identification of SNP's (single nucleotide polymorphisms) that identify genes associated with host resistance and pathogen virulence and shedding.
- Identification of environmental sites, both physical and biological, which provide pathogen reservoirs and sites of amplification.
- Knowledge of the interrelationships among production practices, animal well- being and pathogen shedding.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

The ecology of microbial populations in live animals is a very significant aspect of food safety. Not only is the work significant as a stand-alone research area, it is also provides important input to a number of other research areas, as evidenced by the strong overlap with many other program areas. In general, the research objectives were well defined and many of the objectives were met or suggested important new research directions.

ARS research in this area has demonstrated that the diversity of organisms in the gut has a very significant impact on numerous virulence factors of food-borne pathogens. Understanding the influence of feed, drugs and other factors on the overall ecology of gut micro flora will provide important insight into controlling antibiotic resistance, transfer of virulence factors, protective areas and overall levels of pathogens. For example, the identification of rumen protozoa as an important ecological niche for *Salmonella* is a significant finding and research aimed at understanding this relationship, as well as, finding other "protective" microbial interactions will be important to reducing or eliminating carriage.

The emphasis on food producing animals is important and certainly warranted given the food-borne disease risk. However, similar risks occur in fruits, nuts and vegetables and these issues

need to be addressed as well. Unlike live animal control efforts, the diversity of matrix and environmental factors associated with produce complicates research efforts in this area.

Given the emphasis on ecology, and in particular, on various gut environments, there is little being done on populations and population dynamics. Methods are focused on existing, narrowly focused technology (SNP's, rDNA, etc) and little is being done to look beyond these technologies. Additionally, quorum sensing has been identified as an important indicator for finding or causing pathogens to adapt or survive. Also, see section 1.2.8.

Research into the environmental fate of, disposition, metabolism, excretion and persistence in animals, for numerous chemical contaminants such as perchlorate and polybrominated diphenyl ethers remains an important food safety issue. This information is important to providing baseline information and filling data gaps for risk assessment, risk analysis and other data inputs for evaluating public health concerns. Predicting new chemical hazards in the environmental or in feed is often very difficult, yet sufficient infrastructure needs to exist to assess and measure newly indentified chemicals or chemical toxins. Therefore, a strong research program in both maintaining analytical capabilities and developing new analytical platforms remains a critical priority.

Component 1.1: Pathogens, Toxins and Chemical Contaminants Preharvest

Problem Statement 1.1.4 Intervention Strategies

Outputs

- Bacterial metabolic targets that allow interventions to be closely focused to specific metabolic pathways.
- Interventions targeting specific metabolic endpoints.
- Vaccines that decrease shedding of epizootic pathogens at the time of slaughter.
- Effective interventions that also increase animal or bird productivity.
- Effective modifications of production practices that particularly address animal welfare concerns and /or dietary components in reducing pathogens.
- Interventions that prevent colonization or exclude pathogens from the gut.

Review Team Rating:

Medium - Low Impact

Review Team Assessment:

In order to enhance the safety of food at the time of consumption, there is a need to control contamination at every step of the food chain or system. Control of pathogen contamination at the pre-harvest stage may be accomplished through interventions that limit sources of contamination, reduce prevalence and levels of pathogens, and minimize transfer of contamination to animals, water and foods. One target of pathogen control pre-harvest is to reduce colonization and shedding of zoonotic pathogens by food producing animals. As no single intervention for pathogen control is available, a useful approach is to implement multiple strategies, including interventions that can be used just prior to slaughter. In order for interventions to be more effective, there is a need to better understand the epidemiology and ecology of pathogens in the environment, animals, foods and humans.

The accomplishments as provided to the review panel raised several concerns. Some of the work is not applicable to the outputs defined; other accomplishments describe work that is not recent, novel, nor necessarily effective. Briefly summarizing some of the accomplishments and panel concerns: The work on the progression of *Salmonella* infection in swine was viewed as egregious and does not belong in this program. The identification of *Salmonella* genes for survival in the swine stomach is interesting; however, usefulness to the eventual development of an intervention is questioned. The effects of feed withdrawal and transportation on *Salmonella* contamination of market-age pigs is not new information, and specific intervention strategies arising from this work are not offered. The observation that orange peels and pulp (currently fed to cattle) and their oil extracts exhibit anti-pathogenic activity *in vitro* is interesting, however, effectiveness *in vivo* may not be realized and it may be economically and technologically infeasible to use their oil extracts in cattle feed. Findings that an alfalfa diet during poultry molting may reduce presence of *Salmonella* in eggs was reported in the 2004 NP Review and no further development reported here. Combining control of ambient temperature during pre-refrigeration storage of eggs

with proper refrigerated storage (as is current practice) limited *Salmonella* growth in eggs; this is not an unexpected observation, however, what is the magnitude of potential hazard or risk reduction associated with this additional intervention? Spraying eggs with chemical polymers in combination with biocides provided better sanitization of broiler hatching eggs against *Salmonella* without negative effects on hatchability when tested experimentally – this is an interesting and potentially valuable finding if effective commercially. Another accomplishment describes a novel probiotic, active against *Salmonella* and *Campylobacter* in poultry, which has been commercially available since 2007 – the work for this product would have been conducted prior to this current research program cycle; however, information of value would be the evidence of effectiveness in reductions of *Salmonella* in broilers chicken meat today. Aluminum sulfate and sodium bisulfate treatment of poultry litter reduced *Campylobacter* in chickens, however, it is not clear whether this finding is novel? Ractopamine, which is approved for use in feedlot cattle and finishing swine to increase muscle mass, decreased shedding of *E. coli* O157:H7 in cattle – while an antimicrobial effective would be a useful side-effect to the primary use of the product, it is not clear whether work on ractopamine as an antimicrobial agent, should be a priority considering that use of such compounds in animal feeding is a controversial issue. Good production practices for control of *Toxoplasma* and *Trichinella* infections were provided to the swine industry – this work was for the most part previously reported in 2004; however, such programs are useful to producers. Virus-like particles that may affect severity of the disease were identified in *Cryptosporidium;* this observation is of interest but of unclear food safety implications.

