National Program 108
Food Safety Report
2000-2004

EXECUTIVE SUMMARY

Research Program Report

The divisons of administrative responsibility within the National Food Safety Program 108 are Preharvest (Dr. Robens) which includes Component 1.1 Preharvest Microbial Pathogens, Component 2. Mycotoxins and aspects of Component 3. Chemical Residues. Postharvest (Dr. Lindsay) includes Component 1.2. Postharvest Microbial Pathogens, and aspects of Component 3. Chemical Residues. These divisions follow the NP Action Plan for 2000-2004. The Research Program section of this Report was prepared following the Program Components and their Goals.

Executive Summaries

Component 1. Microbial Pathogens
Component 1.1. Preharvest: Reports 01-28.

Executive Summary Microbial Pathogens - Preharvest

The safety of meat and poultry products starts with healthy animals, and even healthy animals may carry zoonotic pathogens which can affect humans. Preharvest research was conducted in 6 areas (sub-components): methodology (1.1.1); epidemiology and ecology (1.1.2); ecology, and the host-pathogen relationships (1.1.3); intervention strategies (1.1.4); feed withdrawal and transportation (1.1.5); and manure (1.1.6). The food producing animals primarily addressed are cattle, both beef and dairy, swine, and poultry, including broilers, layers and turkeys.

The ARS preharvest food safety research program addresses how farm production products and practices can make animal food products safer. Control of the commonly recognized food safety pathogens, that is, *Salmonella*, *Campylobacte*r, and *E. coli* O157:H7 in cattle, swine, and poultry, the primary sources of meat, milk and eggs for the American diet, is a difficult challenge because these pathogens are not species specific for the most part. Reservoirs are present and amplification can and does occur in more than one species and in more than one environmental niche so it is highly unlike that complete eradication can ever occur. Not all objectives of the Action Plan were met however significant progress was made, and producers now know what products and practices may be useful, and more importantly, what is not effective in controlling these pathogens in their birds and animals.

The most effective control strategies developed are for pathogen control of Salmonella in broiler chickens, where a combination of pathogen control in breeder birds, use of a competitive control product, and biosecurity programs coupled with the short length of grow out prior to slaughter (about 6 weeks) can be expected to result in very low to no contamination with Salmonella. Contrast this with beef production where animals live outside all of their lives, are subject to all types of contamination, may be moved 2-3 times prior to entering the feed yards, and the production cycle is close to 2 years. Here the ARS research program has superbly described the

relationship of *E. coli* O157:H7 with its animal hosts, and has developed an intervention method for beef industry use that is now under regulatory agency review.

Other particular program achievements to help assure pathogen control include:

Poultry - developed new rapid detection methods for Salmonella in eggs; identified biologically active cytokines from naturally occurring Salmonella immune lymphokines (SE-ILK) which provides the information for the poultry industry to produce birds that are highly resistant to infection by *Salmonella*; and developed normal competitive exclusion cultures that prevent Salmonella infections in newly hatched chicks.

Swine - identified two porcine genetic markers with a heritable phenotype associated with disease resistance to *Salmonella*; elucidated the role of lairage in the incidence of Salmonella in pigs at slaughter; developed a normal porcine-derived competitive exclusion bacterial cultures that prevents Salmonella infections in newborn pigs and decreases mortality and necessary medication. Regulatory issues are being resolved prior to commercial development.

Cattle - developed rapid and sensitive tests for *E. coli* O157:H7 and related serotypes and highly specific serological tests for anti-O157 antibodies; demonstrated the failure of numerous production practices to change the incidence of O157; demonstrated the seasonality O157:H7 occurrence, and the multiple sites of high *E. coli* prevalence in the animal and the environment; established the need for a live animal treatment to kill pathogens just prior to slaughter since eliminating exposure would be almost impossible; demonstrated that sodium chlorate given orally to cattle selectively kills pathogens without harming beneficial bacteria.

Antibiotic resistance - described basic mechanisms and selective pressure involved in the evolution and transfer of antibiotic resistance genes among pathogens and commensal bacteria; maintained the animal arm of NARMS, the only national program for surveillance of resistant bacteria in animal in the U.S. which provides accurate information to help assure continued drug; initiated the Collaboration on Food Safety and Animal Health Epidemiology (CAHFSE), a USDA interagency epidemiology program (ARS/APHIS/FSIS) for ongoing, periodic microbiological and antibiotic resistance surveillance in food producing herds/flocks.

Component 1.2 Postharvest: Reports 29-56l
Food Safety Research Information Office (FSRIO) Report 78.

Executive Summary Microbial Pathogens - Postharvest

The reduction and control of microbial pathogens in/on meat, poultry, seafood, and fruit and vegetable food products is the most pressing food safety issue today. During this past 5-year research cycle the postharvest program focussed its efforts in 5 areas (sub-components): methodology (1.1.2); production and processing ecology (1.2.2); intervention strategies-processing control technologies (1.2.3); effects of intervention strategies (1.2.4) and risk assessment (1.2.5). These areas of focus were derived from extensive stakeholder input.

In brief: ARS took the lead to develop methods that have regulatory, industry and research use. A variety of new, improved and innovative methods were developed to detect, differentiate, type, and quantify numerous foodborne pathogens including: Campylobacter, Salmonella, E. coli O157:H7 and related E. coli, Listeria, Yersinia, C. perfringens, B. cereus; Cryptosporidium, hepatitis A, Noroviruses and Fusarium species, and products of their growth such as toxins. New sampling methods were also developed. Specialized on-line sensing systems were also developed to detect contamination, physical and pathophysiological defects. The focus of development was utility and cost-effectiveness for the end user; combined with, user-friendly, rapidity, increased sensitivity, improved discrimination power, balanced with the ability to be automated when combined with the need for high-through-put. Technologies were transferred to the end users, mainly FSIS and FDA who will work with ARS to refine them for (automated) day-to-day use

Utilizing many new, improved and innovative methods described above: data were obtained on the ecology of specific pathogens on foods, and within the production and processing environment. Additionally, research facilitated the identification of critical control points during food production and processing; allowed development of alternative HACCP systems, and led to the design and validation of alternate pathogen intervention strategies. One critical outcome was the need to balance the cost of the intervention strategy, practicality of use by the food processing industry, and any effects on food quality.

The aim of intervention strategies is the reduction and/or control of pathogens and food spoilage organisms, however, unexpected consequences such as adaptive responses may occur which needed to be elucidated. The Programs investments in whole and partial genome sequencing and annotation of Arcobacter, Campylobacter, Clostridium, Escherichia, Listeria, and Salmonella species significantly enhanced the ability to conduct this research. Allied to this were investments in developing single and pan-microarray's.

The Food Safety Program does not conduct risk assessments per se for regulatory use. Data derived from ARS studies were transferred to US regulatory agency stakeholders, to foreign regulatory agencies, and the food industry world-wide to serve as the scientific basis to establish standards, regulations on performance standards, compliance guidelines, and in risk assessments. Under the umbrella of predictive microbiology the Program also developed models and/or methods that predict the behaviour of pathogens in foods that have been determined to present a higher risk of causing foodborne illness. The program has extensive collaborations both nationally and internationally.

The development of the Food Safety Research Information Office (FSRIO) web site has greatly impacted the national and international research community, commodity organizations and the general public by providing consumers, educators and others with food safety resource information.

Component 2. Mycotoxins

2.1 Grain Crops, Tree Nuts, Cotton: Reports 57-71.

2.2 Forage Crops: Tall Fescue: Reports 57-71

Executive Summary Mycotoxins

The potential for the presence of mycotoxins in crops is not only a direct food safety problem, but it threatens the competitiveness of U.S. agriculture in the world market. Major goals are to control aflatoxin in peanuts, corn, cottonseed, tree nuts, and figs; fumonisins in corn; and deoxynivalenol in wheat and barley through an understanding of the biology of plant-fungal interaction, and toxin production in the field. Mycotoxin research was conducted in 7 areas (sub-components) under 2.1: fungal and toxin methodology and identification (2.1.1); crop fungal relationships (2.1.2); production practices and expert systems (2.1.3); breeding resistant crops (2.1.4); biocontrol strategies (2.1.5); pest management - insect transmission and predation (2.1.6); and toxicity evaluations and mechanisms of action of Fusarium toxins (2.1.7). Under 2.1 3 areas (sub-components were addressed: fungal and toxin methodology and identification (2.2.1); biocontrol strategies (2.2.2); and toxicity evaluations and mechanisms of action of ergot alkaloids (2.2.3).

