Annual Report 2000-2001

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN)

Executive Summary

In 1996, the Commissioner of the Food and Drug Administration, Dr. David Kessler, and the President of the University of Maryland, Dr. William Kirwan, met to discuss opportunities for cooperative interactions that would be productive for both institutions. The result of this and a number of subsequent meetings was the April 15, 1996 signing of a Memorandum of Understanding (MOU) that established a cooperative venture, the Joint Institute for Food Safety and Applied Nutrition (JIFSAN). JIFSAN was initially established as a cooperative venture between the University and the FDA Center for Food Safety and Applied Nutrition (CFSAN). Later, the MOU was amended to include the FDA Center for Veterinary Medicine (CVM).

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is a jointly-administered multidisciplinary research and education program. Dr. David R. Lineback (University of Maryland) is the Director and Dr. Paul Mazzocchi (University of Maryland) and Dr. Arthur Miller (CFSAN, FDA) are Associate Directors. Dr. Norris Alderson is the CVM representative.

FDA's broad goals within the collaboration are to expand food safety, human nutrition, and animal health sciences research and education programs that are necessary to provide the Agency with expertise and knowledge needed to recognize and effectively deal with emerging food safety issues and to enhance regulatory review capabilities. Collaborative activities involve research, education, and outreach in microbial pathogens and toxins, food constituents and applied nutrition, animal health sciences: food safety, and food safety risk analysis.

JIFSAN provides a neutral environment in which experts from industry, consumer and trade groups, international organizations, government agencies, and academia pool their resources and ideas to contribute to the scientific base for the development of sound public health policy. Members of the JIFSAN Advisory Council provide advice, vision, and support critical to advancing the Institute's mission of cooperative research and education/outreach. Visiting scientists are encouraged from all sectors. The interactions of FDA, the University, and visiting scientists help ensure that federal regulatory and scientific personnel remain in the forefront of food safety issues. This also provides visiting scientists, faculty and staff insight into regulatory processes. Opportunities for undergraduate and graduate students to work with FDA scientists as interns enhance students' understanding of regulatory processes and will provide them with valuable practical experience. Collaborative research projects contribute to the science

undergirding current and future regulatory issues and activities that impact on public health policies.

Risk analysis (risk assessment, management, and communication) is one focus of JIFSAN programs. This effort promotes the development of risk-based, scientifically-supportable safety standards. These standards can deliver the intended degree of measurable public health protection and can be used to identify priorities to effectively apply available resources. JIFSAN is developing new approaches to information management related to risk analysis. With oversight from the interagency Risk Assessment Consortium (RAC), a Food Safety Risk Analysis Clearinghouse is being developed at JIFSAN. This provides a mechanism to collect and disseminate available data and methodologies from government, academia, and industry. The intent of the Clearinghouse is to provide a centralized information source in areas of risk analysis related to food safety with initial emphasis on microbial pathogens and their toxins. The unique feature of this clearinghouse model lies in the examination and documentation of state-of-the-art methods, data sources, and current results of on-going risk assessments so that a more complete and up-to-date picture of risk assessment is assembled.

The development of partnerships with external constituencies is one of the major avenues JIFSAN uses to expand the science base available for addressing public health policy issues. University and FDA scientists have begun collaborative research efforts with other organizations.

An internal collaborative research program provides seed funding to University of Maryland faculty to support research projects that are closely aligned with FDA's research needs. FDA collaborators on each project help provide additional scientific expertise and insight into the public health impact of the research.

The new CFSAN office and laboratory building is adjacent to the University of Maryland in College Park enabling FDA and the University to share many resources, such as major instrumentation and library facilities. Programs initiated by JIFSAN have demonstrated that the benefits to be achieved by this partnership are substantial.

Trade initiatives have put food safety high on the international agenda. JIFSAN is actively involved in developing collaborations with international organizations to facilitate cooperative research and education programs and the exchange of scientists. In addition, JIFSAN has been designated a Pan American Health Organization/World Health Organization Food Safety Collaborating Center that focuses on risk assessment in food safety and mycotoxin analysis.

The MOU established a set of relationships that closely link the University with CFSAN and CVM by committing to the sharing of facilities, personnel, and intellectual resources when appropriate. Thus, FDA personnel will have access to University facilities such as libraries and may be appointed as adjunct or research faculty in recognition of their involvement in cooperative programs in research, teaching, mentoring, and direction at the graduate and undergraduate levels. FDA will support and utilize major

instrumentation facilities (electron microscopy, nuclear magnetic resonance spectroscopy, mass spectrometry) on the campus and those facilities will house appropriate University of Maryland and FDA personnel. These and other synergistic relationships outlined in the MOU will allow both institutions to remain state of the art in a number of areas where duplicative efforts would be less than successful.

Subsequent to the signing of the MOU, FDA and University personnel developed an Umbrella Cooperative Research and Development Agreement (CRADA) and a multi-party CRADA template. These tools were implemented or designed to facilitate the development of collaborative research beyond the internal competitive research program described later and to provide a mechanism to address issues related to shared resources.

Support for the operation of JIFSAN was provided by FDA and the University. FDA provided a cooperative agreement for $6.5M for five years starting on September 30, 1997. The University provided support in several ways including the return of 100% of the Designated Research Initiative Fund (DRIF) funds from the cooperative agreement to JIFSAN, providing space and administrative support to the program in the form of personnel, and providing space for instrumentation facilities.

The actual operation of JIFSAN commenced with its initial funding on September 30, 1997. Dr. Paul Mazzocchi, Dean of the College of Life Sciences, as Principal Investigator on the FDA-JIFSAN cooperative agreement, served as Acting Director of JIFSAN while the search for the permanent Director was conducted. Dr. David Lineback became Director in November 1998.

Progress Report

During the fourth year of operation for JIFSAN, the administrative structure was strengthened, several education and outreach programs were developed and accomplished, research programs were continued and new ones initiated, and contacts were developed to build partnerships/strategic alliances to plan and initiate additional research, education, and outreach programs. Progress in these areas will be outlined with specific examples included.

The non-competitive base funding for the fourth year was $2,747,000.

Administrative Structure

A unique administrative structure is needed for JIFSAN to allow it to most effectively use resources while planning, organizing, and accomplishing multidisciplinary, multi-institutional programs in research, education, and outreach. An effective way to do this is to utilize, to the greatest extent possible, the administrative structures available in the University of Maryland as one of the major partners in JIFSAN. The structure and policies of a major land-grant university offer the flexibility needed to enable JIFSAN to create and operate strategic alliances involving multiple partners and multiple funding sources.

Events related to JIFSAN's administrative structure and function include:

- Ms. Judy Quigley began work as Coordinator of Conferences and Communications on March 12, 2001.

- Ms. Mary Grimley, who had been partially funded as JIFSAN's Budget Officer, assumed full-time responsibilities and moved into one of the JIFSAN offices (former conference room) on March 30, 2001.

- Both office space and computer laboratory space (approximately 500 sq. ft.) is utilized by the JIFSAN Food Safety Risk Analysis Clearinghouse operations at the VA-MD Regional College of Veterinary Medicine at College Park. This space is used to develop and operate the Clearinghouse and to support risk analysis activities.

- An initial draft of a strategic plan for JIFSAN was revised and is nearing completion. Implementation of elements of the strategic plan has been initiated.

- Dr. Arthur Miller (CFSAN, FDA) became Associate Director upon the departure of Dr. Samuel Page on assignment to the World Health Organization (WHO) in Geneva, Switzerland.

- Presentations concerning JIFSAN and its programs were made at the Annual Meeting, National Center for Food Safety and Technology, Oak Lawn, IL; Food Safety in Europe (Poster), London; Biosciences Research/Technology Review Day (Poster), University of Maryland, College Park; "Diet, Nutrition, and Health" Symposium, 12th International Congress of Food Science and Technology, Seoul, Korea; and to the Senior Food Safety Team, Sodexho-Marriott Corporation, Gaithersburg, MD.

- Dr. Catherine E. O. Woteki, former Undersecretary for Food Safety, U. S. Department of Agriculture, joined the faculty of the College of Agriculture and Natural Resources in a half-time appointment as a Senior Research Scientist on July 1, 2001. A portion of this position is funded by JIFSAN. She will assist with development of the applied nutrition aspects of JIFSAN's programs and will advise the Director on numerous issues.

- The Director participated in a Bipartisan Congressional Retreat (March 9-11, 2002) as an invited expert resource person in the area of developments in biotechnology and the implications for American society. This included impacts on health, medicine and the ethical ramifications. He also served as a member of the panel at the two EPA hearings on Starlink™ corn. He serves as a member of the International Advisory Board (ISAB) of the Central Food Research Institute (KEKI), Budapest. Hungary.

The JIFSAN Advisory Council

Central to the operation of JIFSAN is an Advisory Council composed of members from private sector business, government agencies, academia, and representatives of consumers' interests. This group provides guidance to JIFSAN in developing research, education, and outreach programs to address problems in food safety, nutrition, animal health sciences, and risk analysis. The Advisory Council was scheduled to meet on September 12, 2001 to discuss five internal research projects in progress, needs of the food industry for individuals trained in food safety, and the draft strategic plan. The meeting was cancelled due to the tragic incidents of September 11th. A meeting has been scheduled for February 2002. Contact with several of the Advisory Council representatives was maintained by the Director throughout the year.

Members of the Advisory Council include:

- Private sector industry

  Unilever Bestfoods NA (Dr. Richard Lane)
  Coca-Cola Company (Dr. Michael Carakostas)
  Campbell Soup Company (Dr. George Evancho)
  Dean Foods Company (Dr. George Muck)
  Frito-Lay (Dr. Steve Saunders)
  General Mills (Mr. Frederick Hegele)
  Gerber Products Company (Dr. Nicholas Hether)
  Hershey Foods Corporation (Dr. Stanley Tarka)
  Kellogg Company (Dr. Tracie Sheehan)
  Kraft Foods (Mr. Ron Triani)
  McCormick and Company (Dr. Hamed Faridi)
  McNeil Specialty Products Company (Dr. Leslie Goldsmith)
  M&M/Mars (Dr. Steven Rizk)
  Mead Johnson Nutritionals (Dr. Thomas Ferguson)
  Monsanto Company (Dr. Jerry Hjelle)
  Ocean Spray Cranberries (Dr. Y. Steve Henig)
  Odwalla (Ms. Linda Frelka)
  Procter and Gamble Company (Dr. Keith Triebwasser)
  Tropicana Products (Dr. Jay Shulman)

- Representatives of Consumers' Interests

  Ms. Carol Tucker Foreman (Consumer Federation of America)
  Ms. Laurie Girand (Safe Tables Our Priority)
  Ms. Linda Golodner (National Consumers League)
  Dr. Kristen McNutt (Consumer Choices)

- Academia

  Dr. Lester Crawford (Center for Food and Nutrition Policy, Virginia Polytechnic & State University)
  Dr. Michael Doyle (University of Georgia)
  Dr. Julie Miller Jones (College of St. Catherines)
  Dr. Sanford Miller (Center for Food and Nutrition Policy, Virgina Polytechnic & State University)
  Dr. Michael Pariza (University of Wisconsin)
  Dr. Stephen Taylor (University of Nebraska)
  Dr. Connie Weaver (Purdue University)

- Government

  Dr. Michael Roberts (Central Science Laboratory, Department of Environment, Food and Rural Affairs, UK)

- Individuals

  Dr. Gilbert Leveille (Leveille Associates)

Research Initiatives

Research is a major focus of JIFSAN. Collaborative research supports the goal to develop a strong science base to address ongoing and increasingly complex public food safety issues.

JIFSAN is involved in research in a number of ways:

- By supporting core facilities that benefit FDA and University scientists and their collaborators,

- By funding a competitive internal research program,

- By developing cooperative programs with external constituencies.

- By facilitating programs funded from multiple institutions and other granting sources.

- By supporting scientists working on JIFSAN programs.

By the very nature of the research enterprise, i.e. the time required for building functioning multidisciplinary collaborative research teams and the highly competitive nature of obtaining external research funding, establishing externally-funded research programs for JIFSAN is still in the development stage and will require time.

JIFSAN Competitive Internal Research Program:

A collaborative research program was established in 1998 by providing seed funding of $25,000 each for four research projects. Each project requires collaboration between at least one University of Maryland faculty member and one or more FDA collaborators. The latter help provide additional scientific expertise and insight into the public health impact of the stated research. These projects contribute to the science for current and future regulatory issues and activities that impact on public health policies, and are aligned with the FDA's research needs:

- Development of sampling and detection methods for the identification of pathogens and toxins.
- Further development of an understanding of antibiotic drug resistance.
- Development of methods to characterize the composition and bioactivities of natural constituents of foods, including micronutrients and beneficial non-nutrients.
- Developing messages pertaining to food safety and the adoption of safe food handling practices.

The system for funding grants in the internal research program was standardized to be support for a graduate student plus operational support (a total of $30,000) or for a postdoctoral associate plus operational support (a total of $40,000). Operational support is $10,000 in each case. Proposals may be for three years, but are funded for only one year at a time. Continuation is contingent upon a satisfactory annual progress report, a proposal for continuation of the research, and availability of funding.

Of the five projects funded in January 1999, only two were proposed for three years. Progress on these two projects was reviewed and they were continued for a third (final) year. Of the five projects funded in January 2000, one was proposed for two years and three were proposed for three years. Progress on these three projects was reviewed and all three were continued for a second year. Five new projects were funded in July 2001 with one these being for a single year and the remaining four for three years. A sixth project was partially funded since it was of high quality and had additional support already committed.

Projects funded during the reporting year follow and are described in more detail in Appendix A.

Third (final) year projects scheduled to be completed in May 2001:

- Immunoaffinity Hollow Fiber Ultrafiltration for High Throughput Screening/Residue Analysis in Food Safety. Cheng Lee (UM) and Mary Trucksess (FDA).

- Effects of a Variety of Stress Factors on the Immune Systems of Poultry and Subsequent Infection of Shell Eggs by Salmonella. Wenxia Song (UM) and Richard Raybourne (FDA).

- Surveillance of Poultry and Other Stock for Carriage of Multiresistant Enterococcus. Lewis Carr, Sam Joseph (UM), and David Wagner (FDA)

- Mechanisms of Chemoprevention by Dietary Carotenoids and Their Metabolites in the Prevention of Chronic Disease in Humans. Frederick Khachik (UM), Eugene Mazzola, Shirley Blakely, and Andrija Kornhauser (FDA).

Third year projects (completing January 2002):

- Immunologic Sequela Following Oral Exposure to a Foodborne Toxin. Carol Pontzer (UM), Richard Raybourne, and Mary Ann Principato (FDA)

- The Missing Connection: Isolation and Concentration of Micoorganisms on Biocapture Surfaces. Jonathan Bundy, Catherine Fenselau (UM), Mary Carson, and David Wagner (FDA)

  Funding for this project ended January 30, 2001. Results were included in the Ph.D. dissertation of Jonathan Bundy and were summarized in the 1999/2000 Annual Report for JIFSAN

- Identifying Knowledge Gaps and Improving Communication Strategies to Reduce Food Safety Risks. Mark Kantor, Cynthia Tuttle, Robert Feldman (UM), Toija Riggins, and Alan Levy (FDA).

  This project was funded in January 1999 for one year and a no-cost extension was approved in January 2000 to facilitate completion of the objectives of the study.

- The Detection of Foodborne Pathogens in Biofilms Using Antibodies, Lectins, and Fluorescent Dyes. Ronald Weiner, Sam Joseph, Lewis Carr (UM), and Ben Tall (FDA).

Second year projects (funded in January 2000):

- Viral Immunosuppression and the Infection of Shell Eggs by *Salmonella enteritidis*. Robert Heckert, Wenxia Song (UM), Richard Raybourne, and Uma Babu (FDA).

