Annual Report 2001-2002

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

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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). Initial partners in the cooperation were 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; Dr. Paul Mazzocchi (University of Maryland) and Dr. Arthur Miller (CFSAN, FDA) are Associate Directors. Dr. David Batson 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, with both domestic and international emphases, in microbial pathogens and toxins, food constituents and applied nutrition, animal health sciences: animal health and food safety, food safety risk analysis, and economics.

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), JIFSAN operates a web-based Food Safety Risk Analysis Clearinghouse. 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 toxins. The unique feature of this clearinghouse model resides 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.

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 public health impacts of the research.

The new Harvey W. Wiley Federal Building, CFSAN's office and laboratory facility, is located 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 analysis and food contamination monitoring. This designation is in the process of being renewed. Designation of JIFSAN as a member of the FAO Network of Excellence on Food Quality, Safety, and Nutrition (F.S.Q.N.) is in progress.

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 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 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 a permanent Director was conducted. Dr. David Lineback became Director in November 1998.

Progress Report

During the fifth year of operation for JIFSAN, several education and outreach programs were continued, developed and/or initiated; 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 fifth year was $2,782,800.

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:

- Office and computer laboratory space (approximately 500 sq. ft.) have been utilized by the JIFSAN Food Safety Risk Analysis Clearinghouse 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. Two additional rooms plus storage space were obtained by JIFSAN this year. These are immediately adjacent to JIFSAN's current offices and, following some renovations, will be used to house the Clearinghouse operations. This will centralize JIFSAN's operations into one location increasing operational effectiveness.

- Dr. Wendy Fineblum resigned as Coordinator of the Risk Analysis Clearinghouse in June 2002. Dr. Cristina McLauglin (FDA, CFSAN) was detailed to JIFSAN for four months to serve as Interim Coordinator of the Clearinghouse. Currently, a search for a Coordinator is in progress.

- The first iteration of a strategic plan for JIFSAN was completed and is being implemented.

- Dr. Catherine E. 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. This position was partially funded by JIFSAN. Dr. Woteki served in an advisory capacity to the Director. She left on January 1, 2002 to become Dean, College of Agriculture, Iowa State University.

- Dr. Bern Schwetz, Senior Science Advisor to the Commissioner of FDA, began an IPA appointment with the University in July 2002 and serves as a member of the JIFSAN Working Group and in an advisory role to the Director.

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 met on February 26, 2002 to discuss six internal research projects in progress plus three additional research projects involving a specific research initiative and two research partnerships. Discussions were also held concerning needs in research, education/outreach, and the strategic plan in terms of future directions and emphases. Members of the Advisory Council made the following comments and suggestions.

- Some members would like to assist in the review of proposals for projects to be funded through the JIFSAN Competitive Internal Research Program.
- New collaborative research projects are needed in the following areas:

  - Pathogens – Currently we are just scratching the surface
  - Allergenicity – Inadvertent unlabeled allergens in the food supply
  - Food security – Threat analysis work and exchange of information
  - Nutrition – Labeling
- More JIFSAN student intern involvement in future FDA Science Forums
- Can industry and consumer groups' funds be combined with JIFSAN funds to support a mutually agreeable research project? If so, responses should be solicited from industry.

A meeting of the Advisory Council has been scheduled for November 8, 2002. The Director maintained contact with several Advisory Council representatives 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)
  Frito-Lay (Dr. Steve Saunders)
  General Mills (Mr. Frederick Hegele)
  Gerber Products Company (Dr. Nicholas Hether)
  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 (Ms. Susan Waltman)
  Monsanto Company (Dr. Jerry Hjelle)
  Procter and Gamble Company (Dr. Keith Triebwasser)
  Tropicana Products (Dr. Jay Shuman)

- 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. 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 (Cargill, Inc.)

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 as Principal Investigator (PI) and one or more FDA collaborators. The latter help provide additional scientific expertise and insight into public health impacts of the 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.
- Developing scientific information and understanding of food safety issues important to risk analysis.

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 was $10,000 in each case. This was changed, effective with proposals funded in July 2002, to be $30,000 per project to be used for either a graduate research assistant or a postdoctoral associate. 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 request and 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 the three remaining projects was reviewed and all three were continued for a third 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 for one year since it was of high quality and had additional support already committed. Progress on these four projects was reviewed and all four were continued for a second year. Six new projects were funded in July 2002. One of these was funded for one year while the other five were for three years.

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

Third year projects (scheduled for completion in January 2002):

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

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

Third year projects (funded in January 2000):

- 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).

Second-year projects (funded July 2001):

- 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 Sherman (FDA) (replacing Linda Suydam).

- 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).

First-year projects (funded July 2002):

- Evaluation of the potential toxicity of soybean isoflavones in development and aging. Monica Giusti, Mary Ann Ottinger (UM), and Sherry Ferguson (FDA) (funded for one year)

- Study of nisin and sublancin in a strategy for protection of the United States food supply from pathogenic bacterial spores introduced through bioterrorism. Norman Hansen (UM) and Laila Ali (FDA).

- Facilitating needed drug approvals for aquaculture: In vitro metabolic profiles to characterize and predict drug residues in finfish. Andrew Kane, Renate Reimschuessel (UM) and Badar Shaikh (FDA).

- Moving whole-cell biosensing from a qualitative to quantitative tool: Development of a dynamic cell immobilization mechanism. Y. Martin Lo (UM) and Mahendra Kothary (FDA)

- Safety inspection of fresh cut fruits and vegetables using spectral sensing and machine vision techniques. Yang Tao (UM), Robert Buchanan, Yoonseok Soon (FDA) and Yud-Ren Chen (USDA).

- Influence of pre-harvest antibiotic pesticide treatment on the microflora of apple and pear blossoms, leaves, fruit, and cider and its implications for food safety. Christopher Walsh (UM), Arthur Miller and S. Brian Eblen (FDA).

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. The one listed below, and more fully described in Appendix B, was active during the reporting period.

> 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. Postdoctoral Research Associates have been hired to work in five broad areas of research, where significant knowledge gaps or the lack of appropriate scientific data, methods, or models exist. 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.

JIFSAN Senior Research Scientist:

Dr. Frederick Khachik is a senior research scientist and adjunct professor with joint appointments in the Department of Chemistry and Biochemistry and JIFSAN. He has been partially supported by JIFSAN since it began operation. Dr. Khachik has established an international reputation for his research in the area of carotenoids.

During the past year, he received a $1.2 million grant from the National Institutes of Health to study the effects of two dietary carotenoids, lutein and zeaxanthin. These carotenoids accumulate in the human retina and other eye tissues and may prevent age-related macular degeneration. His patented method for producing rare carotenoids was one of the University of Maryland's inventions of the year for 2000.

Among his publications in 2002 listing an affiliation with JIFSAN are the following:

1. F. Khachik, L. Carvalho, P.S. Bernstein, G.J. Muir, A.-Y. Zhao and N.B. Katz. 2002. Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. Exp. Biol. Med. 227:845-851.

2. F. Khachik, F.F. de Moura, D.-Y. Zhao, C.-P. Aebischer and P.S. Bernstein. 2002. Transformations of selected carotenoids in plasma, liver, and ocular tissues of humans and in nonprimate animal models. Investigative Opthalmology & Visual Sci., 43:3383-3392.

3. O. Kucuk, F. H. Sarkar, W. Sakr, F. Khachik, Z. Djuric, M. Banerjee, M.N. Pollak, J.S. Bertram and D.P. Wood, Jr. 2002. Lycopene in the treatment of prostate cancer. Pure Appl. Chem. 74:1443-1450.

Leveraging:

One of the basic 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.

   Research associated with the following project ended September 2002 and is described in Appendix C. A one-year no-cost extension was granted to provide time to finish several manuscripts and complete the final report.

Fourier Transform Near Infrared (FT-NIR) Rapid Determination of Food Integrity, L.E. Rodriguez-Saona (UM), F.S. Fry and E.M. Calvey (FDA,CFSAN)

Support for this project comes from an Army Cost-Reimbursible Research Contract that was initiated in August 1998.

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 since CFSAN has relocated to its new facilities in College Park.

Risk Analysis/JIFSAN Food Safety Risk Analysis Clearinghouse

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. 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 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.

Dr. Wendy Fineblum served as Coordinator of the Clearinghouse until her resignation in June 2002. Dr. Cristina McLaughlin (FDA CFSAN) has been detailed to JIFSAN for four months (July 2002 – November 2002) to serve as Interim Coordinator.

Progress for the Food Safety Risk Analysis Clearinghouse included:

A Food Safety Risk Analysis Clearinghouse Newsletter was initiated in July 2002 and a second issue is planned for November. The Newsletter serves the purpose of informing the research community of new additions to the Clearinghouse, upcoming events, and to feature a special article or presentation. The number of

researchers that are registered in the database has increased from 22 to about 150. The Newsletter is posted on the Clearinghouse website (http://www.foodriskclearinghouse.umd.edu).

Prior to July 2002, the reported usage statistics included hits by Clearinghouse staff. This was corrected and, in spite of this adjustment, usage from July 2002 to November 2002 increased by 80%. The estimated number of repeat visitors increased from about 6,000 in July to 17,000 in November. These repeat visits to the site were from people requesting pages they had cached or viewed. This increase in use is attributed to efforts by the Clearinghouse staff in making considerable changes and improvement to the format and content of the site, and to the active participation of promotional activities via the Clearinghouse Newsletter and promotional displays during conferences.

Another result of recent changes to the Clearinghouse was the inclusion of the site on ISI Web of Knowledge Current Web Contents ™. ISI Current Web Contents ™ is a prestigious database with strict selection criteria in evaluating data collected in a web site. Their evaluative criteria for Web sites include reviews of factors such as: authority, accuracy, currency, navigation and design, applicability and content, scope, audience level and quality of writing. ISI editors have visited the site, reviewed it, developed a standardized descriptive record, written an abstract and created a link from CC Connect to the Clearinghouse site.

Several new sections have been added to the Clearinghouse such as a section on nutrition and labeling, a section devoted to acrylamide, announcements, and a comprehensive calendar that highlights risk analysis events across organizations and the Newsletter.