Mycotoxin contamination from aflatoxin, fumonisins and vomitoxin occurs in peanuts, corn, cottonseed, treenuts and wheat creates a potential human health hazard, and it severely decreases the value of the commodities. Preharvest control and rapid, nondestructive identification and quantification of the mycotoxins are the greatest needs. The research results of the mycotoxin program will help protect the public health and ensure the continued economic viability of crops.

Because mycotoxins are unevenly distributed an all plant commodities the challenge is to develop non-destructive, rapid, accurate, sensitive and affordable detection methods compatible with both today's high speed, high volume commerce and protection of the public health. Infrared reflectance spectra (NIR) in a commercially available high volume optical sorter successfully rejected kernels from both aflatoxin and fumonisin contaminated grain. To predict and prevent mycotoxins in corn ARS program scientists developed the data and formulated a computer program that would give useful predictions for the occurrence of fumonisin and aflatoxin occurrence in most Midwest corn hybrids in most years. To prevent aflatoxin in cottonseed the mycotoxin program developed AF 36, a product which when applied to the growing cotton plant, competitively excludes/prevents the growth of the fungus *Aspergillus flavis* in cottonseed. Aflatoxin cannot be allowed in milk from dairy cows consuming cottonseed. AF 36 received Section 3 registration from the EPA in 2003 for use in cotton in Arizona and Texas, and subsequently a similar ARS product for protecting peanuts received rapid EPA approval.

The mycotoxin program has embraced genomics which is providing a quantum leap over previous technologies to understand the formation and role of these toxic fungal metabolites. Cooperative agreements for 2 ARS laboratories with The Institute for Genomic Research (TIGR) to prepare expressed sequence tag (EST) sequencing and annotation of two toxin-producing fungi, and a collaboration of ARS with academia in a successful NRI grant will complete the

sequencing and annotation of the fungal genome in order to speed identification of specific fungal targets for prevention strategies. Already the genomics of gallic acid formation in Tulare walnuts is being elucidated. This natural chemical protective factor which completely suppresses aflatoxin production, is the first example of complete resistance to aflatoxin formation. These gene libraries along with developing proteomics capabilities will allow scientists to decipher how environmental factors affect the fungus, and which genes affects fungal survival in the field. Genes will be used for marker assisted breeding of resistant crops.

Component 3. Chemical Residues: Reports 72-77.

Executive Summary Chemical Residues

Preharvest research within this Component focussed on 2 areas (sub-components): food producing animals (3.1.1) and residue and toxin methodology and identification (3.1.2). Dioxins, a class of particularly toxic chemicals has the potential to adversely affect the health of many Americans even at exceedingly low levels. The research program refined available methodology for picogram detection and quantitation, and used these methods to carry out surveys in cooperation with the FSIS and the EPA of the occurrence of toxic dioxins in the meat supply of American consumers, and to identify sources of animal contamination. To prevent heavy metal contamination program research identified soil series which could reliably produce low cadmium sunflowers, flax and durum wheat and identified soil properties (chloride) which caused higher grain cadmium absorption in some soils.

Postharvest research within this Component was directed to 1 area (sub-component); residue and toxin methodology and identification (3.1.2). Research specifically focussed on the development and validation of both screening and confirmatory method to extract selected veterinary drugs, pesticides, residues, and microbial toxins from foods in the field or in the lab. Research developed advantageous multiresidue and multiclass analytical approaches for veterinary drugs in animal food products and pesticides in agricultural food products; and developed rapid screening approaches for the reliable detection of chemical contaminants in foods at regulatory tolerance concentrations, for example QuEChERS. In developing these methods the focus was utility and cost-effectiveness for the end user; combined with, user-friendly, rapidity, increased sensitivity, improved discrimination power, balanced with the ability to be automated when combined with the need for high-through-put. The technology where possible was immediately transferred to stakeholders and end users for incorporation and implementation into Federal, State and International regulatory compliance programs, in particular USDA-FSIS, industry (worldwide) and to researchers.

Subcomponent Research Report and Impact Statements

The preharvest food safety research program addresses what can be done during animal and plant production, that is, up to slaughter and/or harvesting, to make the ultimate food products safer. There has been significant progress during the 4 years of this program review cycle toward meeting the goals delineated in the National Program Action Plan, all of which contribute to improving the safety of the food supply for the American people

Component 1. Microbial Pathogens

1.1 Preharvest:

The largest component of preharvest food safety is pathogen control in cattle, swine, and poultry, the species that are the primary source of animal food products for the American diet. The research has led to important developments, key examples of which follow in each subject section:

1.1.1 Methodology

Work under this sub-component was designed to develop rapid, specific and accurate methodologies to help successfully understand the ecology, epidemiology, and intervention segments of the research efforts, and to support government agency and producer pathogen control programs.

$ Developed genetic based detection and differentiation methods for *Trichinella* & *Toxoplasma* in pigs. Evaluated methods for the inspection of pigs and horses for *Trichinella* at slaughter. Impact: These methods were critical to 1. establishing and maintaining an export market inspection program in cooperation with AMS, and 2. use in a certification program for these parasites in pork modeled on the National Trichinae Certification Program, and 3. achieving an extremely low incidence of *Trichinella* and Toxoplasma parasites in swine that has significantly increased consumer acceptance of pork products.

$ Generated a panel of monoclonal antibodies as accurate diagnostic reagents for the detection and characterization of shiga toxin producing *E. coli* (STEC) and Salmonella, including type, group and serotype specific antibodies. Impact Several of these antibodies were formatted for diagnostic tests through Meridian Biosciences, Cincinatti Ohio, which marketed them as the Immuno-CardStat EHEC O157 kit. The Meridian test is currently widely used in the US meat industry, by commercial food company's (BurgerKing), and in human clinical tests to identify the presence of STEC.

$ An electrostatic sampling device was evaluated for its ability to detect airborne *Salmonella Enterditis* (SE) in rooms containing infected caged laying hens. Impact This device will provide a low cost, sensitive alternative to traditional detection of SE in the environment of laying flocks.

$ Adapted new rapid detection methods, i.e., the fluorescence polarization test and the rapid lateral flow immunodiffusion test, to identifying SE in contaminated, incubated egg pools. Impact The methods are rapid, useful alternatives to longer, more expensive, traditional bacteriologic culturing methods, thus they can help lower the incidence of SE in laying flocks.

$ Developed a rapid, sensitive and specific fluorescent-based (TaqMan) PCR test for detecting O157:H7 and other shiga toxin-producing *E.coli* in bovine feces and tissues. The test was also modified for simultaneous detection of O157:H7 and pathogenic *Salmonella* strains. Additional TaqMan-based PCR tests to detect and quantify important serotypes of shiga toxin-producing E.coli including O157:H7, O26, and O111. Impact These tests will provide the basis for rapid and specific detection *E. Coli* O157:H7 and related pathogens at various stages of pre- and post-harvest operations. Since these tests can be completed within 8-12 hours, potentially contaminated bovine food products can be identified before they are shipped to retailers and consumers

1.1.2. Epidemiology and Ecology

Under this sub-component pathogen risk factors and antibiotic resistance were assessed to elucidate the ecology of food borne pathogens outside of the host animal and risk factors which contribute to their incidence in food producing animals; however, because of the ubiquitous nature of these bacteria and protozoa, much work remains to effectively use this information to develop successful interventions.

$ Demonstrated that STEC O157 is endemic in US cattle herds and occurs at high fecal prevalence rates in summer months. Isolated STEC O157 from multiple pest fly species trapped on livestock farms, and confirmed the clonality of livestock and pest fly bacterial isolates from a given farm. Also documented high pest fly and livestock fecal prevalence of STEC O157, O111, and O26 and *Salmonella* at county and state agricultural fairs similar to that in commercially reared livestock. Also documented the high prevalence of oral cavity and hide STEC O157 in finished beef cattle. Impact Demonstrates that because of the ubiquitous nature of STEC O157, eliminating exposure will be almost impossible, and establishes the need for a live animal treatment to kill the pathogens just prior to slaughter. Also targets non-fecal sources as important for both within herd livestock transmission and in plant carcass contamination.