- Using a Probabilistic Risk Assessment Model to Study Risk of *E. coli* O157:H7 Contamination in Hard Cheeses. Mohammad Modarres (UM) and Joseph Schlesser (NCFST, FDA).

- Antibiotic Resistance Integrons in Shiga Toxi-Producing *Escherichia coli* and *Campylobacter jejuni/coli*. Jianghong Meng (UM), David White, S. Zhao, and David Wagner (FDA).

- The Evaluation and Removal of Bacterial Biofilms from Food and Food Processing Materials. Paul Schreuders (UM) and Leila Ali (FDA).

First-year projects (funded July 2001):

- Monitoring and Compliance Under Seafood HACCP: An Econometric Investigation. Anna Alberini, Erik Lichtenberg (UM), Dominic Mancini, and Robert Scharff (FDA).

- The Use of Tissue Fluid Correlations to Predict Drug Residue Levels in Edible Tissues. Natalie Eddington (UMB), James Peggins, Keesla Moulton, Jurgen von Bredow, and Pamela Chamberlain (FDA).

- Investigating the Perceived Credibility of FDA's Advisory Committee Meetings as Techniques for Communicating about Food, Drug, Biologics, and Medical Device Issues. Katherine McComas (UM) and Linda Suydam (FDA).

- Comparison of the Effects of Curcumin Supplements in Young and Aged Rats. Bernadene Magnuson, Monica Giusti (UM), Fred Hines, Sabine Francke, and Hamida Alam (FDA).

- Modeling the Antimicrobial Effect of Lactate on the Growth and Survival of *Listeria monocytogenes* on ready-to-eat seafood. Kisun Yoon (UMES) and Richard Whiting (FDA).

- In Vitro Metabolic Profiles to Characterize and Predict Drug Residues in Aquacultured Finfish. Andrew Kane (UM), Badar Shaikh, and Renate Reimscheuessel (FDA).

  This project proposal was partially supported to leverage funding already committed to the investigation.

Specific Research Initiatives:

The Cooperative Agreement allows and encourages the development of specific research initiatives built upon common interests and expertise of FDA staff and UM faculty. Those in existence during the reporting period are listed below and more fully described in Appendix B.

- Mechanistic Assays for the Photoxicity of Cosmetics. Daniel Falvey, Peter Vath (UM), and Wayne Wamer (FDA).

- Developing Methodology to Detect Adverse Events. Johnny Blair, Timothy Triplett, Henry Wu, Song Zhao (UM), and Marilyn Flack (FDA).

- Characterization of Multiple Fluoroquinoline Resistance Among Avian *Escherichia coli* Isolates From North Georgia. David Wagner (FDA/CVM) and Jianghong Meng (UM).

JIFSAN Postdoctoral Research Associate Program:

This program strengthens the science base for public health policy by providing short-term research scientists to work in FDA laboratories.. Five broad areas of research, where significant knowledge gaps or the lack of appropriate scientific data, methods, or models exist, were the focus this year. These areas are: (1) Improved detection methods, (2) Understanding resistance to traditional preservation technologies, (3) Understanding antibiotic drug resistance, (4) Prevention techniques: pathogen avoidance, reduction and elimination, and the (5) Impact of food handling, distribution, and storage on food safety. Five postdoctoral research associates are being recruited to work in FDA laboratories in these selected areas. Due to delays in the recruiting process, only four of the positions have been filled. A new position has also been added with the search process being completed and an individual hired.

Leveraging:

One of the basis tenets for operation of JIFSAN is the leveraging of resources. This includes the development of research partnerships and core facilities.

1. Development of research partnerships

   The development of partnerships with external constituencies is one of the major avenues JIFSAN uses to expand the science base available for addressing public health policy issues. UM and FDA scientists have begun collaborative research efforts with other organizations. These projects will contribute to the science for current and future regulatory issues and activities that impact on public health policies and are closely aligned with the FDA's research needs.

   The following two projects are in progress and are described in Appendix C.

   - FT-NIR Rapid Determination of Food Integrity

     Support for this project comes from an Army Cost-Reimbursible Research Contract to Elizabeth Calvey (Co-PI, FDA) and Bruce Jarvis (Co-PI, UM) that was initiated in August 1998.

   - Prevalence/Quantitation of *Listeria monocytogenes* in Selected Retail Products

This project involves a sub-contract between JIFSAN and the National Food Processors Association Research Foundation.

2. Development of Core Facilities:

   The development of core facilities that will benefit FDA and University scientists and their collaborators is a cornerstone of JIFSAN's cooperative programs and objective to leverage resources. Space that will house CFSAN's research mass spectrometry facility will be provided in a new addition that is under construction for the Department of Chemistry and Biochemistry. The effective use of the arrangements for shared facilities (electron microscopy, nuclear magnetic resonance, and mass spectrometry) will increase when CFSAN relocates to its new facilities in College Park in late 2001 and early 2002.

Risk Analysis

Risk analysis applied to food safety is the assessment, management, and communication of risks associated with our food supply. Risk analysis requires multidisciplinary input to identify, analyze, and ultimately guide the development of science-based policies. JIFSAN has identified risk analysis as a major area of focus.

JIFSAN has been charged with the responsibility of developing and operating a Risk Assessment Clearinghouse (Dr. Wendy Fineblum, Coordinator). In 1999, the name was changed to the JIFSAN Food Safety Risk Analysis Clearinghouse to more closely align with international nomenclature in which risk analysis is the umbrella term that includes risk assessment, risk communication, and risk management. The Clearinghouse is being established to collect and disseminate available data and methodologies from government, academic, and industry sectors domestically and internationally. The Clearinghouse will provide a centralized information source for risk analysis related to food safety with initial emphasis on microbial pathogens and their toxins. The Clearinghouse is guided by the Food Safety Initiative (FSI) Risk Assessment Consortium (RAC) composed of a representative from each of the U. S. Federal Government agencies responsible for ensuring the safety of the food supply.

Progress this year included:

- Links made to 17 completed and ongoing food safety risk assessments.
- Access to electronic references via hyperlinks (FDA's *Vibrio parahaemolyticus* risk assessment) is a Clearinghouse exclusive.
- Data for risk assessment has been provided through links to 22 databases of the following types: commodities, food intake, pathogens, residues and additives, natural resources, livestock and international data

- Links are provided to seven resources of shareware and commercial risk and modeling software.

- A questionnaire template has been developed to help departments of public health glean dose-response information from foodborne disease outbreaks.

- Links to seven relevant tutorials have been provided. Included are Clearinghouse exclusives on the Monte Carlo Process; assessing risk of a natural toxin, using aflatoxin as an example; and the food additive safety assessment process using aspartame as an example.

- Data currently being obtained by the National Food Processors Association (NFPA) on exposure to *Listeria monocytogenes* in five ready-to-eat-foods is being accumulated and prepared for posting in the Clearinghouse upon completion of the study. Data from the USDA/Member Company portion of this study will also be included.

Education and Outreach Programs

The establishment of domestic and international education and outreach programs is of vital importance to JIFSAN. These programs involve aspects of food safety, applied nutrition, animal health sciences, and risk analysis that have been identified as areas of need within the purview of JIFSAN's responsibilities. Identification of these areas is done in collaboration with the JIFSAN Advisory Council. The following efforts were initiated or continued during the reporting period.

JIFSAN at IFT: For the fifth consecutive year, JIFSAN staffed a booth at the Food Exposition at the Institute of Food Technologists' 2001 Annual Meeting in New Orleans. This meeting had an attendance of over 17,000. Many food scientists, nutritionists, and industry representatives had an opportunity to visit with personnel from JIFSAN and to become better acquainted with the JIFSAN programs and mission. Two adjoining booths were staffed in cooperation with personnel from the National Center for Food Science and Technology. This will probably be the last time to display at this meeting. JIFSAN participated (staffed a booth) in the First IFT International Food Safety and Quality Conference and Expo in Orlando, Florida, November 15-17, 2000. While attendance was relatively small due to this being the first time such a conference and expo was held, it is likely that the audience involved in this type meeting will be closer to JIFSAN's interest.

JIFSAN at the FDA Science Forum: JIFSAN staffed a booth at the Annual FDA Science Forum held February 15-16 at the Washington, DC Convention Center. The purpose of this exhibit was to provide an opportunity for a broad cross section of FDA scientists and industry to learn more about leveraging opportunities.

JIFSAN at the National Association of State Universities and Land Grant Universities (NASULGC) Exhibition: JIFSAN staffed a booth, in partnership with the

College of Agriculture and Natural Resources (University of Maryland), at the NASULGC Fourth Annual Agricultural Research and Education Exhibition and Capitol Hill Reception in the Rayburn House Office Building on March 6, 2001. The purpose of this exhibition was to inform Congress (House and Senate members and their staffs) of the benefits of agricultural research and education and showcase some of the exciting cutting-edge science taking place in this area in the Land-Grant System. Information featuring some of JIFSAN's programs in food safety and the Food Safety Risk Analysis Clearinghouse was featured.

International Training Program "Enhancing the Safety of Fresh Produce at the Source through Good Agricultural Practices": This five-day training/information exchange program, cosponsored by JIFSAN and the FDA, was offered in Port of Spain, Trinidad, December 4-8, 2000 and in Petrolina, Brazil, June 4-8, 2001. These programs involved approximately 50 individuals each and served as pilot programs for an international training manual being developed through a subcontract to the University of Arkansas. Evaluations by participants indicated that the training programs were successful.

A draft copy of the training manual "Improving the Quality and Safety of Fresh Fruit and Vegetables: A Training Manual for Trainers," is undergoing final review and will be revised into a completed copy (English and Spanish) in early 2002.

A meeting of the International Advisory Council for this training program was held February 14, 2001.

The Director participated with personnel from FDA CFSAN and the National Center for Food Science and Technology in a trip to Puerto Rico to investigate opportunities for collaborative programs. FDA had already committed JIFSAN to offering this training program to Extension Specialists at the University of Puerto Rico, Mayaguez and to participating with some of these Specialists as trainers in a later offering of the training program, probably in the Dominican Republic. This was more thoroughly discussed and explored during a visit to the Mayaguez campus.

Produce Safety Training Program for Cochran Fellows:

In cooperation with FDA/CFSAN, 14 Cochran Fellows from Guatemala were hosted for produce safety training in the U.S. and in Guatemala. They were joined by 15 Guatemalans who have farm investigation responsibilities for the Guatemalan portion of the meeting that involved intensive training in farm investigation. JIFSAN's primary role was administration of the program and organizing a field trip while the Fellows were in the U.S. The Institute was not involved in the Guatemala portion of the program.

JIFSAN arranged a field trip for a later group of Chilean Fellows while they were in the U.S.

Training Programs and Courses:

1. Food Safety and Genetically-Modified Foods

   A graduate-level food safety course "Special Topics: Food Safety and Genetically Modified Foods" (LFSC 609) has been developed for JIFSAN by Dr. Lucinda Jack. This course includes all aspects of food safety and its regulation, with special emphasis on genetically-modified foods. The first half of the course concentrates on the traditional concerns of food safety and the response of food regulations, with minor reference to genetically-modified organisms. The second half of the course deals with the impact of biotechnology on the food supply. The course was offered on-line for the first time starting in September 2001 as part of the Master of Life Sciences program, College of Life Sciences. This is a content-based Masters degree program for high school science teachers that provides in-depth knowledge of current research areas in the biological, biochemical, and biomedical sciences.

2. Food Safety Risk Analysis Training Program

   There is a growing need for understanding and using risk analysis as a decision making tool to facilitate international trade, to ensure a safe domestic food supply, and to enable industry to maximize their innovative potential. A Professional Development Training Program in Food Safety Risk Analysis is being developed to provide training to national and international audiences that targets the key components of risk analysis. The program is being developed and taught by FDA staff and UM faculty. The program will consist of six core courses and six electives that will be offered for the first time during the coming year. Five of the core courses have been pilot tested with FDA participants.

   The College of Agriculture and Natural Resources, University of Maryland, in collaboration with FDA/CFSAN staff, has been awarded a three-year ($320,000) grant to develop a Food Safety Risk Analysis Distance Training Program. This programs seeks to utilize new and emerging information technologies to train food safety professionals in the fundamentals of risk analysis. By improving access to training in the key theories, methodologies, tools, and techniques of food safety risk analysis, food safety professionals across a wide spectrum of the food safety continuum will be able to more effectively identify and assess food safety risks. The proposed program will be based on courses developed for the JIFSAN Professional Development Training Program in Food Safety Risk Analysis given in the traditional classroom setting.

3. Global Risk Assessment Training Needs

   With JIFSAN's interests in risk assessment training, it became obvious that training needs should be considered on a global basis rather than domestic only. Several organizations (ILSI, FAO, WHO) already have been active with training workshops in this area. As a result of further discussions, a partnership has been formed with

ILSI in investigating the needs/opportunities/curricula/training programs for risk assessment training worldwide.

Two meetings (May 15-16 and September 5-6, 2001) have been held with the objective of considering what has been learned from previous ILSI/FAO/WHO workshops in risk assessment and to determine the most appropriate ways to move forward to help meet the training needs of industry, academia and government in food safety risk assessment.

The core group of people involved in these two meetings are to become a "steering committee" to provide strategic advice on the direction of this project. A draft Global Food Safety Risk Assessment Training Needs Discussion Document resulted from the first meeting and was reviewed at the second meeting. It is currently undergoing revision.

The mission of the steering committee includes: (a) advising on scientific content of training courses in food safety risk assessment, (b) identifying and sharing resources for trainers in food safety risk assessment, and (c) identifying gaps in resources and suggesting ways to fill gaps. Discussions and meetings will continue to develop training materials, as needed, and to develop a potential curriculum that can be used by those teaching food safety risk assessment to meet identified needs.

Conferences and Workshops:

1. A two-day workshop on Transgenic Crops was pilot tested with FDA staff and was well received. It will be developed further for future offerings.

2. A one-day session was held at the Stamp Student Union Building for cooperative Extension Educators from across the nation. This was part of the National Extension Association of Family and Consumer Sciences off-site professional development visits, "Workshops on the Move." Over 700 extension educators visited 63 locations across the Baltimore-Washington region. Approximately 30 extension educators were involved in this session. Seven FDA staff and UM faculty provided updates on selected current issues in microbial aspects of foodborne disease, food toxicology: diet and cancer prevention, food biotechnology, antibiotic resistance, functional food and dietary supplements, and food safety issues from an FDA perspective. An open question and answer period resulted in excellent participation from the extension educators. Subsequent feedback on evaluations received for this program indicated excellent reception.

Seminars co-sponsored by JIFSAN:

1. JIFSAN provided support for the Seminar Course “Pharmaceuticals in the Environment” (CHEM 729) presented during the Spring Semester 2001 (January-May) at the University of Maryland at College Park. These seminars provided a state-of-the art assessment of the status of research on pharmaceuticals in the

environment in the U. S. The funding was used primarily to provide travel expenses for leading scientists engaged in this area to present their research data.

2. Dr. Keith Singletary, Department of Nutrition, University of Illinois , Urbana presented a lecture (May 8, 2001) entitled "Cancer Chemoprevention by Grape Constituents" as a Distinguished Speaker in the Food Science Graduate Program seminar series. This was cosponsored with JIFSAN.

3. A series of JIFSAN seminars were planned and scheduled by FDA/CFSAN at the FDA Building at 200 C Street. These included:

    - "Food Irradiation: An Underutilized Public Health Technology," Dr. Fritz Käferstein, Distinguished Visiting Scientist FDA/JIFSAN & USDA/FSIS (formerly Director, Programme of Food Safety and Food Aid, World Health Organization, Geneva, Switzerland) (October 4, 2000)

    - The Chairman's Action Plan for Codex Alimentarius," Thomas Billy, Administrator, Food Safety Inspection Service, USDA; Chairman, Codex Alimentarius Commission, Washington, D.C. (January 24, 2001).