The above examples of detailed assessment indicate much of the information generated in this work area does not seem capable of producing any practical pathogen control strategy.

Research on pathogen control in the field or pre-harvest is interesting and may be useful in controlling pathogen sources and reservoirs; however, outputs linked to presence of pathogens on eventual food products is not addressed. It is evident that perhaps with the exception of *Salmonella* in poultry production, there is a need for good epidemiological studies in the pre-harvest sector.

The 2004 Assessment panel noted that the epidemiology of *Salmonella* in broilers has been investigated sufficiently, and more intervention strategies need to be a focus; environmental influences have been determined and might suggest increased focus on intervention strategies. It is not clear in the present Accomplishment Report that this program has been refocused as strongly recommended.

Component 1.1: Pathogens, Toxins and Chemical Contaminants Preharvest

Problem Statement 1.1.5 Antibiotic Resistance

Outputs

- Development of food animal surveillance and epidemiology programs, particularly the Collaboration for Animal Health and Food Safety Epidemiology (CAHFSE), together with other USDA agencies to assure early detection of epizootic pathogens and unique antibiotic resistance patterns.
- Information about rates and patterns of spread of AR gene flow, among pathogenic and commensal microorganisms and in different environments of various food producing animals, including animal manure. This information can then be used for population genetic studies to map the spread of specific clones in populations across the U.S., and in risk assessments to help assess the effects of antibiotics in animal medicine.
- Identifying linkages of bacterial AR genes to other genes affecting other aspects of bacterial physiology, particularly pathogenicity factors.
- Combining information on the AR of isolates with genetic or other attributes to obtain specific identification and exclusion characteristics for isolates.

Review Team Rating:

Low – Medium Impact

Review Team Assessment:

The importance of antibiotic resistance cannot be understated with respect to animal agriculture and its relationship to public health. Therefore, it is important that this is part of the research focus. The overall strength of the stated accomplishments is the contributions to surveillance and being able to understand how resistance trends are changing. The interaction of NARMS with FDA and CDC gives it a public health influence. In addition, one project had a focus on commensal microbes which are likely to be important in shifting overall resistance patterns, particularly since the commensal microbes far out number the pathogens. The linkage of ARS research to NARMS is excellent and is an important and effective interagency collaboration. However, it is not clear whether the NARMS surveillance system mainly addresses pathogens in sick animals or whether there is a significant tie to normal animals. To be an effective surveillance system that can be used to track antibiotic resistance trends, normal animals receiving antibiotics is an essential target group. The majority of the impacts cited (beyond NARMS) mainly are related to surveillance. It appears that much of the work is purely descriptive and pertinent to few important pathogens. In many cases it is stated that the results will be used to develop new interventions or ways to avoid resistance. It is not clear how the data descriptive surveillance information will result in these new approaches. In addition, most of the work is not longitudinal and there was no focus on the big “issue”, growth-promoting antibiotics. Much work was on plasmid characterization with no apparent correlation to the program objectives. Overall, the research on antibiotic resistance does not appear to be well coordinated and the selected microbes that are studied seem to be those that are favored in disease based

studies. Also, a stated goal is to understand antibiotic resistance emergence and evolution. While numerous molecular-based studies are mentioned in the impacts, none really appear to target evolution of resistance. The studies on linkages between multiple resistances and also with virulence factors are interesting but are mainly descriptive without an understanding of process. Thus, it is not clear how these data will be used.

Contrary to a statement in the Action Plan, the use of antibiotics in Animal Production (feeds) is one of many contributing factors in antibiotic resistance, and not the only factor, as implied.

Basic research regarding the identification and characterization of genetic attributes was significant. Much of the work has contributed to the limited body of knowledge about the capacity of which pathogens acquire and disseminate antibiotic resistance. It is apparent that more fundamental research about antibiotic resistance is needed in animal and plant production and processing environments. Survey of retail and processing environments indicated a limited understanding of persistence and transference of resistant organisms and resistance genes. The practical development of microarray chip technologies for screening and detection of resistance determinants is significant for governmental and industrial uses.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.1 Detection and Validation

Outputs

Sampling:

- Best practice sample collection protocols for various food systems and matrices.
- Innovative approaches to sample processing, for example, universal separation and concentration steps.
- Mathematical algorithms to maximize the probability of detection.

Detection

- Rapid systems for target amplification (genotypic/phenotypic) to maximize detection potential.
- Multiple platform systems that will be combined with multiple target assays (multi-platform devices for universal contaminant detection).
- Systems that function in real or near real-time, or have the potential to be automated for high throughput.