$ Identified filth flies as mechanical vectors of *Cryptosporidium* oocysts. Impact We have almost no control programs for *Cryptosporidium* in cattle, the source of many of the oocysts entering drinking water sources. Thus effective fly control could provide a much needed initial component for control programs.

$ Using highly specific serologic tests for anti-O157 antibodies provided the first estimate of the basic reproductive ratio (R_o) for E coli O157:H7 in beef cattle. Impact R_o indicates

the ease of transmission of an infectious agent; it is necessary for epidemiologic modeling of infection dynamics, and it make possible more accurate prediction of the effectiveness of interventions, such as vaccination which are necessary for making production decisions.

$ Showed that ionophore antibiotics in cattle had no effect on food borne pathogens or their antimicrobial susceptibility. Demonstrated that the animal growth promoters (bacitracin, flavomycin or virginiamycin) do not select for certain species of enterococcus (commensal bacteria) and do not alter normal bacterial populations. Impact Demonstrates that drug use in improving livestock and poultry production efficiency does not increase bacterial food safety hazards, thus helping to maintain the availability of these drugs to producers and keeping meat prices to consumers near present levels while maintaining safety.

$ Through the animal arm of the National Antibiotic Resistance Monitoring System which is located in ARS, determined the antibiotic resistance of over 35,000 Salmonella isolates and lesser numbers of *Campylobacter, Enterococcus* and *E. coli*. Impact This is the only national program for surveillance of resistant bacteria in animals in the US and it provides critical information regarding the prevalence and distribution of anti-microbial resistant bacteria to support the Public Health Action Plan to Combat Antimicrobial Resistance of HHS/CDC and FDA. It is essential to both animal producers and to developers of animal drugs to have accurate information to help assure continued drug availability.

$ Described a variety of environmental sources of Campylobacter during epidemiological studies conducted both in the U.S. and in Iceland. Impact This research has demonstrated that just one method will never be sufficient to control Campylobacter in poultry. Multiple target interventions will be needed to reduce the incidence of contamination in production flocks.

1.1.3 Ecology and the Host Pathogen Relationship

Under this sub-component sites, researchers worked to understand the mechanisms of colonization, virulence, and pathogen risk factors in the host animal, including genetic risk factors, and mechanisms of pathogen acquisition and carriage. However, much work remains to be done to effectively use this information to develop successful interventions.

$ Demonstrated a clear and positive association between large intestinal *E. coli* O157:H7 infection of live cattle and contamination of beef carcasses. Similarly the level of Salmonella on broilers pre- and post-slaughter and processing were found to be correlated. Impact These studies underpin the entire concept of preharvest food safety, and emphasize the need for on-farm interventions to reduce the risk of pathogens reaching consumers.

$ Demonstrated that predation by rumen protozoa can enhance Salmonella virulence, including in multiple antibiotic resistance strains of DT104. Impact This information targets the rumen as a possible pathogen reservoir, and identifies rumen defaunation (killing of the protozoa) as a control target. Protozoa are not necessary for normal rumen function.

$ Using Suppression Subtractive Hybridization and real-time RT-PCR, to quantify the regulation of swine genes involved in both the innate and cell mediated immune responses of swine during Salmonella infection. Impact This information helps scientists understand the carrier state, and particularly how the differences between host adapted and non host adapted serotypes, *Salmonella cholerasuis* and *S. typhimurium*, affect the porcine immune system, making swine more susceptible to colonization.

$ Identified two porcine genetic markers with a heritable phenotype associated with disease resistance to Salmonella. Impact A swine breeding company is using the research findings to assist in the development of Salmonella-resistant lines of pigs.

$ Identified that a wide variation (5 - 100%) exists amongst beef cattle in the ability of their macrophages to kill *E. coli* O157:H7. Impact This can help identify animals with little ability to clear EHEC infection, allowing their offspring to be eliminated from replacement breeding programs.

$ Determined that passage through the bovine gastrointestinal tract does not result in the development of extreme acid resistance of *E coli* O157:H7, and that increasing percentages of dietary corn also does not increase acid resistance of *E.coli* O157:H7. Impact This helps counter concerns that beef cattle contribute to environmental and foodborne burden of acid resistant *E. coli* to which humans may be exposed.

$ Using RT-PCR established that the avian heterophil, the first host cell type encountered by Salmonella in the intestine of poultry, is capable of rapid changes in cytokine gene expression following receptor mediated phagocytosis of Salmonella. Identified biologically active cytokine components of naturally occurring Salmonella enteritidis - immune lymphokines (SE-ILK) in poultry. Impact This knowledge of cytokine gene expression has been transferred to a broiler breeding company and is being used to produce birds highly resistant to Salmonella.

$ Characterized the varying subpopulations of SE that propagate high incidence egg contamination by ribotyping, LPS analysis and animal infection. Impact The animal biologics industry can use this information to aid in optimizing vaccine efficacy.

$ Analyzed levels of *Campylobacter* spp. translocation to different lymphoid and reproductive organs in inoculated breeder hens and roosters utilizing molecular detection techniques. Impact Since Campylobacter may be stable in rooster semen and the bacteria may be carried to lymphoid organs, this is a source of potential transmission among

poultry flocks. Producers of hatching eggs can now work to eliminate this source of bird contamination.

$ Identified basic mechanisms and selective pressures involved in the evolution and transfer of antibiotic resistance genes among pathogens and between commensal bacteria and pathogens. Impact Scientists can now begin to more clearly understand the persistence of antibiotic resistance and the transfer and exacerbation of resistance to unrelated drugs, and begin to more accurately assess intervention strategies.

1.1.4 Intervention Strategies

Research under this sub-component reduces colonization and shedding of pathogens in food animals. This research has led to promising strategies or supplied the basic information necessary for their development, key examples of which follow:

$ Determined that free living microorganisms (rotifers) can ingest Cryptosporidium oocysts and that *Giardia* cysts can be ingested by free living predatory microorganisms. Impact This information provides a potential method of biocontrol which could reduce chemical disinfectant use, the only method now known to be an effective control for these parasite forms.

$ Used transcriptional gene fusion technology to identify the gene *hha* in cattle and showed that its absence dramatically increased the expression of virulence factors essential for *E. coli* O157:H7 colonization. Impact The *hha*-deficient strains will also provide the basis for developing vaccines or for producing large amounts of intimin or other protein factors for use as immunogen for reducing *E. coli* O157:H7 in cattle.

$ Developed real-time fluorescent technology for rapid and sensitive detection of fecal contamination on animal carcasses. Impact Industry developed both handheld and whole animal carcass-scanning devices using this technology. The detectors are commercially available and are being incorporated into HACCP programs at a limited number of beef slaughteriin facilities to provide a highly valuable system for monitoring and removing fecal contamination which will save these facilities nearly $6 million per year. Savings of over $100 M per year are projected savings as the detectors are incorporated into additional slaughter plants.

$ Completed the first anti-STEC O157 intervention trials using natural infected finished beef cattle. Demonstrated failure of pen cleaning and disinfection, two probiotics, testing and quarantine of fed cattle for nine months, group size (1 vs 10 cattle per pen), and simulated transport and holding for slaughter. Provided evidence that feed change (to alfalfa hay), stress response (systemic dexamethasone), and oral neomycin (antibiotic) therapy may influence (promote or inhibit) fecal shedding. (Only oral neomycin has shown practical utility in reducing STEC O157:H7 fecal prevalence in cattle.) Impact

This knowledge will reduce expensive investment in and utilization of plausible, but ineffective, control strategies for STEC O157 by cattle producers.

$ Developed a defined porcine-derived competitive exclusion culture which was studied in field trials with 34,676 pigs. Impact A 3.53% decrease in nursery barn mortality and decreased medication costs which was observed resulting in cost benefits per farm (5 farms) of~$ 24,000 per year from decreased mortality and medication in treated pigs.