    - "Optimizing Pathogen Control with Cost, Risk and Quality Objectives," Dr. Scott A. Malcolm, Department of Food and Resource Economics and Operations Research Program, University of Delaware (February 14, 2001)

    - "The Center for Computational Epidemiology and Risk Analysis at Tuskegee University," Dr. Tsegaye Habatemariam, Associate Dean of Research and Graduate Studies, College of Veterinary Medicine, Nursing & Allied Health (CVMNAH) and David Oryang, Modeling Activities Leader, Center for Computational Epidemiology and Risk Analysis (March 8, 2001)

    - "An Update in Two Parts: Controlling Foodborne Pathogens (and) Conjugated Linoleic Acid as a Model Nutraceutical," Dr. Michael W. Pariza, Distinguished Professor, Director and Chair, Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin - Madison (April 11, 2001)

    - "Mini Course - Risk Analysis: The Role of International Organizations," Dr. Fritz Käferstein, Distinguished Visiting Scientist FDA/JIFSAN & USDA/FSIS (April 17, 20001)

    - "CFSAN's Role in International Food Safety and Trade," Dr. Catherine Carnevale, Director of the Office of Constituents Operations, FDA/CFSAN (May 30, 2001).

    - "Research Priorities for Transmissible Spongiform Encephalopathies in North America," Dr. Robert Rohwer, Director of the Molecular Neurovirology

Laboratory, Veterans Affairs Medication Center, Baltimore; Associate Professor of Neurology, University of Maryland Medical Center, Baltimore (June 29, 2001)

Meetings Cosponsored by JIFSAN:

1. Ceres Executive Leadership Seminar in Food Safety

    - Module 1 - Public/Private Partnership: Is it just talk? San Jose, Costa Rica, June 23-29, 2001
    - Module 2 - Laws, Regulations, and Ethics, Niagara Falls, Ontario, September 30 - October 5, 2001.

    This seminar series is comprised of four modules given in different locations over a period of two years. It is a program designed to promote leadership for the development of a comprehensive food safety policy. It is designed to assist in the development of professionals in agriculture, health and food safety systems (both public and private) into food safety leaders by providing critical information and improved expertise. Approximately 30-35 fellows are participating in the seminar series. Each will have a mentor and a two-year project dealing with a difficult food safety problem in which the individual is involved or interested in solving.

2. A public meeting (December 5, 2000) on Clearinghouse Data Quality Objectives was hosted by JIFSAN in conjunction with a Society for Risk Analysis (SRA) meeting. A major focus of the meeting was gaining public and professional input on draft Data Quality Objectives for use by the Risk Analysis Clearinghouse. Objectives included : (a) outline of gold standard characteristics for studies to be used for microbiological and antimicrobial resistance risk analyses, (b) determine minimum standards for data submission into the Clearinghouse and to provide descriptive information to accompany data, and (c) produce a general document that will discuss the implications of data characteristics and data quality recommendations with regard to use in microbiological and antimicrobial resistance risk analyses. Funding was used for speaker travel, draft documentation development, meeting facilities, and associated expenses.

3. JIFSAN hosted the annual meeting of the National Area Chapter of the Society of Toxicologists on May 15, 2001. The title of the meeting was "Toxicology for the future, are we training the right expertise?" There is a critical need in toxicology to target academic programs to meet future needs and to attract students to this field. Hosting this meeting at the Stamp Student Union Building, UM encouraged participation by the academic community, including students.

4. Giving Simple Carbohydrates a Complex

    A Georgetown Dialogue "Giving Simple Sugars a Complex" was cosponsored on March 15-16, 2001 by the Georgetown University Center for Food and Nutrition

Policy and JIFSAN. Approximately 60 participants and presenters were involved. Objectives of the Dialogue were to (a) examine the past and current definition of simple carbohydrates, (b) examine the methodologies for measuring simple carbohydrates in foods, (c) understand the role of simple carbohydrates in food production, and (d) discuss the policy and labeling of simple carbohydrates on food packages. Proceedings of the Dialogue will be published.

5. Setting Food Safety Priorities

   JIFSAN was a cosponsor of the food safety conference entitled "Setting Food Safety Priorities: Toward a Risk-Based System" convened by Resources for the Future (RFF), May 23-24, 2001. This conference and workshop was designed to advance discussions about how to better support risk-based priority setting in an integrated food safety system. The first day was aimed at a broad policy and research audience, while the second day was designed primarily for a research audience. Plans are to publish a book based on the presented papers and discussions.

Visiting Scientists under the auspices of JIFSAN:

Dr. Fritz Käferstein (WHO retired) completed his three years (November 1998 – November 2001) as a JIFSAN Distinguished Visiting Scientist and returned to Switzerland in August 2001. Dr. Käferstein is the former Director of the Programme of Food Safety and Food Aid for the World Health Organization in Geneva, Switzerland. He served as a JIFSAN liaison with FDA and USDA FSIS in the areas of microbiological food safety and risk assessment; advised/assisted in the development of the Food Safety Risk Analysis Clearinghouse; and facilitated interactions with international food safety organizations - particularly WHO.

International Cooperation and Visitors:

1. Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Current Issues in Food Biotechnology

   As part of a cooperative agreement with the Central Science Laboratory (CSL), Department for Environment, Food, and Rural Affairs (UK), an annual symposium on various themes relating to food safety and applied nutrition is included. These will alternate between the U.K. and the U.S. The inaugural symposium was held at the CSL in York, U.K., June 20-22, 2000. The Second Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Current Issues in Food Biotechnology was held at the Inn and Conference Center, University of Maryland University College, July 11-13, 2001. Speakers were from the Netherlands, Peoples Republic of China, United Kingdom, and the U.S. Over 100 attended the meeting and participated in discussions in each of the four sessions. A one-page summary of the meeting was furnished to the U.S. delegation to the G8 meeting in Genoa, Italy. Proceedings from the symposium will be posted on the JIFSAN website.

2. Development of collaborative efforts with the Central Science Laboratory (CSL), Department for Environment, Food, and Rural Affairs, UK continued with the second joint symposium described above. Efforts are being made to discover areas of research in which collaborative efforts can be initiated. Following attendance at a conference on "Food Safety Objectives" at Georgetown University, Dr. Renata Leuschner (CSL) spent two days in discussions with scientists from CFSAN, CVM, and the University of Maryland concerning the possibility of JIFSAN hosting a Ph.D. candidate from the University of Leeds, with which CSL has cooperative programs. A project proposal was under development between Dr. Leuschner and scientists at CVM. Dr. Leuschner also requested discussions in the areas of microbiological risk assessment, antibiotic resistance, HACCP, bacteriophage applications, viruses and parasites. Discussions will continue to explore potential research cooperation.

3. A small group of FDA and UM personnel visited AgVictoria in Melbourne to further develop the memorandum of agreement between JIFSAN and the Department of Natural Resources and Environment, State of Victoria. While discussions covered a rather wide range of topics, emphasis was given to training programs in the safe production of fresh produce that AgVictoria has in progress in Victoria and in Indonesia and to areas of horticultural research. A few potential areas of mutual interest were uncovered and are being further investigated for initiation of collaborative efforts. With two members of the group having interests and expertise in veterinary medicine, discussions were also held with personnel at the Victoria Institute of Animal Science at Attwood. Subsequently, Dr. Catherine Ainsworth, Department Head, Veterinary Investigations at Attwood visited with scientists at CVM and the Department of Veterinary Medicine (UM) to discuss areas that had been highlighted as potential areas for collaborative research or scientist exchange. These discussions will continue.

4. Personnel associated with the Canadian Institute for Food Inspection and Regulation (CIFIR), University of Guelph, Guelph, Ontario visited the University of Maryland at College Park to discuss the operation of JIFSAN. Members of the group included Ms. Joan Wakeman [Canadian Food Inspection Agency (CFIA) Regulatory Chair], Dr. Mansel Griffiths [Director, Canadian Research Institute for Food Safety (CRIFS), University of Guelph], Mr. Larry Hillier (Vice-President, Operations, CFIA), Dr. Mary Brodhead (Director, Policy, Planning and Learning, CFIA), Dr. Ted Thomas (Manager, Centre of Expertise, Ottawa Laboratory, CFIA), and Mr. Stephen Desroches (Senior Analyst, Review and Evaluation, CFIA). A rather extensive list of questions had been submitted previously to guide the discussions about issues in the operation and functioning of JIFSAN. This was the second time personnel from CIFIR had visited to discuss similar issues.

JIFSAN Student Internship Program

The JIFSAN Student Internship program is designed to provide University of Maryland undergraduate and graduate students with an opportunity to collaborate with FDA scientists on specific projects related to the JIFSAN mission. This program was

implemented as part of the agreement between the University and FDA to cooperate in educational efforts. These opportunities for students enhance their knowledge of and experience in science, particularly in a regulatory environment, and familiarize them with career opportunities in the regulatory sector of public service. These intern positions may be part-time during the semester and full-time during the summer. Students' participation in the program requires that they be entering their sophomore year and majoring in such disciplines as Biology, Microbiology, Biochemistry, Chemistry, Food Science, Entomology, and Animal Science. In addition, during the initial internship semester the student is a volunteer intern working on a specific project. Upon successful completion of the initial volunteer internship, students can be paid for further work in the project. During all phases of the internship, the students have a variety of ways to obtain academic credit for their internship experience.

Twenty individual University of Maryland students have interned (a total of 49 internships) in CFSAN or CVM laboratories in the last year (September 2000 – August 2001). Eleven (11) interns were new to the program. Students who volunteered for at least one semester were given the opportunity to apply for a paid JIFSAN student position for subsequent semesters. Twenty-six (26) interns were paid for at least one semester of their internship at the FDA. Six (6) interns were paid to work a minimum of thirty hours per week at CFSAN or CVM labs during the Summer 2000. The website listing internships is at http://www.jifsan.umd/internship.htm.

Participation in the JIFSAN Internship Program continues to grow. The UM Office of Science Outreach and Special Programs has worked to increase student, faculty, and staff awareness of the Program through its literature, seminar series, and the annual Internship Day which it sponsors. The JIFSAN Program has been well represented at the Internship Day by Ms. Wendy Buckler or Ms. Christine Hileman, the JIFSAN Program Specialist at the FDA, and/or Dr. E. (Tracy) Dill and Ms. Judy Quigley. One of the strongest features of the Program is the continuing collaborative spirit among the participants.

Future Plans (2001-2002):

Continue development and increase use of the JIFSAN Advisory Council. The next meeting is scheduled for February 25-26, 2002,

Continue development of educational materials and training programs for enhancing the safety of fresh produce at the source of production in countries exporting to the U.S. Training programs are scheduled for Extension Specialists at the University of Puerto Rico, Mayaguez in March 2002. Contacts have been initiated in the Dominican Republic for a training course there and identification of an additional country is being sought in cooperation with the FDA. Discussions are in progress concerning the need for additional training materials, i.e. a "tool kit" for instructors.

Continue internal grants program. Fund approximately five new project proposals effective July 1, 2002.

Host, co-sponsor, and/or conduct a limited number of symposia, workshops, and conferences on topics of importance to the food safety, applied nutrition, and animal health communities including:

> First International Conference on Microbiological Risk Assessment: Foodborne Hazards scheduled for July 24-26, 2002 at the Inn and Conference Center, University of Maryland.
>
> International Workshop on Mycotoxins scheduled for July 22-26, 2002 at the new Food and Drug Administration/Center for Food Safety and Applied Nutrition facilities in College Park, Md.
>
> Third Annual CSL/JIFSAN Joint Symposium in Food Safety and Nutrition: Rapid Diagnostic Methods in Food Safety, June 26-28, 2002 (tentative title and dates), Central Science Laboratory, Sand Hutton, York (UK).

Continue development of Risk Analysis Clearinghouse with emphasis on the inclusion of additional risk assessments and data.

Continue development and pilot offerings with FDA audiences of the courses comprising the JIFSAN Professional Development Program in Food Safety Risk Analysis. Some of the courses will be offered for a second time to an audience beyond FDA staff.

Complete development of JIFSAN strategic plan and initiate use of that plan.

Continue development of international contacts and collaborative programs with an emphasis upon development of active cooperative programs with the Central Science Laboratory (CSL), Department of Environment, Food and Rural Affairs (DEFRA), UK; the Department of Natural Resources and Environment, State of Victoria, Australia; the European Commission, Brussels; and the Joint Research Centre (EU), Ispra, Italy.

APPENDIX A

Projects Funded Through JIFSAN Competitive Internal Research Program

Third (final) year projects completing in May 2001:

- **Immunoaffinity Hollow Fiber Ultrafiltration for High Throughput Screening/Residue Analysis in Food Safety.** Cheng Lee (UM) and Mary Trucksess (FDA).

  Modern agriculture has benefited from the development and use of agrochemicals including insecticides, herbicides, and veterinary drugs. In addition to these exogenous chemicals, many undesirable compounds such as plant toxicants and mycotoxins are known to occur naturally. Thus, there exist critical needs for monitoring residue levels in foods, agricultural commodities, and environmental samples. To perform rapid and sensitive residue screening and confirmation in food safety, advanced bioanalytical techniques for multiresidue analysis of parent compounds and their metabolites are essential.

  The proposed immunoaffinity ultrafiltration combines the strengths of membrane filtration in the ease and speed of separation and concentration with the specificity of antigen-antibody interactions. The food contaminants and residues are selected from their sample matrices by formation in solution of noncovalent immunoaffinity complexes with antibodies raised against targeted compounds. Captured compounds in immunoaffinity complexes are retained and separated from other sample components using membrane ultrafiltration based on their differences in size. The specifically selected compounds retained in membrane ultrafiltration are subsequently liberated from the antibodies by acidification or organic solvent for on-line interfacing with electrospray ionization mass spectrometry (ESI-MS).

  On-line coupling of hollow fiber membranes with electrospray ionization mass spectrometry for continuous affinity selection, concentration and identification of small-molecule libraries. Combinatorial chemistry has been widely employed in the pharmaceutical industry in the efforts towards drug discovery. Rapid and sensitive screening of lead candidates among library compounds has thus imposed significant analytical challenges in recent years. This work involved the development of a continuous affinity capture and concentration system, providing cost-effective and structural analysis of drug candidates in a flow-through format. The system combines the strengths of a hollow fiber dialysis membrane of ease and speed of purification and concentration with the specificity of affinity interactions in solution. The complexes between the lead compounds and the affinity binding proteins are separated from other chemical compounds inside a dialysis hollow fiber as the

result of their differences in size. The affinity complexes are further concentrated inside a second dialysis fiber. The concentrated drug candidates are liberated from the binding proteins in a microdialysis junction and can be directly identified using electrospray ionization mass spectrometry.

Integrated plastic microfluidic devices with ESI-MS for drug screening and residue analysis. For this work, two different plastic microfluidic devices are designed and fabricated for applications in high-throughput reside analysis of food contaminants and drug screening of small-molecule libraries. Microfluidic networks on copolyester and poly(dimethylsiloxane) substrates are fabricated by silicon template imprinting and capillary molding techniques. The first device is developed to perform affinity capture, concentration, and direct identification of targeted compounds using electrospray ionization mass spectrometry. Poly(vinylidene fluoride) membranes sandwiched between the imprinted copolyester microchannels in an integrated platform provide continuous affinity dialysis and concentration of a reaction mixture containing aflatoxin B_1 antibody and aflatoxins. The second microfluidic device is composed of microchannels on the poly(dimethylsiloxane) substrates. The device is designed to perform miniaturized ultrafiltration of affinity complexes of phenobarbital antibody and barbiturates, including the sequential loading, washing, and dissociation steps. These microfabricated devices not only significantly reduce dead volume and sample consumption, but also increase the detection sensitivity at least 1-2 orders of magnitude over those reported previously. Improvements in detection sensitivity are attributed to analyte preconcentration during the affinity purification step, limited analyte dilution in the microdialysis junction, minimal sample loss, and the amenability of ESI-MS to nanoscale sample flow rates.

