

Annual Report 1998-1999

The Joint Institute for Food Safety and Applied Nutrition

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, Appendix I) that established a cooperative venture, the Joint Institute for Food Safety and Applied Nutrition (JIFSAN). JIFSAN was initially established as a cooperative venture between the University and the FDA Center for Food Safety and Applied Nutrition (CFSAN). Later, the MOU was amended (Appendix I) to include the FDA Center for Veterinary Medicine (CVM).

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is a jointly-administered multidisciplinary research and education program. Dr. David R. Lineback (University of Maryland) is the Director and Dr. Samuel W. Page (FDA) is the Scientific Director. 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 before they become crises and to enhance regulatory review capabilities. Collaborative activities involve research and outreach in four major areas of FDA's food and veterinary programs: (i) food safety (related to pathogens, contaminants and toxins); (ii) regulatory science (related to the premarket review process, postmarket compliance and enforcement strategies, international standards and competence, and educational outreach); (iii) nutrition (related to nutrient quality, safety and labeling); and (iv) food industry and consumer behavior.

The new CFSAN office and laboratory building will be 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. JIFSAN provides a neutral environment in which experts from industry, consumer and trade groups, international organizations, government agencies, and academia can pool their resources and ideas to provide the scientific base for the development of sound public health policy. JIFSAN Advisory Council members will provide the advice, vision, and support that are necessary to advance JIFSAN's mission of cooperative research and education/outreach in food safety, human nutrition, and animal health. The interactions of FDA, the University, and visiting researchers will help ensure that the regulatory scientists remain in the forefront of food safety issues. This also provides visiting scientists insight into regulatory processes. Visiting scientists from all sectors are being encouraged. Opportunities for undergraduate and graduate students to work with FDA scientists will also enhance students' understanding of regulatory processes and will provide them with valuable

practical experience. Programs initiated by JIFSAN have demonstrated that the benefits to be achieved by this partnership are substantial. These projects will contribute to the science undergirding current and future regulatory issues and activities that impact on public health policies.

Research in risk analysis (risk assessment, management, and communication) is a major focus of the JIFSAN program. The research 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 paradigms. Under the guidance of the interagency Risk Assessment Consortium (RAC) in the Food Safety Initiative, a Risk Analysis Clearinghouse is being developed at JIFSAN. This provides a mechanism to collect and disseminate available data and methodologies from government, academia, industry, and private sectors. The intent of the clearinghouse is to provide a centralized information source in all areas of risk assessment related to food safety with early emphasis on microbial pathogens and their toxins. The unique feature of this clearinghouse model lies in the examination and documentation of state-of-the-art methods, data sources, and current results of on-going risk assessments so that a much more complete and up-to-date picture of risk assessment is assembled.

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

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

Trade initiatives have put food safety high on the international agenda. JIFSAN is actively involved in developing collaborations with international parties 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, which focuses on risk assessment of contaminants in foods and mycotoxin analysis.

The MOU established a set of relationships that closely link the University with CFSAN and CVM by committing to the sharing of facilities, personnel, and intellectual resources when appropriate. Thus, FDA personnel will have access to University facilities such as libraries and may be appointed as adjunct or research faculty to participate in teaching, mentoring, and research direction at the graduate and undergraduate levels. FDA will support and utilize major instrumentation facilities (electron microscopy, nuclear magnetic resonance spectroscopy, etc.) on the campus and those facilities will house appropriate FDA personnel. These and other synergistic relationships outlined in the

MOU will allow both institutions to remain state of the art in a number of areas where duplicative efforts would be less than successful.

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

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

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

Progress Report

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

JIFSAN also responded to a Federal Register Notice of FDA's intention to noncompetitively supplement the cooperative agreement for up to an estimated \$2 million annually. These funds will provide additional support to JIFSAN for the purpose of addressing emerging health issues and crises that are related to food safety and applied nutrition and animal health sciences, and expanding the current scope to include other agency programs such as cosmetics. Approximately \$700,000 was awarded on September 30, 1999 beyond the noncompetitive continuing award. These additional funds are to be used for cosmetics research; FSI GAPS/GMPS international training programs; a pilot training program in areas of interest to JIFSAN and FDA including biotechnology and risk communication.

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.

Specific progress in strengthening JIFSAN's administrative structure includes:

- The nationwide search for a permanent director was successfully completed when Dr. David R. Lineback was appointed to this position. He began work on November 16, 1998.
- The search for an Assistant to the Director culminated in the hiring of Ms. Shelia Richburg, who began work on February 15, 1999.
- An additional office was obtained, on a temporary basis, for use by JIFSAN. It is primarily used for FDA personnel involved in JIFSAN activities that require their presence at College Park. (While construction of the new CFSAN facility is underway adjacent to the College Park Metro Station, completion and relocation of personnel is not anticipated until late 2001 or early 2002.) Two additional offices will be obtained when renovations in Symons Hall are completed in January 2000.
- A Working Group was established and meets on a weekly basis to discuss and plan JIFSAN programs in research, education, and outreach. This group includes the JIFSAN Director (Chair), Scientific Director (FDA), Deputy Scientific Director (FDA), and CVM representative (FDA). Additional University and FDA personnel are included as appropriate to the discussions in progress.
- Three individuals within CFSAN have been assigned to the Scientific Director of JIFSAN. They are working with Dr. Page on JIFSAN activities within the FDA. This Liaison Staff and their responsibilities are listed in the document entitled JIFSAN Program Management and Coordination Activities (Appendix II) which was recently developed.