$ Showed that oral administration of chlorate in the feed or drinking water selectively kills *E. coli* and *Salmonella*, but not beneficial bacteria, in the gut of food animals (poultry, swine and cattle). An improved product with rumen bypass characteristics performed better for cattle than chlorate itself. Clinical field trials in cattle achieved several log reduction in Salmonella and E. coli. Impact This patented technology has the potential to significantly lower levels of *E .coli* O157:H7 in cattle coming to slaughter; a commercial sponsor is investing heavily in the product and regulatory approval is now being sought.

$ *In vitro* and *in vivo* results with several electro-negative compounds, including nitroethane, demonstrated significant Salmonella control which persists longer than with chlorate. In vitro studies have also shown that these nitrocompounds exert significant bactericidal activity against *Campylobacter*, *Listeria*, and *Yersinnia*, and work extremely well with chlorate. Impact These nitro compounds also are potent inhibitors of ruminal methane production, an inefficient digestive process costing U.S. cattle feeders up to $900,000 per day. Thus use of these compounds may be a cost recoverable control strategy for use in controlling *E. coli* O157:H7.

$ Developed intervention strategies, e.g., vaccination, dust reduction with electrostatic space charge system, and alternative molt procedures, e.g., high fiber diet utilizing alfalfa meal/wheat middlings, that put hens into an egg laying pause yet do not exacerbate an *S. enteritidis* problem. Impact The egg laying industry now has strategies to molt hens whose production is degraded, yet should not incur such criticism from the public.

1.1.5 Feed Withdrawal and Transportation

Under this sub-component research was carried out to decrease the potential for introduction of zoonotic pathogens into slaughterhouses.

$ The prevalence of Salmonella in hogs transported and slaughtered at a commercial abattoir was found to be significantly higher (40%) than for hogs slaughtered on farm (8%). Further investigation demonstrated that holding pens pose a significant risk for Salmonella contamination of pork and emphasized the role that stress, commingling, and environmental contamination have on the dissemination of Salmonella in swine. Impact The pork industries now have an additional control point in the production to consumption continuum to include in good production practices and HAACP programs to decrease Salmonella in pork products.

1.1.6 Manure

Under this sub-component research to reduce transport and dissemination of pathogens through manure was carried out. However, much work remains to effectively use this information to help provide producers, ecologists and action agencies with information and strategies to limit the spread of pathogens from animal manure.

$ Real-time PCR methods were develop for the detection of *Mycobacterium* Avium *paratuberculosis* (MAP) the causal agent of Johnes's disease by using PCR methods. Impact Detection of MAP in low numbers from environmental samples can now be accomplished in less than half-a-day instead of months by traditional culture methods. Improved monitoring of MAP from dairies to the environment will be possible.

$ Using laboratory scale circulating aerators determined that the pathogens, *E coli* O157:H7 and Salmonella declined rapidly in both aerated and stagnant manure waters, however variation in survival among strains of E coli O157:H7 was observed. Also *E. coli* O157:H7 failed to become established in manure water reinoculated repeatedly to simulate fecal shedding in dairy barns. In contrast E. coli O157:H7 can survive for long periods and even multiply in feedlot soils, over a wide range of water and manure content. Impact This information helps determine the potential for pathogen transfer to crops fertilized and irrigated with manure water. In addition this infomation is needed by government agencies to make recommendations for livestock producers handling of manure.

$ Determined that simple plant compounds, such as thymol, trans-cinnamic and ferulic acids commons to temperate forages, were effective at inactivating *E. coli* O157:H7 in bovine feces. Impact The research has identified potential amendments to animal manure to control pathogen transmission and has identified potentially exploitable dietary components for reducing pathogen shedding.

Component 1. Microbial Pathogens

1.2 Postharvest

The reduction and control of microbial pathogens in/on meat, poultry, seafood, and fruit and vegetable food products is the most pressing food safety issue today. Research outlined below describes achievements within this area.

1.2.1 Methodology

Work under this sub-component was directed towards developing methodologies that have both regulatory, industry and research use. ARS was directed to take the lead in commonality of interests between government and stakeholds. Specifically, research led to the development of, or ability to:

- Sampling plans which reduced the amount of material needed for testing.
- Capture, separate, concentrate and enumerate from complex-foods and large volumes, including air.
- Nucleic acid and/or protein extraction without the need for toxic materials.
- New resuscitation, enrichment and growth media.
- New mathematical methods for calculation of bacterial cell density.
- Antibodies using phage display, and new animal hosts for immunization.
- New immunomagnetic beads for combination with new chemiluminescence or time resolved fluorescence detection systems.
- Immunofocus assays negating the need for radioactively labelled compounds.
- Automated, multiple assay formats (96, 360 well).
- Improved/new nucleic acid based methods such as: PCR, RT-PCR, PFGE, MLST, ribotyping, and microarrays.
- Bacteriophages capable of inducing bioluminescence for quantitative sensing.
- Miniaturized biosensors
- Specific biomarkers for use with MALDI-TOF mass spectrometery.
- Animal/plant based cell systems that assess potential for in-vivo disease.
- Measure attached bacteria, and biofilm formation on surfaces
- Automated on-line-sensing-systems for carcass inspection.

Impact:

ARS took the lead to develop methods that have regulatory, industry and research use. Technologies were transferred to the end users, mainly FSIS and FDA who will work with ARS to refine them for (automated) day-to-day use. New (sampling) methods have already been accepted for use by FSIS, while for other technologies their full impact is likely 2-years away. These will include: the ability to capture cells from any sample size and type, and enumerate without, or with minimal culture enrichment, currently the most time limiting step. Investments in genomics and proteomics will allow in vitro discrimination based on virulence-associated

genes to separate potential pathogenic from non-pathogenic isolates, and rapidly identify species/strain differences difficult to analyse by other methods. When combined with cell culture methods this will allow determination of virulence and pathogenesis in vivo, without the use of animals.

The development of inexpensive, micro-fluidic and electronic bio-chips that detect cell growth, metabolites such as toxins, and differentiate living and dead cells in very small samples/volumes will allow industry to incorporate this technology into food packages before shipping. Spoilage could be monitored (by GPS) during transportation, and/or by retailer and consumer though an enzyme based color change.

The technology having the greatest impact for regulatory agencies, industry and the consumer will be on-line sensing technology. Immediate implementation in the broiler industry alone would allow re-deployment >1300 inspectors, and save industry upwards of a $1.0 B/year. Consumers would benefit by increased product safety and quality through improved carcass inspection for contamination, disease, defects. Ultimately this technology can be transferred to any food processing industry. It will find further use to evaluate cleaning and sanitation efficiency; and biosecurity to detect advertent/ inadvertent contaminants.

1.2.2 Production and Processing Ecology

The goal of this sub-component was to elucidate the ecology of pathogens on animal products, seafood, fruit and vegetables, and within the processing environment. This information could be used to identify critical control points or validate parameters of existing critical control points, and lead to the design of control or intervention. Specifically, research (often) using technologies developed/described under the previous Methods Section 1.2.1 assisted in providing, determining or demonstrating:

- The utility and use (in the field) of newly developed methods (Section 1.2.1).
- Baseline data, and pathogen numbers/types in/on various foods, food matrices, and food production and processing environments, including air, and water.
- The positive relationship between pathogen infection of live animals, subsequent carcasses contamination, and further processing.
- Identified additional sources of contamination and cross contamination.
- Carcass mapping protocols for beef carcass process control.
- Pathogens vary in their pathogenic potential. Epidemic strain types harbour specific genes that impart increased fitness to food/food related environments.
- The isolation of multiple strains of a specific pathogen from a single food type or processing environment indicated the diversity, yet directed-adaptability.
- Some pathogens share DNA, other do not. Strains that share were associated with the environment, and appear to be implicated with foodborne illness and disease.
- Specific pathogen types, levels and growth maybe closely associated with types, levels and growth of other non-pathogens.

- § Free-living non-pathogenic commensals may be vectors of pathogens and serve as sources of both external and internal contamination of produce.
- § A high risk of pathogen contamination on fresh produce by airborne transmission especially when production was in close proximity to animal production systems.
- § Difficulty of decontaminating produce due to the inability to access all sites of pathogen attachment on the product surface.
- § Model(s) for mixed population biofilms**.** Non-pathogens are unnecessary for attachment of pathogens, but their presence enhances biofilm formation.
- § Surface affects such as electropolishing stainless steel that increase resistance to bacterial attachment/biofilm formation.
- § Several new compounds for commercial applications for surface sanitation in food processing environments.

