AGRICULTURAL RESEARCH SERVICE FOOD SAFETY REVIEW

Members of the ARS National Program in Food Safety (NP 108) Assessment Panel (NP108) met in Beltsville, Maryland on December 14 and 15, 2004 to conduct a retrospective evaluation of this program. This report summarizes the panel's discussions, written assessments, and overall feedback regarding research and related program administration within NP108. An assessment of impact and relative strengths and weaknesses of research components have been included. The Committee reviewed the program as divided into four topic areas (Microbial Pathogens: Pre-Harvest; Microbial Pathogens: Post-Harvest; Mycotoxins; and Chemical Residues) and has provided an overall assessment of impact (high, medium or low) for these general areas. Specific evaluation scores are provided for each subtopic within these topic areas. Comments are based on the apparent "impact" of the research efforts. Impact was measured both by adherence to the Strategic Plan Priority Objectives, and by changes, improvements and advances made to agency policy/regulations, industry practices and food safety. Quantification of impact should not be construed as an assessment of the value of further efforts in an area; e.g., a program area with a high score for impact does not necessarily need continuation of the same efforts, and a low score does not necessarily mean that continued funding is not warranted. In some cases, a low impact represented failure by a research leader to focus on the Priority Objective; in some cases, it meant that it is justifiably too soon in the timeline of this research for this objective to be effectively implemented and have impact. However, this review should be helpful to the panel that develops the next 5 year Strategic Plan.

OVERALL FOOD SAFETY RESEARCH PROGRAM

The food safety program in ARS has been productive and is making important scientific contributions, as documented by progress during the past five years. The general description of this program appropriately considers the priorities articulated in the near-term microbial food safety focus of the National Food Safety Initiative; consistently, ARS has responded to the needs of the Food Safety Inspection Service with high quality pertinent research that changes the way that FSIS does business. ARS research cited in the past five annual reports has had a high impact on numerous widely diverse divisions in FSIS such as policy and regulations, risk assessments, field operations and the laboratories. There are excellent collaborations occurring between ARS and academic scientists, and these efforts are advancing important knowledge of food safety and control of microbiological, toxigenic and chemical hazards. Several members of the review panel noted that the relationship between FSIS and the ARS National Program Staff has never been better. Additionally, there were highly favorable comments on the service ARS provides FDA's Center for Food Safety and Applied Nutrition (CFSAN) in the areas of milk, egg, seafood and produce safety and security. The program enjoys the excellent leadership provided by NP 108 National Program Leaders Drs. Robens and Lindsay (e.g., Drs. Robens and Lindsay were acknowledged with the CFSAN Director's Citation Award).

Increasingly, the ultimate success of a food safety research program will be measured in terms of impact on public health, and the General Accounting Office (GAO) is already viewing reductions in deaths and illnesses from foodborne pathogens as a critical research outcome. The ARS must therefore improve collaboration with public health agencies (e.g. CDC), utilizing databases such as FoodNet and PulseNet to establish baseline data and measure success of interventions/changes in practices, from these baselines. There is an artificial barrier between addressing microbial pathogens from a pre- and post-harvest perspective, and it is critical that this research area be viewed as a continuum. A statement contained within the review materials (page 58, Vol. 1) eloquently summarizes the direction for integration **"**Directing the research program towards improving public health will require reversing our thinking on the food chain; not farm to plate, but plate to farm: the consumer must be considered first, not the producer or processor…..the artificial barrier between pre- and post-harvest must be reconsidered. Food safety and its associated research must be treated as a single entity." The documentation provided to the committee indicated that "The most critical question asked of the Program was 'quantitatively speaking, how many lives and illnesses have been saved or prevented by research conducted by NP 108'? As currently structured and assessed, NP 108 cannot provide data to respond to this question.

STRATEGIC PLAN AND PROGRAM OVERSIGHT

Activities within NP 108 over the past five years have been guided by a strategic plan. The "5 year Strategic Plan" approach was successful in creating a unified, national direction for ARS Food Safety research, and was successful in supporting achievement of numerous high-impact projects. Success in maintaining this focus appears to have been largely due to the efforts of the National Program Staff. The utilization of a five year plan is a useful concept for providing future research focus, and for encouraging high risk research. The five year plan should be streamlined, and the overall strategic objective should be concise (i.e. 2-3 pages maximum) The five year plan needs to be structured by overall strategic objectives, goals and objectives that can be modified during the five years, and tactics to describe how the goals and objectives will be achieved. In the next five year plan, we recommend that ARS establish an NP108 Task Force/Advisory Committee comprised of relevant stakeholders (e.g., FSIS, FDA, CDC, food industry, academia). The strategic direction and primary goals of the NP 108 program should be developed by the National Program Staff in cooperation with research leaders and an advisory group who can help identify priorities, identify goals or tactics not worthy of resources (not likely to succeed, or being undertaken elsewhere), and who can help identify potentially useful extramural collaborations.

Some Priority Objectives were not achieved and, in some cases, barely undertaken. Several projects, primarily Siblings, seemed only tangentially related to the Priority Objectives and, in some cases, to Food Safety. Lack of cooperation between Centers and, in some cases, within Centers, appeared to result in redundant research efforts and missed opportunities to leverage other researchers' accomplishments.

There is a need for periodic, perhaps annual, reassessment of the Strategic Plan by the National Program Staff and stakeholders to realign priorities when needed; i.e., addition or deletion of specific primary goals in response to high-priority food safety needs, additional funding to specific research tactics as warranted to achieve objectives, reduced funding or redirection of tactics no longer needed or not likely to succeed. This allows for necessary flexibility in the strategic objectives, without promoting the concept of "pet projects". The National Program Staff must have this authority, and the responsibility to avoid "creep" from the Plan in response to "issue *du jour*" or pet research interests. There is also a need for periodic feedback to stakeholders and scientists, demonstrating progress toward accomplishment of the Strategic Plan. This opportunity could be useful in demonstrating to stakeholders the benefits of research accomplishments, demonstrating benefits of successful collaborations between research leaders, and demonstrating rewards of focusing efforts on strategic objectives and effective use of resources.

The Committee's assessment of the management of the NP 108 program is quite simple: The way ARS manages research is untenable, period. The National Program Leaders for the Food Safety Program have been given tremendous responsibility but no authority *vis a vis* research in their Food Safety Program. This program cannot be held accountable under the current system. Researchers, Research Leaders, Center Directors, and Area Directors cannot be independent of research oversight if accountability for a $90M budget is warranted. It is unclear whether the productivity of Research Units, Research Leaders and scientists is ever ranked. It is also unclear whether Area Directors, Center Directors, and Research Leaders are required to facilitate reviews of the research at ARS locations around the country. In the absence of a peer review system which insures research quality, the committee cannot be assured that projects of the highest quality and scientific merit are those for which resources are being directed.