The JIFSAN Advisory Council

Central to the operation of JIFSAN is the formation of an Advisory Council composed of members from private sector business, government agencies, academia, and representatives of consumers' interests. This group will provide guidance to JIFSAN in developing research, education, and outreach programs to address problems in food safety, nutrition, and animal health. Organization of the Council continued with its first meeting scheduled for October 20-21, 1999.

Members of the Advisory Council currently include:

- Private sector industry
 - Bestfoods (Dr. Diani Santucci)
 - Coca-Cola Company (Dr. Michael Carakostas)
 - Campbell Soup Company (Dr. George Evancho)
 - Dean Foods Company (Dr. George Muck)
 - Frito-Lay (Dr. Robert Drotman)
 - General Mills (Dr. Frederick Hegele)
 - Gerber Products Company (Dr. Nicholas Hether)
 - Hershey Foods Corporation (Dr. Stanley Tarka)
 - Kellogg Company (Dr. Tracie Sheehan)
 - Kraft Foods (Mr. Ron Triani)
 - McCormick and Company (Dr. Hamed Faridi)
 - McNeil Specialty Products Company (Dr. Steven Mann)
 - M&M/Mars (Dr. Steven Rizk)
 - Mead Johnson Nutritionals (Dr. Mark Dreher)
 - Monsanto Company (Dr. Jerry Hjelle)
 - Nabisco (Dr. W. Kelly Jones)
 - Ocean Spray Cranberries (Dr. Y. Steve Henig)
 - Odwalla (Mr. Stephen Williamson)
 - Procter and Gamble Company (Dr. Keith Triebwasser)
 - Tropicana Products (Dr. Nancy Green)

- Representatives of Consumers' Interests
 - Consumer Federation of America (Ms. Carol Tucker Foreman)
 - National Consumers League (Ms. Linda Golodner)

- Academia
 - Dr. Michael Doyle (University of Georgia)
 - Dr. Julie Miller Jones (College of St. Catherines)
 - Dr. Sanford Miller (Univ. of Texas Health Sciences Center)
 - Dr. Michael Pariza (University of Wisconsin)
 - Dr. Stephen Taylor (University of Nebraska)
 - Dr. Connie Weaver (Purdue University)

- Government
 - Dr. Peter Stanley (Central Science Laboratory, MAFF, UK)

- Individuals
 - Dr. Gilbert Leveille (McNeil Consumer Healthcare)

Development of the Advisory Council will continue next year with inclusion of additional members from private sector business, academia, government agencies, and representatives of consumers' interests

Risk Analysis

JIFSAN has been charged with the responsibility of developing and operating a Risk Assessment Clearinghouse (Dr. W. Hueston, UM, Director). In September, the name was changed to 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 all areas of risk analysis (assessment, management, and communication) related to food safety with initial emphasis on microbial pathogens and their toxins. The unique feature of the Clearinghouse lies in the examination and documentation of state-of-the-art methods, data sources, and current results of on-going risk assessments so that a much more complete and up-to-date picture of risk assessment is assembled. The Clearinghouse is guided by the Food Safety Initiative (FSI) Risk Assessment Consortium (RAC) composed of a representative from each of the government agencies responsible for ensuring the safety of the food supply. Workshops have been conducted to develop a framework that will serve as a template for the Food Safety Risk Analysis Clearinghouse.

Progress this year included:

- JIFSAN hosted a discussion (April 13, 1999) with Dr. Jean Louis Jouve (Unite Evaluation des Risques de Sante, DG XXIV, European Commission) concerning risk assessment in the European Union/Commission. The focus was on opportunities for collaboration between the EC and JIFSAN with specific reference to the Risk Analysis Clearinghouse. Discussions are continuing with Dr. Arpad Somogyi of DG XXIV who will present a draft proposal, developed by JIFSAN, to the new Director General of DG XXIV in January 2000.
- An interactive discussion on the Food Safety Risk Analysis Clearinghouse was presented at the Toxicology Forum (July 11-16) in Aspen, Colorado. Approximately 65 individuals attended and participated in the discussion. These individuals were primarily from the dietary supplement, food, food ingredient, and food packaging sectors of the private sector. A confidential questionnaire on data need was also made available on the opening day of the meeting in addition to a leaflet describing the Clearinghouse. Comments had to be terminated when the time allotted for the discussion expired. The main theme of the comments concerned peer review, the threshold of quality for submission of data and information, the need for "best practices" guidelines

for risk assessment processes, the importance and commitment to continuously update information, the need for objectivity, and the potential power of the Clearinghouse to communicate information and aid risk assessors.