The Clearinghouse made several additions to its exclusive holdings. The web statistics show that some of these new additions were in the top 50 of 4,089 requested URLS.

Examples of new additions:

- Cost of Restrictions on Gulf Oyster Harvesting for Control of *Vibrio vulnificus*-caused Disease

  Report of a study conducted by the Research Triangle Institute (RTI) for the Food and Drug Administration (FDA) on the costs of various control plans for *Vibrio vulnificus* in Gulf of Mexico oysters. Data are provided in tables and charts on oyster harvests, oysters bound for raw consumption, number of harvesters, processing plants, and shippers, baseline prices, and more

- Dietary Supplement Sales Database (DSPD)

A survey conducted by Research Triangle Institute (RTI) under contract with FDA. The access database contains labeling and catalogue information of approximately 3,000 dietary supplement products sold in the United States. The database provides information on the range of products available for sale in the United States, where these products are sold, what they contain, and what claims are being made about these products. Also available are the final report of the project, which includes information on the procedures used and a description of the database, and a supplement to the final report, which includes detailed records from the data collection process.

- Food Testing Laboratory Database (FTLD)

  A survey conducted by Research Triangle Institute (RTI) under contract with FDA. The access database contains 546 records of private laboratories that test food. It includes the variables needed to support the kinds of analyses FDA expects to undertake - such as location and contact information, economic variables, test capabilities, and Quality Assurance.

- FARETM- Food Analysis and Residue Evaluation ProgramTM

  FARETM is modular software for performing probabilistic microbial risk assessment. It was developed by Exponent (formerly Novigen Sciences, Inc.) at the request of and in conjunction with the US Food and Drug Administration (FDA). Although it uses algorithms developed by FDA for the *L. monocytogenes* risk assessment, FARETM- Microbial is capable of performing risk assessments for a wide variety of food borne pathogens. This program and instructions manual are available to download free of charge from "Tools" section of the Clearinghouse.

FAO/WHO Acrylamide in Food Network

JIFSAN has accepted an invitation from WHO and FAO to operate the FAO/WHO Acrylamide in Food Network. This is currently in development and will be operated through the Clearinghouse. The network will function as a global resource and inventory of ongoing research on acrylamide in food. Additional information is included in the **Future Plans (2002-2003)** section.

Education and Outreach Programs

The establishment of education and outreach programs, with both domestic and international emphases, 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 Participation in Exhibitions: During the past year, JIFSAN staffed a booth (display) at:

- The Second IFT International Food Safety and Quality Conference and Expo in Atlanta, Georgia, February 15-17, 2002.
- Second Annual University of Maryland Business and Technology Mixer, University of Maryland, May 20, 2002.
- International Association for Food Protection (IAFP) 89th Annual Meeting, San Diego, California, June 2002.
- Thinking Globally – Working Locally: A Conference on Food Safety Education in Orlando, Florida, September 18-20.

JIFSAN at the FDA Science Forum: JIFSAN staffed a booth at the Annual FDA Science Forum held February 15-16, 2002 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.

Training Programs and Courses:

Food Safety Risk Analysis Professional Development Training Program

The JIFSAN Food Safety Risk Analysis Professional Development Training Program is being developed to address needs related to training in risk analysis in federal agencies, state governments, academia, industry, trade and consumer groups, and economic, legislative and legal professionals. Recently developed and piloted Core Courses (Overview of Risk Analysis, Introduction to Food Safety Risk Assessment, Introduction to Food Safety Risk Communication, Introduction to Food Safety Risk Management) and Intermediate Courses (Quantitative Risk Assessment, Introduction to Economics for Risk Analysis) were offered at the University of Maryland during the period January through August 2002. Several of the courses were offered more than once. They are taught by experts from FDA, UM, other universities, and the private sector. Approximately 266 participants were involved from industry and government agencies with the largest enrollment being from government agencies. Participants also came from Canada, U.K., and Australia.

Work continued on converting selected courses developed for the JIFSAN Professional Development Training Program in Food Safety Risk Analysis, and given in a traditional classroom setting, into a distance delivery format. This is being done through a three-year ($320,000) grant to the College of Agriculture and Natural Resources, University of Maryland, in collaboration with FDA/CFSAN staff. This program seeks to utilize new and emerging information

technologies to train food safety professionals in the fundamentals of risk analysis.

Conferences and Workshops:

First International Microbial Risk Assessment Conference:

JIFSAN and its Food Safety Risk Analysis Clearinghouse, WHO, the Society for Risk Analysis, members of the Food Safety Risk Assessment Consortium, FAO, Food Safety Research Information Office (USDA), and the Joint Institute for Food Safety Research (JIFSR) jointly sponsored the First International Microbial Risk Assessment Conference at the Inn and Conference Center, University of Maryland University College, July 24–26, 2002. The goals of this conference were to develop a network of key professionals worldwide in microbiological food safety risk assessment, to provide a forum for exchange of new concepts and advances in the field, and to evaluate the current status and future needs and directions of this area. The conference was attended by 189 participants from 17 countries. Proceedings of the conference are to be published.

Seminars Co-sponsored by JIFSAN:

1. JIFSAN cosponsored a Current Issues in Food Safety seminar in January 2002. Approximately 100 individuals from the Montgomery Co. Dept. of Health & Human Services were updated on food safety issues such as biotechnology, bioterrorism, BSE /CJD/CWD and FMD, and microbial pathogens and foodborne disease. These individuals were primarily inspectors for restaurants and front-line people inspecting food establishments for safe and sanitary handling of food. Participants commented that it was one of the best seminars they had attended, and they looked forward to similar seminars in the future.

2. A series of JIFSAN seminars were planned and scheduled by JIFSAN and the CFSAN Staff College at the Harvey Wiley Building (FDA) in College Park, Maryland. These included;

    - "A Model-based Risk Assessment Methodology for Food Safety with Applications to E. coli 0157:H7 in Cheeses,: Mohammad Modarres, Director, Center for Reliability Engineering and Co-Director, Center for Technology Risk Studies, Department of Material and Nuclear Engineering, University of Maryland (April 3, 2002)

    - "Carotenoids in the Prevention of Cancer and Macular Degeneration," Frederick Khachik, Department of Chemistry and Biochemistry, University of Maryland (April 17, 2002)

- "Antimicrobial Resistance of Shiga Toxin-Producing *E. coli* and *Campylobacter*," Jianhong Meng, Department of Nutrition and Food Science, University of Maryland (May 1, 2002)

- "The Effect of Iron Supplementation on Zinc Absorption During Early Lactation," Carolyn Chung, Graduate Program in Nutrition, Department of Nutrition and Food Science, University of Maryland (May 15, 2002)

- "Host-Microbial Pathogen Interaction on the Mucosal Surface;" Wenxia Song, Department of Cell Biology and Molecular Genetics Microbiology Building, University of Maryland College Park, MD (May 29, 2002)

- "Examination of Antibiotic Resistance in Enterococci spp. Isolated from Poultry in the Mid Atlantic Region," Lewis E. Carr, Department of Biological Resources Engineering, University of Maryland (June 26, 2002)

- "GMOs: United Kingdom's Approach to Science and Trade Issues," John Dennis, Head, Food Safety and Quality Central Sciences Laboratory, York England (July 8, 2002)

- "Qualifications of Bacterial Biofilms," Paul D. Schreuders, Department of Biological Resources Engineering, University of Maryland, College Park (July 10, 2002)

- "Microfluidics-Based Bioanalytical Tools for High Throughput Drug Screening and Genomic/Proteomic Analyses," Cheng Lee, Department of Chemistry and Biochemistry, University of Maryland, College Park (July 24, 2002)

- "Determinants of Inspections and Compliance in FDA's Seafood Processing HACCP," Eric Lichtenberg, Department of Agricultural and Resource Economics (AREC), University of Maryland, College Park (September 11, 2002)

Meetings Co-sponsored by JIFSAN:

1. Workshop on Biotechnology-derived Nutritious Foods for Developing Countries: Needs, Opportunities, and Barriers

   A workshop on Biotechnology-derived Nutritious Foods for Developing Countries was co-sponsored by the International Life Sciences Institute North America (ILSI NA) Human Nutrition Institute (HNI), the ILSI International Food Biotechnology Committee (IFBiC), the International Food Policy Research Institute (IFPRI), and JIFSAN. The workshop was held January 15-17, 2002, in Cancun, Mexico. A select group of international experts in nutrition and biotechnology reviewed the current scientific information on nutritional needs in

developing countries and food-based approaches, including biotechnology, which could be used to address these problems. The objectives were to identify nutritional needs that could be effectively met through biotechnology-derived foods; to identify opportunities and areas in which the application of the techniques of biotechnology can benefit nutritional needs; to identify products under development that would meet these needs; to identify opportunities/barriers to development of such products and their acceptance; to identify next steps – research, technology transfer, information dissemination, and/or additional workshop discussions and expert panel deliberations. The proceedings of this workshop are to be published.

2. Ceres Executive Leadership Seminar in Food Safety

   - Module 2 - Laws, Regulations, and Ethics, Niagara Falls, Ontario, September 30 - October 5, 2001.
   - Module 3 – The Food Chain, Los Andes, Chile, April 13-19, 2002.
   - Module 4 – Opportunities and Institutions, Trinidad and Tobago, September 14-20, 2002.

   This seminar series was comprised of four modules given in different locations over a period of two years. All four were co-sponsored by JIFSAN. The objective of this program is 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 participated in the seminar series. Each had a mentor and a two-year project dealing with a difficult food safety problem in which the individual is involved or interested in solving.

3. Ceres Roundtable - Acrylamide: Lessons Learned, Plans Ahead

   JIFSAN served as a cosponsor for the Ceres Roundtable on Acrylamide: Lessons Learned, Plans Ahead held at the Center for Nutrition and Food Policy (CFNP), Virginia Tech University offices in Alexandria, VA on September 9, 2002. The objective of this Roundtable was to look at the issues of acrylamide and compare/contrast these with previous food safety issues such as nitrosamines to determine if there were scientific similarities and lessons to be learned relative to appropriate responses and knowledge needs. This involved a small group of experts examining the issues with other participants entering into the discussions. A publication is to result from this Roundtable.