BUDGET

All members of the committee expressed concern about the sustainability of the NP 108 Food Safety program in light of tremendous budget pressures facing USDA and agricultural research. It appears that the current budget allocation process (or lack thereof) is driven by incremental resource addition as opposed to redistribution of resources to fund priority research. Currently, most budgetary resources are allocated for salaries, leaving little for operating. In light of the program objectives and research goals, it is unrealistic that significant accomplishments will continue to be made in the absence of adequate operating and equipment resources. The situation faced by ARS is not unlike the situation which the Land Grant University System has faced for the past decade. Most, if not all, institutions have engaged in severe budget reductions and realignment of resources under a strategic plan and zero based budgeting. Some maximum percentage of research resources should be allocated to investigatory or support services (allocated by Center, by research area and/or by National Program Staff), and the remainder tied to goals and tactics strongly linked to the Strategic Plan. Rewards and consequences should be created to recognize effective use of the limited research funds. The adoption of a peer-reviewed, competitive approach to research proposals is recommended as it affords

the best mechanism for insuring research of high scientific merit. Several panelists shared concern about the quality of the research being conducted in the absence of a competitive, peer-reviewed process. What assurance can NP 108 provide that the best research is being conducted? While the ARS Food Safety Program budget has been >$90M/year, the actual discretionary research funds (after overhead and pass-through) have been closer to $7M/year, to be distributed among >250 scientists (approximately $28,000/SY). Much of the budget is dedicated (by Congress) to particular Regional Centers or labs, and it will be difficult to redistribute as needed. Tactics (projects or methods to achieve the goals) need to be developed by a panel of Center directors and research leaders, led by the National Program Staff and with a directed objective of cooperation where such cooperation can leverage expertise in different Centers or from extramural sources (and a caution to not create cooperative efforts for their own sake). Budgetary estimates (along with priority in the Strategic Plan and anticipated impact) must be considered in the proposed research tactic, in order to focus resources in an effective manner, and to promote research excellence.

MICROBIAL PATHOGENS PREHARVEST

Research programs within this topic area were ranked as having a medium to high impact (Overall Score 6.5)

1.1.1 Methodology (Overall Impact- 8)

- The development of several direct plating methods for quantitation of *Campylobacter* is probably the single most important methods development that may be directly used by FSIS in the near future to set standards for the poultry industry. The committee viewed this area as high priority research.

- New methods are needed to accurately and rapidly identify *Campylobacter* without lengthy cultural confirmatory procedures. In cooperative studies between the Poultry Microbiological Safety Research Unit (PMSRU) and Idaho Technology Lab (Salt Lake City, UT), targets for DNA probes were identified and used to synthesize probes for *Campylobacter* identification, and these probes were evaluated for their sensitivity and specificity in detecting *Campylobacter* in broiler carcass rinses. This research resulted in a genus-specific real-time assay used for detecting *Campylobacter* in enrichment cultures derived from chicken products. This assay provides a test for the presence of *Campylobacter* in food sample enrichments, a rapid confirmatory tool for individual colony isolates of *Campylobacter*, and a potential means to achieve semi-quantitation of this pathogen without enrichment.

- Researchers and industry need more sensitive and rapid detection methodologies to monitor the microbiological quality of their products. A new automated electrochemical-luminescent technology which detects *Salmonella* from poultry products in 24 hours was evaluated for efficacy in detecting *Salmonella*. The IGEN Pathogen *Salmonella* test was shown to be as efficient as an automated PCR assay for detecting *Salmonella* from raw chicken rinse samples and ready-to-eat chicken products. This study has identified a useful tool which will aid researchers and the poultry industry to more efficiently meet the microbiological needs.

- Because of the fastidious nature of *Campylobacter*, recovery from meat or other sources is difficult. Enhanced methods for recovering *Campylobacter* from chicken carcass rinsates were developed. Use of centrifugation resulted in a >50% increase in the recovery of *Campylobacter*; however, of significance is that each method appeared to select for different populations of *Campylobacter* as antimicrobial testing results were different between the methods. These results are significant in that previous methods led to the isolation and under reporting of *Campylobacter* in samples and illustrates the impact methodology has on analytical results.

- Detecting infected poultry flocks is essential for controlling egg-borne transmission of SE to humans. In a study conducted at the Southeast Poultry Research Laboratory, in collaboration with Diachemix Corporation, a new fluorescence polarization test was evaluated for its ability to detect specific antibodies in egg yolks produced by hens infected with SE. When applied to egg yolk samples from experimentally inoculated laying hens, the new test detected SE infection as often as a conventional antibody assay and produced fewer misleading cross-reactions with samples from hens infected with a different type of *Salmonella*. These results demonstrate that this very rapid new test is an effective alternative to traditional methods for detecting SE infection in poultry.

- This test was also evaluated for its ability to rapidly detect SE in incubated egg contents pools. Both the fluorescence polarization test and a rapid lateral flow immunodiffusion test consistently provided positive results after egg pools were contaminated with very small numbers of SE cells and incubated to allow bacterial multiplication. These results demonstrate that new rapid detection methods can be useful alternatives to bacteriologic culturing for identifying eggs contaminated with SE, although these rapid methods may be less sensitive than traditional methods.

- An ELISA test based on tachyzoite extract was tested extensively and compared with currently used agglutination methods. The test was further adapted for use with meat juices. A commercial version of this test has been submitted for USDA licensing. To develop an assay for stage-specific differentiation of *Toxoplasma*

infection in swine and humans, an 18.3 kDa oocyst-specific protein has been identified which is recognized by humans and swine with oocyst-induced toxoplasmosis. Development of the recombinant antigen-based ELISA is underway. Combined with data generated by the National Retail Meats Survey and serological testing of human serum banks in collaboration with the CDC, an understanding of the epidemiology of infection in humans will be developed.

- Recovery and enumeration of stressed microorganisms from harsh processing or production environments is very difficult and improved methods are needed. The PMSRU developed a sensitive method for recovery and enumeration of stressed *Campylobacter* using a novel medium. More injured *Campylobacter* from processed chicken carcass rinse samples were recovered by the new method compared to conventional plating techniques. This technique uses much less media than conventional methods and may be utilized to enumerate *Campylobacter* in other stressed environmental samples such as poultry litter.

- *Clostridium perfringens* is an important pathogen associated with the poultry production environment and is difficult to enumerate. A membrane filtration method was shown to be efficacious in enumerating *C. perfringens* from broiler cecal samples. The method which was originally used for water testing results in a semi-confirmatory assay that is quantitative and uses only a single membrane filter per plate. This assay was necessary in order to enumerate levels of C*lostridium perfringens* in drug-free reared broilers.

- A method and devices for detecting fecal contamination on beef carcasses were developed, patented, and licensed for commercial development. A USDA CRADA partner has designed and sold hand-held fecal detection devices to five major beef processing companies and they have also designed and sold whole carcass scanners for real-time detection of fecal contamination in slaughter facilities. These devices are being used to reduce the incidence of fecal contamination in beef that can occur during slaughter.