Validation

- Determined under both laboratory, pilot-processing, and commercial-processing conditions, and where appropriate in association with the specific stakeholder end-user, and/or development partner.
- The protocol for method validation for any specific pathogen, toxin or chemical contaminant detection will be different. However, the following generalities hold. The fundamental parameters for method validation will include evaluation of (1) sensitivity and selectivity; (2) accuracy, precision, recovery, and reproducibility; (3) stability and calibration curve (4) cost, robustness, and environmental impact.
- Measurements for each (pathogen/toxin/chemical contaminant) in the matrix will be validated. In addition, the stability of the (pathogen/toxin/chemical contaminants) in spiked samples will be determined.

Review Team Rating:

High Impact

Review Team Assessment:

ARS undertakes innovative, sometimes world leading, research into the detection and validation of specific, sensitive methods with the potential to be rapid and cost effective. For example, the development of microfluidic chips to replace classical agar culture is an exciting prospect. Similarly, the development of hetero-nanorods to detect *Salmonella* is ground breaking and we await a description of its sensitivity and robustness for food use. An extensive range of detection

methods are being developed but particularly for PCR methods, some evaluation could be made of whether it is sufficient to detect both live and dead cells, or if it is more beneficial to know which cells are still alive, particularly following a food treatment or factory cleaning procedure. In effect, this is covered by using a brief culture enrichment to improve sensitivity of detection to as little as 1 cell per gram of food, but it does not take into account whether viable but non-culturable (VBNC) cells may be present.

Ultimately ARS must decide if it should focus on the most promising key detection technologies/sensor systems depending on the food matrix, point of use in the food chain and whether it is to be used for product quality, pathogen/toxin/chemical tracking or forensics during outbreak investigations.

The work showing non-specific attachment of *Brochothrix* and *Carnobacterium* during IMB capture of *Salmonella* or *E. coli* shows the limitations of the technique. The data obtained are useful but perhaps the project could now be terminated and all IMB procedures reviewed to assess whether non-specific binding will impact on other analyte detection.

The importance of the collaboration with Purdue University is recognized and could be emulated elsewhere.

Method development was strong, but validation was not evident; the process used for identifying methods to be validated and the method validation protocol and objective should be defined. This should be aligned or at least consistent with international standardization bodies such as ISO or AOAC International.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.2 On-line Sensing Systems that Assist Processing, and Have Application in Food Security Antibiotic Resistance

Outputs

- Commercial implementation for detecting pathophysiological abnormalities, and feces/ingesta in poultry combined and integrated with on-line washing/ intervention systems developed by ARS.
- Development, evaluation, validation, refinement, and commercial or near- commercial implementation of computerized, on-line detection/sensing systems for whole beef and pork carcasses; feces and defects on apples and other fresh fruits and vegetables; physical hazards (bones, glass, plastic, stones and metal) in foods; and physical defects in shell eggs.
- Development, evaluation, validation, refinement and commercial implementation of head-gear systems; for use by small producer/processing operations. Expand their use to include other areas, for example: small and medium size processing facilities, cleaning and sanitation issues both in production and retail facilities.
- Development of innovative sensing capabilities for use in military and food security applications. Any work should specifically be coordinated with needs in the Food Security problem 1.2.9 of the Action Plan.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

Since the burden for inspection responsibilities have shifted from the regulatory agencies to producers and processors, the need for effective and cost efficient on-line, computerized sensing systems placed in processing environments will assist and improve the inspection system and minimize variability and error. Technologies developed or tested that use optical light sensors, real-time imaging (infrared, optoelectronic, multispectral) indicated true promise for industrial applications. Detection of bacterial biofilms, product flaws and foreign materials, fecal contamination, and wholesomeness demonstrate innovation and has relevance for industry and consumer health. Research was practical and valuable.

Good technology transfer is evident.

The studies demonstrated a significant collaboration between industry and government, and inter-governmental collaboration as well.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.3 Production and Processing Ecology

Outputs

- Determine why certain, and often very specific pathogens (types/subtypes) persist not only on food but in the food processing environment.
- Determine why certain types/subtypes are better adapted to production environments rather than the food processing environment, and vice versa.
- Determine correlations between specific genetic types or clonal groups and virulence characteristics; and any relationships between the virulence characteristics and human infections.
- Understand biofilm formation structure and composition both on foods and within processing, and transportation environments. Elucidate any differences between biofilms found on product, those found on equipment producing the product, and containers. Specifically addressing the formation and behavior of pathogens in biofilms associated with raw fruits and vegetables, both conventionally and organically produced: and elucidating the differences in biofilms on containers and equipment used in the produce industry.
- Determine the genes associated with altered physiological states, adaptation to extrinsic/intrinsic stresses, and quorum sensing.
- Provide baseline data to regulatory agencies to write Performance Standards.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

ARS feels that their mandate is not to conduct baselines. However, the research needs identified that this was an important action for ARS to pursue. Considerable work was done to identify points of contamination. The outputs of the projects provided substantive insight to the public health regulatory agencies, particularly FSIS. Many of the outputs were conveyed into guidance to small plant operations and into policy development by FSIS.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.4 Processing Intervention Strategies

Outputs

- Evaluate, develop, and validate through both laboratory, pilot-plant processing and commercial processing facilities the effect of single and combinations of intervention technologies (multi-target approach) on pathogen reduction. Ensure that lethality/intervention treatments do not negatively impact product quality.
- Determine whether combinations of non-thermal technologies can be incorporated in the hurdle concept; and determine whether single or combinations of non-thermal technologies are more effective if used in combination with traditional interventions.
- Increase fundamental understanding of the mechanisms, modes and sites of action at the cellular level of various intervention (inactivation) processes, and combination(s) thereof.
- Develop methods to prevent the growth of pathogenic and spoilage microorganisms in minimally preserved, brined, and fresh-cut foods. Develop predictive models for growth survival and inactivation of pathogens critical to Problem Statement 1.2.7.
- Utilize the inactivation data to model pathogen and non-pathogen behavior in complex food systems. These types of studies are fundamental to developing HACCP systems and regulations, and are critical to Problem Statement 1.2.7.
- Develop of post-harvest interventions options for small and very-small FSIS regulated plants.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