4. National Capital Area Chapter of the Society of Toxicology

   JIFSAN was a co-sponsor of a meeting of the National Capital Area Chapter of the Society of Toxicology held in May 2002 at the Stamp Student Union, University of Maryland in College Park. More than 110 people from government

agencies, the private sector and local universities, including the University of Maryland, attended the symposium.

JIFSAN was a co-sponsor of the National Capital Area Chapter of the Society of Toxicology 2002 Fall Symposium "Current Issues and Strategies for Food Safety" held in Washington on December 6, 2001. The workshop was an important step in facilitating discussion between scientists with a wide range of experience to bring forward various perspectives on current issues related to ensuring the safety of our food supply.

International Cooperation and Training Programs:

1. International Good Agricultural Practices (GAPs) Training Program "Enhancing the Safety of Fresh Produce at the Source through Good Agricultural Practices"

This five-day training program, co-sponsored by JIFSAN and the FDA, was offered to Cooperative Extension Specialists at the University of Puerto Rico, Mayaguez Campus, March 2002; in the Dominican Republic, July 2002 for which two of the individuals trained in Puerto Rico participated in the instruction; and in Mexico, August 2002. These train-the-trainer programs involved approximately 50 individuals each and are taught by a team of six to eight experts from FDA CFSAN, University of Maryland, Clemson University, and Mississippi State University. The program emphasis is on applying good agricultural practices (GAPs) and Good Management Practices (GMPs) to the production of raw fruits and vegetables with reduced microbial and chemical loads. Evaluations by participants indicated that the training programs were successful in conveying information needed to improve the safety of fresh fruits and vegetables at the source of production prior to export to the U.S.

A training manual "Improving the Quality and Safety of Fresh Fruit and Vegetables: A Training Manual for Trainers" was completed through a sub-contract to the University of Arkansas. English and Spanish versions of the manual are posted on the JIFSAN website (http://www.jifsan.umd.edu) for use by others involved in similar training programs throughout the world.

2. Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Rapid Diagnostic Methods in Food Safety

As part of a cooperative agreement with the Central Science Laboratory (CSL), Department for Environment, Food, and Rural Affairs (UK), an annual Joint Symposium on Food Safety and Nutrition, with a different topic selected each year, alternates between York, UK and College Park, MD. The Third Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Rapid Diagnostic Methods in Food Safety was held at the Central Science Laboratory, Sand Hutton, York, UK, June 26–28, 2002. For the first time the symposium was held in collaboration with the Joint Research Centre (EU,

Ispra, Italy) and the Food Standards Agency (UK). The symposium attracted approximately 140 individuals, 20 poster presentations, and 10 exhibitors from 18-20 countries.

3. International Workshop on Mycotoxins

The FDA and JIFSAN organized and cosponsored an International Workshop on Mycotoxins, July 22–26, 2002 at the Center for Food Safety and Applied Nutrition (CFSAN) in College Park, Maryland. Other cosponsors included FAO, IAEA, USDA, WHO, UNEP, OICD, CSL-UK, PAHO, ITP, EC, and AOAC. There were 122 registrants from 45 countries. The long-term objective of this workshop is to reduce human and animal exposure to mycotoxins through (a) increased awareness of health risks associated with mycotoxin contamination, (b) accessibility to training and detection methods, (c) knowledge of conditions leading to mycotoxin formation, (d) regulation and monitoring programs and (e) compliance with international trade standards. The scope of this five-year project includes the development of training materials in a format that will allow for the conduct of this training, initially in the United States and subsequently at satellite regional training locations worldwide.

4. Development of International Collaborations

The Director visited with Dr. Robert Premier and colleagues in the Department of Natural Resources and Environment, State of Victoria, (Melbourne, Australia), July 9th, to continue development of a collaborative research project. A proposal from Dr. Jianghong Meng, Department of Nutrition and Food Science, UM was discussed and further developed. This would involve the application and evaluation of rapid methods for detecting pathogenic organisms in fresh produce. This may become part of an Australian investigation into following the microbiological bacterial levels for a number of vegetable lines from farm to market.

The Director accompanied Dr. Thomas Fretz (Dean, College of Agriculture and Natural Resources, UM) and Dr. Saul Sosnowski (Director, International Programs, UM) to the University of Costa Rica, August 19-23. Discussions were held concerning opportunities to develop cooperative efforts between the two institutions. This included discussions between Dr. Lineback and faculty in food science concerning potential areas for cooperation. He also discussed two projects related to the International Training Program in Good Agricultural Practices being done under sub-contract by JIFSAN to the University of Costa Rica.

Over the past couple of years, JIFSAN has conducted discussions with personnel from the Canadian Institute for Food Inspection and Regulation (CIFIR). This Institute is in its third year of operation and is similar to

JIFSAN. It is a collaborative program between the University of Guelph and the Canadian Food Inspection Agency (CFIA). Personnel from CIFIR have visited JIFSAN twice and a recent meeting between the two Institutes occurred in Guelph (September 24, 2002). This involved Dr. Lineback, Director; Dr. Paul Mazzocchi (UM), Associate Director; Dr. Arthur Miller (FDA, CFSAN), Associate Director; Dr. Elizabeth Calvey (FDA, CFSAN), Deputy Associate Director; and Dr. David Lei (Head, Department of Nutrition and Food Science, UM) from JIFSAN; and Dr. Jim Pettit, Director; Ms. Joan Wakeman, CFIA Regulatory Chair; and colleagues from CIFIR and the University of Guelph. Conference calls are held on a rather regular basis between the Director of JIFSAN and the two CIFIR leaders. Discussions focus on operational issues encountered in establishing and operating CIFIR and JIFSAN. A verbal agreement was reached at the September meeting to explore the possibility of developing a Memorandum of Agreement for cooperation between JIFSAN and CFIA with one goal being to establish an annual joint symposium in an area of mutual interest alternating between Canada and the U.S.

Other Activities:

FDA/Maryland Day

The Center for Food Safety and Applied Nutrition (CFSAN), FDA moved to a new facility adjacent to the University of Maryland at College Park campus beginning in October 2001. FDA staff will now have increased access to facilities at the UM. To facilitate this move, assist FDA staff in becoming more familiar with the UM campus and facilities, and to further strengthen the partnership, an FDA/Maryland Day was held November 3, 2001. Tours of the campus and UM facilities occurred with FDA staff being guests of the UM at an afternoon football game.

Senior Seminar in Public Relations (Comm 483) Class Project

Through contacts and arrangements made by the CFSAN/FDA Liaison Staff, Dr. Katherine McComas' (UM) Senior Seminar in Public Relations class, as their semester project, developed a public relations campaign designed to promote CFSAN and JIFSAN to the UM College Park. The class conducted a research study that provided basic information for campaigns. Members of the class worked as teams to develop four proposals that included a situational analysis, a program outline, and prototype public relations materials that would be used in the proposed program. These were presented to CFSAN and JIFSAN in an oral report session and in written format at the end of the Spring 2002 semester (May 2002).

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 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-eight (28) different University of Maryland students have interned (65 internships) in CFSAN or CVM laboratories during the last year (September 2001 – August 2002). Students who volunteered for at least one semester were given the opportunity to apply for a paid JIFSAN student position for subsequent semesters. Twenty-two (22) different students were paid for a total of 43 internships (semesters). The website listing internships is at http://www.jifsan.umd/internship.htm.

Participation in the JIFSAN Internship Program continues to grow. Dr. Kaci Thompson of the UM Information Resource Center (College of Life Sciences) has worked to increase student, faculty, and staff awareness of the Program through the literature, seminar series, and the annual Internship Day which the Center sponsors. JIFSAN has actively and regularly participated in the Internship Day. One of the strongest features of the Program is the continuing collaborative spirit among the participants.

Future Plans (2002-2003):

- Continue efforts to expand the JIFSAN internship program and to increase the number of competitive positions available. Expansion of internship opportunities to include non-laboratory projects is planned.

- 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 tentatively planned for Mexico, Peru (Andean countries) and one other location during 2002-2003. A formal evaluation of the training program will be conducted.

- Continue internal grants program. Fund approximately five new project proposals effective July 1, 2003.

- 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:

  Fourth Annual CSL/JIFSAN Joint Symposium in Food Safety and Nutrition: Risk Analysis, June 11-13, 2003 (tentative title) at the Inn and Conference Center, University of Maryland University College, Adelphi, Maryland.

- Continue development of Risk Analysis Clearinghouse with emphasis on the inclusion of additional risk assessments, data, and related information of value to risk assessors.

  Data, obtained through a sub-contract to the National Food Processors Association (NFPA), on the prevalence and quantities of *Listeria monocytogenes* in retail samples of smoked seafood, prepared seafood salads, bagged salads, Hispanic-style soft cheese, and blue veined and soft mold-ripened cheeses will be entered into the Clearinghouse data base. Additionally, data from a larger study in other ready-to-eat foods, sponsored by USDA CSREES and several industry partners and used by NFPA for a risk assessment that is now completed, may be available to be included.

- JIFSAN has been requested to operate the FAO/WHO Acrylamide in Food Network (redesignated as Acrylamide Infonet in November 2002) and has accepted this responsibility. This arose as one of the recommendations from the FAO/WHO Expert Consultation on Acylamide in Food, June 25-27, 2002 in Geneva. The network will function as a global resource and inventory of ongoing research on acrylamide in food. It will encompass formal research, surveillance/monitoring, and industry investigations. The network's aim is to allow all interested parties to share relevant data as well as information on ongoing investigations. It is also designed to serve as a discussion forum for active researchers and others in the field. The operation of the network will be done through JIFSAN's Food Safety Risk Analysis Clearinghouse.

- Continue development and offerings of the courses comprising the JIFSAN Professional Development Program in Food Safety Risk Analysis.

- Continue development of international contacts and collaborative programs.