1.1.2 Epidemiology and Ecology (Overall Impact-7)

- 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

- Campylobacter work conducted in Iceland should also be done in the U.S.

- A clear and significant relationship between infections of live cattle with *E. coli* O157:H7 and contamination of beef carcasses has been documented and several potential intervention strategies to reduce fecal shedding of STEC O157 were evaluated. Unfortunately, none of the interventions tested to date have shown practical utility in reducing STEC O157 fecal prevalence in cattle. A large collaborative field project (involving more than 10,000 cattle from multiple states) with the National Animal Health Monitoring System (NAHMS) on the epidemiology of STEC O157 in U.S. feedlot cattle was completed. It was shown that cattle are at highest risk of STEC O157 fecal shedding in late summer and early fall.

- Causes of the strong observed seasonality of STEC O157 occurrence in livestock, food, and human infections resulting in high summer prevalence peaks and low winter troughs are an enigma, but ambient temperature is believed to play a role. In collaboration with Louisiana State University, the summer fecal prevalence of STEC O157 in 13 subtropical Louisiana dairy cattle herds, and the prevalence each season over one year in five Louisiana dairy herds was measured. Summer point prevalence was 6.6% of 791 cattle, while prevalence by season was 0.6% in winter, 7.6% in spring, 6.6% in summer, and 0.63% in fall. These findings demonstrate that STEC O157 fecal shedding is "seasonal" in dairy cattle in the "aseasonal" subtropical Louisiana environment, suggesting that STEC O157 seasonality is complex and is not solely a function of ambient temperature

- Studies demonstrated that the hide surface and the oral cavity of finished beef feedlot cattle may have higher STEC O157 prevalence than bovine feces. This finding has significant implications for reduction of STEC O157 meat contamination at slaughter (i.e., emphasizing hide and mouth as carcass contamination sources).

- Understanding infectious sites and mechanisms of *E. coli* O157:H7 in cattle is an important step for designing methods to detect and prevent colonization and for reducing human foodborne disease caused by this important pathogen. Scientists at the National Animal Disease Center (NADC), Ames, IA, and a visiting scientist from the School of Veterinary Medicine Hanover, Germany, conducted experiments to determine if *E. coli* O157:H7 could be found at sites other than the intestines and feces in experimentally infected calves. Results indicate that *E. coli* O157:H7 was present in the gall bladder of infected cattle and that the bacteria caused “attaching and effacing” lesions in the gall bladder similar to the lesions caused by *E. coli* O157:H7 in cattle intestines. These observations suggest that the gall bladder could be a source of *E. coli*

O157:H7 contamination in meat and that the gall bladder should be more carefully examined as a possible site of persistent *E. coli* O157:H7 infection in cattle. Persistence in the gall bladder is significant information. This may explain discrepancies in intervention therapies. Animals may be culture "negative" simply because they are not shedding into the feces at the time of testing, and then when stressed, may begin to shed from the persistence site in the gallbladder.

- Epidemiological studies conducted on 58 *T. gondii* infected swine farms demonstrated that oocysts could not be detected by bioassay in soil from the infected farms, but a significant risk of infection correlated with the presence of cats on the farms. Additional studies demonstrated that pig infections resulted from oocysts in the environment being transported into confinement barns on contaminated footwear; boot hygiene is a critical issue associated with *T. gondii* infection in confinement barns.

1.1.3 Ecology and the Host Pathogen Relationship (Overall Impact 6)

- Luminescent *Salmonella* research important for determining colonization. Research on biophotonic imaging of luminescent *Salmonella* (and other human pathogens) administered to broilers/layers will be an invaluable research tool for developing and interventions that can reduce/eliminate human pathogens. It will demonstrate *in situ* real time "visualization" of the protective effect of interventions which have demonstrated efficacy against Salmonellae (and other pathogens) in field trials. The project will also fill in gaps in our knowledge of commensal colonization of chickens with human pathogens as well providing for rapid assessment/visualization of pre-harvest strategies that could reduce human pathogens in poultry/eggs presented for slaughter/ processing.

- Should be evaluating plants in pre-harvest as well as post-harvest
- There is a significant preharvest component to fruits and vegetables and it should be included in the action plan; we are aware that ARS is working in this area and it is surprising that results were not included in the review materials.
- Immunological aspects including cytokine production
- Identified basic methods of antibiotic transfer resistance

1.1.4 Interventions (Overall Impact 6)

The committee viewed reducing the high incidence of *Salmonella* in poultry as a high priority for research, along with intervention methods for *Campylobacter*. Future efforts directed toward addressing data gaps of risk assessments should be considered, and collaborations between ARS, ERS and academia should be fostered.

- Sodium chlorate supplementation was found to reduce *E. coli* 0157:H7 fecal shedding in cattle and a patent (U.S. Patent No. 6,475,527) was awarded for this technology. Specially formulated preparations of select nitrocompounds that effectively and selectively killed pathogenic *E. coli, Salmonella* and *Campylobacter* in the gut of food animals were discovered. Patent applications have been filed for the use of select nitrocompounds to reduce gut and environmental concentrations of pathogenic bacteria and for the use of a secondary plant compound to treat infections by shiga-toxin producing bacteria. The committee agreed that this type of research was important and should be continued with a focus on residue studies leading to FDA approval of this intervention.

- Based on audits of swine management practices and the prevalence of *Toxoplasma* in pigs raised under various management systems, a series of good production practices (GPP) were established encompassing rodent control, general hygiene, biosecurity, feed sources and storage**.** Pork producers participating in the study implemented these practices and were given follow-up audits. Most farms improved in audit scores following the producer education effort; implementation of boot hygiene resulted in 57 of 58 farms becoming *Toxoplasma* negative over the course of 2 finishing cycles**.** The developed pre-harvest food safety plan was delivered to the cooperator (Farmland Foods) and has been made available to the entire swine industry. APHIS and the National Pork Board are considering how to use these findings in a Certification Program modeled on the National Trichinae Certification Program.

- Scientists established the capability of an Experimental Chlorate Product (ECP) to prevent enteropathogen colonization of market-age broilers, newly-hatched chicks, and turkeys. Broilers provided ECP orally had significant lower concentrations of *Salmonella* and *E. coli*, as well as decreased mortality, and fewer lesions associated with the poultry disease called Necrotic Enteritis caused by *Clostridium perfringens.*

- A high fiber, low energy molting diet was developed that results in an additional laying cycle for hens without increasing susceptibility to SE organ and gut infection. Hens provided a molt diet containing 100% alfalfa, when compared to hens molted via feed deprivation, had equivalent ovary weight regression but re-entered into egg production sooner, and produced more eggs with less body weight loss with decreased incidence of SE infection, presumably due to decreased stress. This accomplishment is important because it provides the poultry industry with an alternative molting strategy

1.1.5 Feed Withdrawal and Transportation (Overall Impact 6)

- Should be classified under ecology, not enough projects to demonstrate productivity, we understand feed withdrawal projects will be coded separately

- Marketing stress experiments (transportation and mixing) performed on nonshedding, *Salmonella*-infected swine (carrier-state) suggested an increase risk of fecal shedding of *Salmonella* if pigs are not immediately slaughtered upon arrival at processing plant. Further studies confirmed that marketing stress plays a significant role in the fecal shedding of *Salmonella* by asymptomatic carrier pigs and that the amount of feed in the digestive tract affects fecal shedding of *Salmonella.* These results will impact the pork industry's efforts to implement a strategic plan to maintain a safe national food chain and compete for new international markets.