When applied at adequate levels or intensities, traditional as well as novel food processing technologies are able to inactivate microorganisms. However, in order to maintain product characteristics, functionality, and eating and nutritional quality, and provide variety in the human diet, certain foods may be exposed to processing treatments of reduced intensities. Postharvest technologies and strategies to eliminate, reduce, or suppress growth of pathogens are needed for foods in order to enhance their safety and maintain quality and other properties or characteristics. Successful selection of antimicrobial processing and pathogen control technologies and interventions requires adequate understanding of their modes of action and effects on the microbial ecology of a food product.

Overall the list of accomplishments for meat products and produce is impressive.

In general, important advances were made in thermal and certain non-thermal technologies for pathogen reduction in animals, meats, and produce, especially as single interventions; several studies addressed the first and second outcomes listed above.

The work on pathogen reduction on produce generated interesting and potentially useful information, as this area required major attention in the past 2-3 years. The work on application of non-thermal pathogen inactivation technologies is also interesting and potentially useful. Although some work was done, only limited research addressed the remaining outputs listed above.

Specifically, very limited or no research addressed:

- Combinations of antimicrobial intervention technologies (multi-target approach).
- Combinations of non-thermal technologies.
- Effect of lethality/intervention treatments on product quality.
- Fundamental understanding of the mechanisms, modes and sites of action at the cellular level of various intervention (inactivation) processes and combinations.
- Methods to prevent the growth of pathogenic and spoilage microorganisms in minimally preserved, brined, and fresh-cut foods.
- Progress in these areas may have been reported under different areas.
- It is not clear how much of the work was performed in the laboratory, pilot plant or commercially, as stated in the outputs.
- While some outputs were linked to commercial applications, others should be linked to future needs for advancements that may lead to applications, or determination that the effort should be terminated due to lack of future prospects.

The majority of studies met their objectives of development, validation of innovative intervention technologies that have practical application in the postharvest and processing environments. The findings in general have practical relevance for industrial use and public health enhancement. There was some studies that did not take into account the cost efficiencies, consumer acceptance and sensory attributes.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.5 Omics

Outputs

- Improved science-based risk assessment decisions to control foodborne pathogens. In particular, allowing comparisons (molecular systematics) of strains, providing information on genes that contribute to pathogenicity in human and/or animal hosts
- Allow the study of expression of genes of interest, including, but not limited to genes involved in virulence and/or viability (growth and survival) in foods.
- Increase understanding of population genetics and epidemiology, by assisting development of improved ways of molecular tracking of pathogens through the food chain.
- Allow incorporation of sequence information into the design and optimization of nucleic acid arrays and chips.
- Facilitate gene expression studies for identification of sequences expressed or repressed in response to external cues such as those likely to be encountered by the specific bacterium in the environment, foods, and/or humans.
- Make possible more rapid and less costly diagnostic methods and vaccine development, and allowing analyses of resistance to antimicrobials, toxins, and disinfectants.
- Reveal any novel genes associated with specific ecological niches, and allow for comparative genomic analyses to determine critical areas for targeting strategies for interventions and controls.
- Identify regions of the genome that may have variations in the rate of nucleotide substitution or in the rate of intergenic recombination.

Review Team Rating:

High Impact

Review Team Assessment:

This program area should be noted to be genomics. Other aspects of Omics do not appear to be represented. Overall, there is good productivity in the development of potential diagnostic test using microarray chips. Also, several studies resulted in the development of typing schemas for some pathogens, which is a goal for the program. It will be important to translate these test systems into useful diagnostic tools. None of the techniques are rapid and high throughput. But the new data are valuable. *Listeria*, *Campylobacter*, and *Clostridia* are the most intensively studied microbes in these studies. *Campylobacter* clearly is one of the most prevalent food borne bacterial pathogens. The *Listeria* work did not appear to be well coordinated. The degree of focus on *Clostridium* might not be warranted. The tools for gene expression studies are in place and this is noted in the impacts sections. However, what seems to be lacking is an understanding

of how the gene expression studies will be performed and interpreted. There is no mention to *Salmonella* and *E. coli* in the Omics section. This might be because of extensive work in academic institutions but is an important gap. Also, there is no mention of viruses. It is recognized that ARS is attentive to FSIS's requests and needs.