- On September 1, 1999, JIFSAN hosted an open meeting entitled "Clearinghouse Priorities for Microbial Risk Assessment" attended by 180 registrants. The morning session consisted of presentations of five microbial risk assessments currently in progress, with a focus on their principle data needs and what data they could offer to the Clearinghouse. The afternoon session focused on successful models for accessing and collecting existing data. A panel discussion with three international invitees and the day's speakers identified critical issues and commented on public input.

The National Food Processors Association (NFPA) announced their agreement to use the Risk Analysis Clearinghouse as the repository for the data in their upcoming quantitated data collection to help establish baseline exposure estimates for *Listeria monocytogenes*. Details of this data sharing will be worked out.

- On September 2, 1999, a JIFSAN-organized and -sponsored invitation-only workshop entitled "Clearinghouse Workshop on Microbial Risk Assessment Data" was held. Participants included government, industry, academic, and international risk assessors. A facilitated approach was used to help prioritize the work of the Clearinghouse staff with respect to Clearinghouse web implementation and data issues. The draft framework outline was reviewed and clarified.
- Dr. Wendy Fineblum was hired as Coordinator for the Risk Analysis Clearinghouse. She has Ph.D. and DVM degrees and began employment on July 6, 1999.

With Dr. Fineblum's coordination, the website (www.foodriskclearinghouse.umd.edu) for the Risk Analysis Clearinghouse is being developed. Plans are to open the Clearinghouse to Risk Assessment Consortium members on December 1, 1999 and to the public shortly after that, i.e. after receiving additional input and evaluation from the RAC members.

As part of JIFSAN's educational and outreach efforts in Risk Analysis, an international workshop entitled "Fumonisin Risk Assessment Workshop" is scheduled for January 10-12, 2000 at the Inn & Conference Center, University of Maryland at College Park. The meeting is cosponsored by JIFSAN, the FDA, USDA, and the World Health Organization (WHO).

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 key public issues.

JIFSAN will be involved in research in a number of ways:

- By supporting core facilities that will benefit FDA and University scientists and their collaborators.
- By funding small research programs through the Cooperative Agreement.
- By developing cooperative programs with external constituencies.
- By facilitating funding mechanisms 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 the research programs of JIFSAN will require time.

A collaborative research program was established in the summer of 1998 by providing seed funding of \$25,000 each for four research projects. Each project requires collaboration between at least one University of Maryland faculty member and one or more FDA collaborators. The latter help provide additional scientific expertise and insight into the public health impact of the stated research. These projects 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:

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

The first four projects funded in May 1998 were reviewed on the basis of a progress report and a proposal for continuation of the research. All four were funded for a second year. In addition, five new projects were funded in January 1999. Three of these were proposed for three years and two were for one year only. During the year, the system for

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

Projects currently funded include:

Second year of funding:

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

Modern agriculture has benefited from the development and use of agrochemicals including insecticides, herbicides, and veterinary drugs. In addition to these exogenous chemicals, many undesirable compounds such as plant toxicants and mycotoxins are known to occur naturally. Thus, there exist critical needs for monitoring residue levels in foods, agricultural commodities, and environmental samples.

The goal of this research is to develop immunoaffinity hollow fiber ultrafiltration, which integrates membrane separation with the specificity of antigen-antibody interactions, for multiresidue analysis of drugs, pesticides, toxicants, and mycotoxins in foods, agricultural commodities, and environmental samples. Immunoaffinity extraction of targeted compounds in a hollow fiber membrane, together with the highest level of analyte confirmation provided by mass spectrometry detection, represents an automated, cost-effective, high-resolution, and high-throughput bioanalytical screening and monitoring tool in support of food safety in the areas of risk analysis, animal health sciences, and rapid detection of microbial pathogens and naturally-occurring toxins.

During the first year, the system was tested with barbiturates and wafarin compounds. The antibody only reacts with the barbiturates that contain a six-membered heterocycle. The negative electrospray ionization (ESI) mass spectrum clearly indicated the specific m/z ions corresponding to the captured barbiturate compounds and clearly illustrated the screening power of immunoaffinity hollow fiber ultrafiltration. Enrichment in the concentration unit was clearly supported and the near complete dissociation of immunoaffinity complexes in the microdialysis junction right before the electrospray process was indicated for a mass loading of 4×10^{-16} mole for each barbiturate analyte. By replacing the monoclonal antibody with human serum albumin, the wafarin compounds are selected from their sample matrices by formation of affinity complexes with human serum albumin in

solution. The negative ESI mass spectrum displayed four out of five warfarin compounds originally present in the sample solution. Based on the multiresidue analysis of barbiturates and warfarins, the antibodies with cross-reactivities toward mycotoxins and insecticides are under investigation for simultaneously screening and analyzing a wide range of food contaminants and residues in a single hollow fiber.

The proposed research plan for the second year includes development of a microfabricated immunoaffinity ultrafiltration system in a single integrated platform, the combination of miniaturized device with MS detection for rapid analysis of food contaminants and residues, and the use of miniaturized device for rapid cleanup of food samples for off-line HPLC-MS analysis. Research collaborations will be established in the analysis of mycotoxins using the microfabricated immunoaffinity purification device.