1.1.6 Manure (Overall Impact 6)

- The committee agreed that this is going to continue to be a priority issue and should not be de-emphasized. However, we believe that this research area should be realigned under ecology where the research may have more of an impact and may be perceived as a higher priority by scientists doing this work. There is important research ongoing directed at aerated manure waters and dairy waste water work.

MICROBIAL PATHOGENS-POSTHARVEST:

Research programs within this topic area were ranked as having a medium to high impact (Overall score 7)

General Comments:

- The distinction between pre- and post-harvest research needs to be reconsidered. Food safety extends from the farm to consumption. Food safety research should reflect this reality and be integrated across the various steps in the food chain. This is particularly important in the steps immediately prior to processing

- For microorganisms of concern, ARS should consider adding the bacteria *Enterobacter sakazaki* and *Mycobacterium paratuberculosis*; and the protozoan *Cyclospora*;
- Since NIH has limited or no active projects on the basic pathogenesis of *Vibrio parahaemolyticus* or *Enterobacter sakazaki*, any good proposals put forth by ARS scientists on the characteristics of these organisms would be helpful;

- ARS has, of course, been heavily involved in the numerous discussions on research priorities for TSEs and perhaps may wish to capture those proposed and active initiatives here, unless another program is more appropriate;
- The Federal food safety research programs in general have lost critical mass in the area of bacterial toxins. This program may serve as an impetus for a new initiative in this critical area;
- Microbial CT agents of concern in the classified vulnerability assessments of the food/agriculture sector documents, collaboratively written by FSIS and FDA, should be addressed;
- When recognizing the research to address the constraints of industry and regulatory agencies, section (d) on decontamination should include not only foods but the processing environments (*e.g.,* TSEs, *Bacillus anthracis*, also remember the fate of the Chicago milk processing plant associated with the huge *Salmonella* outbreak in the early 1980s);
- When recognizing the research to address the constraints of industry and regulatory agencies, section (e) on improved food handling, distribution, and storage techniques should explicitly include the distribution/retail environments and perhaps the need for scientific support of the FOOD CODE;
- When mentioning the food products addressed, ARS should consider including game meats, which have split regulatory responsibility per mutual agreement between FDA and FSIS;
- Under newly recognized thrusts, ARS should consider catfish, the microbial contamination of dried agricultural commodities (*e.g., Enterobacter sakazaki* and infant formula; milk powders; dietary supplement safety);
- Further investment by ARS in the long term goal(s) of on-line detection and biosensor technology is important to the food safety regulatory agencies, especially during periods of the Department of Homeland Security counter terrorism heightened alerts (*i.e.*, orange and red alerts);
- Specific mentioning that ARS is partnering with FSIS and FDA on the Shell Egg Safety Plan, particularly the research needs section, would be appropriate;
- Specific mentioning that ARS is partnering with FDA on the new Produce Safety Initiative, particularly the research needs section, would be appropriate.
- Some of these suggestions may also be applicable to the Preharvest Microbial Pathogens topic.

1.2.1 Methodology (Overall subtopic impact rating 7): Efforts to improve methodologies have benefited the regulatory agencies and the industry. Testing and inspection do not make foods safe, but research should continue to focus on detection capability. More emphasis should be place on minimizing culture enrichment time and, to the extent practical and scientifically defensible, develop real-time, on-line sensing technologies. Understanding the important role of virulence markers would enable a better evaluation and reaction to isolates detected through testing
Good adherence to some program objectives, such as 1.2.1.1, parts of 1.2.1.3 (particularly toward subtyping of species), 1.2.1.5, and particularly 1.2.1.7 in poultry. Some progress toward 1.2.1.2 (real time detection with biosensors). Not much progress in “sampling

methodologies to determine the most appropriate locations, sites and frequency for sampling" (1.2.1.3); may build off of ecology studies in the future. Limited but significant efforts toward 1.2.1.6.

Overall, a high score for impact in areas such as:

- novel use of genetic and other tools for detection and subspecies differentiation, not only of high importance pathogens like *L. monocytogenes*, but also economically important pathogens such as parasites and viruses;
- attention to the need for detection methodology to be rapid, rugged, sensitive and cost effective for large and small operator use;
- predictive models capable of assessing "safe harbors" for food processing operations;
- on-line sensing technologies that are effective at production speed and, while developed for a specific purpose, appear adaptable to other analytes; and particularly for transfer of these methods to development of ecology and intervention strategies.

Some concerns:

- Some of the projects appear to be adapted to the objectives, rather than driven by the objectives.
- Duplicate (or related) efforts at different facilities, but without indication of collaboration; (e.g., reports 31 and 36, and reports 42, 45 and 46a).

- (29) O157 positive but H7 negative and *stx* negative are rare, the predomininant cause of false positives; regional aspects of O157 prevalence due to variability in day-to-day sampling; helped establish industry recommended protocol to allow plants to benchmark their hygienic status.
- (30) well hypothesized and conducted *E. coli* indicator organism study that failed—these studies are very difficult but good approach
- (34) *Campylobacter* and *Listeria* typing methods (multilocus sequence typing [MLST] and flagellin gene sequence) developed to study in chicken plant epi (sources, stability, genetic exchange).

- A new biosensor-based method was developed by the ARS to detect heat-resistant toxins in foods such as ham, milk and eggs should help researchers and inspectors detect toxins that cause gastroenteritis. The method specifically detects chemical signals from *Staphylococcus aureus* enterotoxins A (SEA) and B (SEB) and provides information about their specific biological activities. Other research efforts are using advanced technologies to develop methods to screen, detect and confirm multiple chemical residues--such as veterinary drugs and pesticides--in food products.

1.2.2 Production and Processing Ecology (Overall Rating 6)

- Interventions need to be developed with a view to a total systems approach.
- Baseline surveys are needed at ARS. ARS' role should be to assist with study design and monitoring.

- Research has benefited the industry by developing additional data to verify process controls. Understanding the ecology of microorganisms is very useful to develop appropriate intervention strategies, but the data must be collected and analyzed in a manner that provides the industry useable information. Collecting baseline data is an appropriate research objective. More emphasis should be placed on identifying pathogenic strains that pose the most significant human health risk and identifying their source and epidemiology.