Impact:

The technologies assisted processors monitor microbial loads on animal hides and carcasses at several points during processing, as a means of verifying process control. Prior to this technology there was no established protocol to accomplish this goal. Implementation of industry-wide standard protocols for microbiological testing would allow individual plants to benchmark their hygienic status and evaluate the efficacy of current on-line antimicrobial interventions.

Prevalence data and tracking results indicate that minimizing contamination early in processing can lead to large reductions in contamination of the final product. Pathogens can be transferred from a slaughter plant to a further processing plant by way of raw product and subtypes can persist in the further processing environment for extended periods of time. This impacted industry approaches regarding the source of pathogen contamination on carcasses and initiated studies of antimicrobial interventions with specific targets.

The phylogenetic relationships of many pathogens both between and within Genus and species is incorrect. Certainly, not all strains pose the same risk to human health. Epidemic types and fitness may partially account for their involvement in human illness and disease. This provides the regulatory agency's the potential to reduce the size and number of food safety recalls (due false positives), allows detection and quantitation of only critical isolates, while providing maximum protection to the consumer.

The presence of multiple strains emphasized the need to develop further DNA sequence based methods for finer comparisons, better source-tracking and epidemiology for recalls. Adaptability to various environments are genetic traits associated with altered physiological states, or adaptation to extrinsic/intrinsic stresses. Physical/chemical structures on the surface of the pathogen often facilitate increased attachment/resistance to interventions. This impacts decisions as to type and efficacy of decontamination/ sanitation practices.

1.2.3 Intervention Strategies -Processing and Control Technologies

Under these two sub-components the goals were to develop intervention strategies through innovative processing technology that will: aid regulatory agencies in establishing the basis in regulations for HACCP programs; and aid both the large and small processor in carrying out good manufacturing practices for: animal, seafood products and plant products. Research assisted in providing, determining or demonstrating:

- § Efficacy/effect of dietary changes and carcass electrical-stimulation on subsequent, processing times, safety, and quality attributes.
- § Efficacy of GRAS chemical compounds for use as antimicrobial alternatives.
- § Efficacy of altered scalding/washing regimes, and their effect on product safety and quality.
- § Electroflotation technology improved the quality of process water, water based processing liquids such as brines, and manure lagoon water.
- § Evaluated efficacy of various antimicrobial compounds and hide-wash-cabinet, and chemical dehairing for utility in hide decontamination.
- § Reduction in levels of both spoilage bacteria and pathogens in meat products using hydrodynamic pressure processing was only partly effective, and only if used in combination with other stresses.
- § Limited effectiveness of Vacuum Steam Vacuum (VSV) technology in poultry processing. Redirected for application in processing hot-dogs with positive impact.
- § Effectiveness of using Radio Frequency Electric Fields (RFEF) was varied and product dependent.
- § Doses of Ionizing Radiation (IR) required for processing various foods (plant and animal derived) were determined. Changes in quality and sensory properties were also evaluated. The effectiveness is improved in combination with additional intervention strategies.
- § Many physical and chemical intervention/control strategies for produce initially through efficacious, proved ineffective, or difficult to implement. Negative effects on quality, and safety (residues) were often apparent.
- § Innovative areas such as using competitive exclusion on produce were equivocal. Bacteriophage were however, shown to have potential as a biocontrol strategy.
- § New BL-2 food processing suites were designed and built. Their utility was varied, and product dependent. Future use is likely to address food security issues.
- § Depuration and/or high pressure for decontaminating shellfish was either equivocal or ineffective.
- § Antimicrobial strategies for meat and poultry were ineffective in finfish processing. Effective cleaning and sanitation were critical to clean operations and enhanced safety and quality.
- § Thermal processing times, temperatures, under various conditions to ensure the safety of a variety of foods: including cooked meat and poultry products, ready-to-eat foods, and juices.
- § Computer programs that model different processing systems in order to predict temperature distributions and pathogen lethality.
- § Methods using acid and heat to prevent the growth of pathogens and spoilage microorganisms in minimally preserved brined and fresh cut products.

Impact:

Simple and cost effective changes in diet and feed withdrawal, combined with electrical stimulation, minimizes fecal contamination during slaughter, increasing safety, while producing more tender meat, thus increasing quality. Dietary changes have been incorporated by integrators, and two international processing equipment manufacturers are marketing carcass stimulation equipment.

Simple changes in water flow during scalding are effective in reducing contamination and cross contamination, thus reducing the need for higher water temperatures and energy use without affecting product safety and quality. Use of GRAS compounds increased product safety and quality, reducing the need for chlorine and phosphates, both significant trade barriers.

Industry is committed to installing hide wash cabinets and utilizing some form of antimicrobial compound or regime (steam) since it will benefit the producer, and provide the consumer with a safer product. Sodium hydroxide and chlorinated water were found to be highly effective antimicrobials, but their use has trade issues. Chemical dehairing although efficacious and approved by industry and FSIS could not be readily implemented by many producers/processors. The use of CPC (found in mouthwash) was novel, and could be readily used in combination with dehairing.

Implementation of electroflotation technology offers the ability to simultaneously improve the quality of processing waters and fluids through removal of suspended solids, while reducing the microbial load. This substantially reduces the amount of fresh water required to maintain water volumes which is a significant saving to industry. The power energy costs to maintain the technology are also minimal < 3kWhr/1000L.

There have been some collaborative agreements with industry, and patents applications for various technologies. However, in most cases these were product specific. For example, VSV will have application in formed meat products, and possible harder skinned (formed) fruits (lemons) and vegetables (carrots), while RFEF could have application in the low pH juice industry. Application of RFEF in for example the liquid egg would ensure commercialization.

Radiation biology research has provided timely data for use by Federal regulatory agencies and industry to aid in evaluations of petitions to allow a variety of irradiated foods. The use of IR remains controversial, especially where food, for example ground beef, is being provided to children through the School Lunch Program.

The inability to obtain a new irradiator has frustrated advancement of the research program, and necessitated collaborations with industry partners particularly in E-beam studies.

Despite concerted efforts by a number of labs the development of generic intervention technologies/strategies for decontamination of produce remains largely unfulfilled. Tissue sensitivity, residues, impenetrable tissues and biofilms were critical issues to ineffectiveness. Specific applications were developed and implemented, for example using chlorine for sanitizing

seed for the sprouting industry. The use of chlorine dioxide appeared to hold significant (generic) promise especially for sensitive fruits such as strawberries. Acidified sodium chlorite (SANOVA) for fresh-cut produce (lettuce), and bacteriophage for packaged fresh fruits also show promise for commercialisation.

The concept of new and/or improved commercial depuration and pressure processes to eliminate bacteria and viruses in shellfish has raised considerable interest among the seafood industry and regulatory agencies. However, the studies demonstrate the ineffectiveness of either commercial process, and highlight the continued potential health concerns with these foods
Thermal processing data were transferred to regulatory agency stakeholders (FSIS/FDA), foreign regulatory agencies, and the food industry world-wide to serve as the scientific basis to establish standards/regulations on performance standards, compliance guidelines, in risk assessments, and food inspection programs. The predictive equations were incorporated as part of a user-friendly USDA pathogen modeling (computer) program (PMP) and contributed to ComBase, an international microbial modeling database. (see under sub-heading 2.6 Risk Assessment).

In summary, alternative/new technologies to process foods all have the ability to inactivate microorganisms to varying degrees. The major issue is that for most, the treatment intensities required for inactivation often result in adverse functional and/or sensory properties, significantly reducing food quality. Thus, as single intervention strategies each of the technologies are not efficacious, especially when combined with high costs and energy expense. Since safety cannot be guaranteed at a reasonable cost most technologies are of limited practical use for the food processing industry.