Publication status

1. Y. Jiang, P.-C. Wang, L. E. Locascio, and C. S. Lee. 2001. Integrated plastic microfluidic devices with ESI-MS for drug screening and residue analysis, Anal. Chem. 73:2048.

2. Y. Jiang and C. S. Lee. 2001. On-line coupling of micro-enzyme reactor with micro-membrane chromatography for protein digestion, peptide separation, and protein identification using ESI-MS, J. Chromatogr. A 924:315.

3. Y. Jiang and C. S. Lee. 2001. On-line coupling of hollow fiber membranes with ESI-MS for continuous affinity selection, concentration, and identification of small-molecule libraries, J. Mass Spectrom. 36:664.

4. Y. Jiang, J. Gao, J. Xu, L. E. Locascio, M. Gaitan, and C. S. Lee. 2000. High throughput screening/residue analysis and high speed protein digestion using miniaturized membrane reactors/separator, in "Proceedings of the Micro Total Analysis Systems 2000 Symposium," Van Den Berg, A., Olthuis, W., Bergveld, P. (eds.), Kluwer Academic Publishers, p. 485.

5. P.-C. Wang, D. L. DeVoe, and C. S. Lee. 2001. Integration of polymeric membranes with microfluidic networks for bioanalytical applications, Electrophoresis, in press.

- **Effects of a Variety of Stress Factors on the Immune Systems of Poultry and Subsequent Infection of Shell Eggs by Salmonella.** Wenxia Song (UM) and Richard Raybourne (FDA).

  Salmonellae are gram-negative bacteria that cause gastroenteritis and enteric fever. *Salmonellae* carried by chickens and shell eggs are a major source of human intestinal infections. *Salmonellae* invade the host by inducing their own uptake into the intestinal epithelial cells. In the host body, *Salmonellae* have the ability to survive and replicate in mononuclear phagocytes, which leads to systemic infection. The mucosal immune system of intestines is the first line protection from *Salmonella* infection. To find new strategies for *Salmonellae* prevention, we have analyzed *Salmonella enteriditis* (SE)-induced mucosal immune responses in young chicks and effect of an immunosuppressing virus and vaccination on chicks' immune response against SE.

  Chicks (one-day old) were orally inoculated with SE. SE-specific IgA was detected two weeks post inoculation and peaked four weeks post inoculation. By eight weeks, when most of the bacteria resided inside host cells, the SE-specific IgA returned to the basal level. SE inoculation increased the numbers of IgA secreting and carrying cells and epithelial associated T lymphocytes in the chicken intestine. Introducing chicken anemia virus that are commonly carried by farm house chickens with SE together significantly inhibited SE-induced mucosal immune responses, including a reduced level of SE-specific IgA, decreased numbers of IgA secreting and carrying cells and epithelial associated T cells. Thus, orally inoculated SE is able to activate the humoral and cellular immune responses in the mucosal surface, and chicken anemia virus weakens these immune responses.

  To test the ability of Salmonella vaccines to generate the mucosal immune responses, chickens that were vaccinated twice with an attenuated live *Salmonella* or heat-killed *Salmonella* were orally inoculated with SE. We found that the chickens vaccinated with heat-killed Salmonella, but not the chickens vaccinated with attenuated live *Salmonella,* increased the level of SE-specific IgA in the chicken intestine mucus in response to SE inoculation. This indicates that heat-killed Salmonella is more effective in generating mucosal protection.

- **Surveillance of Poultry and Other Stock for Carriage of Multiresistant Enterococcus.** Lewis Carr, Sam Joseph (UM) and David Wagner (FDA).

  SPECIFIC AIM #1 (From prior funding period): Environmental surveillance of poultry for *Enterococcus* spp. and determination of resistance

  Of the more than 1000 bacterial isolates recovered from over 70 commercial broiler and roaster poultry farms of the Delmarva Peninsula, 541 *Enterococcus* isolates have been identified and confirmed during year one of funding. Subsequent isolates from years two and three are under review and number over 550 isolated from more than 80 farms. These isolates have come from swabs of fecal material found on poultry transport containers (PTCs) as well as litter from farms. A full protocol for authoritative identification to species has been developed and is currently being applied to all isolates.

  Using this identification schema, we have found ~60% of our isolates to be *E. faecalis*. *E. faecium* was the next most common isolate (18.6%). The *E. faecium* designation of four biotypes is on the basis of biochemical reaction differences for the fermentation of the carbohydrates raffinose and sucrose. Other isolates of note include those that fall into one of the five groups (I-V) which were not definitively identified to species due to the substantial, but not unexpected deviation from established biochemical profiles derived from established species.

Identification	Percent of Total
E. faecalis	209 (38.6)
E. faecalis-atypical	122 (22.6)
E. faecium biotype 1	11 (2.0)
E. faecium biotype 2	47 (8.7)
E. faecium biotype 3	19 (3.5)
E. faecium biotype 4	24 (4.4)
E. gallinarum	13 (2.4)
E. casseliflavus	1 (.20)
E. durans	1 (.20)
Group I	2 (.37)
Group II	59 (10.9)
Group III	23 (4.25)
TBD	10 (1.85)

  Antimicrobial susceptibility testing for all 541 isolates from year one is complete and results of isolates from years two and three are being tabulated. From the analysis of the year one isolates, uniform resistance to low levels of

aminoglycosides (amikacin, apramycin, gentamicin, kanamycin, and streptomycin) and all classes of cephalosporins (ceftiofur, ceftriaxone, cefazolin and cephalothin) is apparent in all species of *Enterococcus*. Examining resistance of the two species that constitute greater than 80% of the isolates (*E. faecalis*, *E. faecium*), differences in resistance appear in nitrofurantoin (1.8%, 84.5%), penicillin (0.7%, 73.2%), and the lincosamide clindamycin (2.1%, 56.3%). Resistance to tetracycline was high in both species with 95%-75% of isolates resistant. Macrolide resistance (erythromycin, clarithromycin) was consistent within the species with higher rates found in *E. faecalis* (69.5%) than in *E. faecium* (36.7%). Resistance to the fluoroquinolones ciprofloxacin and lomefloxacin was consistent within *E. faecium* (88.7%, 86%) but not *E. faecalis* (25.3%, 100%). Synercid resistance was found to be remarkably high at 60.9% of all the *E. faecium* isolates. High-level aminoglycoside resistance was evident in both species with high level streptomycin resistance found in 39.5% and 25% of isolates while gentamicin resistance was seen at relatively low levels in *E. faecalis* (5.6%) and not detected in *E. faecium*. No unexpected vancomycin resistance has been detected. The high level of resistance in this organism suggests that this reservoir of resistance may compromise the therapeutic potential of quinupristin-dalfopristin for humans.

In an effort to expedite the workload, the Vitek® identification system has been incorporated to verify the grouping patterns of new isolates into manageable classes. Recently reported molecular methods that have proven to be more discriminatory and consistent will also be employed in the near future. Selected isolates (n = 179) have been screened against a new panel of antibiotics which include the production antibiotics bacitracin, flavomycin, salinomycin, tylosin, lincomycin, and virginiamycin. The panel also extends the range of the antibiotics vancomycin, erythromycin, penicillin, tetracycline, and gentamicin and adds kanamycin and streptomycin at high concentrations.

Analysis of the resistance patterns of *E. faecium* to this new panel reveals several important details. Briefly, resistance to these production drugs was prevalent, with resistance rates of 79%, 71%, 35%, and 35% for penicillin, tetracycline, erythromycin, and high-level streptomycin, respectively. Resistance to bacitracin (99%), flavomycin (97%), lincomycin (93%), and tylosin (23%) was observed at levels of 256, 32, 32, and 32 μg/ml, respectively. No high-level gentamicin or vancomycin resistance was observed, although resistance to streptogramin, virginiamycin (62.5% at ≥4 μg/ml), corresponded closely with resistance to the human analogue, quinupristin-dalfopristin (60%). The resistance profile of this collection of *E. faecium* isolates that originate from the commercial production environment suggests that resistance develops and predominates with the use of antimicrobials in the production environment and may mirror the extent of use within the region. While debate continues on the origin of *E. faecium* resistance from the human population, this data supports the assertion that

agricultural usage of antimicrobial agents selects for resistance determinants that could exacerbate the resistance problem of enterococci from the human microbiota.

SPECIFIC AIM #2 (From prior funding period): Analysis of relatedness of resistant isolates

Comparative ribotype analysis of 53 human and 132 poultry isolates was done using the Qualicon RiboPrinter™. Human isolates were obtained from multiple medical centers across the U.S. and poultry isolates were collected from the Delaware-Maryland-Virginia peninsula. RiboPrint™ patterns from both human and poultry sources showed extensive diversity and several large clusters of strains. Interestingly, the *E. faecium* populations within humans and poultry were nearly distinct. One poultry isolate fell in a large cluster of human isolates and one small cluster contained one human and one poultry isolate. While these data suggest that *E. faecium* isolated from poultry are unlikely to be the source of human clinical enterococcal isolates, more analysis is needed with a larger group of isolates.

A commitment of time and resources by the Center for Veterinary Medicine has been made to evaluate clonality of isolates. This will involve the application of the repetitive extragenic palindromic (REP) PCR methodology in order to quickly gauge the clonality of isolates.

SPECIFIC AIM #3 (From prior funding period): Viability of *Enterococcus* spp. in the feed production process

Nine isolates of *Enterococcus* spp. have been isolated and characterized from feed that did not contain growth sub-therapeutic antibiotics. Generally, these isolates are more sensitive to antimicrobial agents, but high-level aminoglycoside resistance has been observed in two isolates. Research will be continued in this area.

Summary: The data generated to date demonstrate that the *Enterococcus* isolates that we have recovered from the poultry environment possess broad resistance to antimicrobial agents. We plan to perform clonality studies of resistant isolates in the near future to support population studies of resistance in *Enterococcus* in addition to the surveillance of targeted resistance genes within sub-populations of the genus. The analysis of this tremendous volume of data is incomplete, but ongoing.

Publication status

a. Publications:

1. J. R. Hayes, A. C. Mcintosh, S. Qaiyumi, J. A. Johnson, L. L. English, L. E. Carr, D. D. Wagner, and S. W. Joseph. 2001. High Frequency Recovery of Quinupristin-Dalfopristin-Resistant *Enterococcus faecium* Isolates from the Poultry Production Environment, J. Clin. Microbiol. 39 (6): 2298-2299.

2. S. W. Joseph, J. R. Hayes, L. L. English, L. E. Carr, and D. D. Wagner. 2001. Implications of Multiple Antimicrobial Resistant Enterococci Associated with the Poultry Environment, Food Additives and Contaminants. In press.

b. Presentations

J. R. Hayes, D.D. Wagner, L.L. English, L.E. Carr, and S.W. Joseph. "Antimicrobial Resistance Profiling of *Enterococcus faecium* from Commercial Poultry Production Environments," General Meeting, American Society for Microbiology, Orlando, FL., May 2001.

- **Mechanisms of chemoprevention by dietary carotenoids and their metabolites in the prevention of chronic diseases in humans.** Frederick Khachik (UM), Eugene Mazzola, Shirley Blakely, and Andrija Kornhauser (FDA).

Mechanistic studies of chemoprevention by carotenoids has focused on up-regulation of the intercellular expression of gap-junction communication proteins and the induction of detoxication or phase 2 enzymes by carotenoids and their metabolites. The inhibitory effects of a mixture of nine prominent dietary carotenoids (multicarotenoid) on organ site carcinogenesis in C57BL/6J mice have also been investigated. This study has been conducted in collaboration with Dr. Martin Lipkin and Dr. Nitin Telang at the Strang Cancer Research Laboratory at the Rockefeller University, N.Y. Multicarotenoid consisted of lutein, zeaxanthin, lycopene, neurosporene, gamma-carotene, alpha-carotene, beta-carotene, phytofluene, and phytoene. These were extracted, isolated, and purified from various natural products by crystallization and chromatography. The metabolites of lutein, zeaxanthin, and lycopene were prepared by total and partial synthesis.

Gap-Junctional Communication Assay. This study has been conducted in collaboration with Professor John Bertram at the Cancer Research Center of Hawaii. A mixture of nine dietary carotenoids, designed to be similar in composition and molar ratio to that found in a "healthy diet", has been

observed to inhibit the induction of carcinogen-induced neoplastic transformation in 10T1/2 cells. This was the same system in which it had been shown that single carotenoids have the ability to inhibit neoplastic transformation. The results so far have been confusing; over the dose range of 10^{-5} to 10^{-6}M, concentrations previously shown with single agents to be active, the incidence of transformed foci was increased. This increase was approximately 4-fold (from six transformed foci in carcinogen-only treated controls, to 24 foci in cultures also treated with carotenoids) at the concentration of 10^{-5}M. These experiments are currently being repeated and extended to an examination of the influence of this carotenoid mixture on connexin 43 expression. If these results are indeed repeatable, under conditions where single carotenoids again show effectiveness, the studies have the potential to shed light on the very worrying evidence from two beta-carotene intervention trials conducted in smokers, that supplemental beta-carotene increases lung cancer risk. The activity of carotenoids oxidation products as modifiers of differentiation in human keratinocytes also has been investigated. Studies have so far confirmed the ability of lycopene, and its oxidized derivative 2,6-cyclolycopene-1,5-diol, found in the human diet and in plasma, to modify markers of differentiation in HaCaT cells.

Induction of Phase 2 (Detoxication) Enzymes by Carotenoids. Detoxication (phase 2) enzymes including quinone reductase [QR; NAD(P)H: (quinone acceptor) oxidoreductase, EC 1.6.99.2] are transcriptionally induced in many mammalian cells by low concentrations of a wide variety of chemical agents and such induction is associated with reduced susceptibility to chemical carcinogenesis. In collaboration with Dr. Paul Talalay and Jed Fahey (Brassica Chemoprotection Lab., Johns Hopkins University), it has been shown that carotenoids and their metabolites have considerable ability to induce phase 2 enzymes and may have potential significance as means of detoxifying xenobiotics and acting as chemopreventive agents.

Inhibitory effects of multicarotenoid on organ site carcinogenesis in C57BL/6J mice. This project was carried out in collaboration with Dr. Martin Lipkin and Dr. Nitin Telang at the Strang Cancer Research Laboratory at the Rockefeller University, N.Y. Rodent models have provided important clinically relevant leads for colon, breast, and prostate carcinogenesis and its prevention by natural and synthetic compounds. C57BL/6J strain of mouse exhibits low incidence of spontaneous breast and colon cancer. Administration of Western-style diet (high fat, low calcium and low vitamin D) results in accelerated growth of precancerous lesions in colon and breast of these mice. The above colon model has been investigated as well as cell abnormalities in other organs such as breast, brain, and liver in mice fed a Western diet with and without multicarotenoid mixture (MCM) of nine dietary carotenoids). 20 C57BL/6J female mice at 4-5 weeks of age were randomized by weight and allocated into two treatment groups of ten each. Group I received control Western-style diet (AIN-76A) and group II received control Western-style

diet plus MCM. Freshly stored diets (slightly in excess of 5 g with or without MCM) at -70°C were thawed and provided to each animal for three days of their supplies (2.5 mg total carotenoid/day). After six weeks, five animals from each group were sacrificed by carbon dioxide asphyxiation and all the remaining animals were similarly sacrificed after 24 weeks. Tissues (liver, colon, breast, and brain) were excised and fixed for histopathology and frozen at -70^{o}C until analysis. Histopathology includes biomarkers for hyperproliferation, hyperplasia and incidence of precancerous lesions. The histology is currently in progress and is performed at the Strang Cancer Research Laboratory. The uptake of carotenoids by various tissues (colon, breast, liver, brain) has been determined by extraction and HPLC analysis.