  Dr. Renata Leuschner (CSL, DEFRA, UK) will participate as a Visiting Scientist at FDA CFSAN from October 7-31, 2002. She will collaborate with Drs. Steven Musser and Wallace Andrews at CFSAN and interact

with Dr. Catherine Fenselau (UM) in evaluating the use of mass spectrometry to differentiate strains of Salmonella.

- Continue development of a Memorandum of Agreement for cooperation with the Canadian Institute for Food Inspection and Regulation (CIFIR), University of Guelph. This will include planning of the First Joint CIFIR/JIFSAN Symposium tentatively scheduled for September 2002 in Guelph in the area of food safety.

- Continue development of a cooperative research project with the Department of Natural Resources and Environment (DNRE), State of Victoria, Australia. This will be in the area of bacterial contamination of fresh produce. Plans are for Dr. Jianghong Meng to visit Dr. Robert Premier and colleagues at DNRE in Melbourne in January 2003 to continue discussions and finalize development of the project.

- JIFSAN has been part of and facilitated coordination of an ad hoc Acrylamide Working Group; composed of food industry, trade association, academic and government representatives in discussions concerning issues raised by reports of the occurrence of acrylamide in food products. JIFSAN and the National Center of Food Safety and Technology (IIT/FDA, Argo, IL) are organizing a workshop entitled "Acrylamide in Food: Scientific Issues, Uncertainties, and Research Strategies" to be held October 28-30, 2002 at the O'Hare Ramada Plaza Hotel in Chicago. Approximately 170 invited experts will participate in discussions in five subject areas (Working Groups) to identify data gaps in the scientific knowledge underlying the occurrence of acrylamide in a wide variety of foods. The workshop will focus on science and identify research needs that can be included in a coordinated research agenda.

APPENDIX A

Projects Funded Through JIFSAN Competitive Internal Research Program

Third year projects (completing January 2002):

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

  Results from this research project were cited in last year's annual report.

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

  Results from this research project were cited in last year's annual report.

Second year projects (funded in January 2000):

- **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 the risk of *Escherichia coli* O157:H7 contamination in rennet-coagulated cheese, in particular hard cheese (cheddar). A secondary objective is to develop a model that also supports regulatory decision-making and establishes a formal and systematic way to define the needs for additional research.

  To evaluate the risk of *E. coli* O157:H7 in the cheese-making process, this research has used probabilistic model-based tools. Significant goals include determining risk significant activities or events, finding best control strategies, defining areas that need more data in order to improve risk estimates and reduce uncertainties, and calculating expected societal impacts due to exposure to *E. coli* O157:H7.

  A number of probabilistic model-based tools and techniques used in various other engineering disciplines are being adapted for research in food safety applications, i.e. foodborne pathogens in cheeses. A food safety risk assessment involves a number of different steps: 1. screening to determine whether the risks from a particular microbial foodborne hazard has reached a threshold of concern; 2. estimating the frequency of occurrence of a particular hazard and its magnitude occurring at a specific

location or locations along the farm to fork path (public health); 3. determining risk-significant contributors to develop control mechanisms and define needs for more data; and, 4. providing the results of the risk assessment to regulatory decision makers.

The probabilistic risk assessment model to study the risk of *E. coli* O157:H7 contamination in hard cheese is being developed using a risk-modeling concept called Master Logic Diagram (MLD); this modeling process has been limited to rennet-coagulated cheeses. A high-level MLD model has been developed that includes three steps: production, distribution, and consumption. Then each of these stages has been divided into successively more abstract steps, and each step will then be modeled taking into account the most critical variables.

Previous work focused on gathering literature on *E. coli* contamination in cheese. This information has been reviewed and incorporated into the production phase of the risk model. A database for assessing the statistical growth behavior of *E. coli* at each step in the cheese-making process in the production phase has been completed. This database focuses almost exclusively on *E. coli* O157:H7; however, due to the limited availability of data for this specific strain, some generic data from other strains have also been incorporated. Significant effort has been placed on understanding the literature and the modeling process used for this phase.

Current work has also focused on gathering literature relevant to the distribution and consumption phases. Much data has been found concerning the times and temperatures of storage during the distribution phase. Various equations and models have been investigated to model the steps included in this phase, including the Gompertz, Baranyi, and three-phase linear models. One of the more promising options for modeling the distribution phase may be the Gompertz equation, which predicts microbial concentration at a given time. However, the Gompertz model tends to overestimate the maximum population density and only models microbial concentration based on time. It is obvious that *E. coli* concentration is affected not only by time, but also by temperature and pH. Current research is focusing on how to improve upon this equation to more accurately model the distribution phase.

Research has also focused on dose-response models for the consumption phase. The literature reveals several dose-response models, including: Log-logistic, Log-normal, Simple exponential, Flexible exponential, Beta-poisson, and Weibull-gamma. It appears that the Beta-poisson function may be the most promising model to perform the dose-response analysis, as it is most frequently used for foodborne pathogens. This functional form assumes that a single organism is capable of infecting and inciting

illness in an individual; in addition, this model also assumes that organisms operate independently within the host.

It is challenging to model *E. coli* O157:H7, as there is no human clinical trial data available. In order to model *E. coli* O157:H7, a dose-response envelope method has been utilized from previous researchers' work. This envelope method characterizes the uncertainty about the probability of symptomatic illness at an ingested dose level based on bounding estimates. The upper and lower boundaries use data from surrogate pathogens that share some of the virulence factors exhibited by *E. coli* O157:H7. Enteropathogenic *E. coli* (EPEC) and *Shigella dysenteriae* represent the lower and upper bounds, respectively. This method results in upper and lower boundaries that envelop the E. coli O157:H7 dose-response function.

Current work is focusing on recreating this envelope model with the addition of more data to update the upper and lower boundaries. Research is also focusing on finding other surrogate pathogens that may provide better bounds. In addition, further research may reveal that the envelop method could be applied to one of the other dose-response models and provide an even better estimate of the probability of illness at a given ingested dose level.

Finally, data on the annual consumption of various cheeses has been collected, as well as information concerning statistics on the various public health outcomes resulting from illness due to *E. coli* O157:H7. Also, data concerning the number of persons in various susceptible populations has been collected.

Future goals for this project include integrating all of the information and data collected for distribution and consumption phases into the previous work (production phase). The basic steps have been outlined for these two phases, and models have been found in the literature for these various steps. For example, the information gathered on the Gompertz equation and envelope-method for dose-response modeling will be starting points to model the distribution and consumption phases; however, each of these models have the potential for being improved upon to model the various steps even more accurately. The final step will be tying all of the steps together in each phase, and finally integrating all of the phases into one complete model.

- **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* other than serotype O157 isolated from animals, humans, and food.

     As discussed in last year's progress report, antimicrobial resistance was widespread among *E.* coli isolates of "serotype" O157. Similar results were found for isolates of serotypes O26, O103, O111, O128, and O145. Strains of these serotypes have been implicated in infections with Shiga toxin-producing *E. coli* (STEC). Approximately 50% of the 137 isolates from humans were resistant to ampicillin, sulfamethoxazole, cephalothin, tetracycline, or streptomycin, and approximately 25% were resistant to choramphenicol, trimethoprim-sulfamethoxazole, or amoxicillin-clavulanic acid. Approximately 50% of the 534 isolates recovered from food animals were resistant to sulfamethoxazole, tetracycline or streptomycin. Of 195 isolates that possessed Shiga toxin–related virulence genes, approximately 40% were resistant to sufamethoxazole, tetracycline, or streptomycin. The data indicated that antimicrobial resistance in *E. coli* was likely the result of using tetracyclines, sulfa drugs, cephalosporins, and penicillins in clinical medicine and food animal production.

  2. Antimicrobial-resistant *Campylobacter* isolated from retail raw meats

     Campylobacter is the most common cause of foodborne gastroenteritis. Resistance to antimicrobials used for the treatment of campylobacteriosis has increased. However, until recently, no method for antimicrobial susceptibility testing has been standardized. Thus, it is difficult to compare results from various studies due to a large number of testing variables as well as different interpretive criteria used. Experiments were conducted to compare Etest and agar dilution methods for antimicrobial susceptibility testing of *Campylobacter*. The data demonstrated that MICs (Minimum Inhibitory Concentrations) obtained by Etest were not in complete agreement with MICs generated by agar dilution. Although Etest has proven to be a satisfactory testing method, its use for *Campylobacter* susceptibility testing requires further standardization for certain antimicrobial agents. This study has contributed to the NCCLS guidelines for the standard susceptibility testing method for *Campylobacter*.

     Also, antimicrobial susceptibility of 378 *Campylobacter* isolates from retail meats was examined. The most common resistance among the *Campylobacter* was to tetracycline (82%), followed by doxycycline

(77%), erythromycin (54%), nalidixic acid (41%), and ciprofloxacin (35%). *Campylobacter* resistant to antimicrobial agents used for treating human campylobacteriosis are common in some retail meats. The high prevalence of resistant isolates on retail chicken and turkey meats suggests that *Campylobacter* foodborne illness acquired from these meat types may be less responsive to anti-infective therapy. Efforts to maintain the effectiveness of these drugs must address the public health consequences of their use in agriculture.

Publications and presentations:

1. Schroeder, C.M., C. Zhao, C. DebRoy, J. Torcolini, S. Zhao, D.G. White, D.D. Wagner, P.F. McDermott, R.D. Walker, and J. Meng. 2002. Antimicrobial resistance of *Escherichia coli* O157 isolated from humans, cattle, swine, and food. Applied and Environmental Microbiology 68:576-581.

2. Ge, B., S. Bodies, R.D. Walker, D.G. White, S. Zhao, P.B. McDermott, and J. Meng. 2002. Comparison of Etest and agar dilution for antimocrobial susceptibility testing of *Campylobacter* isolated from retail meats. J. Antimicrobial Chemotherapy 50:487-494.