- Good efforts toward objective 1.2.2.1, ecology of pathogens on raw materials (animal/produce). Has and will enhance intervention strategies at the pre-/post-harvest interface. But far less success on other objectives, i.e., ecology in processing environments - reason unknown. Efforts seem to have been redirected toward genetic diversity of pathogens and mechanisms of that diversity. While a significant realization (i.e., "not all strains pose the same risk to human health"), this does not significantly advance the objective – i.e., unless the subtype ecologically behaves differently than others in the species, any of the species can be used to model ecological behavior.

- (29) Prevalence and tracking data for *E. coli* O157 indicated contamination was occurring early in beef slaughterhouse processing; seasonal effect of prevalence; hides were major source of contamination; transfer rate of O157 from hide to carcass surface determined—pre-evisceration carcass prevalence did not mirror hide prevalence, again not supporting regional differences.
- (30) seasonal and geographic determination in prevalence of O157:H7 determined in plant processing environment; the latter conflicts with (29)—has this been resolved?; confirmed beef hides are source of contamination; tested injected marinades but no results stated; developed method to ship samples that preserves pathogen viability—could this approach account for different conclusion on regional variability?

1.2.3 Intervention Strategies-Process and Control Technologies (Overall Ranking 5):

From the perspective of many on the review panel, this area of research should receive the maximum research emphasis. Research to minimize fecal contamination during slaughter; technologies to improve processing water quality and the use of pathogen modeling programs for ready-to-eat processes have assisted the industry in its ability to reduce pathogens on food products and simultaneously achieve public health gains. Prevalence data indicate significant improvements have been made. Modeling would be enhanced if more products are assessed for incorporation into the modeling data and software selections. These inoculated-pack studies would not only enhance existing models, but would greatly assist regulators and industry in their on-going assessments of the safety of manufacturing practices. Research on interventions would be enhanced

through industry involvement so that results can be used in accelerating the approval of the interventions by FSIS. ARS currently does not consider validation of new processing equipment a research priority; however, members of the review panel see this as of extreme importance.

- Some research leaders need to develop better collaboration with industry, particularly with respect to designing and developing projects. The review revealed that research was performed by ARS on interventions that industry knew didn't work or would not be used. This is an example of what must be changed in the future to insure the continued credibility of ARS research.

- Information that was gained on carcass mapping was a good example of collaboration with industry with a high impact outcome

- Need to have industry input in terms of research design; this could be achieved through peer review.
- Should be aligned with public health needs and not to exclude validation of equipment and new technology in the action plan
- Encourage targeted research based on industry regulatory agency consortium as a model for setting up the strategic priorities or action plan e.g. sprout interventions, steam processing of cantaloupes
- Need more innovation and development of new interventions and strategies.

Mixed impact. Use of developed methods and understanding of microbial ecology of incoming animals led to some high impact interventions for post-slaughter handling of animals (1.2.3.1). Significant efforts related to objectives 1.2.3.2 – 1.2.3.11 had limited/no impact because of limited effectiveness (new technologies) or consumer perception (irradiation). This is similar to results experienced by industry. Only one report cited had results related to 1.2.3.6 (genetic engineering of produce for extended shelf-life). Good impact realized from thermal inactivation studies and related mathematical models ("safe harbor" processes – see 1.2.5).

- (29) Methodology used to verify process control in plant.
- (31) real time detection of fecal matter on apples by automated fluorescence technique (JIFSAN collaboration)—automated, high throughput; also used for chickens; allows for redeployment of inspector resources; also positive effect for juice/cider pathogen reduction and safety;
- (33) no difference in microbial load on carcass of feathered or featherless broiler carcasses during at end of processing; 3 tank, counter flow scalder effective at reducing *Salmonella* in final third tank; counterflow scalding effective.
- (34) storing chicken transport crates for 48hrs before reuse reduces *Campylobacter* contamination;

1.2.4 Effect of Intervention Strategies (Overall Impact 4): Genetic analysis to identify virulence and persistence of pathogens in the production environment may prove to be highly beneficial in developing improved processing, sanitation

and decontamination strategies. To date, this promise has been largely unfulfilled. The continuum between food safety and food quality research should not be overlooked. Rarely are microbial interventions adopted without considering food quality, organoleptic evaluation, practicality of use and economics. This requires all disciplines to work together before intervention technologies are accepted and implemented.

Several reports detailed expression of stress-related responses, but most dealt with genetic expression only. Few (one) assessed the importance of these responses relative to processing operations and food safety. No effects on policy/regulation, food processing operations or consumer safety. This area of research is in its infancy.

- (29) Microbial interventions (which ones?) shown effective in reducing O157 to undetectable levels.
- (30) cetylpyridinium chloride (CPC) successful as a model hide was to reduce overall microbial load; parameters of application determined; hide wash cabinets with effective sodium hydroxide or chlorinated water adopted by large beef producer;
- (33) sealing cloacae of broiler carcasses prior to processing virtually eliminates *Campylobacter* contamination; trisodium phosphate (TSP) reduces post-chill broiler carcass *Salmonella* contamination; FSIS approved use of acidic calcium sulfate; herbal extracts being tested—potential to reduce cost, environmental impact of chlorine based washes.
- (34) in package pasteurization via steam or hot water effectively eliminates pathogens from fully cooked and packaged RTE poultry products

- The goals are worthy in congruence with other detection or ecology and interventions; the impact has not been recognized
- Agency needs to monitor how long they continue to invest without any impact results
- Genomics should be a stand alone area and should interact with other objectives

1.2.5 Risk Assessment (Overall Impact 7)

Very useful data have been generated to perform appropriate risk assessments, but much more are needed to reduce assumptions and uncertainties. Developing data to enhance risk assessments should be funded as on-going research. Some of the key data needs are in the following areas;

- Improved understanding of the linkage between human health risk and specific food vehicles
- Improved understanding of the dose-response relationship between pathogens and consumers, including the effect of food type on the relationship
- Improved data for assessing transfer rates from processing surfaces to food
- Improved understanding of the impact of retail, restaurant and consumer operations on the overall contribution to cases of foodborne illnesses

Research highlights included:

- (29) O157 prevalence and tracking data used in FSIS risk assessment models.

- (30) Provided FSIS with comprehensive non-O157 STEC carcass contamination in U.S. (*i.e.*, baseline prevalence).
- (32) Baseline studies on food contact and non-food contact surfaces before and after sanitation procedures in egg processing plants—FSIS risk assessment; also internal contents of unprocessed and processed shell eggs during prolonged storage determined—the former remained low indicating low cost for egg plant sanitation;

Enhancements to the PMP and creation of ComBase and CEMMI have had high impact in 1) establishing "safe harbors" for food processing and 2) establishing a unified approach and international forum for predictive model development and risk assessments. However, this research area is in its infancy.

COMPONENT 2. MYCOTOXINS

Research programs within this topic area were ranked as having a medium to high impact.