Although after 24 weeks, nearly all carotenoids were highly bioavailable in colon and liver, the major carotenoids in brain were lycopene, lutein, and β-carotene. ζ-Carotene predominated in the breast tissues while lutein, lycopene, γ-carotene, and α-carotene were detected only in low concentrations. Because epidemiological studies have associated the high consumption of carotenoid-rich foods with a lower risk of cancer, a mixture of prominent serum carotenoids (multicarotenoid) would be expected to be more effective in cancer chemoprevention than a single carotenoid. C57BL/6J Female mice appear to serve as an appropriate model for investigating the efficacy of individual or purified mixtures of dietary carotenoids on target organ carcinogenesis. While there is some evidence that individual or certain combinations of dietary carotenoids may protect specific organs against carcinogenesis, supplementation with a wide range of carotenoids would be expected to provide a collective protective effect and may prove to be a more effective chemoprevention strategy. Future studies with this animal model should provide mechanistic and phenomenological leads to understand the efficacy of multicarotenoid supplementation in cancer chemoprevention.

Publication Status

a. Publications

1. J. S. Bertram, T. King, L. Fukishima, and F. Khachik. 2000. Enhanced activity of an oxidation product of lycopene found in tomato products and human serum relevant to cancer prevention, In: Antioxidant and Redox Regulation of Genes, C. K. Sen, H. Sies, and P. A. Baeuerle (eds), Academic Press, San Diego, Chapter 18, pp. 409-424.

2. T. A. Scholz, T. R. Hata, L. K. Pershing, W. Gellermann, R. McClane, M. Alexeeva, I. Irmakov, and F. Khachik. 2000. Non-invasive Raman spectroscopic detection of carotenoids in human skin, J. Invest. Dermatology 115 (3): 441-448.

3. S. R. Blakely, E. Grundel, and F. Khachik. 2000. "Vitamin E enhances lutein bioavailability in Zucker lean but not obese female rats," FASEB Journal 14 (4): A235.

4. P. S. Bernstein, F. Khachik, L. S. Carvalho, G. J. Muir, D. Y. Zhao, and N. B. Katz. 2001. "Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye, Exper. Eye Res. 72 (3): 215-223.

5. O. Kucuk, F. H. Sarkar, W. Sakr, Z. Djuric, M. N. Pollak, F. Khachik, Y. W. Li, M. Banerjee, D. Gringnon, J. S. Bertram, J.S.; J. D. Crissman, E. J. Pontes, and D. P. Wood Jr. 2001. "Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy, Cancer Epidemiology, Biomarkers, and Prevention 10: 861-868.

6. F. Khachik. 2001. A simultaneous process for extraction, isolation, and purification of lutein, zeaxanthin, and several rare carotenoids from marigold flowers, Lycium Chinese mill, and green plants, US Patent, 6,262,284 B1, July 17.

7. F. Khachik, L. Carvalho, P. S. Bernstein, G. J. Muir, D. Y. Zhao, and N. B. Katz, 2001. Chemistry, distribution, and metabolism of tomato carotenoids and their Impact on human health, Proceedings of the International Symposium on the Role of Tomato Products and Carotenoids in Disease Prevention, New York, April 10, 2001 (will be published by the New York Academy of Medicine).

b. Abstracts and Invited* Presentations

1. *F. Khachik, "Distribution, Bioavailability, and Metabolism of Lycopene in Humans," Prostate Cancer Prevention 2000: The Role of Nutrition, Organized by Faculty of Medicine, University of Toronto, Toronto, Canada, March 3-4, 2000.

2. *F. Khachik, F. "Distribution of Carotenoids in Human Serum and Tisssues," First South East Asia and Pacific Regional Meeting on Carotenoids, Bangkok, Thailand, August 2-5, 2000.

3. *F. Khachik, "Update on Carotenoid Analysis," First South East Asia and Pacific Regional Meeting on Carotenoids, Bangkok, Thailand, August 2-5, 2000.

4. J. Humphries, R. Graham, G. McIntosh, R. Worsley, and F. Khachik, "The Role of Carotenoids in Human Health," First South

East Asia and Pacific Regional Meeting on Carotenoids, Bangkok, Thailand, August 2-5, 2000.

5. *F. Khachik, “Bioavailability and Metabolism of Dietary Carotenoids," International Conference and Exhibition on Nutraceuticals and Functional Foods, Houston, Texas, USA, September 14-17, 2000.

6. *F. Khachik, "Mechanistic Studies on Carotenoids and Their Metabolites in the Prevention of Chronic Diseases," International Conference and Exhibition on Nutraceuticals and Functional Foods, Houston, Texas, USA, September 14-17, 2000.

7. F. Khachik, L. Carvalho, N. Telang, E. Fumio, and M. Lipkin, "Bioavailability and Tissue Disposition of Major Dietary Carotenoids in C57BL/6J Mice Administered a Formulated Multicarotenoid Mixture," Gordon Research Conference on Carotenoids, Ventura, California, USA, January 14-19, 2001.

8. F. Khachik, L. Carvalho, P. S. Bernstein, G. J. Muir, D. Y. Zhao, and N. B. Katz, "Distribution of Carotenoids and Their Metabolites in the Ocular Tissues of Humans, Quails, and Frogs: Useful Non-primate Animal Models for Investigating the Metabolic Transformation of Macular Carotenoids," Gordon Research Conference on Carotenoids, Ventura, California, USA, January 14-19, 2001.

9. *F. Khachik, L. Carvalho, P. S. Bernstein, G. J. Muir, D. Y. Zhao, and N. B. Katz, “Chemistry, Distribution, and Metabolism of Tomato Carotenoids and Their Impact on Human Health,” International Symposium on the Role of Tomato Products and Carotenoids in Disease Prevention, New York, New York, April 10, 2001.

Third year projects (completing January 2002):

- **Immunologic Sequela Following Oral Exposure to a Foodborne Toxin.** Carol Pontzer (UM), Richard Raybourne and MaryAnn Principato (FDA)

Staphylococcal enterotoxins (SE) are exoproteins produced *S. aureus* that act as superantigens and have been implicated as a leading cause of foodborne disease and toxic shock. Little is known about how these molecules penetrate the gut lining and gain access to both local and systemic immune tissues. To model movement in vitro of staphylococcal enterotoxins we have employed a monolayer system composed of crypt-like human colonic T-84 cells. SEB

and SEA showed comparable dose dependent transcytosis in vitro, while TSST-1 exhibited increased movement at lower doses. Synthetic peptides corresponding to specific regions of the SEB molecule were tested in vitro to identify the domain of the protein involved in the transcytosis of SE. A toxin peptide of particular interest contains the amino acid sequence KKKVTAQELD, that is highly conserved across all SE. At a toxin:peptide ratio of 1:10, movement of SEB across the monolayers was reduced by 85%. Antisera made against the SEB peptide recognized native SEB and also inhibited SEB transcytosis. Finally, the conserved 10 amino acid peptide inhibited transcytosis of multiple staphylococcal enterotoxins, SEA, SEE and TSST-1. These data demonstrate that this region of the staphylococcal enterotoxins plays a distinct role in toxin movement across epithelial cells. It has implications for the prevention of staphylococcal enterotoxin-mediated disease by design of a peptide vaccine that could reduce systemic exposure to oral or inhaled superantigens. Since the sequence identified is highly conserved, it allows for a single epitope blocking the transcytosis of multiple SE. A large in vivo trial of an anti-enterotoxin vaccine in mice challenged with oral toxins was conducted during the summer of 2001.

Publication status

a. Abstracts of presentations

1. J. W. Shupp, S. Weitzel, and C. H. Pontzer. 2001. Epithelial Transcytosis of Staphylococcal Enterotoxin: Inhibition by Synthetic Peptides, FASEB J. 15:A1008.

2. Y. Karmazyn, M. Principato, M. Robi, C. Pontzer, and R. Abe. 2001. Histological Examination of Morphological Alterations and Induction of Apoptosis in Young Mice, Due to Ingestion of Staphylococcal enterotoxin B, FASEB J. 15:A371.

- **The Missing Connection: Isolation and concentration of Microorganisms on Biocapture Surfaces.** Jonathan Bundy, Catherine Fenselau (UM), Mary Carson and David Wagner (FDA)

  Funding for this project ended January 30, 2001. Results were included in the Ph.D. dissertation of Jonathan Bundy and were summarized in the 1999/2000 Annual Report for JIFSAN.

Publication status

a. Publications

1. J. L. Bundy, and C. Fenselau. 2000. Lectin and Carbohydrate Affinity Capture Surfaces for Mass Spectrometric Analysis of Microorganisms, Anal. Chem. 73:751-757.

2. J. L. Bundy. Development of Biocapture Surfaces for Mass Spectrometric Analysis of Microorganisms, Ph.D. Dissertation, University of Maryland, 2001.

b. Presentations

1. J. L. Bundy, Development of Biocapture Surfaces for Mass Spectrometric Analysis of Microorganisms, Seminar Presented to the Center for Veterinary Medicine, Food and Drug Administration, October, 2000.

2. J. L. Bundy, Development of Biocapture Surfaces For Mass Spectrometric Analysis of Microorganisms, Seminar Presented to the Chemical and Analytical Sciences Division, Oak Ridge National Laboratory, September, 2000.

3. J. L. Bundy, C. Fenselau, and M. Carson, Biocapture Surfaces for Mass Spectrometric Analysis of Microorganisms, 15th International Mass Spectrometry Conferences, Barcelona, August 2000.

4. J. L. Bundy and C. Fenselau, Carbohydrate Affinity Capture Surfaces for Mass Spectrometric Analysis of Microorganisms, Presented at the 48th ASMS Conference on Mass Spectrometry and Allied Topics, Long Beach CA, June, 2000.

- **Identifying Knowledge Gaps and Improving Communication Strategies to Reduce Food Safety Risks.** Mark Kantor, Cynthia Tuttle, Robert Feldman (UM), Toija Riggins and Alan Levy (FDA).

  This project was funded in January 1999 for one year and a no-cost extension was approved in January 2000 to facilitate completion of the objectives of the study.

  How food safety professionals and general consumers perceive the relative importance of various practices, behaviors, and situations that impact the risk of foodborne illness was the objective of this study, including knowledge of certain relationships between foods and pathogens. A web-

based survey instrument was administered using a 5-point rating scale for most questions to address perceptions, attitudes and awareness among professionals about microbial risks associated with food handling practices and the food distribution system. After pilot testing and publicizing to professionals via electronic listservs, the survey instrument was uploaded on a website using the server of the College of Agriculture and Natural Resources at the University of Maryland. The survey instrument was completed on-line. Data were automatically collected in a Microsoft Access database and analyzed using SAS.

A total of 645 respondents (489 F and 156 M) with college degrees (33% B.S., 48% M.S., 20% doctoral) were included in the initial analysis. Perceptions of food safety risks and awareness of food/pathogen relationships were similar for all education groups, but subjects with doctoral degrees tended to rate certain potentially hazardous food handling practices as being less risky. Respondent perceptions about the relative importance of various practices, and their principal advice relative to safe handling, were remarkably consistent with conventional food safety guidelines. For the consumer version of the survey, a telephone survey is being developed that will be administered to approximately 800 low-income consumers by the University of Maryland Survey Research Center.

Whether there are misconceptions about risks or major gaps in awareness among consumers is expected to be assessed by comparing results of professional and consumer surveys. Through an enhanced understanding of risk perceptions of professionals and consumers, it may be possible to increase the effectiveness of health education messages and programs aimed at reducing the incidence of foodborne diseases in the population.

Publication Status

M. A. Kantor, A. S. Levy, C. R. Tuttle, C. R., and T. A. Riggins. Attitudes, Awareness, and Perceptions of Food Safety Risks Among Professionals (Poster), Abstract 73B-4, 2001 Annual Meeting of the Institute of Food Technologists, New Orleans.

- **The Detection of Foodborne Pathogens in Biofilms Using Antibodies, Lectins, and Fluorescent Dyes.** Ronald Weiner, Sam Joseph, Lewis Carr (UM) and Ben Tall (FDA)

All of the proposed objectives of this project, as shown below, have been or are in the process of being completed. In the applied area, a new technology (Calcofluor- UV protocols) has been developed that can be used to better detect biofilm contamination of foods as products of pathogens. This technology can be developed into an important tool for

FDA food inspectors. A prototype method is currently being used to examine poultry cages in commercial poultry processing plants on the eastern shore of Maryland. Also, it has been discovered that a ubiquitous and formidable foodborne pathogen, *Salmonella typhimurium* DT-104, can induce the formation of a capsule leading to biofilm formation and better survival in the environment. This can have profound consequences on food safety.

Two recently published journal articles, cited below, have resulted from this work and a third has been submitted. The first deals with the mechanisms by which microorganisms adhere to surfaces. The second reports initial findings on the rugose (biofilm-forming) state of *Salmonella typhimurium* DT-104 and the conditions under which it forms biofilms that enables the pathogen to resist removal from food containers. The submitted paper is on the use of lectins to find and presumptively identify specific foodborne bacterial pathogens in biofilms.

Accomplishments in meeting objectives:

1. First year
    a. Antibody probes were made against Salmonella DT 104 and *Hyphomonas* MHS-3.

    b. Specific lectins against the exopolysaccharide (EPS) and biofilms of four foodborne pathogens (added *Salmonella* DT-104 to the study) were found.

    c. The fluorescence of Calcofluor was determined when bound to the EPS of bacteria. Its sensitivity was measured at various dilutions on uniform biofilms and, at optimum concentration, against biofilms of varying age and development.

    d. Beyond the original proposal, the following was accomplished:

        1. The ability of the pathogens to form biofilm on various surfaces such as Teflon™, glass, aluminum, plastic and steel was checked.

        2. Conditions under which *Salmonella* DT-104 is likely to form a biofilm were examined.

2. Second year
    a. The probes were tested in microcosms in single species

    b. Probes were tested, using multispecies microcosms.

c. Beyond the original proposal, the following was accomplished:

1. The effects of temperature on the ability of *Salmonella* DT-104 to form biofilms that resist removal and disinfecting continued to be examined.

2. Using genetic and physiological approaches, the factors that influence capsule and biofilm formation in *Salmonella* DT-104 were studied.

3. Third year

a. Calcofluor and lectin fluorescence are being field tested and compared with known titers.

b. Each of the three types of probes are being tested at production facilities and processing plants on various, random surfaces and materials to compare efficiency of the probes against standard HACCP culturing procedures

c. An inexpensive, easy to use, field testing kit for presumptive identification of biofilm contamination and foodborne pathogenic species identification will be provided.

Publication status

1. E. Quintero, S. Langille, and R. Weiner. 2001. Exopolysaccharide capsules of marine, prothescate bacterial specifically bind complexed and colloidal gold, J. Industrial Microbiol. & Biotechnol. 27:1-4.

2. Y. A. Anriany, R. M. Weiner, J. A. Johnson, C. Ericksson de Rezende, and S. W. Joseph. 2001. *Salmonella enterica* Serovar Typhimurium DT104 Displays a Rugose Phenotype, Appl. Environ. Microbiol. 67:4048-4056.