3. Schroeder, C.M., J. Meng, S. Zhao, C. DebRoy, J. Torcolini, C. Zhao, P.F. McDermott, D.D. Wagner, R.D. Walker, and D. G. White. 2002. Antimicrobial resistance of *Escherichia coli* O26, O101, O111, O128, and O145 from animals and man. Emerging Infectious Diseases 8: (in press)

4. Ge, B., D.G. White, S. Zhao, P.F. McDermott, R.D. Walker, and J. Meng. 2002. Antimicrobial-resistant *Campylobacter* isolated from retail raw meats. 102nd Annual Meeting Am. Soc. Microbiol. Salt Lake City, UT.

- **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 project is nearing the completion of its third (and final) year. The project was intended to examine the relationship between bacterial biofilms and the substrate on which they were grown.

Current Results

Results for *E. coli* indicate that cleaning of a food-processing material should be performed within short time intervals, 3-6 hours. Biofilms at this time are small, and tend to be reversibly attached. Therefore, they are fairly easy to remove. Brushing the material should be avoided, since it may cause scratches that protect future biofilms from removal. Since biofilms do not grow well on polyethylene or glass, these materials are recommended for use. In case stainless steel is the only choice to be used, polished stainless steel, or brushed to a surface roughness of 2B, should be used. Biofilms grow to large values within 12 hours on polished stainless steel and thereafter detach, while they grow slower and detach at a later stage with an increase in roughness. Excessive roughness, such as that due to machining with a flycutter, should be avoided since biofilms grow to large sizes, and are resistant to detachment.

Ultrasound is a good means to remove young biofilms, particularly in combination with 200 ppm chlorine that both contributes to detachment and kills the biofilms. Applying the treatments one after another increases the risk of recovery and regrowth of the biofilm, especially when cleaning scribed surfaces. When establishing a HACCP plan, investigating the food-processing material for damages, such as scratches, should be included. In addition, special precaution should be taken in the interface between two materials since biofilms tend to increase in size differently depending on material.

Continuing Research

The research in this project is continuing by examining the interactions of the bacteria and plant materials. Specifically, we are examining the growth of *E. coli* on lettuce leaves. Unlike the man-made materials, foodstuffs are not flat. Therefore, we are performing 3-dimensional reconstruction of the surface of the leaf and the bacterial patches using confocal laser scanning microscopy.

Other Outcomes

This research has contributed to the support of four graduate students. They are

Andrea Lomander	PhD	2002
Ning Yang	MS	2001
Yingli Fu	Current Doctoral Student	
Sang Jun Lee	Current Masters Student	

In addition, two undergraduate capstone design projects have been generated by this Project.

Publications:

Lomander, A., Schreuders, P.D., Russek-Cohen, E., and Ali, L., "A Method for Rapid Analysis of Biofilm Morphology and Coverage on Glass and Polished and Brushed Stainless Steel," *Transactions of the ASAE*, 45 (2), 479-487.

Presentations (based on results from this project):

1. Lomander A. **Schreuders P.D.**, and Ali, L.H., "The Development of Methods for the Evaluation and Removal of Bacterial Biofilms," Northeast Agricultural/ Biological Engineering Conference, Lancaster, PA, August 1-4, 1999.

2. Lomander A., **Schreuders P.D.**, and Ali, L.H., "Analytical Techniques for the Evaluation of Biofilms on Food Processing Materials," Northeast Agricultural/ Biological Engineering Conference, Ithaca, NY, July 30 – August 2, 2000.

3. Lomander, A., and **Schreuders, P.D.**, "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. **Schreuders, P.D.** and Yang, N., "Multifluorophore Image Analysis," Northeast Agricultural/ Biological Engineering Conference, Guelph, ON, Canada, July 8-11, 2001.

5. Ali, L.H. and **Schreuders, P.D.** "Developing Collaborative Efforts between the Food and Drug Agency and the University of Maryland," Washington, DC, December 12, 2001.

6. **Schreuders, P.D.**, Lomander A., and Ali, L. "Quantification of Bacterial Biofilms," Food and Drug Administration, College Park, MD, July 10, 2002.

7. **Schreuders, P.D.**, Lomander, A., and Ali, L.H., "Morphological Responses by Biofilms to Surface Defects Created in Stainless Steel," Annual Meeting of the Institute for Biological Engineering, Baton Rouge, LA, 2002

8. Lomander, A., Ali, L.H., and **Schreuders, P.D.**, "A Method for Rapid Analysis of Biofilms on Glass and on Polished and Brushed Stainless Steel,: 2002 FDA Science Forum, Washington, DC, February 20-21, 2002.

9. Lee, S.J., **Schreuders, P.D.**, Lomander, A., and Russek-Cohen, E. "Quantification of Biofilm Structure on Different Surfaces and After

Treatment with Different Sanitizers," Annual Meeting of the Institute for Biological Engineering, Athens, GA, 2003.

10. Fu, Y and **Schreuders, P.D.**, "Confocal Imaging and 3D Image Reconstruction of Microbial Biofilms on Lettuce," Annual Meeting of the Institute for Biological Engineering, Athens, GA, 2003.

Second-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).

  Analysis of the data documenting inspection and compliance with HACCP of seafood processing plants from the onset of the HACCP requirements to 2002 is continuing. The goals of this analysis are twofold: (1) to study the criteria implicitly used by FDA for setting inspection priorities and (2) to study the influence of inspection on compliance with the HACCP regulation. The criteria implicitly used to set inspection priorities are of interest in light of criticisms of the seafood HACCP program raised by the General Accounting Office and in light of FDA's own stated priorities. The influence of inspection on compliance is of interest for determining the effectiveness of FDA's implementation of the HACCP regulation.

  Initial analyses have been based on an aggregate definition of inspection and compliance status. Specifically, a panel dataset has been created that follows all plants from 1998 to 2002, with observations taken at annual intervals. An indicator (INSPECT) has also been created that takes a value of one if the plant was inspected on account of at least one product or process in that year, and another key variable (HACCPANY) if the plant was found to be in violation with respect to any HACCP requirement (recordkeeping, written plan in place, etc.).

  Based on these aggregate variables, a Markov chain model[1] has been estimated that predicts the probability of an inspection as a function of whether the plant was inspected in the previous year, its compliance status in the previous year, indicators for FDA region or, in alternative specifications, the average number of plants per inspector in that region (a measure of the resources available to FDA in that region), and the kind of establishment (of interest because of criticisms raised by GAO). We also include among the determinants of an inspection the size of the plant, measured by the number of employees, and indicators of whether the plant processes a product identified by FDA as high risk (e.g., histamine-

[1]Our Markov chain model is a logit model of the form $\Pr(Insp_{it} = 1) = \Gamma(a + Insp_{i,t-1}\beta + Compl_{it}\gamma + \mathbf{x}_{it}\delta)$, where $\Gamma(\bullet)$ is the standard logistic cdf.

producing species, aquaculture products, consumer-ready-to-eat products, smoked products, and breaded products).

Attention is restricted to those plants that are required to have HACCP in place, to the years 1998-2001. Plants in US territories are excluded from the analysis, and when a plant is required to have a HACCP plan, but has failed to develop one, this is being treated as an instance of non-compliance.

This statistical model is intended to test a theoretical model of targeting of high-risk plants subject to budget constraints. The results indicate that many of the covariates have statistically significant coefficients, although the overall performance of the model is modest, as the model predicts correctly only 60.66% of the observations. This leads to a conclusion that some targeting exists, that resource constraints exert a significant influence on priority setting, but that perhaps factors that were unable to be observed exert significant influence on FDA priority setting.

At this time, the plant records are being geocoded, so that distances from/to FDA district headquarters and a spatial density of plants can be computed. Including this variable should improve the ability to control for inspector workload and thus agency resource constraints. Models of inspection and compliance, based on a variant of the bivariate probit model, that allow for correlation between inspection and compliance status also are being run. Duration models of the time between inspections also have been estimated. The duration models broadly confirm the findings of the Markov chain models.

Once the aggregate modeling has been completed, all of the aforementioned models will be re-estimated using individual inspection and compliance records instead of aggregate indicators. This procedure should reduce losses in predictive power due to aggregation. The focus will be on products identified by FDA as high risk (and thus high priority): breaded products, histamine-producing species, consumer-ready-to-eat products, smoked products, and aquaculture products.

- **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).

Purpose: The purpose of this project is to provide the initial validation for the use of tissue-fluid correlation data as a tool to be used by food safety personnel to accurately predict whether a particular animal has tissue drug residues which are in violation of FDA-approved tolerances. The following section briefly summarizes the work completed under the various specific aims of the proposal**.**

Experiments: Studies were performed with male Sprague Dawley mice administered a single IP dose of gentamicin. Blood was collected by heart puncture and the following tissues were collected at each time point: muscle, brain, liver, bile, kidney, spleen, heart, lungs, bone marrow, muscle and reproductive organs. In addition, a pharmacokinetic and systemic bioavailability of gentamicin study in young Holstein steers was performed. Blood, urine, ultrafiltrate and muscle biopsy samples were collected at various time points. Following a 2-week washout the animals were dosed IM (4 mg/kg) and samples collected. We have developed an LC-MS/MS method for gentamicin in plasma and urine and are developing a physiological model to predict tissue residue levels using gentamicin as our model "drug".

Significance: Testing for drug residues in edible tissues from food producing animals normally occurs following slaughter. Meat with residues that exceed tolerance is declared adulterated and must be destroyed. Our work is focused on using physiological modeling techniques to determine if easily obtainable biological fluid (saliva, plasma or urine) correlate to drug residues in other tissues (e.g., muscle). *The significance of this work is that if a rapid method could be develop that would sample the biological fluids and directly correlate to edible tissues this could provide for rapid, inexpensive preslaughter screening tests of animals for violative drug residues.* The use of these rapid tests would allow more animals to be monitored to ensure greater food safety. The preslaughter testing would also allow animals to be kept from slaughter until drug residues deplete to safe levels by normal routes of excretion.

- **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 Sherman (following move of Linda Suydam) (FDA)

  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 ensuing FDA decisions. In addition, the degree to which knowledge of the waiver process influences perceptions of impartiality is examined. Finally, 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 is examined. The 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. This project's findings will comprise a scientific foundation on which to base future communication efforts aimed at improving the credibility of, and satisfaction with, FDA policy making.