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

Salmonella enteritidis (*S. E.*) carried by chickens and shell eggs has become a major source of human intestinal infections. Despite the tremendous efforts made by the poultry industry, no effective measurements for elimination of *S. E.* colonization have been generated. Since the rate of horizontal transmission among chickens and egg-laying hens is very rapid, general hygiene measurements are not as effective as desired. The purpose of this study is to examine variables affecting the immune response of hens against *S. E.*, especially under stress conditions. Hens with a weak immune system are likely to be more susceptible to *S. E.* infections. Activating the hens' immune system, such as by immunization, can prevent or eliminate the infection. This study will advance our ability to identify the factors that can up-regulate or down-regulate the immune system of hens, leading to approaches for inhibiting the colonization of the intestine and the reproductive tissues by *S. E.*, thereby leading to a decreased incidence of contaminated shell eggs and reduction of exposure to consumers.

The specific aim for the first year was to establish the methods for studying the immune system of chickens. The project had three major goals: 1.) Determine the level of antibody secretion and the *S. E.*-specific antibody secretion; 2.) Establish appropriate measures for cell mediated immunity including the level of cytokine secretion; and 3.) Morphologically observe the activation or the suppression of the immune system.

Using an enzyme-linked immunosorbent assay (ELISA), the concentration of IgG, IgA and IgY were determined at the nanogram level. Using the outer-membrane proteins of *S. E.* as antigens and commercially available anti-*S. E.* antibodies as positive controls, a method was established to determine the

secreting level of *S. E.*-specific antibodies in serum. Using these methods, it was possible to analyze the humoral immune response of hens against *S. E.*

Using histochemical analysis of spleen and intestine of hens and antibodies, the germinal center where B cells are activated in spleen was identified. These methods will allow the direct analysis of the maturation and activation of the immune systems of hens during *S. E.* infection. A *S. E.* strain was generated that constitutively expresses green fluorescent protein (GFP). Using the fluorescent *S. E.*, it will be possible to follow the colonization and invasion process of *S. E.* in hen's intestine and in a cell culture system.

A method was established to determine the proliferation of hen splenic lymphocytes that are activated by mitogens or cytokines. The optimal temperature, incubation time and medium were identified for the assay. Supernatants and cells from these cultures have been frozen and will be analyzed for cytokines. A method to determine the cytotoxicity of Natural Killer (NK) cells has been established. The cytotoxicity of NK cells from hens was determined by its ability to kill target cells.

To test these methods in *S. E.*-infected hens, a group of egg-laying hens has been immunized with paraformaldehyde-inactivated *S. E.* The immune responses of the immunized hens are being analyzed.

Thus, during the first year of this grant, the targeted specific aim was accomplished. Using the methods that were established during the first year, it will be possible to study the immune response of hens against *S. E.* It will be possible to examine both humoral and cellular immune responses, analyze cytokine secretion which regulates immune responses, and observe the process of *S. E.* invasion and the formation of germinal center in spleen and intestine.

During the remaining two years of the proposal two specific aims are stated. The first is to investigate the immune response of hens against *S. E.* Using the methods established during the first year, the immune response of hens receiving different doses of *S. E.* in different phases of the hens' production cycle will be examined. The immune response of hens at a time just after receiving the bacteria will be compared versus a designated time long after the inoculation. The second specific aim is to investigate the effect of stresses on the spread of *S. E.* among hens and the immune response of hens. Using the methods established in the first year, how various stresses influence the immune response of hens against *S. E.* will be investigated, as well as how the changes in the hens' immune system relate to the spreading rate of the bacteria. The following stress factors will be examined: a. temperature; b. air quality; c. lighting; and d. personal space.

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

Several species of *Enterococcus*, commonly carried by poultry, rarely cause infection in humans from their common site in the intestines, but can cause severe illness if contamination of the bloodstream occurs. The major problem with enterococci is that they are usually resistant to many antimicrobial agents used in treating bloodstream infection. Naturally resistant to penicillins, they acquired gentamicin resistance in the 1970's, leaving vancomycin as the only effective agent in most cases. In 1986, vancomycin resistance appeared in Europe and shortly thereafter in New York. Vancomycin-resistant *enterococcus* (VRE), usually *E. faecium* and *E. faecalis*, has spread throughout the U.S., although the prevalence rate is still higher in the eastern parts. The University of Maryland Hospital in Baltimore has reported a prevalence of from 12 to 20% of high-level VRE throughout the hospital.

The resistance patterns are troubling since many of the products used are either structurally related to or are commonly used therapeutic drugs for humans. Thus, poultry might be a source of resistant enterococci or other resistant pathogens that may be transmissible to humans through consumption of poultry products.

The specific aim for the first year of this project was to determine the presence and prevalence in farm stock of *Enterococcus* spp. which are resistant to agents commonly used in human therapy. Special emphasis was placed on resistance to antimicrobials that are also used in raising or treating stock.