The genomic sequencing work on *Campylobacter* and *Arcobacter* is recognized as well-leading and will have a tremendous impact on future virulence and epidemiological studies. It is surprising that little is being reported in this section on *Salmonella* or *E. coli* O157. Much of the research findings are on a single microorganism (genus, speies, or strain) so overall utilization remains to be seen.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.6 Safety and Health

Note: The single CRIS project addressing this research was transferred to NP107 just after implementation of the NP108 2006-2010 Action Plan.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.7 Risk Assessment

Outputs

- Determine the influence of the food matrix on the infective dose of a specific pathogen, and the subsequent biological response. Examine the uncertainties that surround dose-response models derived from animal studies, and determine which models more accurately function as surrogates for humans. Determine if dose-response data from epidemiological investigations are ultimately better.
- Develop and validate models that consider the inherent complexities if intrinsic and extrinsic parameters; or the macro- and/or micro-environment in food matrices. Determine the strategies needed to extrapolate model predictions to more complex foods.
- Determine and elucidate objective measures to assess model performance versus observation. Develop alternate responses or options, for example iterative approaches for specific food applications.
- Determine what strategies should be used to predict the distribution of lag times, and the worst-case scenario, since lag time has the highest uncertainty in predictive modelling.
- Develop data, and use these data for the development of predictive models for growth survival and inactivation of pathogens critical in minimally processed, brined, and fresh-cut produce.
- Determine the strategies for converting and integrating ARS models into fully functional process risk models.
- Compile quantitative food microbiology data into a database so that they can be exploited for model development and validation, especially when resources are limited.

Review Team Rating:

High – Low Impact

Review Team Assessment:

This program resulted in the successful development and validation of models for several food/pathogens/processes that are relevant to current problems in the food industry and with public health impact. The outputs were made available to end users for use in risk assessment, policy-making, and food processing. The quantitative data were archived in ComBase and models incorporated into the USDA-Pathogen Modeling Program (PMP), benefitting a global community of risk assessors and risk managers. Novel research resulted in new models to describe survival and growth of low numbers of Salmonella in the presence of native microflora on chicken skin, a major outcome of this program, and microbial surface transfer models to describe and predict cross-contamination with pathogens (*Listeria monocytogenes*, *E. coli* O157:H7 or *Salmonella*) during mechanical slicing of deli meats. These achievements are notable, and have high impact in the area of risk assessment and applications for regulatory decision-making. However, these achievements are essentially in only two of the anticipated

output categories, that of “Develop and validate models that consider the inherent complexities if intrinsic and extrinsic parameters; or the macro- and/or micro-environment in food matrices.” and “Compile quantitative food microbiology data into a database so that they can be exploited for model development and validation, especially when resources are limited.”

For the majority of anticipated outputs in this work theme, no accomplishments, nor progress, are reported and some explanation for shortfalls in these objectives should be provided. These are critical areas of research and model development necessary to advance microbial food safety risk assessment and new predictive/analytical tools that will be useful for both risk assessors and risk managers.

The modeling efforts met the goal, there should be more emphasis on developing new models, and continued development of existing models. The models also need to be tested and validated as described in the Action Plan.

The question remains as to how data gaps are identified, and then integrated into the risk program.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.8 Pathogenicity

Outputs

- Isolation and characterization of virulence factors such as toxins, adhesions and invasions, and determination of how they interact, for example, do various factors behave synergistically or antagonistically?
- Utilize genomics and bioinformatics to classify pathogens based on specific virulence factors, rather than by name or by non-virulent associated phenotypic traits. This will improve the evaluation of safety currently focused on organisms that have differential pathogenicity.
- Determine the ecological basis for predominance, persistence and succession along the food chain to prioritize points for intervention application. (See Problem Statement 1.2.3)
- Utilize genomics and bioinformatics information to understand why evolutionary shifts occur, and identify the selection pressures responsible for the shifts.
- Utilizing bioinformatics to make predictions, for example on protein structure and function.
- Determine whether quorum sensing occur in foods, or on surfaces (biofilms), and if so, whether it is factor in determining or regulating virulence in foodborne pathogens
- Determine through analysis of gene/protein expression profiles the responses to intrinsic and extrinsic stressors imposed during production, processing and storage.
- Determine if stress-regulatory-response-systems may also be targets for the design of innovative antimicrobial agents.
- Though understanding virulence, develop improved detection and identification methods that enable differentiation of virulent from non-virulent.
- Initiate studies through collaborations with other Federal agencies, affiliated research establishments and Universities to determine the infectious dose of various pathogens and their toxins, in various food matrices, both pre and post stress.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

The recognition of the potential importance of quorum sensing and biofilm formation to pathogenicity of foodborne microorganisms and resistance to food processing stressors is an important step forward. Similarly, the finding that Stx-encoding prophage increased the survival of *E. coli* O157 when incubated with the soil dwelling ciliate, *Tetrahymena pyrifromis*, shows the impact of ecology on pathogenicity. It is noteworthy that *Tetrahymena* are biofilm predators,

again emphasizing the importance and impact of biofilm formation and ecology on the food chain.

An example of biofilm resistance to environmental stressors is shown with the work that *E. coli* O157 is as much as 10,000 times more resistant to hydrogen peroxide than non-biofilm cells. The phenomenon of biofilm phenotype-resistance is well known but in this instance ARS were able to utilize their expertise in genomics and proteomics to describe the expression of peroxide-stress-genes which should lead to the design of intervention strategies.

The finding of a single, secreted kinase that serves as a major virulence factor for *Toxoplasma gondii* opens the possibility of identifying, using gene target detection, if there are virulent and avirulent strains in the food chain.
The research described was novel and cutting edge, excellent work.

However, as exemplified by the *Tetrahymena* work, biofilms are rarely mono-species and regularly comprise polymicrobial communities with the ability to produce an extensive range of quorum sensing pheromones and the ability to "cross-talk" between genera and phyla. Indeed, one ARS study shows that non-*E. coli* O157 strains can augment biofilm formation by *E. coli* O157 strains. This is a start along the polymicrobial route. An example for ARS to consider is with *C. jejuni* where previous studies have shown that this important pathogen survives well in drinking water supply biofilms comprised of complex communities of microorganisms. *C. jejuni* is a fecal pathogen and would not be expected to form biofilms on its own on foods or in processing factories when fecal contamination occurs in the food chain.