During the past year, participant observation was conducted at nearly one dozen advisory committee meetings. In addition to becoming familiar with the advisory committee process, field notes were used to generate themes and questions for interviews and surveys. A pilot study of meeting participants also was conducted. Surveys were mailed following one FDA advisory committee meeting to the 47 people who participated, or registered to participate, in the open public hearing portion of the meeting. Twenty-three questionnaires were returned, yielding a 52% adjusted response rate. The questionnaires were designed to gather both closed and open-ended responses to assist in developing the larger questionnaire, which will be disseminated in the second year of the project. Twenty-two (22) in-depth interviews with FDA officials were completed. Officials interviewed included members of the Advisory Committee Council, Advisory Committee Executive Secretaries, and Press and Public Affairs Officers. Each of these interviews was conducted in-person, audio-taped, and at least one hour in duration. Finally, a 10-year content analysis of stories appearing in the *New York Times, Washington Post, USA Today,* and *Los Angeles Times,* written about conflicts of interest in science, was completed.

Presentations:

1. McComas, K.A., and Simone, L.M. Media Coverage of Conflicts of Interest in Science. *Paper presented at the 2002 Association for Education in Journalism and Mass Communication Annual Convention,* Miami, FL, August 7-10.

2. McComas, K.A., and Simone, L.M. Conflict of Interest, Credibility, and Advisory Committee Meetings: A Pilot Study. *Paper to be presented at the 2002 Society for Risk Analysis Annual Meeting, New Orleans, LA,* December 8-11.

- **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).

One major short-term animal experiment has been completed and the long-term chronic exposure study for this project currently is being initiated. Results from the short-term study do not support the hypothesis that curcumin inhibits the development of age-associated pathological changes in rats; however, age significantly affected the reduction of early colonic cancer lesions by curcumin. There was no significant effect of the curcumin diet on the incidence or severity of the following age-related histopathological changes in the AOM-treated rats: chronic progressive nephropathy (CPN); chronic cardiomyopathy (CCM); polyarteritis nodosa (PN); hyperplastic and degenerative liver changes. In all age groups (young, adult, aged), the incidence of rats with thyroidal C-cell hyperplasia was higher in non-curcumin than the incidence in curcumin-fed rats. Similarly, the incidence of young and aged rats with testicular interstitial cell hyperplasia was higher in non-curcumin than the incidence of young and aged rats with testicular interstitial cell hyperplasia in curcumin-treated. However, since the degree of severity of these lesions was higher in the curcumin-fed rats, the biological significance of these trends remains uncertain. Further evaluation of tissues from this study is underway.

Curcumin supplementation resulted in a significant reduction of preneoplastic colonic lesions, aberrant crypt foci, in young and aged rats but had no effect in adult rats. The mechanism(s) for this age-related difference is under investigation. The measurements of expression of cyclooxygenase COX-1 and COX-2, using multiplex RT-PCR and LabChip technology, were established and optimized. Preliminary results suggest that curcumin has little effect on COX2 expression in the kidney regardless of age, but does appear to reduce COX2 expression in the liver of all age groups. Curcumin had little effect on COX-1, as expected.

The laboratories of the principal investigator and the FDA collaborators have given several presentations of the results from this project. Manuscripts of the work completed are now being prepared for publication. Additional studies are in progress.

Presentations:

1. S. Francke-Carroll, J.E. Montgomery, F.A. Hines, and B.A. Magnuson. The effect of a dietary curcumin supplement on the development of histopathological age-associated changes in male azoxymethane-treated F344 rats. Society of Toxicologic Pathology. Denver, Colorado. June 2-6, 2002.

2. Y. Kwon, J. Montgomery, M. Malik and B. Magnuson. Aging alters the inhibition of colonic aberrant crypt foci by curcumin. American Institute for Cancer Research Annual Research Conference, Washington, DC. July 11-12, 2002.

3. M. Malik and B. Magnuson. Semi-quantitative multiplex PCR using LabChip technology. CE in Biotechnology and Pharmaceutical Industries. Practical Applications for Analysis of Proteins, Nucleotides and Small Molecules. August 17-19, 2002 Washington, DC

- **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)

  Objective 1: Determine the antimicrobial effect of lactate on *Listeria monocytogenes* in ready-to eat, smoked fish during refrigerated storage

  We have tested the antimicrobial effect of 2 % PURASAL P Opti.Form 4™ (3.3% of 60% solution, combination of 1.85% potassium lactate and 0.13% sodium diacetate) on *Listeria monocytogenes* inoculated on smoked salmon stored at 4ºC, 10ºC, and –20ºC. The use of 2% Opti.Form 4™ completely inhibited the growth of *L. monocytogenes* at 4ºC for 37 days. It also extended the lag phase up to 9 days and caused a significant delay in the growth of *L. monocytogenes* on smoked salmon stored at 10ºC compared to control. The populations of *L. monocytogenes* declined steadily during storage of –20ºC after 1 month but 2% Opti.Form 4™ further lowered the survival of *L. monocytogenes*. In addition, the resulting growth data on smoked salmon fits more closely to the growth curve of *L.monocytogenes* developed in static broth stored at both 4ºC and 10ºC.

  Objective 2: Develop the model to describe both growth and survival of *L. monocytogenes*.

  The effect of pH on the growth kinetics of *Listeria monocytogenes* Scott A was compared in static versus agitated broths stored at 4°C and 10°C with or without 2% PURASAL P Opti.Form 4™. At pH 5.5, a listeriostatic effect was observed by 2% Opti.Form 4™ at both 4°C and 10°C, despite agitation. At pH 6.0, 2% Opti.Form 4™ addition completely inhibited the growth of *L. monocytogenes* in only static broth stored at 4°C up to 19 days, while 3% Opti.Form 4™ (5% of 60 % solution) is needed to suppress the growth of *L. monocytogenes* in static broth stored at 10ºC during 13 days of storage. At pH 6.5, 2% Opti.Form 4™ addition significantly inhibited the growth of *L. monocytogenes* at both 4°C and 10°C, but the inhibitory effect of Opti.Form 4™ was much greater at 4°C. At pH 7.5, no significant antimicrobial effect of 2% Opti.Form 4™ was observed at both 4°C and 10°C. In this study, we found agitation significantly decreased the lag time and increased the growth rate of *L.monocytogenes* in broth stored at 4°C at all tested pH. A similar, but less obvious, trend was observed in broths stored at 10°C, indicating that agitation in previous growth model with broth could be one factor for some overestimation of microbial growth

when compared to the food. In addition, both pH and storage temperature significantly affect 2 % Opti.Form 4™ effectiveness on the growth of *L.monocytogenes* in broth.

The present data clearly indicate that 2% (3.3% of 60% solution) PURASAL P Opti.Form 4™ can be used to control the growth of *L. monocytogenes* on food products at pH 6.5 or below and can be expected to greatly enhance the safety of refrigerated, ready-to-eat smoked fish. However, strict temperature control, lower than 4ºC is required to maximize an antimicrobial effect of PURASAL P Opti.Form 4™.

Publication/Presentation:

R.A. Barratt, K.S. Yoon and R.C. Whiting. 2002. The effect of pH and agitation on the growth of *Listeria monocytogenes* in brain heart infusion (BHI) broth containing combined potassium lactate and sodium diacetate stored at 4°C and 10°C. 89th IAFP Annual Meeting Abstracts. P:51 San Diego, CA, June 30- July 4

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

  The partial support of JIFSAN for one year and results obtained were used as the basis for obtaining JIFSAN funding for a 3-year project effective July 1, 2002 (see next section).

First-year projects (funded in July 2002):

- **Evaluation of the potential toxicity of soybean isoflavones in development and aging.** Monica Giusti, Mary Ann Ottinger (UM), and Sherry Ferguson (FDA).

  Preliminary experiments have shown that genistein and its metabolites are transferred into the egg yolks of birds receiving genistein supplements, with a double significance:

  1. It demonstrates the potential for isoflavone exposure of embryo during development.
  2. It shows that eggs can be isoflavone carriers and additional sources of isoflavones for the human diet.

  In cases where the effects of isoflavones are desirable, these eggs could be considered of added value, but more evidence is needed to determine if the concentrations accumulated are enough to be of significant biological activity.

This one-year study will evaluate the transfer of soy isoflavones into the egg. In this study, the investigators want to strengthen the evidence for the deposition of genistein and its glucoside derivative into the egg yolk, and expand it to include other isoflavones, simulating the proportions found in the regular diet.

(This is a revision of a proposed three-year project that was suggested by the JIFSAN Research Proposal Review Committee to enable the investigators to gain sufficient data to prepare a proposal emphasizing foods from animals fed soy diets as sources of isoflavones in the human diet, i.e, human exposure from these sources.)

- **Study of nisin and sublancin in a strategy for protection of the United States food supply from pathogenic bacterial spores introduced through bioterrorism.** Norman Hansen (UM) and Laila Ali (FDA).

  This laboratory has recently made a potentially important discovery that may prove useful in strategies to combat the threat of bioterrorist introduction of pathogens into the U.S. food supply. While studying the inhibitory properties of two antimicrobial peptides, nisin and sublancin, it was discovered that a simultaneous combination of the two inhibitors displayed a novel activity that neither possesses when used alone. The novel activity is that spores of the bacterium *Bacillus cereus* are inhibited from undergoing germination. This is surprising, because when either nisin or sublancin is used alone germination occurs, and inhibition results later in the outgrowth process. This novel activity has obvious application to the serious new threat to food safety, which is the intentional introduction of pathogenic bacterial spore formers such as *Bacillus anthracis* and *Clostridium botulinum* into the food supply by bioterrorists. The biological similarity of *B. cereus* spores to these pathogenic spores argues that nisin/sublancin mixtures may provide protection of the food supplies against this kind of terrorist attack. The proposed research is directed toward a more thorough understanding of the inhibitory effect in *B. cereus* spores, which will be employed as a non-pathogenic model of the pathogenic spores of *B. anthracis* and *C. botulinum*. Experiments will determine whether the inhibition of *B. cereus* spores is an irreversible, or "sporicidal" effect, and to elucidate aspects of the target and molecular mechanism of action in the spore. Of particular interest will be the mechanism by which the inhibitory effects of the two agents combine to inhibit spore germination, whereas neither inhibits germination when used alone. The ability of the nisin/sublancin mixture to inhibit spore germination in a model food system will be tested. Whereas the discovery of the nisin/sublancin effect is timely because of the bioterrorist threat, an understanding of this novel mechanism could be broadly useful in

enhancing food safety, because spore-forming bacterial pathogens are a perennial problem.