Of the 935 bacterial isolates recovered from over 70 commercial broiler and roaster poultry farms of the Delmarva Peninsula, 432 presumptive *Enterococcus* isolates have been identified. These isolates have come from swabs of fecal material found on poultry transport containers (PTCs) as well as litter from farms. A full protocol for authoritative identification of species has been developed and is currently being applied to all isolates.

Antimicrobial susceptibility testing for all isolates is in progress. Consistent patterns of resistance are evident in the 48 isolates tested. Uniform resistance to amikacin, apramycin, ceftiofur, ceftriaxone, kanamycin, tetracycline, streptomycin, and the first generation cephalosporins cefazolin and cephalothin is apparent. To a lesser extent, resistance is seen to lomefloxacin, nitrofurantoin, ofloxacin, and sulfamethoxazole. Paired resistance to the macrolides clarithromycin and erythromycin is present in 39.6% of isolates. Although low level gentamicin resistance is seen in 41.7% of isolates, no isolate as yet possesses high-level gentamicin resistance. Interestingly, an isolate has demonstrated intermediate resistance to vancomycin. The genetic element that confers this resistance is being characterized.

The data generated to date demonstrate that the *Enterococcus* isolates that have been recovered from the poultry environment possess broad resistance to antimicrobial agents. A single isolate will be the most immediate focus of molecular study because of its intermediate resistance to vancomycin. The plan is to perform pulsed-field gel electrophoresis studies of resistant isolates in the near future due to the suggestive vancomycin resistance in *Enterococcus*. This and future data will be compared to past studies of resistance in both agricultural and clinical settings to detect trends in the development of resistance as well as to analyze the efficacy of future resultant controlled drug usage mandates.

In the continuing research, the prevalence of *Enterococcus* sp. will be evaluated from sources that represent potential control points in the transmission of foodborne disease. This group of bacteria has a facility to rapidly develop resistance to the latest antimicrobial agents and transmit them to other bacteria, especially following the use of the same or related drugs on farm animals. Resistance to antibiotics routinely used to treat gram-positive human infections as well as commonly used antibiotics in agriculture will be assayed, with particular attention paid to gentamicin, vancomycin, and virginiamycin/Synercid resistance. Resistant isolates will be further typed to compare with other strains for epidemiological purposes. Molecular methods, especially pulsed-field gel electrophoresis, will be the definitive methods to assign relatedness of isolates. Three specific aims are cited: 1.) What is the prevalence of *Enterococcus* spp. and the associated resistance patterns from poultry sources? Timely observation of trends, which might potentially negatively impact human health or the regional poultry industry, are critical and are addressed through continuous surveillance. 2.) What relatedness exists between the resistant isolates of *Enterococcus*? These data will represent the demographic changes of the enterococcal population regionally and might allow the opportunity to intercede and prevent the spread of resistant biotypes. 3.) What is the prevalence of *Enterococcus* in feedstuffs? In lieu of recent data implicating poultry feed as a source of contamination with vancomycin-resistant enterococci, an analysis into the effectiveness of feed pelletters to kill enterococci is warranted.

- **Bioavailability, Metabolism, and the Role of Dietary Carotenoids in Human Health.** Dr. Frederick Khachik (UM), Dr. Andrija Kornhauser and Dr. Shirley Blakely (FDA).

Carotenoids are one of the major constituents of fruits and vegetables whose high consumption has been associated with a reduction in the incidence of chronic diseases. However, with the exception of beta-carotene, the bioavailability, metabolism, and tissue distribution of other major dietary carotenoids in humans with regard to function and mechanism of action in disease prevention have not been investigated to date. This study will

determine the qualitative and quantitative distribution of carotenoids and their metabolites in the tissues of rodents in an effort towards establishing their functional role in human health. The results from these studies will ultimately allow the investigators to determine the bioavailability and the nutritional benefits of carotenoids and their metabolites in humans. At present, this proposal focuses on qualitative and quantitative distribution of dietary carotenoids and their metabolites in the tissues of rats supplemented with various doses of a major dietary carotenoid, namely, lutein. This project will also involve the improvement of current analytical methods for the detection of these nutritive food components in tissues of rats that can ultimately be applied to donor tissues of humans.

The study was initially designed to determine the bioavailability of lutein, vitamin E, and vitamin C in blood and tissues (liver, brain, retina) of rats supplemented with these antioxidants and their combination. Based on extraction and analysis of the antioxidants and their combinations in the blood and tissues of the rats, it was decided to focus the effort on the extraction and analysis of lutein, vitamin A, and vitamin E in the plasma and liver of the supplemented rats.

Plasma concentrations of lutein do not appear to be dose dependent for either the obese or lean group of rats. At low and high lutein dose levels, the plasma concentration of supplemented vitamin E does not appear to be affected by lutein in both obese and lean rats. However, the lutein plasma concentrations seem to be dose dependent when both the obese and lean rats are supplemented with this compound together with vitamin E.