There are various studies worldwide on the use of quorum sensing antagonists to prevent biofilm formation e.g. in the marine environment and clinical infections. Consideration should be given to initiating similar studies in the food chain, utilizing the chemistry expertise with ARS.

It will be important to determine whether the ability of salt to positively influence biofilm forming ability of *C. jejuni* and *C. coli* is due to a physiological effect or a physico-chemical charge effect on the cell membrane attachment processes.

Component 1.2: Pathogens, Toxins and Chemical Contaminants Postharvest

Problem Statement 1.2.9 Food Security

Outputs

- Develop best practice sample collection and analysis system for foods of concern.
- Validate methods and diagnostics (nucleic acid based, serological, analytical assays) specifically developed for the detection of select agents (biological agents, toxins, chemical residues and pesticides) in various food matrices.
- Develop systems and models that validate the behavior of pathogen in foods, and the effect of various intervention strategies on select agents.
- Identify, develop and validate decontamination systems for various food commodities, with particular reference to quantity, level and type of contamination. Develop simulation models for strategies of containment, decontamination and disposal of both of the affected food; the production and processing facilities, within the distribution system, and environmental fate, persistence and impact of the select agent.

Review Team Rating:

Medium Impact

Review Team Assessment:

The panel strongly recommends that this research activity be referred to as "Food Defense" as opposed to "Food Security". The term Food Security is internationally used to indicate adequacy of the food supply.

According to the report the Program was limited to areas of expertise with in the food safety group, and often the use of surrogate organisms, since BL-3 facilities were not available. Also the majority of research conducted was in response to specific requests from defense/security related agencies (DHS/FBI/DoD); and some projects were specifically funded by DHS. Unfortunately the details of research considered "classified" have not been included within this document. Furthermore, although difficult to assess technology, transfer of outcomes was indicated with statements such as: "The technology was transferred to the appropriate agencies." "The information was also transferred to other requesting defense agencies." "This information was transferred to the appropriate agency, and will help to provide effective food defense." Etc.

It was not evident where best practice sample collection and analysis system for foods of concern was addressed. A number of activities addressed various methods of analysis of select agents or surrogates. Also there were some significant decontamination findings.

The use of a qPCR test to detect the minute amounts of castor plant DNA that would be present in a food, if adulterated with a crude preparation of ricin is an innovative approach and serves as an example of an approach that could be useful if false positives could be controlled. It would be useful to see a comparison with the assay for ricin that was also reported here as well as the many available rapid and reliable kits.

Microbial toxins also were the focus of several studies. Specific mAbs analysis for BoNT is needed and promising, and the approach to assay the degradation of the substrate attacked by BoNT is innovative and useful. The study of SEA and SEB for food defense may be misplaced but should be useful for food safety applications. The same may hold for study of Shiga toxin heat resistance.

Concerns were expressed that other agencies have well established tests for select agents such as ricin and BoNT, and appropriate ARS individuals should meet with key personnel from DHS, DoD, FBI etc. to determine useful work that would not be redundant.

The studies on *Yersinia pestis* and YPST cover growth, inactivation and detection in various food matrices. Some of the observations seem obvious such as the sensitivity to gamma irradiation but in general the need for information on these microorganisms in food is great. Other decontamination studies on toxins such as ricin, BoNT, and Shiga toxin are needed because of the dearth of information available. The findings frequently seem quite pedestrian but are essential for food defense. The microfiltration removal of *B.anthracis* spores seems so predictable (since it does take out bacteria of the size of those spores) that the research is difficult to rationalize. Finally even though T2 toxin is recognized as a select agent, the research on T2 mycotoxin done in this group may be far more relevant to food safety than it is to food defense.

In summary, although it was not evident where best practice sample collection and analysis systems for foods of concern were addressed, many of the activities addressing various methods of analysis of select agents or surrogates in food matrices have promise. The comprehensive evaluations of *Y. pestis* and YPST in food matrices also are valuable. No modeling was mentioned in either growth or decontamination; nevertheless, there were some significant decontamination findings.

Component 2: Mycotoxins and Plant Toxins

Problem Statement 2.1.1: Toxin Methodology and Identification

Outputs

- Rapid, accurate, sensitive and potentially affordable detection methods that are compatible with high speed, high volume commerce, that can be linked to optical sorters, and that are preferably non-destructive.
- Spectral analysis of contaminants to identify key optical signals that can be used for identification.
- Expand the use of genetic based determinants in PCR based tests including microarrays to rapidly identify mycotoxigenic fungi in contaminated food products.
- Novel mycotoxin-binding materials such as molecularly imprinted polymers, novel carbohydrates, and improved antibodies that bind mycotoxins.

Review Team Rating:

High Impact

Review Team Assessment:

USDA/ARS has continued as a world leader in mycotoxin research. Research in the 6 program areas identified in the Food Safety Action Plan are variously divided between 7 different research Centers (Beltsville, MD, Peoria, IL, New Orleans, LA, Athens, GA, Davison, GA and Stoneville, MS). Many of the toxigenic fungi produce toxins that are highly carcinogenic (e.g., aflatoxins) and some are even identified as select agents. Some of the work has a high degree of difficulty and complexity and crosses multi-disciplinary lines of investigation. The warning set forth in the Action Plan (p. 30) should be remembered, *"When plants cannot make their own necessary protective chemicals (against insect and mammalian herbivores) the need may be met by endophytic fungi. In developing genetic solutions (to control toxigenic fungi) we must be careful to not inadvertently enhance factors which may be responsible for pest resistance in plants, and which simultaneously may have unanticipated consequences in animals and humans."*

ARS scientists in the mycotoxin research program are productive as evidenced by many papers in the peer reviewed literature (approximately 16% of publications for this rating period), much of their work is highly innovative and often they are pioneering new areas of investigation. Their work is proactive for during this reporting period they have identified new species of toxigenic fungi (pub 1298) and new toxins. Their work is by necessity global for the toxigenic fungi and the foods they infect travel without passports.