- **Facilitating needed drug approvals for aquaculture: In vitro metabolic profiles to characterize and predict drug residues in finfish.** Andrew Kane, Renate Reimschuessel (UM) and Badar Shaikh (FDA).

  There is a vital need to expand the repertoire of therapeutic drugs for veterinary use in aquaculture. However, very few drugs are currently approved by the FDA for use in aquaculture species, compared with traditional farm species. In order to facilitate the drug approval process for cultured fish species, it is desirable to establish species of "crop groupings" based on similar drug enzymatic metabolic profiles between species. The metabolic profiles are related to different species' drug excretion rates and tissue residues; these factors determine the appropriateness of the use of certain therapeutic drugs in aquacultured species destined for human consumption. Studies of mammalian drug metabolism *in vitro* are predictive of the fate of a particular drug, and are valuable in demonstrating or confirming that the animal possesses a particular metabolic capacity. The study will use multiple fish species to investigate drug metabolism *in vitro*, determine drug residue patterns *in vivo*, and establish relationships between species-specific *in vitro* metabolic signatures and residue profiles. Both phase I (cytochrome P-450 dependent) and phase II (conjugation) pathways of drug metabolism will be studied in selected freshwater and brackish water aquacultured fish species to ascertain the differences or similarities in their levels and abilities to biotransform model substrates and a model veterinary drug. The drug and metabolite residue profiles determined *in vitro* will be compared with data obtained from *in vivo* experiments. Similarities (or dissimilarities) between species will determine the likelihood of deriving species groupings, based on their metabolic profiles and tissues residues, to foster the approval of needed therapeutics in aquaculture.

- **Moving whole-cell biosensing from a qualitative to quantitative tool: development of a dynamic cell immobilization mechanism.** Y. Martin Lo (UM) and Mahendra Kothary (FDA)

  The goal of this study is to enable quantitative whole-cell biosensing by developing a novel dynamic system for the immobilization of stress-responsive luminous bacteria. To date, many genetically-engineered strains containing selected stress-responsive *E. coli* promoters fused to the *Photohabdus luminescens lux*CDABE reporter have been developed. Use of the five-gene *lux* reporter system allows facile monitoring of gene expression because all components necessary for light production are present in the cell. The bioluminescence reporter has advantageous properties, such as real-time response, excellent sensitivity, and large

dynamic range, because the product of its pathway, light production, can be easily detected. Moreover, not only do the responses of an organism to environmental insult supply instantaneous light signals, they also provide insight into the molecular mechanisms of toxicity because the responses also include repair mechanisms specific for the damage occurred. The responses of this collection were found to be biologically appropriate when stressed by oxidative damage, internal acidification. DNA damage, protein damage, super-stationary phase, and sigma S stress. The pattern of stress-inducible responses has been shown to be capable of yielding a characteristic stress fingerprint specific to the types of damage sustained by the cell, indicating a great potential for detecting and assessing the presence and severity of toxic compounds in food. However, the applicability of these bioluminescent cells is greatly hindered due to lack of control over the total number of cells in a suspending culture, limiting the light signals to a qualitative rather than a quantitative tool. Hence, there is a pressing need for robust and effective procedures that enable rapid incorporation of these or similarly constructed biosensing strains into whole-cell biosensors. A systematic approach of the evaluation of interactions between cells and the supporting matrix, as well as the immobilization capacity of different crosslinking agents, have been developed by the Food Bioprocess Engineering Group. It is anticipated that, via the collaboration with M.H. Kothary who provides outstanding expertise in microbiology, especially in evaluating cell viability and sensitivity, a dynamic cell immobilization mechanism that enables single-layer immobilization of the cell can be developed.

The proposed project consists of three major research stages: (1) establishment of the cell immobilization mechanism, (2) verification of sensing capacity and cell viability, and (3) quantitative analysis of sensing signals.

- **Safety inspection of fresh cut fruits and vegetables using spectral sensing and machine vision techniques.** Yang Tao (UM), Robert Buchanan, Yoonseok Soon (FDA) and Yud-Ren Chen (USDA).

  Fresh fruits and vegetables are an important component of a healthy diet. Recent outbreaks of foodborne illnesses associated with fresh produce have raised concerns about food safety hazards from fresh and fresh-cut fruits and vegetables. According to FDA's *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*, fecal contamination is a major source of human pathogens associated with fresh produce. A detection technique is desired for prompt removal of the potential food safety hazards prior to processing to reduce the risk of cross-contamination. However, no intervention technologies are currently available to effectively detect contaminated and defective produce that may harbor human pathogens. This project will develop optical sensing

techniques, which will be applicable to instantly detect contaminations and defects in fruits and vegetables. The outcome of this project will provide the fresh fruit and vegetable industry with advanced technology to improve the safety of fresh fruit and fresh-cut supplies.

The overall goal of this project is to develop optical sensing and imaging techniques to detect major visible and invisible contaminations and defects. This safety-oriented optical sensor technology will reduce microbial harborage and cross-contamination by detecting and removing potential food safety hazards from the processing and packaging stream.

Specifically, the objectives of this study include:

1. To identify and characterize contaminations and defects on ready-to-eat fresh fruits and vegetables based on spectral sensing;
2. To determine the efficacy of using multi-spectral imaging techniques to identify various fecal contaminations on fresh produce; and
3. To test and validate multi-spectral imaging techniques to detect potentially problematic defects that could harbor pathogens, such as cuts or cracks in fruits and vegetables, where it is difficult for washing water to reach.

- **Influence of pre-harvest antibiotic pesticide treatment of the microflora of apple and pear blossoms, leaves, fruit, and cider and its implications for food safety.** Christopher Walsh (UM), Arthur Miller and S. Brian Eblen (FDA).

The hypothesis that application of antibiotic pesticides alters the native bacterial microflora of preharvest orchard fruit, creating ecological niches for human enteric pathogens will be studied in this project. This orchard management practice may be a factor contributing to the increased foodborne illness associated with consumption of unpasteurized fruit juices. In particular, the focus will be on the effects of bacteriocides that are used commercially by apple and pear growers to control fireblight, caused by the plant pathogen *Erwinia amylovora*. Bacteriocides are used commercially in conventional orchards but are not allowed in organic plantings. While considerable research has been conducted to control this plant pathogen under both management systems, little is known of the effects of these control measures on existing apple and pear orchards at the Western Maryland Research and Education Center in Keedysville. The effects of organic and conventional management schedules on the changes in microflora in apple and pear flowers, leaves, fruits, and juices will be compared. A series of longitudinal field studies over two growing seasons are proposed to monitor the effects of these standard orchard management practices on the microbial

flora in trees, fruits and juices. It is expected that results from this study will serve as the science base for providing food safety guidance to orchard growers and unpasteurized juice processors. This proposal is a leveraging opportunity to add a food safety component to a Maryland Center for AgroEcology grant.

APPENDIX B

Specific Research Initiative

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

Fluoroquinolones were introduced in 1995 for veterinary use in the U.S. and worldwide for treatment of avian colibacillosis caused by *Escherichia coli*. This study was to investigate the genetic determinants responsible for decreased susceptibility to fluoroquinolones observed among *E. coli* isolates from colisepticemia-diseased birds. 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* were determined via broth micro-dilution. Of the 100 isolates, 59% were resistant to nalidixic acid and sarafloxacin, while 34% displayed resistance to difloxacin. Decreased susceptibility to enrofloxacin (9%), ciprofloxacin (3%), and gatifloxacin (1%) was observed. Amplification and DNA sequencing of the quinolone-resistant determining region, revealed that point mutations occurred in the encoding genes: *gyrA* (S83L), *gyrB* (G436A), *parC* (G56C) and *parE* (H497T). Organic solvent tolerance test was performed to screen potential expression of multi-drug resistant efflux pumps. Of the 59 fluoroquinolone-resistant *E. coli*, 12 isolates were tolerant to cyclohexane. 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:

Fourier-Transform Near-Infrared Rapid Determination of Food Integrity, L.E. Rodriguez-Saona (UM), F. S. Fry and E.M. Calvey (FDA,CFSAN)

Rapid and cost-effective techniques for the use by regulatory agencies, the army, and the industry are required in order to increase surveillance. This issue was addressed by investigating the use of Fourier-transform near-infrared (FT-NIR) spectroscopy and multivariate analysis to aid in the implementation of safety monitoring procedures. Support for this project comes from the Combating Terrorism Technology Support Office through an Army Cost-Reimbursable Research Contract to Dr. Elizabeth Calvey (FDA) and Dr. Bruce Jarvis (UM) that was initiated in Agust 1998. This project was funded for a total of three years (1998-2001) with two subsequent no-cost extensions through September 2003. The second no-cost extension provides additional time to prepare the final report for the research sponsors and finalizing manuscripts in progress.

This research has shown that multivariate models, based on FT-NIR spectral data, can be developed for the prediction of castor bean meal (containing the highly cytotoxic protein ricin) contamination in flour-based products. The FT-NIR technology has also been tested for the rapid detection (< 3 min) of sugars in apple and orange juices. The research has had favorable interest by the juice industry, being presented at the Technical Committee for Juice and Juice Products (TCJJP) biannual meeting. In order to test the applicability of the techniques developed, the investigators worked with a major food company in developing models for the detection of surrogate adulterants in flour samples. By combining multivariate analysis and FT-NIR spectroscopy, it was possible to identify samples whose spectra do not match those of a control sample. In addition, the feasibility of the use of FT-NIR for classification, identification, and sub-typing of bacterial contamination in solutions was evaluated. A simple membrane-based procedure to produce a thin bacterial film was developed that has yielded increased sensitivity and allowed the rapid discrimination among closely related strains by principal component analysis.