1.2.5 Effect of Intervention Strategies

Under this sub-component research was directed towards elucidating and understanding the effects of intervention strategies. Interventions may result in a variety of effects both to the food, and to the microorganisms (resistance). Research which addressed Sections 1.2.2 (Ecology) and 1.2.4 (Intervention Strategies) within the Action Plan was directly related to, or impacted this Section to provide, determine or demonstrate:

- Investments in whole and partial genome sequencing and annotation significantly enhanced the ability to conduct this research.
- Genes or cassettes involved in adaptation to a wide range of intrinsic and extrinsic stressors were identified and characterized
- Stress-adaptation genes were typically found in higher numbers in pathogens.
- Adaptation to one stressor was not a predictor of adaptation to another: that is resistance to one stressor did not necessarily provide cross protection or cross sensitisation to another.
- Adaptation to stressors was often Genus, species or strain specific.
- Adaptation to stressors was often determined by the availability of specific food components.
- Products of certain stress adaptation genes also appear to be virulence factors.

- § Some pathogens increase their ability to survive stress (fitness) through cell aggregation; formation of biofilms; alteration in cell structure (from a rod to a cocci); induction of sporulation; or (possible) selective encapsulation in protozoa.
- § Different types of quorum sensing systems (cell-to-cell signalling) are associated with adaptation to stress, and subsequent growth and survival.
- § Aggregation, biofilm formation, and quorum sensing systems may play significant roles in allowing pathogens share to DNA, and hence adapt to stress.

Impact:

Comparative genetic analysis will provide clues to virulence, pathogenicity, and the persistence of pathogens in/on animals and plants; in the production environment; in foods; and in food processing environments. Utilization of microarrays (developed in section 2.1) will facilitate analysis of these genes and their products for differences in expression and function. Products of stress-adaptation genes or quorum sensing systems are potential targets for future antimicrobial development. Sanitation/decontamination strategies must now be targeted to those pathogen cells that are protected by aggregates/biofilms/cell states, in order to be effective.

1.2.6 Risk Assessment

Research under this sub-component addressed the need for quantitative measurement of pathogens at critical points during food processing in order to provide the necessary data to carry out risk assessment; develop and validate predictive microbial models; and identify areas where interventions are most critical. Research determined, developed models and/or methods that:

- § Predict temperature distributions and thermal death rates for pathogens and non-pathogens in various foods, and with other extrinsic and intrinsic factors.
- § Predict the relative growth of the pathogen from spores at temperatures applicable to the cooling of cooked products.
- § Predict competition between bacteria used in the production of acidified pickle products.
- § For evaluating the performance of models utilizing the concept of an “acceptable” prediction zone. The method measures global/regional prediction accuracy.
- § Predict growth in raw, sterile, and cooked foods over the entire growth temperature range.
- § Describe the behavior of high risk pathogens (L. monocytogenes; E. coli O157:H7) in higher-risk foods identified by FDA/FSIS risk assessments.
- § Predict the incidence and distribution of pathogens as a function of PCR detection time score and sample size.
- § Predict the dose-response of consumers to ingestion of pathogens as a function of dose, strain prevalence, and strain virulency.
- § Predict non-thermal death kinetics; effects of irradiation; time-to-toxigenesis, time to turbidity.
- § Predict the distribution of E. coli O157:H7 in raw ground beef.
- § Process risk model that predicts the change in pathogen incidence and number across unit

operations of the retail-to-table chain
- § Predict the risk of illness from consumption as a function of the uncertainty of the processing parameters in the unit operations.
- § Predict pathogen transfer potential from surfaces within processing environments.
- § Indicate that models developed in broth are not necessarily good predictors of behavior in foods.
- § Allowed enhanced development of the PMP, and the international database (Combase)
- § Allowed the formation of CEMMI

Impact:

All microbial models were incorporated into a regularly updated Pathogen Modeling Program (PMP) available at www.arserrc.gov/mfs/CRIS041.htm or on CD. Each year, the PMP is downloaded approximately 6,000 times by more than 25% of the US food industry, and to users in over 35 countries. The models are user friendly. Users are able to input specific information about their food products, and then receive predictions of pathogen behavior via easy to read graphical output. Further enhancements include facilitating input of PMP data into risk assessment software, and incorporating an Expert System to provide users with more precise interpretations of PMP information.

The PMP has become invaluable as a predictive tool to rapidly provide accurate estimates of pathogen survival, allow food processors to formulate foods to include acknowledged intrinsic barriers, assess the microbial risk of a particular food and design reduced thermal processes that ensure safety against pathogens while minimizing quality losses. Currently the PMP is being translated into Spanish and Chinese. Several other European countries (France and Finland) are currently discussing translation capabilities.

In collaboration with the U.K. Institute of Food Research and the UK-Food Standards Agency, the data under-pinning the PMP and the equivalent (U.K. Food MicroModel) have now been archived in a public database, ComBase (www.combase.cc). ComBase also includes thousands of records from the literature, as well as from numerous international institutions, and industry, resulting in over 32,000 records of predictive microbiology information. ComBase will accelerate the development of new models, the validation of existing models, and the production of risk assessments.

As of the Fall 2004 the Combase consortium will formally include Food Science Australia and U. Tasmania joined as the Australian Food Safety Center of Excellence (AFSCE). The new partnership adds a enhanced geographical perspective creating a UK-US-Australia triangle. Inclusion of the AFSCE will also assist in developing networks with Asian activities, especially assisting ongoing relationships with China; and in developing productive associations with the International Committee on the Microbiological Safety of Foods (ICMSF) and CODEX, thus elevating Combase in international microbial risk assessment.

The Center of Excellence for Microbial Modeling and Informatics (CEMMI) was formed to encapsulate all the programmatic efforts www.arserrc.gov/cemmi. The goals are: facilitating

model development among researchers and industry; extending model development (informatics) through a “data warehouse” function (Combase); communicating through national and international symposia and the internet; working with users of predictive microbiology; enhancing uniformity of experimental designs; linking modelers with industry; and defining researchable data gaps.

Food Safety Research Information Office (FSRIO)

The primary objective of the Food Safety Research Information Office (FSRIO) funded through NP108 but located at the National Agricultural Library (NAL) is to serve as the central source for information on past, present and proposed national, and where appropriate, international research activities related to food safety. Some of the major accomplishments of FSRIO were:

- Developing a Web site (www.nal.usda.gov/fsrio) that provides a searchable database and reference services to serve both the research community and the general public.
- Integrating the Joint Institute of Food Safety Research (JIFSR) data set into the FSRIO to enhance and sustain the Federal food safety research inventory.
- Producing resource lists and fact sheets on specific research topics.
- Being proactive through future initiatives: launching version 2.0 of the research projects database and a newly designed Web site in early 2005. The version will also establish a systematic method of collecting data from USDA and other federal research agencies based on its new database architecture.
- Establishment of cooperative agreements with other academic institutions to assess research initiatives using information from its research projects database.

Impact:

FSRIO has greatly impacted the national and international research community, commodity organizations and the general public by providing consumers, educators and others with food safety resource information. Integration of the JIFSR database avoided federal duplication of effort with saving of over $500,000/year.

Component 2. Mycotoxins

Mycotoxin contamination from aflatoxin, fumonisins and vomitoxin occurs in peanuts, corn, cottonseed, tree nuts and wheat; this segment of the Food Safety program addresses their control in those crops and seeks to obtain information and assess the biological effects of the toxins. The research has led to important developments, key examples of which follow in each section:

2.1 Mycotoxins in Grain Crops, Tree Nuts and Cotton

2.1.1 Fungal and toxin methodology and identification

Under this component, methods to detect and quantify both fungi and their mycotoxins were developed. Because mycotoxins are unevenly distributed in any contaminated plant commodity, the greatest need is a methodology to determine the contamination of the entire tested lot. Further the challenge is to develop non-destructive, rapid, accurate, sensitive and affordable detection methods compatible with both today's high speed, high volume commerce and protect the public health.

$ Developed rapid, easy to perform and quickly learned fluorescence polarization immunoassays for measuring the fumonisin and zearalonone mycotoxins in maize and deoxynivalenol in wheat. Impact The assays offer environmentally friendly alternatives to traditional instrumental methods, and ELISAs, and they will be useful screening tools to determine mycotoxin contamination.