2.1 Mycotoxins in Grain Crops, Tree Nuts and Cotton

2.1.1 Fungal and toxin methodology and identification (Impact Rating 7)

- Research is focused on detecting and quantifying molds and mycotoxins. Recognizing that various molds and their mycotoxins are unevenly distributed among grains, tree nuts and cotton and within a given commodity molds and mycotoxins may be found only in certain sub-samples of a test lot, the challenge is to develop rapid, sensitive and non-destructive measurements. Substantial progress has been made towards achieving these goals.

- The technologies developed in this component have immediate application in the grain and further-process food industries. Government, industry, and academic programs have been influenced by the findings and development of assays to screen grains for mycotoxins. Use of these assays in grain handling and processing should result in a reduction or elimination of target mycotoxins in foods as they reach the consumer.
- Developing more sophisticated technology
- Technologies are immediately applicable to industrial settings

- Rapid monoclonal assays for quantifying fumonisin and zearalenone in corn and deoxynivalenol (DON) in wheat were developed. This fluorescence polarization immunoassay provides a more rapid alternative to the traditional instrumental or ELISA assays. Multiple antibodies developed for several mycotoxins including fumonisins, zearalenone and DON, and the incorporation of these antibodies into rapid ELISA test kits. Good success. Multiple publications. Two CRADAs established. Reagents prepared to demonstrate the feasibility of surface plasmon

resonance technology and the development of fumonisins resistant corn. SBIR grant awarded for further commercialization.

- Studies designed to examine critical control points in corn resistance/susceptibility to aflatoxin appeared to lack focus. The only apparent significant success was the use of a high volume optical sorter based on NIR to separate aflatoxin and fumonisins contaminated grains. The accomplishment of stated goals, i.e., identification of corn resistance factors, mechanisms of competitive interference, significance of hyphal incompatibility, etc., appears to be far off. Limited publications. Other than sorting studies, limited technology transfer.

2.1.2 Crop fungal relationships (Impact 8)

- Research in this component is focused on interactions between fungi and plants during growth and mycotoxin production by fungi in or on plants. The overall goal of one project is to identify microorganisms that can be used to control the growth of *Fusarium verticillioides* and *Neotyphodium caenophialum*, which produce fumonisins and ergot alkaloids, respectively, in corn and forage grasses. Specific virulence factors, host/fungus compatibility and bacterial enhancing factors were identified. A biological association between *F. verticilliodes* and corn was determined. Bacterial strains (*Bacillus mojavensis*) that have disease-protecting traits were identified. Identified a specific fungal inhibitor and genes responsible for inhibition. Numerous publications reported. Multiple CRADAs established. Some material transfer agreements.

- Another study determined the chemical, biochemical and physical basis for resistance of the Tulare variety walnut to infection by *A. flavus.* Biocompetitive microorganisms capable of suppressing growth of *A. flavus* and aflatoxin production in walnuts, pistachios and almonds were also investigated. Resistance was attributable to natural chemical inhibitors (tannins) in the seed coat. Possible application of findings to grape diseases. Significant publications reported. No technology transfer reported.

2.1.3 Production practices and expert systems (Impact 7)

- Progress toward providing benefits to the grower and consumer was evident in all projects within this component. The impact of successfully predicting mycotoxin occurrence in grain crops and biocontrolling aflatoxigenic *A. flavus* would be substantial in terms of providing safer products to the consumer, whether in the U.S. or in other countries.
- This research component comprises preharvest cultural practices and expert systems designed to reduce aflatoxin formation. The rehydration procedure used to facilitate cracking of closed shell pistachios was shown to result in high aflatoxin levels, resulting in a recommendation that the practice should be abandoned because of the potential for aflatoxins to exceed regulatory guidelines.

- Research is also focused on developing insect-oriented management strategies to reduce mycotoxins in corn. Introducing insect resistance by genetic engineering and developing a farmer-based program for managing mycotoxins are research goals. Using a tobacco plant model system, advances have been made in identification of genes responsible for conferring resistance to caterpillars and aphids. Application to field use was studied and significant publications reported. One CRADA established.

- A third focus is on development of methods to manage aflatoxigenic fungi through better understanding of the etiology and epidemiology of contamination. Field tests showed that multiple atoxigenic strains have potential for controlling aflatoxigenic strains. Practical aflatoxin management tools established and put in use. Significant technology transfer undertaken. Over 30,000 and 5,000 acres are under treatment in AZ and TX, respectively, in 2004.

2.1.4 Breeding resistant crops (Impact 6)

- This component has made progress toward the goal of providing knowledge of crop varieties that will not support the growth and toxin production by mycotoxigenic fungi. Work on genomics holds promise for providing knowledge to develop strategies for controlling the growth of mycotoxigenic fungi on selected grains and oilseeds. Reasonable progress has been made toward these objectives. The lack of practical application of research results to stated objectives was problematic. Linkages to food safety are not clear. Development of relationships with plant biologists (versus crop scientists) may be necessary to forward a food safety agenda.

- Crop and fungal genomes will be necessary to develop interventions and crop resistance to infection and mycotoxin production are the focus of this component. Gene sequencing of *Fusarium verticillioides* to elucidate the gene cluster and the biochemical pathway for fumonisin production has been completed. This library is being used to identify genes that regulate fumonisin production as well as the ability of *F. verticillioides* to infect corn.

- Study focuses on collection/development of information on genetic/biochemical pathways to fumonisins formation in corn. Discovered 15-gene cluster that correlates with fumonisins formation. This study has made significant progress in gene identification, etc.; however, there is a lack of practical application of this information to reducing fumonisins formation in corn/maize. Significant publications reported. Technology transfer has focused on research and material transfer. Project may benefit from redirection.

- Study focuses on determining the effect the *Fusarium* contamination has on wheat and barley diseases and the magnitude of economic impact. Identified and

characterized multiple genes associated with trichothecene formation. Multiple publications reported. There is a lack of practical application of research results to stated objectives. Stated objectives have not been met and focus appears to be only on gene identification, therefore this research is viewed as low impact. Technology transfer has focused on research and material transfer.

- Research aimed at aflatoxin control has focused on the genetic role in aflatoxin formation and possible interruption of aflatoxin synthesis. Cloned DNA library established. Identified inhibitors used in breeding activities, developed non-toxigenic strain of *Aspergillus flavus* for use in AZ, TX and CA. Information obtained can be used in seed control and fungal survivability. Numerous publications reported. Significant technology transfer established. This is a high impact research area.

- Research is focused on the identification of chemicals, proteins and genes that could be used in plant breeding and genetic engineering to reduce *A. flavus* infection and aflatoxin formation in cottonseed. Extensive technology transfer operations have been carried out including patents, plant breeders, genetic engineering, academic research, industrial operations. Limited publications reported. Prototypes of transformed cotton produced showing template plant transformation approach. Potential application to peanuts demonstrated. Potential anti-fungal peptide discovered that could be used in plant disease control. Anti-fungal proteins discovered in corn. The impact of this research is high.