Detecting Contaminants in Flour and a Flour-Based Product

Methodology was developed and evaluated for the rapid detection of castor bean meal (CBM) in flour and a flour-based product. The method is intended to be a prototype to develop a more general approach to detect food tampering. FT-NIR spectra were obtained by using a diffuse reflection-integrating sphere. Flour spiked with caffeine, crystalline sugar or cornmeal (1-20% w/w) was 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 (MSC) 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, or 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}).

Changes in the residual moisture content of samples can interfere with the predictive ability of the NIR calibration model. Models were developed with samples of wheat flour contaminated with castor bean meal at different water activity levels in order to account for prediction errors due to different moisture levels of the samples. The wheat flour contaminated with castor bean meal (CBM) was placed on petri dishes to maximize the relative humidity equilibration with the salt solutions. Thirty samples containing CBM levels ranging from 0 to 2.2% were placed in a desiccator to accurately control the water activity (a_w) levels. The a_w levels evaluated included ~0.05 (calcium sulfate), 0.2 and 0.4 (potassium acetate), 0.6 (magnesium nitrate), 0.8 and 0.9 (sodium chloride). Cross-validated Partial Least Square (PLS) regression models were generated to quantify the CBM contamination at different a_w levels in a wheat flour matrix. The prediction error (SECV) estimated from the test objects in the calibration set (internal validation) ranged from 0.20 to 0.31% for the individual a_w models. Multivariate analysis gave coefficient of determination (R^2) of >90% and negligible bias effect for wheat flour contaminated with castor bean meal. A model was generated by including the complete data set (260 samples) and gave estimates for SEV of 0.36 and 0.02% and R^2 of 84 and 99.9% for CBM contamination and a_w in wheat flour, respectively. The PLS regression loadings displayed similar absorption than those previously reported for wheat flour contaminated with castor bean meal with bands that correlated with amide modes and residual oil.

The feasibility of the use of the spectral data generated by FT-NIR spectroscopy and multivariate analysis for the detection of adulterants in flour products was evaluated. A major food company provided a blinded set of flour samples (labeled as sample #1, #2, #3, #4 and #5). The samples included a flour control and flour containing two different contaminants at two levels of contamination. FT-NIR spectra were obtained by using the diffuse reflectance-integrating sphere. Prior to calibration, the FT-NIR reflectance data was mean-centered and baseline corrected. Soft Independent Modeling Class Analogy (SIMCA) was able to cluster the different samples, which confirmed that all the samples contained differences in their chemical composition. By looking at the class projections, it was inferred that sample #3 was the flour control and samples #1 and #5 contained the higher levels of the contaminant. To test the hypothesis, mixtures were prepared of the samples #3 with #1and #5, respectively, at 1:1 and 1:4 ratios. Based on the experimental approach, it was possible to deduce that sample #3 was the control, samples #2 and #5 contained the same contaminant at levels of 1% and 5%, respectively, and that samples #4 and #1 contained the same contaminant at levels of 1% and 5%, respectively. By combining multivariate analysis and FT-NIR spectroscopy, samples whose spectra do not match those of a control sample were able to be identified. In order to quantify the level of

contamination, a Partial Least Squares (PLS) Regression model can be constructed and used to predict the levels at which the contaminant is present.

Rapid Detection of Sugars in Juices

A simple analytical procedure for the rapid determination of individual sugars in fruit juices was developed. Different NIR detection devices and sample preparation methods were tested 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. PLS regression was used to create calibration models that were cross-validated (leave-one-out). The prediction ability of the models was evaluated on fruit juices and compared with HPLC and standard enzymatic techniques. The PLS regression 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.

Rapid Detection and Identification of Bacterial Strains

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, *Pseudomonas aeruginosa*, *Bacillus amyloliquifaciens*, *Bacillus cereus* and *Listeria innocua*) were filtered to harvest the cells and eliminate the aqueous matrix, which has a strong NIR signal. FT-NIR spectra were obtained by using a diffuse reflection-integrating sphere. Principal component analysis (PCA) showed tight clustering of the bacterial strains at the information-rich spectral region of 5200-4000 cm^{-1}. The method reproducibly distinguished between the different *E. coli* isolates and conclusively identified the relationship among a new isolate and one of the test species. This methodology may allow for the rapid assessment of potential bacterial contamination in liquids with minimal sample preparation.

In order to evaluate pathogenic strains while addressing safety concerns, the effect of ethanol treatment to kill the microorganisms was evaluated. Bacteria including strains of *Escherichia coli* spp., *Pseudomonas aeruginosa*, *Bacillus* spp. and *Listeria innocua* were evaluated. The bacterial cells were treated with ethanol (70% v/v) and then concentrated on an aluminum oxide membrane to obtain a thin bacterial film. This simple membrane filtration procedure generated reproducible FT-NIR spectra that allowed for the rapid discrimination among closely related strains. PCA and SIMCA

of transformed spectra in the 5200-4000 cm^{-1} region exhibited clusters that discriminated between bacteria species at levels of ~1 mg wet cells weight (~ 10^6 CFU/mg). Factors such as bacterial film thickness, and stage of growth substantially affected the FT-NIR spectra and diminished the ability of PCA to differentiate among strains; this underscores the importance of developing robust sampling protocols. Ethanol treatment of bacterial cells had an effect on the spectral features and the SIMCA modeling performance but allowed the clarification of pathogenic strains with increased safety. The bacterial films appear stable even after three months, provided that the spectral range is narrowed to 5100 – 4400 cm^{-1} to build the chemometric models. Analysis of apple juice samples inoculated with different *E. coli* strains at ~10^5 CFU/mL showed FT-NIR spectral features consistent with bacteria contamination and SIMCA correctly predicted the presence of the different *E. coli* strains. FT-NIR in conjunction with multivariate techniques can be used for the rapid and accurate evaluation of potential bacterial contamination in liquids with minimal sample manipulation and hence limited exposure of the lab worker to the agents.

The use of FT-NIR spectroscopy and multivariate pattern recognition techniques was evaluated to address the need for a fast and sensitive method for the detection and classification of *Bacillus* spp. The complex cellular composition of bacteria yields FT-NIR vibrational transitions that in some instances permit discrimination. Multiple isolates of three *Bacillus* species: *B. cereus*, *B. thuringiensis*, and *B. subtilis* were studied through repeated trials. After growth, the bacterial cells were either re-suspended in saline solution (0.9%) or in ethanol (70%) to address safety concerns when handling pathogenic strains. As stated above, the cells were then concentrated by filtration on an aluminum oxide membrane to form a thin uniform bacterial film. SIMCA of transformed spectra in the 5000-4000 cm^{-1} region exhibited clusters that permitted accurate species-level classification and strain specific discrimination of the isolates. This methodology shows promise for the rapid and accurate classification (with minimal sample manipulation) of *Bacillus* species of public health concern and the agriculturally beneficial strains that are otherwise very similar to the pathogens.

In order for the FT-NIR methodology to become a standard typing tool for bacteria species, additional research needs to address topics such as limits of sensitivity, generation of a library of major foodborne pathogens, evaluation of other multivariate classification methods, and test whether the model can distinguish mixtures of bacteria in liquids.

Scientific Publications in Refereed Journals:

1. Rodriguez-Saona, L.E., McLaughlin M.A., Fry, F.S., Calvey, E.M. 2001. Rapid analysis of sugars in fruit juices by FT-NIR. Carbohydrate Research, 336: 63-74

2. Rodriguez-Saona, L.E., F.M. Khambaty, Fry, F.S. and Calvey, E.M. 2001. Rapid detection and identification of bacterial strains by Fourier Transform Near-Infrared spectroscopy. J. Agric. Food Chem. 49:574-579.

3. Rodriguez-Saona, L.E., Fry, F.S. and Calvey, E.M. 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.

Other Publications:

1. Rodriguez-Saona, L.E., Khambaty, F.M., Fry, F.S., Calvey, E.M. 2002. Discrimination of Bacterial Strains by Fourier-Transform Near-Infrared Spectroscopy Using an Aluminum Oxide Membrane. In Proceedings of the Conference on "Spectroscopic Properties of Biological Materials". Environmental and Industrial Sensing Symposium. International Society for Optical Engineering. Newton, MA.

2. Rodriguez-Saona, L.E., Khambaty, F.M., Fry, F.S., Calvey, E.M. 2001. A novel approach for the rapid discrimination of bacterial strains by FT-NIR spectroscopy. In Proceedings of the Conference on "Photonic Detection and Intervention Techniques for Safe Plant, Animal and Food". International Society for Optical Engineering. Boston, MA.

Presentations (Name of presenter in bold-face, ** Invited Speaker):

1. **Rodriguez-Saona, L.E.,** Fry, F.S., Calvey, E.M. 2002. Application of aluminum oxide membranes for the rapid classification of bacterial strains using Fourier Transform Near-Infrared spectroscopy and multivariate analysis. Poster presented at the Ann. Mtg., Inst. Food Technol., Anaheim, CA, June 15-19.

2. ****E. M. Calvey**, L. E. Rodriguez-Saona, F. S. Fry, F.M. Khambaty, and M. M. Mossoba. 2002. Applications of FT-NIR and FT-IR for rapid testing of Food Contaminants. Third Joint CSL/JIFSAN Symposium on Food Safety and Nutrition. Rapid Diagnostic Methods in Food Safety, June 26-28.

3. ****E. M. Calvey**, L. E. Rodriguez-Saona, F. S. Fry, and F.M. Khambaty. 2002. FT-NIR Rapid Determination of Food Contaminants. JIFSAN Advisory Council Meeting. College Park.

4. ****Rodriguez-Saona L.E.,** McLaughlin M.A., Fry F.S., and Calvey E.M. 2002. FT-NIR rapid determination of sugars in fruit juices. Technical Committee for Juice and Juice Products (TCJJP) biannual meeting. Crystal City, VA. May 2.