$ Dual near infrared (NIR) reflectance spectra in a single pass through a commercially available high volume optical sorter simultaneously and successfully rejected kernels from aflatoxin and fumonisins contaminated grain. Impact Use of this technology which examines all product, overcomes the very serious sampling problem of unequal distribution of mycotoxins. Although more work will be necessary to improve the accuracy and specificity of this technology to detect very low amounts of mycotoxin contamination, this development is a first very important step in making available an online technology to eliminate mycotoxins from infected commodities, in order to meet FDA human food standards.

2.1.2 Crop fungal Relationships

Research in this component addresses plant/fungal interaction during fungal growth and toxin production.

$ Determined that *Fusarium verticillioides* is transmitted from seed to corn plant to seed as an endophyte, and the fungus grows more readily on reproductive and immature tissues than old vegetative tissue. No deleterious effects on corn yield were found in a 3 year study and only infrequent negative effects on plant growth under ideal field conditions;

but under stress, fumonisin accumulation is accelerated. Impact This information on fungal transmission provides a basic understanding of the very complex fungal endophyte/crop interaction which is necessary to develop effective strategies to prevent mycotoxin accumulation in crop plants.

$ Determined that the very effective aflatoxin resistance in the Tulare variety of walnut is due to high levels of hydrolyzable tannins in the seed coat which persists throughout the growing season, in contrast to other varieties. Fungal tannase hydrolyzes tannins to form gallic acid which affects fungal response to oxidative stress and is a potent inhibitor of aflatoxin synthesis. Impact This information explains the basis of a naturally occurring antifungal/mycotoxin mechanism and points to prevention of aflatoxin biosynthesis by disruption of oxidative stress response pathways, and it will help guide genetic selection of resistant varieties of other nut crops.

2.1.3 Production practices and expert systems

Research in this component comprises cultural practices and expert systems designed to effect preharvest practices to reduce aflatoxin formation.

$ Developed the data and formulated a computer program that would give useful predictions for fumonisin and aflatoxin occurrence in most corn hybrids in most years. Impact This program provides farmers a valuable tool for a comprehensive mycotoxin management program. It has been made available to producers in an easy to use format

$ Showed that the rehydration procedure to facilitate cracking of closed shell pistachios results in exceptionally high aflatoxin levels. Impact This knowledge indicating that rehydration should be abandoned will help pistachio producers meet the very low regulatory guidelines for aflatoxin.

$ Demonstrated that the second phase of contamination of cottonseed, that is between boll opening and harvest, was the most important factor predisposing the crop to aflatoxin contamination, rather than the first phase as was traditionally emphasized. Impact This knowledge highlights the importance of harvesting cotton early, and not allowing the cotton crop to get “wet” and it also helps cotton breeders target events in plant growth and development for reducing aflatoxin susceptibility of their crop.

2.1.4 Breeding resistant crops

This component obtains knowledge of the crop and fungal genome that will be necessary to develop interventions and crops resistant to mycotoxins. It is the component where the greatest progress has been made in setting the stage for providing farmers with crop varieties that will not support fungal growth and toxin production.

$ Developed a *Fusarium verticillioides* Expressed Sequence Tag (EST) library that includes 86,400 individual sequences that correspond to over 11,000 unique genes, and elucidated the gene cluster and the biochemical pathway required for fumonisin production. This library is used to identify genes that regulate both fumonisin production and the ability of *F. verticillioides* to cause maize ear rot. Impact This gene library will provide information, targets, and/or tools that can be used to develop mycotoxin and maize ear rot control strategies. This gene library will also allow scientists to decipher how environmental factors affect the fungus, which genes are turned on during the plant-fungus interaction and toxin production, and which genes affects fungal survival in the field environment, information which is critical if fungal infection and toxin production in crops is to be prevented.

$ Screened EST libraries for scab resistant wheat (*Fusarium graminearum*) and identified novel trichothecene resistance genes. Also biochemically characterized transgenic lines of wheat and barley that carry the TRI 101 or PDR 5 gene and have increased resistance to Fusarium head blight. Impact These genes and resistant lines of grains are critical for development and commercialization of Fusarium head blight resistant corn, wheat and barley which do not produce vomitoxin; commercial development is underway.

$ A cloned DNA library of *A. flavus* which was prepared and sequenced for TIGR using EST technology to identify unique genes that the fungus uses to accomplish all its biological and physiological functions. Impact This gene library will allow deciphering of how environmental factors affect the fungus, which genes are turned on during the plant-fungus interaction and aflatoxin production, as well as in fungal survival in the field environment. Allows identification/ characterization of a complex set of genes involved in fungal virulence, aflatoxin formation signaling pathways between the fungus and the environment and fungal reproduction/survival, processes which need to be understood if fungal infection and aflatoxin production in crops is to be prevented..

$ Proteomics was used to identify in corn several (12) fungal resistance related characteristics and stress responsive proteins/genes in resistant lines. Impact The genes will be used for marker assisted breeding of crops resistant to aflatoxin through both DNA/gene based and protein/antibody probe based methods.

2.1.5 Biocontrol technologies

Research in this component has been highly successful in developing biocontrol strain technologies to prevent aflatoxin in cotton and peanuts, and some promising trials have been carried out with corn and tree nuts.

$ Developed atoxigenic strain technology to both reduce aflatoxin contamination in affected crops and associated costs to an agricultural industry. Established baseline levels of the atoxigenic biological control fungus AF36 which provides a basis for determining influence of AF36 on natural mycoflora and fungal communities when

applied over large expanses. Compiled all of the information and interpreted the data which was used to obtain EPA approval of AF 36. Developed a commercial scale production system for this fungal biological control agent. Specific technologies were transferred to industry, the Arizona Cotton Research and Protection Council, including simple strain identification, starter culture procedures, scale-up procedures, quality control procedures and methods to assess efficacy. Impact Over 30,000 acres of crops will be treated in collaboration with ARS in AZ in 2004, and 5000 acres will be treated in Texas where some communities have severe problems with aflatoxin. Up to $18 million in annual cottonseed losses by AZ industries alone could be prevented by use of atoxigenic strain technology in biocontrol of aflatoxin. Prevention of the losses throughout the million plus acres of cotton and corn in affected parts of South Texas would have an even greater affect.

$ Data covering several years of studies with a biocontrol *A. flavus* strain to prevent aflatoxin in peanuts were compiled into an application to EPA for Section 3 registration of the biopesticide. Impact EPA granted Section 3 registration for Afla-Guard for use in controlling aflatoxin contamination of peanuts. The product is manufactured for sale by Circle One Global, and is being applied commercially to the 2004 peanut crop in Georgia and Alabama. Use of Afla-Guard could significantly reduce losses due to aflatoxin contamination of the southeastern US peanut industry which is greater that $25 million per year while increasing the safety of peanuts for consumers.

$ Demonstrated that the patented isolate of *Bacillus mojavensis* is a natural endophyte with plant enhancing and disease protecting traits, and that all strains of this biocontrol bacterium are endophytic and can reduce fumonisin in corn as well as losses from seeding blight Impact This isolate can form the basis of a biocontrol product to prevent the growth of *Fusarium moniliforme* and production of fumonisin in corn.

2.1.6 Pest management/insect transmission and predation

Research in this component characterizes plant/fungal/interactions that affect the degree of A. flavus contamination and aflatoxin formation.

$ Demonstrated that Bt corn often had significantly reduced levels of mycotoxins compared to non-Bt corn, but that the degree of benefit was dependent on the timing and makeup of the insect pest complex in the particular crop year. Impact This information is critical in predicting the usefulness of genetically engineered corn and in providing corn producers with information on the specific benefits that they can expect from planting Bt corn.

$ Developed a new lure which provides an additional tool for controlling the codling moth (CM), a destructive agricultural pest, that is associated with high aflatoxin in tree nuts. US patents on this CM kairomone lure were issued. Impact The CM kairomone improves the efficacy of pheromones and lowers significantly the cost of mating

disruption of CM through conventional application methods. The lure is now being used in formulations that include both lure and pesticides

2.1.7 Toxicity evaluations and mechanism of action of Fusarium toxins

FDA has established guidelines for mycotoxins in the US, but their toxicity continues to be assessed by the world community. Continued assessment of certain aspects of mammalian toxicity is necessary.