2.1.5 Biocontrol technologies (Impact 6)

- It is estimated that $18 million in annual cottonseed losses in Arizona could be prevented using AF36 technology. Prevention of losses in corn and cotton in Texas is predicted to be even greater. Knowledge gained from the work done using *B. mojavensis* will become the basis for developing a strategy to control the growth of *Fusarium moniliforme* and fumonisin production in corn. Progress toward meeting this objective is substantial.

- Research focused on the development of a non-toxigenic strain of *Aspergillus* that could be used for aflatoxin control in the field similar to studies for cotton in AZ, TX, and CA. *A. flavus* strain obtained. Formulation technique patented. CRADA established. EPA approval for intended use obtained. Actual effectual application not reported. Multiple publications reported.

2.1.6 Pest management/insect transmission and predation (Impact 7)

- This is an example of a research area which is forcing the issue of food safety, which appears to be of secondary impact.

- Research included the identification of chemical plant volatiles (including sex pheromones) that disrupt insect pest behavior. Some potential chemicals identified that prevent aflatoxin biosynthesis. Limited success shown. Very limited publications reported. Application of kairomone lures for codling moths patented and commercialized. One CRADA established.

2.1.7 Toxicity evaluations and mechanism of action of *Fusarium* toxins (Impact 9)

- High impact projects focused on defining biological mechanisms, predicting extent of mycotoxin modified response to exposure, and extent that food-processing procedures can minimize the detrimental effect of mycotoxin exposure in humans. Cultured cell-lines, plant models and GM mice are useful in determining effect of fumonisins. Significant toxicological information, evaluated and applied to multiple locations worldwide as well as food preparation procedures. Addressed effect of potential use of biological and chemical agents in bioterrorism. Conducted training and participated in analytical expertise transfer in Central America. Showed that "hidden" fumonisin reaction products not produced in significant amounts, nixtamalization process results in toxicity reduction for fumonisins and reaction products. Significant publications reported. Technology transfer included patents, CRADA and cooperative work agreements including the Institute of Nutrition of Central America and Panama.

ADDITIONAL COMMENTARY

Although this program focuses on food safety issues, it is strongly recommended that USDA combine the food safety program with a support program that focuses on food and agriculture security/defense issues. This would include studies addressing rapid, user-friendly field detection methodologies, prevention operations, and remediation/decontamination procedures for select agents (including mycotoxins as well as biological, chemical and radiological materials) that could be used in intentional adulteration of agricultural commodities and foods.

3.0 CHEMICAL RESIDUES:

The overall impact of projects in this research area was judged to be high, particularly with respect to dioxin analysis.

3.1.1.1 Food Producing Animals (8)
Residue and Toxin Methodology Identification

- Dioxin is a priority research area for FSIS, FDA, EPA and CDC. It is also of concern to producers because of safety and international trade concerns. The Panel believes that dioxin will continue to be an important trade issue and supports continued dioxin research if public health or trade is threatened. In 2004, the European Union (EU) recommended ongoing monitoring of the background level of dioxins, furans and dioxin-like PCBs in both feed and food. This will

likely emerge as a global trade issue in the future, and ARS is positioned to provide key leadership in this area.

- The Unit has a reputation as the "State of the Art Laboratory" for dioxin analysis in food. The Panel feels it was important to have a United States Department of Agriculture laboratory taking the lead with surveys of food products for dioxin.

- The Unit scientists have had an impact through presentations at major scientific meetings and through publications in leading journals in the field. Unit scientists have also provided scientific information on dioxins to ARS National Program staff, FSIS scientists, and the National Academy of Sciences committee on the Implications of Dioxins in the Food Supply.

- The reduced time and cost for dioxin sample cleanup is a major impact. Unit scientists have identified sources of dioxin in the livestock environment (PCP treated wood, dioxin contaminated mineral supplement). They have also demonstrated that PCP treated wood requires an alternative disposal method because it releases dioxins/furans when combusted. Studies elucidating the metabolic pathways of dioxins and brominated flame retardants will be used by pharmacologists, toxicologists, regulators and risk assessors. Likewise the study of the bioavailabilty of dioxin in dairy cattle which demonstrated a biphasic withdrawal rates and the dioxin bioconcentration in milk and tissues was a significant study adding to our body of knowledge on dioxins

- Producers, regulatory agencies, and consumers appreciate the approach the AMACRU has taken to dioxin research. The Unit scientists have developed cheaper, more sensitive methods for dioxin analyses. They have identified dioxin release from the combustion of pentachlorophenol treated wood which demonstrates the need for an alternate disposal method that will not cause the release of pentachlorophenol. They have not only identified dioxin residues in food products, but have worked to identify the source of the dioxin residues to eliminate them from the food chain: (1) high levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/DFs) in cattle exposed to pentachlorophenol treated wood and swine with elevated levels of PCDD/DFs exposed to a dioxin containing mineral supplement. Additionally the Unit scientists have looked at metabolic agents to reduce dioxin tissue residues in animals exposed to dioxin. This is in accordance with the Unit mission, "reduce the negative impact of foreign chemicals in food producing animals and in the processing of food." The Unit plans to look for sources of dioxin in turkeys and beef cattle that could impact public health or trade. The Panel is supportive of this.

- The Panel recommends increased collaborations with EPA and USGS to include laboratory exchanges. The EPA is currently looking at endocrine disruptors in the environment and has not yet developed the framework for regulating this class of chemicals. The Panel noted there seemed to be little collaboration between academicians and ARS scientists working on endocrine disruptors. The Panel

thinks the Unit should seek input from other scientists working on endocrine disruptors to benefit study design, data analysis, and grant proposals.

- The Panel believes the AMACRU is the logical unit to look at the fate of endocrine disruptors, antibiotics, and other agriculture related chemicals (and their metabolites) in manure, soil, and water. The Panel thinks there should be statistical and epidemiological input into the current (and future) on-farm study design(s) to maximize the useable data of this "real-world look" at the fate of estrogenic compounds in the environment. The Panel thinks the work demonstrating Atrazine's competitive inhibition of phosphodiesterase is both relevant and significant.

- The Panel understands that the initial choice of sulfa antibiotics for their research on antibiotic movement through soils was because of the historical use of sulfas and availability of radiolabeled sulfas. Studies should also be conducted on other drugs/compounds used more commonly in production. The Panel supports the Unit conducting laboratory studies of the fate of a number of chemicals/compounds in different soil types to develop models that could serve as hypotheses for how other chemical compounds will behave in soil. The Unit should also test their hypotheses in agricultural production units and determine if their laboratory models mirror what is happening in similar soil types in the production environment. The Panel also supports the objective of comparing screening tests for endocrine disruptors with LC/MS. A validated screening test for endocrine disruptors would facilitate this research. The development of "clean-up" methods should facilitate this.