Appropriate sampling and proper attention to matrix effects and extraction efficiencies all had to be addressed in mycotoxin methods development. ARS scientists have pioneered statistical sampling for large volumes (eg., lots shipped in barges and railroad tank cars) of product. To

protect the public health and to facilitate commerce they have developed rapid detection methods (with field deployability) and have transferred the technology. Often methods are developed not as an end point but by necessity for studies such as those of toxigenic fungi and the crops they infect, discovery of new toxigenic strains of fungi and of new toxins, development of mitigation strategies and in genetic studies.

Outputs have been met.

Component 2: Mycotoxins and Plant Toxins

Problem Statement 2.1.2: Crop/Fungal/Insect/Toxin Relationships

Outputs

- Determining the effects of weather on toxin accumulation.
- Identify and develop commercially practical synthesis for specific pheromones and kairomones that can help control insect pests of tree nuts
- Developing mechanisms and products for preventing fungal introduction by insects.
- Determination of the sources and mechanisms of plant accumulation of toxic amounts of heavy metals or other toxins in food crop plants.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

Research in this component is designed to provide understanding between the very complex interactions between fungi, insects, crops and the environment during both fungal and plant growth and maturation. The work is important to assist in development of effect mitigation strategies. The work is interdisciplinary and several important accomplishments are listed and include the discovery that fumonisin contamination can occur in crops other than maize, identification of gene clusters responsible for synthesis of two lesser known mycotoxins by Fusarium, microarray used to identify genes required by *F. verticillioides* to cause ear rot in corn and determined functions of genes in this species required for ear rot, studied interactions of *F. verticillioides*, fumonisins and maize, for transgenic corn found new broad spectrum insect resistant genesthat may be active against mycotoxigenic fungi (note: determine whether potential exists for allergenic protein). A number of these studies use what appear to be novel approaches such as development of a robotic molecular molecular evolutionary apparatus to develop a more effective insecticidal peptide (more information needed, CRADA anticipated) and use of array analysis to identify putative regulatory gene(s) for a secondary metabolite pathway of maize that produces a compound responsible for broad spectrum insect and disease resistance. This work looks quite promising. Other studies involve natural control such as evaluating efficacy of fungal and plant secondary metabolites from new sources active against corn insect pests associated with mycotoxin contamination. There is much work on development of transgenic plants for insect control.

Component 2: Mycotoxins and Plant Toxins

Problem Statement 2.1.3: Production Practices and Expert Systems

Outputs

- Identification of cultural crop production and handling practices that can assist in the reduction of mycotoxins in crops. This includes effects of various management strategies, e.g., rotations, tillage, and cover crops, and herbicide-resistant crops under different weather conditions, and insect control
- Formulation of user-friendly computer programs to provide useful predictions for mycotoxin occurrence.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

The goal of this area is to have effective strategies to prevent plant or fungal toxin accumulation on crop plants. It appears that much progress has been made. Most notably a web site has been developed and made available for a predictive computer program for mycotoxins in Midwest field corn. ARS developed and validated a high speed optical sorting system for removing fungal damaged and toxin contaminated kernels of grain with high sorting rates. This should reduce amount of grain that must be discarded. The finding that application of herbicides such as Round-up and Liberty have no adverse impact on growth, development, yield or mycotoxin incidence in earlier maturing hybrid corn. Models were developed for predictions of aflatoxin levels in cottonseed prior to harvest based on timing of rainfall and temperature (has model been validated?).

Recording of negative results can be valuable as exemplified by the study of Potassium fertilizer not reducing aflatoxin.

Component 2: Mycotoxins and Plant Toxins

Problem Statement 2.1.4: Breeding Resistant Crops

Outputs

- Identification of unique fungal genes for specific biological and physiological functions for use in highly sensitive PCR based tests (including microarrays).
- Completion of the identity of the gene clusters and biochemical pathways required for the production of common mycotoxins.
- An understanding of how environmental factors affect the fungus, which genes are turned on during the plant-fungus interaction and mycotoxin production, and which are essential to fungal survival in the field environment.
- An understanding of the effect of the plant on the growth and sporulation of the fungi and on mycotoxin production under various crop production and stress conditions.
- Use of identified genes for marker assisted selection of corn resistant to mycotoxins.

Review Team Rating:

High Impact

Review Team Assessment:

Development and enhancement of intrinsic crop resistance to colonization by fungi to control subsequent production of mycotoxins requires fundamental knowledge of the plants and the fungus. Genomics can assist in supplying the knowledge that will address the ultimate goals. Techniques have been developed and refined to study unique fungal genes. Transgenic plants and selected fungi have been used to achieve the outputs. A number of innovative approaches have been used and outputs have been transferred to plant production groups. The fungi selected for study produce mycotoxins of importance to food safety, be it at the production phase of the food chain, e.g., aflatoxin, trichothecene, fumonisin, ochratoxin A, patulin. Direct impact on food safety is relatively subtle in that mycotoxin contaminated corn and other grains should not reach the consumer as final food products, consequently the control of mycotoxin production through plant breeding specifically affects the production of crops and their subsequent quality.