Second year projects (funded in January 2000):

- **Viral Immunosuppression and the Infection of Shell Eggs by *Salmonella enteritidis*.** Robert Heckert, Wenxia Song (UM), Richard Raybourne, and Uma Babu (FDA)

In the last 20 years there has been an increase in human food-poisoning outbreaks attributable to *Salmonella enteritidis* (SE) in the United States. Epidemiological studies of this increase have indicated that grade A shell eggs are an important source of SE. Despite the tremendous efforts made by the poultry industry, no effective measures for elimination of SE colonization have been developed. In addition, the process by which laying hens become infected with SE and subsequently produce contaminated eggs remains unclear.

The humoral immune responses after infection with SE have been extensively studied for diagnostic purposes. However, the fundamental mechanism of mucosal resistance to infection and clearance of SE from the gut has received scant attention. Protection from infection by SE through humoral mechanisms alone is unlikely, with it being a facultative intracellular bacterium. There is enough evidence in various animal models that cell mediated immunity plays a major role in controlling Salmonella infection. In chickens, thus far, there is lack of detailed knowledge on the immune mechanisms involved in defense against Salmonella infection.

This investigation has shown that there is a link between the immune status of the bird and the ability of that bird to resist Salmonella infection. Is has been shown that, early in the bird's life, both infectious bursal disease virus (IBDV) and chicken anemia virus (CAV) infect and cause a severe immunological disturbance. This is clearly seen as a decrease in the ability of the bird to produce antibodies both systemically and in the intestinal mucosa (where the organism colonizes and invades) and in the bird's normal population of immunological cells. Furthermore, the investigators believe that IBDV and CAV are very widespread in the poultry industry in general and that many birds are affected resulting in some degree of immunosuppression.

These experimental studies showed that *Salmonella enteritidis* phage type 4, when inoculated into 1-day-old SPF chickens, resulted in the cecal carriage of Salmonella up to 20 weeks post-inoculation (PI). Co-infection with either IBDV or CAV resulted in an increase in numbers of Salmonella present in the birds that were infected at any one time, but not an extension of the duration of infection. The antibody isotype-specific responses to Salmonella infection were poor, as indicated by low IgA and IgM responses in the serum. IgG responses against Salmonella were better, commencing from four weeks PI and being maintained in a significant percent of inoculated chickens up to 20 weeks PI, but then declining. This overall weak immune response was shown to be further depressed when chickens were also infected with either IBDV or CAV. In the intestinal tract, the IgA responses against *S. enteritidis* were clearly higher in the control chickens than the immunocompromised chickens at most time points. The rise in IgA in the intestinal contents was seen to

parallel the reduction of cecal carriage of Salmonella in the immunocompetent chickens, indicating that the IgA response has a role in the clearance of Salmonella from the gut.

In chickens, it has been shown that local cell mediated immunity plays a major role in controlling Salmonella infections. Local immunity in the gut of chickens mediated by T-cells was verified in this study by the demonstration of proliferation of CD_4^+, CD_8^+ and CD_3^+ T cells. By immunohistochemistry, T cells in the lamina propria were mostly found near the lining epithelium and a large number of intra-epithelial lymphocytes. In those chickens infected with CAV, there was a dramatic change in the populations of CD_4^+ and CD_8^+ cells in the early weeks after viral infection, which then returned to normal when the birds became older. These observations indicated that T cell mediated immunosuppression (as induced by viral infection) also enhanced intestinal colonization and organ invasion by *S. enteritidis.*

Overall, what was observed in these studies was a transient suppression of immune function as manifested by decreased intestinal IgA levels and decreased cellular immune responses due to infection by immunosuppressive viruses. These effects were most pronounced for several weeks after the viral infection, but then returned to normal levels when the birds approached maturity. At the time the birds were producing eggs, we did not observe any shedding of Salmonella from the infected or co-infected birds and therefore failed to show that infection by immunosuppressive viruses induced higher shedding of Salmonella into the egg. However, the earlier effects of increased Salmonella shedding due to immunosuppression were clear and could have an effect on the amount of Salmonella shed into the bird's or farm's environment.

Since viral immunosuppression, either T cell mediated (e.g. chicken anemia virus) or B cell mediated (e.g. infectious bursal disease virus) occurs early in the life of commercial chickens, any Salmonella intervention strategies should take this into account.

This JIFSAN project laid the foundation for a successful competition for a National Food Safety Grant. This grant overlapped with the final year of the JIFSAN grant and allowed the investigators to begin studying various intervention strategies, one of which was vaccination to overcome the weak immune response observed with natural Salmonella infection. To date these studies have supported the earlier conclusion that natural infection produces weak and transient immune responses that are ineffective at preventing or clearing a Salmonella infection. Immunization with a killed vaccine was found to provide a better immune response than using a modified live vaccine. This immunity is better with respect to intestinal IgA (which is the main barrier to Salmonella colonization of the

intestinal tract) and better in producing cellular immunity (that which is responsible for eliminating the infection). Other methods of increasing the immunity of the hatched chick, such that it is better able to resist Salmonella infection at hatch, are currently being explored.

- **Using a Probabilistic Risk Assessment Model to Study Risk of *E. coli* O157:H7 Contamination in Hard Cheeses.** Mohammad Modarres (UM) and Joseph Schlesser (NCFST, FDA)

  The objective of this research is to develop a probabilistic risk assessment model to study risk of *Escherichia coli* O157:H7 contamination in rennet-acid coagulated cheese and, particularly, hard cheese (Cheddar). The objective is also to support regulatory decision-making and establishing a formal and systematic way to define the needs for additional research.

  In this research, a number of probabilistic model-based tools and techniques developed and used in various engineering disciplines are being adapted for applications to food safety and, particularly, foodborne pathogens in cheeses. In this approach, food risk assessment involves the steps of screening to 1) determine whether risks from *Escherichia coli* O157:H7 reach a threshold of concern; 2) estimate frequency of occurrence of particular hazards and their magnitude occurring at a specific location or at all locations (public risk) along the farm-to-fork path; 3) determine risk-significant contributors to develop control mechanisms and identify data gaps and target research that should have the greatest value in terms of public health impact; and 4) provide the results of the risk assessment to regulatory decision makers.

  Scientifically-based risk assessments are needed to support policy decisions regarding public health concerns associated with foodborne pathogens. As was recommended in the FAO/WHO Consultation in Rome in 1997, scientific risk assessment should be the basis for making risk management decisions involving health and safety aspects of food standards.

  One of the most important recommendations of this consultation was to develop quantitative methods of risk assessment for biological hazards to facilitate and improve the application of risk management strategies such as Hazard Analysis Critical Control Points (HACCP).

  Particularly, the probabilistic model-based tools being developed allow determination of risk significant activities or events, best control strategies, areas where more data improve risk estimates and reduce uncertainties, and expected societal impacts due to exposure to *Escherichia coli* O157:H7.

According to the plan of execution, where the research project was divided into three phases (Production, Distribution, and Consumption), currently the research is in "Distribution" (phase II) which focuses on the analysis of the risk during transportation and distribution of cheese to the consumer.

Research activities accomplished. The literature on *Escherichia coli* O157:H7 contamination of cheeses has been reviewed. A substantial amount of data has been gathered. This has allowed the development of a database to characterize the dynamic growth behavior of *Escherichia coli* O157:H7 during the cheese production process.

A model-based approach was developed to assess the risk from E*scherichia coli* O157:H7 during the production phase. This logic model guides the process of data gathering, synthesis, and risk analysis. Some preliminary risk assessment has been performed.

A risk-modeling concept called "Master Logic Diagram" (MLD) has been adapted to the general cheese-making process. Due to the diversity of cheeses and the different cheese-making processes involved, the MLD has been limited to Rennet-Acid Coagulated Cheeses. In this MLD model, the manufacturing process has been divided into successively more abstract steps, and each step has been modeled taking into account the most critical variables, such as temperature and time, affecting the growth of *Escherichia coli* O157:H7.

The cheese-making process has been divided into the constituent steps and the interrelationship shown between the different steps. Models have been created for each of the elements of the cheese-making process. These models are created to estimate the contamination level of each element.

A generic model has been created for the Rennet-Acid coagulated cheeses, and only some representative types of cheeses such as Cheddar, Brick, Cottage, Emmental, Camembert, and Tilsit may be applied. However, the model has been developed in such a way that it can be easily adapted for use in modeling a variety of other cheeses.

Basically, for the production phase three different probabilistic models were developed.

- Cheddar case: This application allows the estimation of the final contamination in a cheddar cheese production process.

- Rennet acid case: This application allows the estmation of the final contamination in a Rennet-Acid Coagulated cheese production process when specific knowledge about the

manufacturing process, such a starter culture and type of salting used is available.

- Rennet acid general case: This application allows the estimation of the final contamination in a Rennet-Acid Coagulated cheese production process when specific knowledge about the manufacturing process is not available.

The MLD model is based on some risk (contamination) multiplicative factors, which have been determined using the databases available in the open literature; however, when more data is obtained (more is being sought) a more representative and well-defined probabilistic distribution can be developed.

Ongoing activities: According to the plan of execution, where the entire research project was divided into three phases (Production, Distribution, and Consumption), currently the research is in the middle of phase II. This focuses on the analysis of the risk of cheese transportation and distribution to the consumer. The work to be done in the period September 2001 - January 2002 is oriented to complete phase II. The worked planned for after January 2002 includes risk estimation due to "Consumption" of cheeses (phase III).

The model used to describe the growth of *Escherichia coli* O157:H7 during phase II is based on the Gompertz equation. A database for assessing the statistical growth behavior of *Escherichia coli* O157:H7 for important steps during the transportation and distribution phase is being completed. This preliminary database has been oriented almost exclusively to the strain O157:H7; however, due to the limited available data for this specific strain, some generic data for *Escherichia coli* are being used.

The validation and adaptation of generic data constitutes the next stages of research. Elicitation techniques using expert opinions and use of the Bayesian approach to combine expert opinion with actual data are being studied.

Expected products: When the model is completed, it could be used not only to predict the amount of *Escherichia coli* O157:H7 in a specific cheese-making step, but also could be used to identify risk-significant contributors and conditions to prevent the growth of this pathogen. The process can be extended to many other types of food.

Additionally, it will allow risk analysts to identify data gaps and to target research that potentially has the greatest value in terms of public health

impact, and providing the results of the risk assessment to regulatory decision makers.

- **Antibiotic Resistance Integrons in Shiga Toxin-producing *Escherichia coli* and *Campylobacter jejuni/coli*.** Jianhong Meng (UM), David White, S. Zhao, and David Wagner (FDA)

  1. Characterization of antibiotic resistance among *E. coli* O157 isolates from animals, humans, and food

     A total of 361 *Escherichia coli* O157 isolates recovered from humans, cattle, swine, and food during the years 1985 to 2000 were examined to better understand the relative contributions of antimicrobial use in human and veterinary medicine, on the development of antimicrobial resistance among this organism. Based on broth microdilution results, 220 (61%) of the isolates were susceptible to all 13 antimicrobials tested. Ninety-nine (27%) O157 isolates, however, were resistant to tetracycline, followed by 93 (26%) to sulfamethoxazole, 61 (17%) to cephalothin, and 48 (13%) to ampicillin. Highest frequencies of resistance occurred among swine isolates (n = 70) where 52 (74%) were resistant to sulfamethoxazole, 50 (71%) to tetracycline, 38 (54%) to cephalothin, and 17 (24%) to ampicillin. Based on the presence of Shiga toxin genes as determined by PCR, 210 (58%) of the isolates were identified as Shiga toxin-producing *E. coli* (STEC). Among these, resistance was generally low; yet 21 (10%) were resistant to sulfamethoxazole, and 19 (9%) to tetracycline. Based on latex agglutination, 189 (52%) of the isolates were identified as *E. coli* O157:H7, among which 19 (10%) were resistant to sulfamethoxazole, and 16 (8%) to tetracycline. No statistically significant changes in antimicrobial resistance over the fifteen-year period were observed for *E. coli* O157 isolates. The selection pressure imposed by use of antimicrobials in the animal production environment most likely appears to be a driving force in the emergence of antimicrobial resistance among STEC and non-STEC O157.

  2. Antimicrobial-Resistant *Campylobacter* Isolated from Retail Raw Meats in the Greater Washington Area

     Campylobacter is the most common cause of bacterial gastroenteritis in the United States, mainly transmitted through food. Resistance to antimicrobials used for the treatment of campylobacterosis has increased. The present study investigated antimicrobial resistance among *Campylobacter* isolates recovered from retail raw meats from 1999-2000 in the Greater Washington Area. All 378 *Campylobacter* isolates (194 *C. jejuni*, 153 *C. coli*, and 31 other *Campylobacter*), from 159 retail raw meats (chicken, turkey, pork and beef), were determined

using agar dilution method to seven antimicrobial agents: chloramphenicol, ciprofloxacin, doxycycline, erythromycin, gentamicin, nalidixic acid, and tetracycline. The most common resistance among the *Campylobacter* isolates was to tetracycline (75.9%), followed by doxycycline (72.8%), erythromycin (43.7%), nalidixic acid (33.1%), and ciprofloxacin (28.8%). Two *C. coli* from turkey were resistant to chloramphenicol. None of the isolates demonstrated resistance to gentamicin. *C. coli* exhibited higher resistant rates to erythromycin, ciprofloxacin, and nalidixic acid than *C. jejuni* ($p<0.05$), and isolates from turkey also showed significantly higher resistant rates ($p<0.05$) than isolates from chicken to all the antimicrobial agents tested except for gentamicin. Multi-drug resistance was commonly observed, with 80.7% of the isolates resistant to ≥ 2, and 25.1% to ≥ 4 antimicrobials. *Campylobacter* isolates resistant to antimicrobial agents used for treating *Campylobacter* infection in humans are common in retail meat products from the Greater Washington DC area.

Publication status

a. Presentations:

1. B. Ge, D. G. White, S. Zhao, P. F. McDermott, R. D. Walker, and J. Meng. 2001. Antimicrobial-resistant *Campylobacter* isolated from retail raw meats in greater Washington area, The 11th Intl. Workshop on Campylobacter, Helicobacter and Related Organisms, Freiburg, Germany.

2. B. Ge, S. Bodeis, R. D. Walker, D. G. White, S. Zhao, P. F. McDermott, and J. Meng. 2001. Comparison of Etest and Agar Dilution Methods for Antibiotic Susceptibility Testing of *Campylobacter* Isolated from Retail Meats, Annual Meeting of National Antimicrobial Resistance Monitoring Program, Rockville, MD.

- **The Evaluation and Removal of Bacterial Biofilms from Food and Food Processing Materials.** Paul Schreuders, S. Joseph, A.Lomander (UM) and Leila Ali (FDA)

  This research involves the development of a method for examination of *E. coli* biofilms on materials frequently used in the food processing industry.

Objective 1. Development of a method for growing biofilms in lab scale.

This objective has been completed. A method for growing biofilms on rigid substrates (such as stainless steel and glass) and on films (such as polyethylene sheeting) has been developed. The culture occurs in a tank with culture medium circulated at a low flow rate to prevent localized nutrient depletion. The tank with its content is kept at 25 °C.

316 Stainless steel, glass slides, and polyethylene sheets are being examined. The slides from stainless steel have different surface morphologies: polished to a bright finish, brushed to a matte finish, grooved with a fly cutter, and scribed with an industrial diamond. These surfaces provide a variety of materials and surface treatments.

Objective 2. Development a general protocol for the staining and analysis of bacterial biofilms.

One indicator of bacterial mortality is the loss of integrity in the membrane surrounding the bacterium. This loss of integrity is being evaluated using the stain/counterstain pair SYTO-16/Propidium Iodide. Differences in color make it possible to distinguish between live and dead bacteria in the biofilm.