- The Panel recommends the Unit also continue their laboratory studies on the fate of chemicals/compounds in manure (composted *vis a vis* liquid manure) and develop models for the behavior of chemicals/compounds (and their metabolites) in manure. The Unit should similarly test in the field (or collaborate with other scientists to field test) hypotheses generated from their laboratory manure research. The Panel recommends a strong focus on amelioration/remediation of chemicals/compounds that can have a negative impact on the food/environment when the Unit identifies that the chemicals/compounds move through soils unaltered.

- The Panel recommends that the AMACRU consult with its stakeholders (industry, producer groups, and regulatory agencies – FSIS/FDA) with regards to the development of residue tests. The Panel is not aware of any food safety issue with Ractopamine; it has been given a zero withdrawal time by the FDA; a test has been developed (and patented) for detecting Ractopamine; and therefore, the Panel believes there is little need for additional research on Ractopamine as a residue issue. The Panel does see the relevance of looking at the use of Ractopamine and other compounds as a possible remediation for dioxin residues. The Panel is also not aware of any public health, trade, or residue issue with Zilpaterol. In the future, industry, FDA, and/or the FSIS may ask for a test to

detect Zilpaterol; however, the Panel is not sure of what risk Zilpaterol poses and its relevance.

- The food safety chlorate project is an excellent example of a collaborative approach that has the support of producer groups, industry, consumers, and regulatory agencies. Representatives of the National Cattlemen's Beef Association and EKA Chemicals have praised the work conducted by scientists at the Unit for their metabolic/tissue residue study using radiolabeled chlorate. The reduction of shedding of pathogens in food producing animals should be a major priority and is of great interest to producers, industry, consumers, and regulatory agencies.

- The Panel believes that brominated flame retardants (BFRs) are an emerging issue. The AMACRU has found BFRs in pork, beef, ground beef, chicken, and a local bison. Polybrominated Diphenyl Ethers (PBDEs) are flame retardants currently used in polyeurethane and in coatings on fabrics and furniture. The PDBEs have dioxin-like toxicity. They have been found in umbilical cord blood and human breast milk. The Panel recommends AMACRU scientists consult with producers and regulatory agencies before analyzing a portion or all of the 510 adipose samples from the recent survey of dioxins in U.S. meat and poultry for PBDEs.

- The Panel believes the fescue toxicity project was at a standstill for want of radiolabeled ergovaline. Fescue toxicity may not be as big of a concern in the United States as it is in other countries. The Panel recommends that resources be diverted from this project.

- The Unit scientists work on the fate of endocrine disruptors, antibiotics, and other chemicals through soils has great potential impact, as does the fate of chemical compounds in composted manure and un-composted manure. The reporting of three different sulfas moving freely (and unaltered) through differing soil types with over 90% recovery was surprising. It has implications for sulfas entering the groundwater. Unit scientists have presented their findings at national meetings. Because of their collaboration with soil scientists they should be able to elucidate the fate of a number of agricultural chemicals in various soil types. The research on composting demonstrated degradation of estradiol and testosterone. The impact of this could be huge as it comes at a time when the EPA is focusing increased attention on endocrine disruptors in the environment.

- The Unit scientists have made antibodies to a number of chemical compounds and organisms both for residue/microorganism detection and sample clean-up. They have presented their findings at national and international meetings and collaborated with scientists and regulators all over the world. The only patent ever received by the AMACRU was for a monoclonal antibody, cell line and immunoassay.

- Because of the AMACRU's prestige in conducting metabolism/tissue residue studies, the FDA-Center for Veterinary Medicine sponsored a study by Unit scientists of the suspected carcinogen, Nitrofuazone. Nitrofurazone products were used in every food animal veterinary practice in the United States. Unit scientists reported meat and milk residues of nitrofurazone and its biotransformation products occurred following labeled use. Manufacturers removed the food animal indications on their nitrofurazone products and the use of nitrofurazone in food animals became illegal.

- The Unit worked with both FDA and industry on various aspects of Ractopamine metabolism. Some of the Unit's work was used in a pivotal study submitted to the FDA for approval of Ractopamine. This is significant as Ractopamine was approved on December 22, 1999, by the FDA for finishing swine. It now has widespread use in the swine industry as a leanness enhancing (growth promoting) agent.

- Because of its prestige as "The Metabolism Laboratory for Tissue Residue Studies", AMACRU was asked to conduct tissue residue studies necessary for FDA approval of sodium chlorate as a "drug" to reduce the fecal carriage and shedding of gram negative human pathogens (such as *Escherichia coli* O157:H7 and *Salmonella sp.*) in cattle. This study has tremendous potential impact. The reduction of fecal carriage and shedding of pathogens on the farm could significantly reduce foodborne illness.

- The AMACRU scientists recently reported that Atrazine competitively inhibits phosphodiesterase; but does not bind estrogen receptor sites. Phosphodiesterase inhibition causes a build up of cyclic adenosine monophosphate (cyclic AMP) which ultimately leads to an increase of aromatase which converts the androgen, testosterone, to the estrogen, estradiol. Thus, Atrazine can have an indirect estrogenic effect. The Panel believes the current work on Atrazine is significant and could have a large impact. In a recently revised EPA human health risk assessment on Atrazine, it states: "Atrazine, a systemic herbicide that blocks photosynthesis, is currently one of the two most widely used agricultural pesticides in the U.S. Approximately 64 to 75 million pounds (lbs) of active ingredient (ai) are applied per year. About three-fourths of all field corn and sorghum are treated with Atrazine annually for weed control. Seventy percent (70%) of the Atrazine applied to corn and sorghum is used prior to emergence (preemergence), and thirty percent (30%) is applied postemergence."

- The AMACRU has supported a pre-harvest food safety initiative with the current metabolism study on radio-labeled chlorate. This chlorate residue study is necessary for FDA approval of sodium chlorate which can reduce human pathogens in the gut flora of livestock and poultry when included in their feed or water. Scientists also conducted a metabolism study of the nitrofurazone family (suspect carcinogen) for the FDA which led to the ban of this family of compounds in food animals. The AMACRU worked on Ractopamine at the

request of FDA and performed Ractopamine analyses to support a pivotal pharmacokinetic study of Ractopamine.

- Chemical residues may be underfunded. The cost of the equipment required to for chemical/residue analyses is staggering. Residue labs should seek collaborative relationships and extramural funding to procure equipment needed to maintain their "state of the art" status *vis a vis* chemical/residue detection. No one can run a "state of the art" metabolic or analytical chemistry laboratory without "state of the art" instruments.

- It is critical to producers to have a laboratory in ARS that has the best possible instrumentation for analyzing the environment and food for chemicals and toxins. Funding is insufficient for scientists in the AMACRU to conduct quality research that is high impact, and instruments, instrument maintenance, reagents, supplies/consumables, etc. used by AMACRU scientists in conducting their research are expensive. The Unit could pursue extramural funding to upgrade instruments and increase their budget.