$ Specific molecular endpoints of fumonisin-induced disruption of sphingolipid biosynthesis and downstream mechanisms of action were determined using cultured cells, plant models, and genetically modified mice. Impact Study results were the basis for developing mechanism of action based bioassays for 'fumonisin like' activity in foods and other matrices and provided critical data for science-based risk assessments world wide which are now the basis for regulatory decisions to protect consumers.

$ Identified the critical step that significantly reduces fumonisin levels in masa and torilla chips prepared from contaminated corn under commercial conditions. Also determined that traditional Mayan tortilla production methods reduce total fumonisins; showed that chemical analyses accurately predicted biological activity of the masa and tortilla chips, and validated toxicological endpoints using a rat feeding bioassay. Showed that frying and baking did not significantly affect measurable fumonisin levels or toxicity of fumonisin-contaminated cornmeal. Impact The findings are essential for the FDA and WHO to make solid recommendations regarding safe levels of fumonisins in food and for guiding industry, particularly with nixtamilization, to best achieve these safe levels.

2.2.1 Mycotoxins in Forage Crops

Mycotoxins may also be present in forage crops primarily from endohytic fungi, and they often significantly benefit plant growth and production.

- There were insufficient resources to make significant progress in this area, however, work with collaborators has established the complete alkaloidal chemical spectrum of seven patented strains of novel endophytes as well as 21 additional non-patented novel endophytes. Also purified several non-ergot alkaloid chemicals for testing in an in vitro assay for nematode toxicity. Impact These findings will help define the best grass-endophyte combinations for specific locations relative to tolerance to biotic and abiotic stresses and will be economically important viable alternatives for enhancement of grasses.

Component 3. Chemical Residues

Research in this component determines sources and the bioavailablilty of chemical residues to which food producing animals may be exposed.

3.1 Preharvest

3.1.1.1 Food Producing Animals

$ Determined the bioavailability and bioaccumulation of certain brominated and chlorinated dibenzodioxins using laboratory animals as well as samples of naturally contaminated feed and food products. Impact Studies on the transfer of dioxins from feed and food ingredients into animal product, such as milk, provide important information to regulatory agencies when assessing the potential risk of contamination, as well as producing data on background contamination.

3.1.1.1 Residue and toxin methodology and identification

$ Worked with the Food Safety and Inspection Service to analyze dioxins, furans and co-planar PCBs in meat and poultry samples from four animal classes, beef, pork, chicken and turkey. Impact This study analyzes current levels of dioxins in domestically produced meat and poultry and compares these new results with those from a previous surveys; it will allow FSIS, FDA and EPA to make the best possible recommendations for keeping the levels of dioxins at safe levels in the American diet

3.1.2 Toxins in food crops for direct human consumption

Research in this component seeks to determine the mechanism of plant accumulation of toxic amounts of heavy metals in food crop plants and determines how best to reduce this toxic metal accumulation.

$ Elucidated factors affecting the interaction between soil and crops so that interventions could be developed, either avoidance of certain soil series, use of soil amendments, and/or development of crop varieties which do not absorb the toxic trace elements. Identified soil series which could reliable produce low cadmium sunflowers, flax and durum wheat and identified soil properties (chloride) which caused higher grain cadmium absorption in some soils Impact This knowledge gave US sunflower marketing companies the ability to purchase and market lower cadmium non oilseed sunflower kernels which both protect the public health and have a European market for $30 million per year.

$ Illustrated which properties of rice strongly affect the potential for risk from grain cadmium even when the rice is grown on soils with high zinc and cadmium levels. Natural nutrients of rice induce deficiency of iron, zinc and calcium; and cadmium is 10-

20 fold higher in animals consuming rice than when adequate dietary levels of iron, zinc and calcium are supplied, showing that rice is unique. Impact This information is being used to encourage regulation of cadmium in rice rather than in all crops, and to have different limits for crops which do not have concomitant zinc accumulation. European Union failure to consider these aspects of dietary and crop cadmium risks caused large over-estimation of risk from dietary cadmium and could have caused significant economic dislocation to many countries, including a 30% increase in the cost of fertilizers.

3.2 Postharvest

3.2.1 Residue and toxin methodology and identification

Under this sub-component research was directed towards developing chemical and immunological based methods to detect and quantify drug and contaminant residues in animals tissues and fluids, plant products, and food matrices. A critical objective was to develop both screening and confirmatory methods for use by regulatory and action agencies, producers, industry and researchers. Specifically, research led to the development of methods to:

- Using LC, fluorescence and MS^n (LC-FL-MS^n) for the analysis of 8 fluoroquinolone (FQ) antibiotics in a variety of matrices
- A confirmatory analytical method for 11 lactam antibiotics using LC-MS^n.
- A quantitative and confirmatory method for 10 lactams using LC/MS-MS.
- A rapid extraction and cleanup technique recovered lactams below U.S. tolerance level and has high sample throughput of 30-40 samples/8 hr.
- A LC-MS^n method to analyze 8 beta-agonists and 6 thyreostat growth promoters.
- Quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for multiple pesticide residues in foods.
- The ‘analyte protectants’ concept in the GC analysis of pesticides to improve quantitation, reduce GC maintenance, and save time in sample preparation.
- Fast GC/MS resulting in 4-fold increase in speed of pesticide analysis.
- Efficient cleanup dispersive SPE for residues using a sorbent to retain sample matrix interferences rather than the analytes.
- Simple, rapid and sensitive fluorescence screening methods to detect enrofloxacin and tetracyclines.
- Lanthanide-sensitized time-resolved luminescence (TRL) methods for screening trace amounts of fluoroquinolones or tetracyclines in tissues with detectable concentrations below U.S. tolerance levels, as required for monitoring in the E.U. Incorporated into a portable TRL detection instrument for field applications.
- A sensitive, simple and rapid solid-matrix TRL approach for tetracyclines in milk and other liquid samples. This innovative approach is patent application pending.
- Non-instrumental latex agglutination assay that can detect 10 ng of Staphylococcus aureus toxins, SEA and SEB in 1g of food samples. Improved sensitivity to 1 ng/g was attained using a BIAcore biosensor.

- § High throughput, rapid screening for spectinomycin residues at 5 –100 ng/g.
- § Subcritical water-based extraction and sample cleanup technique for avoparcin with 100% recovery.
- § Subcritical water based technology for the extraction of triazine pesticides (atrazine, simazine, and propazine) from fat samples and their analysis using GC/MS. All three pesticides are described by the EPA as possible carcinogens.

Impact:

This impact of this research is best articulated by the expanded scope of analyses, combined with reduced costs, easy of use, rapidity, efficiency etc. For example:

Using the QuEChERS method a single analyst can extract hundreds of pesticides from over 100 samples per day costing $1 in materials/sample. The method is currently being validated and transferred for AOAC approval.

New methods for fast GC/MS are the most simple, rugged, and least expensive way to shorten analytical runtime in GC/MS using common instruments. Their use will generate savings in materials, equipment, and labor costs. This method also meets FSIS needs to analyze over 60 pesticides in large number of samples per year.

Combining individual screening methods into one procedure increases efficiency to detect various classes of drugs in the same extract. This approach is easier and cheaper than immunoassays, faster than bioassays, and can be done in a slaughterhouse or laboratory, saving time and expense.

Development of the field portable TRL fluorometer provides a rapid, on-site test for tetracycline and fluoroquinolone residues in slaughter houses or farms, which would reduce or eliminate false violative samples from being transported to FSIS laboratories, thus, saving transportation costs. Reduction by 10% of the 10,000 tests by FSIS, would generates savings of $0.2M per year.

Screening 10% of milk tankers for tetracycline using rapid solid matrix TRL would save 6.4 million man hours, as well as $1.6 million from elimination of solvent.

FSIS expressed the need for alternate detection methods for SEA/SEB to current commercial assays. The latex assay costs less than $1 per test compared to $12 – 20 per sample using commercial test kits. Similarly, the cost of materials for the biosensor analysis is <$1 per test and its semi-automated system also reduces labor costs.

The subcritical water extraction method was the first instrumental, cost effective method for avoparacin isolation and quantitation in meats.