A protocol has been developed that allows the analysis of these images using a low-light black-and-white GBC-CCD video camera mounted on an epifluorescence microscope is used to obtain gray scale images of the biofilms grown of the slides. The acquisition times are sufficiently short (< 5 seconds) so that photo bleaching of the fluorophores is not significant. Two different analyses are performed to examine the percentage of a surface that is covered with biofilm. The first analysis determines the average percent coverage of live and total biofilm on each slide. Three metrics are computed for the individual biofilm patches; patch area (*A*), patch perimeter (*P*), and circularity (*C*). Circularity is the ratio of the measured perimeter of a biofilm patch to the perimeter of a circle with the same area as the biofilm patch. A perfect circle has the circularity of 1 and other shapes have values for *C* greater than 1. A peer reviewed paper describing this research is in press.

Black-and-white analysis of the color images, while effective, is both time consuming and labor intensive. Therefore, in parallel with the research, computer software is being developed to aid in the study of cell function. The Multifluorescence Image Analysis software is a color based program. It segments and measures particles in an image based on the color of the stains. Segmentation of the image will be accomplished using thresholding based in the HSV color space (Hue, Saturation, Value). The program is currently being calibrated using a wide range of biofilm images,

with the hope that we will be able to automate the analysis. A paper describing this work is in preparation.

Objective 3. Investigation of growth of biofilms from the E. coli over time on different materials and different surface morphologies, using different sanitation methods.

Much of the experimental data describing biofilm growth has been gathered. Sampling of the biofilms is performed at 3, 6, 12, 24 and 48 hours. A five minute exposure to the following cleaning/sanitizing methods has been used: rinsing in deionized water, ultrasound, chlorine, phenol, and ozone. Chlorine of the concentrations 50 and 200 ppm have been used, and phenol 0.5 and 1%. The ozone work should be completed in the near future. The analyses of the resulting data are currently being performed and the analysis of the effectiveness of ultrasonic cleaning is largely completed.

The preliminary results for ultrasonic cleaning indicate that the *E. coli* biofilms do not actively increase in size on any material until after about 12 hours of age. The effectiveness of ultrasonic cleaning has been found to be independent of material (polyethylene, glass or stainless steel). However, there appear to be significant differences linked to the surface morphology.

Interestingly, when the individual biofilm patches were examined, reductions in the patch's areas were most evident in the largest patches and least evident in the small biofilm patches.

Publication status

a. Publications:

A. Lomander, P. D. Schreuders, E. Russek-Cohen, and L. Ali, 2002. A Method for Rapid Analysis of Biofilm Morphology and Coverage on Glass and Polished and Brushed Stainless Steel, *Transactions of the ASAE* (In Press)

b. Presentations:

1. A. Lomander, P. D. Schreuders, and L. H. Ali. "The Development of Methods for the Evaluation and Removal of Bacterial Biofilms," Northeast Agricultural/ Biological Engineering Conference, Lancaster, PA, August 1-4, (1999).
2. A. Lomander, P. D. Schreuders, and L. H. Ali. "Analytical Techniques for the Evaluation of Biofilms on Food Processing Materials," Northeast Agricultural/ Biological Engineering Conference, Ithaca, NY, July 30 – August 2, (2000).

3. A. Lomander and P. D. Schreuders. “Sanitation of Biofilms from *E. coli* O157:H7 on Food Processing Materials,” Northeast Agricultural/ Biological Engineering Conference, Guelph, ON, Canada, July 8-11 (2001).
4. P. D. Schreuders and N. Yang. “Multifluorophore Image Analysis,” Northeast Agricultural/ Biological Engineering Conference, Guelph, ON, Canada, July 8-11 (2001).
5. P. D. Schreuders, A. Lomander, and L. H. Ali> “Morphological Responses by Biofilms to Surface Defects Created in Stainless Steel,” Annual Meeting of the Institute for Biological Engineering, Baton Rouge, LA, (2002). (Accepted)

First-year projects (funded July 2001):

- **Monitoring and Compliance Under Seafood HACCP: An Econometric Investigation.** Anna Alberini, Erik Lichtenberg (UM), Dominic Mancini, and Robert Scharff (FDA).

  Abstract: The FDA has instituted a HACCP-based regulatory regime in the U.S. seafood industry in response to increases in the number of food pathogens, public concern about food safety, growth of the food industry and international trade in food products, and tightening constraints on FDA enforcement capabilities. Under HACCP, seafood processing firms are required to establish preventive measures, monitoring regimes, and procedures for corrective action to address potential food safety hazards. FDA does not approve HACCP plans or conduct routine reviews; instead, it relies on annual spot inspections and examination of firms' records to ensure compliance. The design of this inspection program and the accompanying system of penalties for violators are essential determinants of success for this regulatory program.

  The purpose of this research project is to provide information that can help improve the design of this regulatory program by investigating the determinants of plant compliance with seafood HACCP regulations to date. Identification of plant characteristics correlated with non-compliance can help FDA improve inspection targeting, thereby improving the level of food safety provided while simultaneously alleviating pressure from budgetary and personnel constraints. Criticism of the seafood HACCP program expressed in a recent (2001) General Account Office report as well as other venues, underscores the importance of addressing these issues. The results of this research should also provide information useful in FDA's recently announced mid-course correction of the seafood HACCP program.

The study will utilize data collected by the FDA's Office of Seafood for its evaluation of the seafood HACCP program. This project is funded for one year.

- **The Use of Tissue Fluid Correlations to Predict Drug Residue Levels in Edible Tissues.** Natalie Eddington (UMB), James Peggins, Keesla Moulton, Jurgen von Bredow, and Pamela Chamberlain (FDA).

  Abstract: Less than 1% of slaughtered animals are routinely monitored for drug residues. The regulatory methods used to measure drug residues in tissues from cattle are complex and time consuming. In addition, because tissue drug residue testing normally occurs post-slaughter, meat with residues that exceed tolerance is declared adulterated and must be destroyed. The current system of testing is inadequate to provide maximum assurance of food safety.

  The use of rapid, inexpensive pre- or post-slaughter screening tests (similar to those used for milk) based on detection of drug residues in some easily obtained biological fluid (saliva, plasma or urine) would allow more animals to be monitored and ensure greater food safety. Pre-slaughter testing would allow animals with predicted violative residues to be held back until drug residues deplete to legal limits by normal routes of excretion. The pharmacokinetics of a number of agents have been characterized by tissue distribution studies and subsequent physiologically-based modeling. These models are useful in predicting tissue levels following single or multiple dose(s). By evaluating the relationship between drug administration, tissue uptake and, more importantly, tissue elimination, whether an animal is likely to have tissue residues that exceed tolerances can be predicted better. For this project, tissue exposure will be assessed by the development of physiologically-based pharmacokinetic flow models characterizing tissue distribution and elimination of the test agents. In addition to providing insight on tissue distribution and disposition, these physiological flow models can also assist in extrapolating results from one animal species to another, i.e. swine, sheep, etc.

  This project proposes first to develop and validate a physiologic based pharmacokinetic model using rats, and then to modify the model for application to beef cattle. These models will be developed by correlating drug levels in some easily sampled biological fluids, i.e. urine, saliva, or blood, with drug residues that are present in edible tissues. Prototypic drugs such as gentamicin, florfenicol, oxytetracycline, enrofloxacin or ivermectin will be used during model development. These drugs, commonly used to treat infectious and parasitic diseases in food-producing animals, have long depletion times (>28 days) which increases the potential for treated animals to inadvertently enter the human food supply. Drugs such as gentamicin, which has no approved use in cattle, are problematic

because of their off-label use in dairy cows and bob veal calves, and because residues can persist in the kidney for months. These models may also be useful for back extrapolating an estimate of the dose administered and the time of administration based on a measured concentration in tissue.

- **Investigating the Perceived Credibility of FDA's Advisory Committee Meetings as Techniques for Communicating about Food, Drug, Biologics, and Medical Device Issues.** Katherine McComas (UM) and Linda Suydam (FDA)

  Abstract. Among many techniques available for seeking input and expertise on food, drug, biologics, and medical device issues at the U.S. Food and Drug Administration are FDA's advisory committees. Although a principal component of the FDA decision-making process for years, advisory committees have recently received criticism regarding the impartiality of its pool of experts. This project investigates the extent to which conflict-of-interest considerations influence the perceived credibility of FDA advisory committees, as well as the credibility of ensuring FDA decisions. In addition, this project will determine the degree to which knowledge of the waiver process influences perceptions of impartiality. Finally, this project examines the extent to which conflict-of-interest considerations influence satisfaction with advisory committee meetings as techniques for helping FDA obtain the best scientific advice on policy decisions. The proposed research design includes participant-observation of advisory committee meetings, and questionnaires and focus groups conducted with individuals who have attended advisory committee meetings. It also includes interviews with experts who have served on FDA advisory committees and FDA officials, as well as a content analysis of media coverage of FDA advisory committee meetings. The results of the study will offer insight into the influence of conflict-of-interest considerations on the perceived credibility of FDA advisory committees. As such, these findings will comprise a scientific foundation on which to build future communication efforts aimed at improving credibility and satisfaction.

- **Comparison of the Effects of Curcumin Supplements in Young and Aged Rats.** Bernadene Magnuson, Monica Giusti (UM), Fred Hines, Sabine Francke, and Hamida Alam (FDA).

  Abstract: Curcumin (diferuloylmethane) is a major component of turmeric and is commonly used as a spice, food-coloring agent, and dietary supplement. For the latter, the reported potential health benefits of curcumin include prevention of aging, promotion of liver health, prevention of various cancers, and anti-inflammatory effects. For these purported health benefits, the primary consumers using curcumin supplements are likely to be middle-aged to elderly individuals. However, preclincal

studies on the absorption, metabolism, and efficacy of curcumin supplementation have been conducted using young animals. In this project, tissues collected from young and aged animals treated with or without curcumin will be evaluated. The objective of the light microscopic examination of tissues will be to determine 1) the toxic or beneficial effects of feeding curcumin to a population of young animals by comparing young controls to young treated animals, 2) the toxic or beneficial effects of feeding curcumin to a population of aged animals by comparing aged controls to aged treated animals, and 3) the difference, if any, in the toxic or beneficial effects of feeding curcumin to young and aged animals. In addition to evaluating tissues by light microscopic methods, levels of curcumin metabolites will be measured and gene expression changes related to aging will be evaluated. The results of these studies may provide valuable information on the safety, efficacy and health benefit claims of curcumin supplements.

- **Modeling the Antimicrobial Effect of Lactate on the Growth and Survival of *Listeria monocytogenes* on ready-to-eat seafood.** Kisun Yoon (UMES) and Richard Whiting (FDA)

Abstract. Refrigerated seafood is growing in popularity in response to consumer demands for fresh convenience food that is minimally processed and read-to-eat. The presence, growth, and survival of *Listeria monocytogenes* during retail and home storage is a particular concern, since this organism is frequently associated with fresh, frozen, and mainly ready-to-eat seafood products including smoked fish. *L. monocytogenes* is difficult to control in seafood because it is a psychrotrophic microorganism and is resistant to diverse environmental conditions such as low pH, pasteurization, and high NaCl concentrations. Furthermore, the severity and case-fatality rate of the disease urgently demand that we apply appropriate preventive measures. In addition, the FDA interpretation of its risk assessment on *L. monocytogenes* was that smoked seafood and ready-to-eat seafood products need new techniques and better information to enhance control of this pathogen.

Predictive modeling involves mathematical expressions describing microbial behavior under various environmental factors such as temperature, pH, water activity, atmosphere, and presence of inhibitory chemicals. A primary model describes how microbial numbers change with time in a specified environment. A secondary model describes the impact of cultural and environmental variables, such as temperature, pH, or the presence of antimicrobials, on the parameters of the primary model. These two models provide a good description of microbial behavior at different physical and chemical conditions and can be used to forecast the shelf life and safety of food products.

Sodium lactate is currently used in the meat industry for its flavor enhancing and shelf life-extending capabilities for a wide range of cured, uncured, and fresh meat and poultry products. A number of studies have reported antibacterial effects of lactates. While sodium lactate has been shown to influence pathogens' behavior, including L*isteria, Salmonella, Staphylococcus aureus* and *Clostridium*, its antimicrobial effect on growth or survival of *L. monocytogenes* as a function of temperature has not been modeled in either microbiological broth or food products. In this study, the antimicrobial effect of lactate on *Listeria monocytogenes* will be determined in ready-to-eat, smoked fish and crab cake during refrigerated storage. Then, a model will be developed to describe both growth and survival of *L. monocytogenes* based on the Baranyi model. In addition to the standard regression models, Zwietering's approach will be evaluated then to develop secondary level modeling, the Gamma model, for the growth of *L. monocytogenes* as a function of lactate concentration. Using the dimensionless ratio approach in the Gamma model, new environmental factors affecting microbial growth, such a sodium lactate, can easily be added into the existing models.

This project will provide scientific information needed for HACCP analysis and microbial risk assessment of ready-to-eat seafood to diverse interest groups such as FDA, the seafood industry, retailers, and consumers. The newly developed models will be a tool to estimate the effect of lactate on both growth and survival of *L. monocytogenes* at various storage temperatures at the retail store. In addition, demonstrating the feasibility of the Gamma model to allow addition of a new control factor into existing models, without repeating all of the previous modeling studies, would be an advance in modeling techniques.

- **In Vitro Metabolic Profiles to Characterize and Predict Drug Residues in Aquacultured Finfish.** Andrew Kane (UM), Badar Shaikh, and Renate Reimschuessel (FDA)

Abstract. There is a vital need to expand the repertoire of therapeutic drugs for veterinary use in aquaculture. Compared with traditional farm species, very few drugs are currently approved by the FDA for use in aquaculture species. In order to facilitate the drug approval process for cultured fish species, it is desirable to establish species groupings, based on similar drug enzymatic metabolic profiles and drug residue patterns between species. The metabolic profiles are related to different species' drug excretion rates and tissue residues. These factors determine the appropriateness of therapeutic drugs used in aquacultured species destined for human consumption. Studies of mammalian drug metabolism *in vitro* are predictive of the fate of particular drugs, and are valuable in demonstrating or confirming that the animal possesses a particular metabolic capacity. This study will investigate drug metabolism *in vitro*,

in multiple fish species, and a model to predict drug residue patterns in edible tissues. Both phase I (cytochrome P-450 dependent) and phase II (conjugation) pathways of drug metabolism will be studied in selected freshwater and brackish water fish species to ascertain the differences or similarities in their levels and abilities to biotransform model substrates and veterinary drugs. The drug and metabolite residue profiles determined *in vitro* will be compared with data obtained from *in vivo* drug residue experiments. Similarities (and dissimilarities) between species will determine the likelihood of deriving species groupings based on their metabolic profiles and tissue residues.

This project proposal was partially supported to leverage funding already committed to the investigation.

APPENDIX B

Specific Research Initiatives

- **Mechanistic Assays for the Phototoxicity of Cosmetics.** Daniel Falvey and Peter Vath (UM), and Wayne Wamer (FDA)

  The long-term objective of this research is development of non-animal assays that predict the risks associated with the use of cosmetic ingredients on sun-exposed skin. It is hypothesized that photophysical measurements, characterizing the formation and decay of a potential photosensitizer's excited states under biologically relevant conditions, and *in vitro* photobiological measurements, characterizing a potential photosensitizer's cytotoxicity and cellular targets, provide the mechanistic information needed for estimating acute and chronic phototoxic risks. A series of photophysical and photobiological studies have been carried out aimed at examining potential harmful effects of cosmetic ingredients that are commonly applied to sun-exposed skin. Work to date has focused on various retinoids: all trans-retinol, retinol acetate, and retinol palmitate. All three retinoids are commonly found in cosmetics, particularly wrinkle reduction and moisturizing creams.