The liver concentration of lutein in obese and lean rats, supplemented with the low dose of this compound, appear to be similar. This is also the case for the obese and lean group supplemented with lutein at the high dose. Once again the absorption of lutein by the liver does not appear to be dose dependent. Based on the preliminary examination of the lutein and vitamin E liver concentrations, there appears to be no obvious interaction between these nutrients when supplemented together. However, these data can only be fully interpreted once the statistical analysis of the results is completed.

This shows that rats supplemented with purified lutein absorb this compound into plasma and liver and that rodents can be used as an inexpensive model to study the potential benefit of carotenoids in the prevention of chronic diseases such as obesity, cancer, and cardiovascular. Nonetheless, future supplementation studies should be conducted with a purified mixture of dietary carotenoids to confirm the bioavailability of a wide range of these compounds in plasma and selected target tissues.

The proposal for the two-year continuation moves to a different focus. The title is: **Mechanisms of chemoprevention by dietary carotenoids and their metabolites in the prevention of chronic diseases in humans. Dr. Frederick Khachik (UM), Dr. Eugene Mazzola, Dr. Shirley Blakely, and Dr. Andrija Kornhauser (FDA).**

Dietary carotenoids are one of the most abundant classes of nutrients in fruits and vegetables that are found at relatively high concentrations in human serum, fluid, and tissues. High consumption of carotenoid-rich fruits and vegetables as well as high blood carotenoid levels have been associated with a reduction in the incidence of chronic diseases such as cancer and cardiovascular. Although 34 carotenoids, including eight metabolites, have been identified in human serum, fluid, and tissues, only beta-carotene has been extensively studied for its potential health benefits in the prevention of chronic disease in humans. The investigators have recently shown that other major dietary carotenoids such as lutein, zeaxanthin, lycopene, and their metabolites can protect the genetic damage to cells more effectively than beta-carotene. Compounds that can prevent or reverse this genetic damage are called chemopreventive agents; these impart their action by several known mechanisms of chemoprevention.

In this proposal, the biological activities of action for 10 major dietary carotenoids and five of their metabolites, individually and in mixtures, will be investigated in several *in vitro* and *in vivo* models of chemoprevention. These are:

- a. intercellular gap-junctional communication proteins in an *in vitro* model,
- b. the detoxication (phase 2) enzymes in an *in vitro* model, and
- c. an *in vivo* anti-inflammatory model.

The studies will be further extended to a series of synthetic analogs of lycopene metabolites to investigate their structure/activity relationship in comparison with lycopene. Among 72 epidemiological studies, 57 have reported inverse associations between tomato intake or blood lycopene level and the risk of cancer at a defined anatomic site; 35 of these inverse associations have been shown to be statistically significant.

The project will be conducted in two parts. The first part involves preparation of major dietary carotenoids by isolation from edible plant sources as well as total synthesis of the oxidative metabolites of lycopene, lutein, and zeaxanthin. In addition, several structurally related analogs of lycopene metabolites will also be prepared by total synthesis. The second part of the study will examine the chemopreventive properties of the dietary carotenoids and their metabolites in various *in vitro* and *in vivo* models of carcinogenesis.

The proposed study will enable the investigators to determine structural requirement and chemopreventive potency of dietary carotenoids and their metabolites. Based on these findings, a multicarotenoid supplement consisting of a mixture of dietary carotenoids and their metabolites could be developed by scientists for clinical chemoprevention trials of chronic diseases such as cancer and cardiovascular.

First year funding (new projects initiated in January 1999):

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

It has been known for some time that exotoxins secreted by certain Gram-positive bacteria profoundly alter the immune system. Among these are enterotoxins produced by *Staphylococcus aureus* of which there are seven serotypes, SEA, SEB, SEC1, SEC2, SEC3, SED, and SEE. These proteins have been shown to exert a three-fold effect on the host organism: as enterotoxins, they induce emesis and diarrhea in humans and non-human primates (1); as exotoxins, they have been implicated in induction of toxic shock (2); and as superantigens, they induce extensive V β -specific T cell stimulation (3) followed by anergy and apoptosis which results in immunosuppression.

At any one time, *S. aureus* is carried by approximately 25-30% of the population. The high carrier rate may be a factor in the prevalence of *S. aureus* contamination of food. Poor personal hygiene and improper holding temperatures of meats and creamy dishes, such as custards and salad dressing, have been implicated as the primary etiologies of foodborne disease caused by *S. aureus*. At temperatures below 60°C, the bacteria will grow and many strains produce enterotoxins. Once ingested, the intact SE can transcytose the intestinal epithelium, gaining access to the peripheral immune system. The symptoms of staphylococcal food poisoning, including nausea, acute vomiting, severe abdominal pain, and diarrhea, begin with 1-6 hours after the food is eaten and resolve with 24 hours. The mechanism responsible for the emetic response to SE appears to be immune-mediated, in that stimulation of T cell proliferation is associated with massive interleukin 2 (IL-2) production. In fact, the symptoms of food poisoning can be mimicked by administration of exogenous IL-2. For some individuals the immune sequela can be severe.