Component 2: Mycotoxins and Plant Toxins

Problem Statement 2.1.5: Biocontrol Technologies

Outputs

- New or modified effective biocontrol organisms and delivery systems that do not introduce toxic factors.
- Establishment of baseline levels of the atoxigenic biological control organisms to provide a basis for determining the influence of applied biocontrol products on natural microbial communities, particularly when applied over large crop acreages.
- Further delineation of the role of endophytic fungi in regulating plant metabolism and in providing effective defense against predators and stresses.
- Determination of the effect of control strain distribution on the ecology of the producing area.
- Safety, efficacy, and stability data necessary for maintenance and expansion of biopesticide registrations with EPA.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

Biocontrol agents can be used at all phases of agricultural processes such as reduction of insect damage to crops, growth inhibition of toxigenic fungi by various means such as by competitive exclusion by non pathogenic or atoxigenic species of bacteria or fungi, inhibition of toxin production by toxigenic fungi. Finding appropriate biocontrol agents often requires extensive studies of potential agents for specific toxigenic fungi and for specific targeted crops, often in specific environments. As noted in section 2.1.1, this often involves intensive methods development and can sometimes yield serendipitous results that can relate to other mycotoxin program areas. Many of these studies are time consuming and expensive. Agents proposed if they show promise in the laboratory or small scale test plots ultimately require applications for use to regulatory agencies such as EPA for approval for environmental use and/or FDA for use in regulated food products, often after extensive and appropriate field tests.

Studies outlined in this section are innovative requiring extensive understanding of multidisciplinary areas such as plant responses, plant and microbial genetics, soil chemistry, etc. These studies include use of antioxidants and enhancement of stress resistance traits in plants to inhibit or suppress aflatoxin production, development of bacterial, yeast, or atoxigenic fungal control agents active against toxigenic fungi, bacterial enhancement of corn plant's natural defense system to Fusarium species (reduction of fumonisins), and identification of a number of safe natural products that enhance effectiveness of commercial fungicides. There is technology transfer for many fo these studies. Outputs have been met or are underway.

Patulin is now recognized as a potent quorum-sensing inhibitor, which may facilitate the colonization of foods by fungi by overcoming resident microbial flora. Therefore, the presence of microbial flora in foods should not be overlooked when considering fungal colonization and production of toxic patulin. This offers the potential for cross-collaboration between fungal and biofilm research groups.

Component 2: Mycotoxins and Plant Toxins

Problem Statement 2.1.6: Toxicity Evaluations and Mechanism of Action

Outputs

- Chemical speciation of toxins following ingestion and elucidation of their specific biological effects with relevant dietary components.
- Mechanism of action based bioassays for mycotoxin-like activity in foods and other matrices.
- Chemical analyses that accurately predict the biological activity of toxins as they are found in prepared food products, and assistance in the development of production procedures to achieve safe levels.
- Specific molecular endpoints of toxins in mammalian systems and downstream mechanisms of action using *in vivo* systems, plant models, and whole animal test systems; and validation of toxicological endpoints in whole animal feeding studies.

Review Team Rating:

High – Medium Impact

Review Team Assessment:

As stated in the program description, these studies are conducted to answer questions for regulatory decision making by FDA and/or other decision making bodies. The studies require toxicological studies using appropriate animal models and often involve determinations of biochemical pathways influenced by the toxin under investigation. In addition, studies are designed to determine whether crop handling, food processing or other manipulations affect toxicity Heavy metals are included in this section although the section title does not reflect this. Several excellent studies included here such fumonisin uptake and accumulation in animal tissues demonstrating that few FB compounds are of toxicological significance, studies of neural tube defects (NTD) in mice for predicting risk in humans, studies showing nixtamalization does not contribute to NTD and may reduce FB in cooked product, extrusion cooking with glucose supplementation reduces FB in commercial maize-based products, further elucidation of biochemical effects of fumonison in animal tissues aiding in development of animal models for risk assessment, and finding that DON or vomitoxin is resistant to degradation during preparation of wheat-based food products under commercially relevant conditions.

For heavy metals, studies were conducted to answer questions about Cd, Pb and As uptake or presence in growing soils or plants. Several studies resulted in significant findings or suggestions for mitigation such as finding that deliberate adding of Fe to soil can reduce Cd phytoavailability, low soil Zn causes increased Cd accumulation in lettuce and other crops, characterized higher bioavailability of Cd in polished rice with effects of flooding and drainage of Cd contaminated rice paddies and found in Thailand high Cd in rice, soybeans and tobacco grown by local residents in an area subject to mine waste waters containing high Cd and Zn

(with supporting epidemiological data of confirmed Cd-induced kidney disease in exposed populations. Data from this latter study convinced the Thai government to test the soil, crops and humans to determine the extent and severity of contamination. Hopefully this resulted in mitigation strategies. Another study showed that Pb accumulates inside the carrot root and thus supports regulatory decisions to limit production of carrots on high Pb soils. Potatoes are insensitive to soil Pb. A last study showed that long term application to Atlantic Coastal Plain soils of poultry litter containing As residues as feed additives did not cause As accumulation in soils most likely due to low levels of adsorbent soil iron oxides and high levels of phosphate which competes for As sorption.

Outputs have been met or are underway.