  Laser flash photolysis (LFP) experiments were carried out on the retinoids to study possible photochemical mechanisms leading to their phototoxicity. Upon irradiation with 355 nm light in acetonitrile both all trans-retinol and retinol acetate give an absorption band at 580 nm, corresponding to the retinyl cation. The LFP experiments were repeated in mixtures of acetonitrile and water. As the fraction of water was increased, the absorption band due to the retinyl cation also increased due to the greater stabilization of the cation by water. However, at a water fraction of approximately 70% the absorption band at 580 nm decreased due to aggregation of the retinoids in the more aqueous solutions.

  In order to carry out the experiments in aqueous solutions, surfactants were added to form micelles. The micelles also more closely resemble the conditions in biological systems since they possess both polar and nonpolar regions. The surfactants sodium dodecyl sulfate (SDS) and cetyl(hexadecyl)trimethylammonium bromide (CTAB) were used to form the micelles in aqueous solution. SDS forms micelles with anionic head groups while CTAB forms cationic micelles. In both cases an absorption band at 580 nm due to the retinyl cation was observed following irradiation with 355 nm light.

  One possible pathway leading to the phototoxicity of retinoids involves the reaction of the retinyl cation with a biological substrate. Addition of tryptophan to all trans-retinol in SDS micelles was found to quench the retinyl

cation while the addition of guanosine monophosphate had no effect on the lifetime of the retinyl cation. Future experiments will continue to look at the effects of other biological substrates, such as DNA and proteins, on the lifetime of the retinyl cation in micellar solutions. LFP experiments will also be carried out on the retinoids in cells.

- **Developing Methodology to Detect Adverse Events.** Johnny Blair, Timothy Triplett, Henry Wu, Song Zhao (UM), and Marilyn Flack (FDA)

  The goal of this project (initiated in late September 2000) is to develop an Internet-based reporting system that will collect high quality data that can then be utilized not only by FDA, but also by the public. This will provide access to risk management information to the primary users of this database. The primary public users are hospitals and other facilities that use medical devices.

  The research is designed to analyze the components of a reporting system which the public users of the data in the reporting system database will find most useful in conducting risk hazard and risk management analysis.

  The University of Maryland's Office of Academic Computer Services (OACS) is developing the software for the project, and the University's Survey Research Center has been researching and developing the physical appearance of the data entry screens, questions to be used to collect adverse event information, and the most useful means of providing the risk management information to the reporting facilities. The results of pilot testing will determine which research concepts provide the most useful information to the reporting facilities so that they can use these data to provide safer patient care.

  The Survey Research Center has completed the initial phase of research. Hospital risk managers, lawyers, and quality improvement specialists were interviewed. From information obtained from these interviews, a web-based form was designed, reviewed, and redesigned. The prototype was given to OACS for development as a website.

  As part of the initial phase the following was determined: a) the most user-friendly data collection format; b) what types of additional data collection items hospitals may/may not be willing to respond to and why/or why not; and c) what data elements were deemed useful by the hospitals.

  The Office of Academic Computer Services (OACS) has completed the Internet-based data entry screen and FDA is conducting beta testing. The Survey Research Center is also obtaining feedback from risk managers concerning the usability of the screens, i.e. the look and "feel" of the interface. Additionally, OACS is nearing completion on the other interfaces and on the search engine.

During the course of this project, it was learned that the software was required to meet all FDA Information Technology development standards and guidelines if it was going to be used to collect information, that is considered confidential, from hospitals. Since the software developed during this project will eventually be used to collect adverse event information from facilities, as required under the Food, Drug and Cosmetic Act, Section 519, the scope of the project changed.

In order to meet the considerably increased programming requirements, additional funding ($100,000) beyond the JIFSAN Collaborative Agreement was provided by CDRH to pay additional costs and necessary enhancements. This is an excellent example of leveraging of resources.

It is anticipated that the project will be completed during the spring of 2002.

- **Characterization of Multiple Fluoroquinoline Resistance among Avian *Escherichia coli* Isolates from North Georgia**. David White (FDA/CVM) and Jianghong Meng (UM)

  Fluoroquinolones (e.g. sarafloxacin) were introduced in 1995 for veterinary use in the United States and worldwide for treatment of avian colibacillosis caused by *Escherichia coli*. This study was undertaken to investigate the genetic determinants responsible for decreased susceptibility to fluoroquinolones observed among *E. coli* isolates from colisepticemia-diseased birds from North Georgia. Fluoroquinolones of human and veterinary significance were assayed, including: gatifloxacin, sarafloxacin, enrofloxacin, ciprofloxacin, difloxacin, danofloxacin, levofloxacin, orbifloxacin, and nalidixic acid. Antimicrobial minimum inhibitory concentrations (MICs) of 100 avian *E. coli* isolates were determined via broth micro-dilution, and interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS). Fifty-nine percent of the isolates were resistant to nalidixic acid, and sarafloxacin, while 34% displayed resistance to difloxacin. Decreased susceptibility to enrofloxacin (9%), ciprofloxacin (3%), gatifloxacin (1%), and levofloxacin (1%) was observed. Amplification of the quinolone-resistance determining region (QRDR) by PCR, followed by DNA sequencing, revealed that point mutations occurred within the DNA sequences of the *gyrA* and *gyrB* genes, which code for DNA gyrase, and *parC* and *parE* genes coding for topoisomerase IV. The potential presence of efflux pump mechanisms was investigated by the use of a preliminary organic solvent tolerance test on the fluoroquinolone-resistant isolates. Twelve fluoroquinolone-resistant *E. coli* isolates were tolerant to cyclohexane. In this study, we report the emergence of fluoroquinolone resistance in *E. coli* from cases of avian colibacillosis from North Georgia. Detection of fluoroquinolone resistance in these birds stresses the need for judicious use of antimicrobials to prevent the development of resistance in other bacterial

pathogens associated with poultry and introduction of antimicrobial resistant bacteria into the nation’s food supply.

APPENDIX C

Leveraging

Development of Research Partnerships:

- **FT-NIR Rapid Determination of Food Integrity**

  Food supplies are among the most vulnerable routes for the delivery of lethal or incapacitating quantities of chemical or biological agents. The goal of this project is to develop methodology for the rapid detection of contaminants (chemical and microbial) in a wide range of foods by using FT-NIR spectroscopy combined with multivariate data analysis techniques. The data obtained can lead to the development of a database to support studies on the natural variation and variation caused from different processing techniques in foods. The results of this project should provide for cost effective screening techniques that can be used by the food industry, FDA, other food safety agencies, and DOD to increase surveillance of the food supply for contaminants, including potential threat agents. The food industry would have "value added" incentives to apply this technology as part of their HACCP and quality assurance programs. Support for this project comes from an Army Cost-Reimbursible Research Contract to Dr. Elizabeth Calvey (Co-PI, FDA) and Dr. Bruce Jarvis (Co-PI, UM) that was initiated in August 1998.

  A fourth year of support for Dr. Luis Rodriques-Saona was obtained. In addition, four FDA staff are involved with the development of the research - Dr. Elizabeth M. Calvey (project director), Dr. Fred S. Fry (chemometrics), Dr. Farukh M. Khambaty (microbiology) and Dr. Magdi M. Mossoba (IR spectroscopy). A fifth FDA scientist, Dr. Michael A. McLaughlin (carbohydrate chemistry) became involved to facilitate the comparison of the FT-NIR method for quantifying sugars in fruit juices with traditional HPLC methods.

  The fourth year support was made possible by incorporating into the collaboration the complimentary research of Dr. Alan Samuels from the Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD. The thrust of additions to the project is to incorporate the FT-IR component that Dr. Alan Samuels is researching including a comparison of a selection of bacterial samples obtained by using a variety of protocols and different instruments in the mid-infrared region; and exploring the applicability of multivariate analysis protocols to bacterial spectra in the mid-infrared region.

  Additionally, talks have begun with interested third parties in the establishment of a CRADA to facilitate the transfer of the technology to field applications.

Rapid Detection of Castor Bean Meal Flour-Based Products: Methodology was developed and evaluated for the rapid detection of castor bean meal (CBM) containing the toxic protein ricin by using Fourier-transform near infrared (FT-NIR) spectroscopy and multivariate techniques. The method is intended to be a prototype to develop a more general approach to detect food tampering. Measurements were made on a FT-NIR system using a diffuse reflection-integrating sphere. Flour spiked with caffeine, crystalline sugar and corn meal, 1-20% w/w, were used as test articles to evaluate the methodologies. Food matrices (bleached flour, wheat flour and blueberry pancake mix) spiked with CBM (0.5-8% w/w) were analyzed. Multiplicative scatter correction transformed partial least squares regression models, using a specific NIR spectral region, predicted CBM contamination in foods with standard error of cross-validation (SECV) < 0.6% and coefficient of determination (R^2) > 94%. Models discriminated between flour samples contaminated with CBM and other protein sources (egg white, soybean meal, tofu, and infant formula). CBM had loading spectra with bands characteristic of amide groups (4880 and 4555 cm^{-1}) and lipids (5800, 5685, 4340 and 4261 cm^{-1}).

Rapid Detection and Identification of Bacterial Strains: The use of Fourier-transform near infrared (FT-NIR) spectroscopy and multivariate pattern recognition techniques for rapid detection and identification of bacterial contamination in liquids was evaluated. The complex biochemical composition of bacteria yields FT-NIR vibrational transitions (overtone and combination bands) that can be used for classification and identification. Bacterial suspensions (*E. coli* HB101, *E. coli* ATCC 43888, *E. coli* 1224, *Bacillus amyloliquifaciens*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Listeria innocua*) were filtered to harvest the cells and eliminate the matrix, which has a strong NIR signal. The use of a simple membrane filtration procedure to produce a thin, uniform bacterial film generated reproducible FT-NIR spectra that can be used for rapid discrimination among closely related strains. The use of Anodisc membranes gave the most reproducible results. FT-NIR measurements were done using a diffuse reflection-integrating sphere. Transformation of the spectra with second derivatives resolved specific FT-NIR features in the information-rich spectral region of 5000-4000 cm^{-1} to allow principal components analysis (PCA) to group the samples into different tight clusters. This methodology may allow for the rapid assessment of potential bacterial contamination in liquids with minimal sample preparation.

Rapid Analysis of Sugars in Fruit Juices: A simple analytical procedure using FT-NIR and multivariate techniques for the rapid determination of individual sugars in fruit juices was evaluated. Different NIR detection devices and sample preparation methods were tested by using model solutions to determine their analytical performance. Aqueous solutions of sugar mixtures

(glucose, fructose and sucrose; 0-8% w/v) were used to develop a calibration model. Direct measurements were made by transflection using a reflectance accessory, by transmittance using a 0.5 mm cell, and by reflectance using a fiberglass paper filter. FT-NIR spectral data were transformed to the second derivative. Partial least squares regression (PLSR) was used to create calibration models that were cross-validated (leave-one-out approach). The prediction ability of the models was evaluated on fruit juices and compared with HPLC and standard enzymatic techniques. The PLSR loading spectra showed characteristic absorption bands for the different sugars. Models generated from transmittance spectra gave the best performance with standard error of prediction (SEP) <0.10% and R^2 of 99.9% that accurately and precisely predicted the sugar levels in juices, whereas lower precision was obtained with models generated from reflectance spectra. FT-NIR spectroscopy allowed for the rapid (~3 min analysis time), accurate and non-destructive analysis of sugars in juices and could be applied in quality control of beverages or to monitor for adulteration or contamination.

Publication Status:

a. Publications

1. L. E. Rodriguez-Saona, F. S. Fry, and E. M. Calvey. 2000. Use of Fourier Transform Near-Infrared Reflectance Spectroscopy for Rapid Quantification of Castor Bean Meal in a Selection of Flour-Based Products, J. Agric Food Chem. 48:5169-5177.

2. L. E. Rodriguez-Saona, F. M. Khambaty, F. S. Fry, and E. M. Calvey. 2001. Rapid Detection and Identification of Bacterial Strains by Fourier Transform Near-Infrared Spectroscopy, J. Agric. Food Chem. 49:574-579.

3. L. E. Rodriguez-Saona, F. M. Khambaty, F. S. Fry, and E. M. Calvey. 2001. A Novel Approach for the Rapid Discrimination of Bacterial Strains by Fourier-Transform Near-Infrared Spectroscopy, Proceedings of SPIE Vol. 4206:22-31.

4. Luis E. Rodriguez-Saona, Frederick S. Fry, Michael A. McLaughlin, Elizabeth M. Calvey. 2001. Rapid Analysis of Sugars in Fruit Juices by FT-NIR Spectroscopy, Carbohydrate Research, in press.

b. Presentations

1. L. E. Rodriguez-Saona, F. S. Fry, Farukh M. Khambaty, and E. M. Calvey. "FT-NIR Rapid Determination of Food Contaminants," EAS, 1999, Somerset NJ.

2. L. E. Rodriguez-Saona, F. M. Khambaty, M. M. Mossoba, F. S. Fry, and E. M. Calvey. "FT-NIR Rapid Determination of Food Integrity," USDA Biosensor Meeting, Beltsville, MD. Feb 2000.

3. L. E. Rodriguez-Saona, F. M. Khambaty, M. M. Mossoba, F. S. Fry, and E. M. Calvey. " Detection and Classification of Bacteria Strains by Fourier Transform Near-Infrared Spectroscopy," 2000 FDA Science Forum. Washington, DC. Feb 2000.

4. L. E. Rodriguez-Saona, F. S. Fry, M. A. and E. M. Calvey. "Rapid Analysis of sugars in fruit juices by FT-NIR: Comparison of Sampling Devices," IFT Annual Meeting, Dallas, TX. June 2000.

5. L. E. Rodriguez-Saona, F. M. Khambaty, F. S. Fry, and E. M. Calvey "Fourier-Transform Near-Infrared (FT-NIR) Spectroscopy for Food and Water Contamination Monitoring," 34th Middle Atlantic Regional Meeting (MARM) sponsored by the American Chemical Society (ACS) May 30 - June 1, 2001.

- **Prevalence/quantitation of *Listeria monocytogenes* in Selected Retail Products**

  The FDA/USDA risk assessment of *Listeria monocytogenes* identified a number of foods for which data on prevalence and quantities of this foodborne pathogen could be strengthened to improve the reliability of the analysis.

  The National Food Processors Association Research Foundation (NFPA RF) is conducting a risk assessment on listeriosis from ready-to-eat foods. This project is funded by USDA CSREES and several industry partners and has been in progress since January 2000. The objective of the project is to evaluate the types and numbers of *L. monocytogenes* present in retail samples of certain ready-to-eat foods, then correlate those data with available consumption data to derive an exposure assessment. The exposure data will be compared with illness data collected by CDC concurrently and in the same geographic locations as the sample collection to assess the risk of listeriosis from consumption of specific numbers or subtypes of *L. monocytogenes*.

  Five product categories that would benefit from additional data collection were identified. These included smoked seafood, prepared seafood salads, bagged salads, Hispanic-style soft cheeses, and blue veined and soft mold-ripened cheeses.

  JIFSAN funded a project with the National Food Processors Association Research Foundation to determine the prevalence/quantitation of *Listeria monocytogenes* in retail products from these five product categories. This

study leverages the study funded under CSREES in that NFPA already has protocols and infrastructure in place. Data from this exposure study are being received and will be posted on the JIFSAN Food Safety Analysis Clearinghouse to provide transparent quantitative exposure data to risk assessors working on *Listeria monocytogenes*.