Remarkably little, however, is known about the fate of SE in the gut or their interaction with local or gut-associated immune tissues. The hazards resulting from these interactions include such diverse sequelae as precipitation and exacerbation of autoimmune disease in susceptible members of the population, and massive reductions in numbers of T cells,

which may be catastrophic for immunocompromised individuals. The degree of association between the levels of oral exposure to SE and increased risk for immune sequelae has not been determined, but toxin exposure levels required to alter immune function may well be below those which induce emetic and toxic shock responses. This research will seek to assess immunologic risk associated with different oral exposure levels of SE. The role of gut-associated lymphoid tissue in oral toxin-induced disease will be delineated. This work will provide improved exposure models for transcytosis of SE and potentially other toxins in food across the gut epithelium. The role of SE from food as inducers of a specific immune sequela, autoimmune disease, is to be examined in an animal model of human multiple sclerosis, and, as a result of these animal studies, biomarkers of human exposure to SE may be developed. Together, these aims strongly support the food safety and risk assessment goals of JIFSAN.

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

Control and detection of microorganisms have been an important regulatory activity for many years. Recent progress in several areas of science - molecular biology, mass spectrometry, and computational power - have opened new and complementary approaches to rapid and reliable characterization of microorganisms. Development of these new techniques has also been of interest to the military, whose funding has supported progress in duplex-binding based mapping of amplified bacterial RNA on microchips with mass spectrometric or fluorescent readout. DOD and DARPA have supported development of electrospray and MALDI mass spectrometry for very rapid characterization of bacteria at the species level and virus at the strain level by recognition of lipid and/or protein biomarkers. For any of the approaches to be implemented, the microorganisms must first be isolated.

In general, scientists have worked with well-characterized samples of microorganisms obtained from cultures. Little attention has been given to the question of fishing the needle out of an atmospheric, aqueous or solid haystack. The classical approach of growing out the microorganisms is time-consuming and intellectually compromised by the possibility of selectivity. A desirable strategy would complement this approach with a more rapid parallel technique.

Affinity binding of microorganisms by plant lectins, mammalian receptors, or carbohydrates is well known and is thought to play a major role in the colonization and invasion of host cells. Often complexation between a lectin and carbohydrates on the surfaces of microorganisms will

occur nonspecifically across several genres. Antibodies, of course, can be developed with higher specificity. Thus, immobilization of these various biopolymers can provide biocapture surfaces targeted either generally or specifically. Such biocapture surfaces serve both to isolate analytes from complex mixtures and to concentrate the sample. The captured sample can be readily released for any sort of subsequent processing or analysis. Thus, the development of biocapture surfaces to isolate and concentrate microorganisms can provide, in a modular form, the missing link between collection of a sample from the food chain and characterization by PCR amplification and/or mass spectrometry of any microbial contaminants.

This two-year research project will develop and evaluate techniques to capture microbial pathogens and toxins from complex matrices that are part of the human food chain. Affinity binding on a biocapture surface will both isolate and concentrate microorganisms for subsequent analysis by a variety of methods, including mass spectrometry. The objectives are to construct bioaffinity surfaces with immobilized lectins, antibodies and carbohydrate ligands already known to bind microorganisms; and to evaluate the efficacy and selectivity of these bioactive surfaces to capture microorganisms, targeted as of interest to the FDA, from a range of food chain milieu. In addition to evaluating specific systems, this work will also test the principle for broader adaptation.

The work will be initiated using dithiobis[succinimidylpropionate] (DSP) to construct the biocapture surfaces whose affinities for Gram-negative bacteria have been confirmed. Successful biocapture surfaces can be elaborated as three-dimensional arrays and tested for improved binding yields. The investigators have shown that concanavalin A, Abrus precatorius lectin, and wheat germ agglutinin immobilized on activated gold surfaces capture *E. coli*, *E. herbicola*, *S. typhimurium*, and *B. sphericus* from buffered urine or aqueous solutions. Each of these three surfaces exhibits a different preferential binding pattern. A multiplexing lectin surface that incorporates all three is currently being constructed. These lectin biocapture surfaces are designed to isolate and concentrate a broad spectrum of microorganisms based on binding to carbohydrate motifs present on the cell surface. The use of immobilized antibodies and antisera against target microorganisms will also be evaluated.

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

A major concern in the food industry is the delivery of foods that are free of spoilage and pathogenic microorganisms. To control contamination by pathogenic bacteria, numerous safeguards are employed from production to delivery to market. These safeguards are primarily predicated on

making the environment unfavorable to the organisms. They include the use of decontaminating agents such as hypochlorites on the processing lines and in chill tanks of poultry processing plants. For the food processing industry under current HACCP guidelines, periodic testing is done for the presence of *E. coli* and *Salmonella* to determine effectiveness of sanitary controls in plants.

Detection and identification of foodborne pathogens, especially quantitatively, are becoming necessary in environments housing meat and poultry. These procedures assist in determining that appropriate measures are being taken to assure compliance to industry standards and government regulations. In developing detection and quantitation methodology and in monitoring the cleaning and decontamination of poultry transport units, the investigators' experience is that detection is frequently complicated because of the presence of biofilms. A biofilm consists of colonies of microorganisms encased in capsular slime, attached to a surface. Capsules normally surround the cell external to the envelope but can also be more localized as in a holdfast. Many are pure capsular polysaccharide (CP), while a few covalently or otherwise bind proteins, fatty acids or other residues.

These film-forming communities of bacteria are frequently encased in a rigidly adhering, viscous material which is difficult to penetrate thus shielding the pathogens from detection. The film provides a more stable environment of higher nutrition and protects the organisms from physical and chemical agents, including antibiotics and disinfectants. It is known to protect and hide bacteria, including foodborne pathogens. Persistence of pathogens in biofilms provides ample opportunity for even further contamination in houses, transport containers, and processing plants, especially in those areas where air is being exhausted or ventilated. For this reason, it is perhaps more advantageous to have reporter systems which can identify the presence of the film itself, which in turn reveals the presence of particular bacteria. Without this type of awareness, safety measurements can sometimes be deceiving and food may not be as safe as it should be for consumption.

The goal of the research is to develop and deliver systems for the rapid, specific and sensitive detection of biofilms of *Campylobacter jejuni*, *Salmonella*, and *Aeromonas hydrophila* on foods and on surfaces in food production, transportation, processing and preparation areas. Both monoclonal and polyclonal antibody probes that can be used for the rapid, specific, and sensitive detection of biofilms of these organisms will be developed. Using Hyphomonas MHS-3, a universal probe will be developed to enable broad-spectrum detection of exopolysaccharide-containing biofilms. Other possible approaches will be explored for biofilm detection including the use of lectins and Calcofluor. The probes

will be tested in microcosms against individual target organisms and then again in multispecies microcosms. After these evaluations are accomplished, field tests for pathogen biofilms will be performed on surfaces made of various materials in processing plants and in production facilities. In particular, poultry transport containers and vehicles, both before and after decontamination will be examined for the presence of biofilms. This system of detection can enable more thorough cleaning, decontamination, and inspection of foods and their environments.

- **Effect of Moderate Dose Iron Supplementation on Zinc Absorption and Metabolism During Lactation.** Dr. Robert Jackson and Dr. Phylis Moser-Veillon (UM) and Dr. Isaac Rabbani (FDA - participation terminated when animal portion of proposal was not funded)

To combat nutritional anemia, caused by iron deficiency, the World Health Organization (WHO) has made the elimination of iron deficiency a major focus of intervention programs worldwide. To achieve this, iron supplementation has been recommended and is now both routinely and widely used. The current WHO recommendation, for lactating women, is 60 mgs of iron per day. In the US, 30 mgs of iron per day are recommended during pregnancy and 15 mgs of iron are recommended during lactation.

There is increasing recognition of the impact a supplemental dose of one nutrient can have on the absorption and/or assimilation of other nutrients. However, very little is known about the effect of moderate doses of iron on the absorption and utilization of other divalent minerals, such as zinc and calcium.

Zinc is an essential nutrient and is involved in as many as 200 different metalloenzymes that participate in carbohydrate, protein, lipid, and nucleic acid metabolism. Zinc plays an important role in milk production, in growth, normal taste acuity, reproduction, and in immune function. Zinc is also important as an antioxidant. Zinc deficiency results from inadequate dietary intake and also from poor bioavailability from cereal-based diets. Zinc deficiency has been found to exist in several countries. Many nutrition experts believe that zinc deficiency is a major public health problem and deserves to be added to the World Health Organization's list of prevalent nutritional problems.

This project chose to study zinc absorption in lactating women for four reasons. First, the competitive inhibitory effects of iron would be more noticeable during lactation. Second, data from animal studies show that zinc absorption in lactating rats is as much as 50% greater than it is in pregnant rats. Studies in humans confirm that zinc absorption is higher during lactation than during pregnancy. Third, women given supplements

during pregnancy frequently continue to take those supplements during lactation. Fourth, the effect of a moderate dosage of supplemental iron (between 60-120 mg/day) on the competitive inhibition of zinc uptake is not known. In view of the widespread use of moderate and high-dose iron supplements in both developing and developed countries, and the increasing documentation of inadequate zinc intake and stores in pregnant and lactating women worldwide, it appears critical to study the effect that moderate dose iron supplementation has on zinc absorption.

The purpose of this one-year study is to ascertain if the consumption of an oral dose of iron by lactating women, similar in size to that delivered by prenatal tablets used in the United States, affects zinc absorption. The knowledge gained from this study will be important for combating micronutrient malnutrition among American women, women in low-income countries, and for providing a scientific basis to make nutrient intake recommendations for lactating women. It will also be the basis for a future long-term supplementation study.

- **Identifying Knowledge Gaps and Improving Communication Strategies to Reduce Food Safety Risks.** Dr. Mark Kantor (UM) and Dr. Toija Riggins (FDA)

Public awareness of the importance of food safety has increased during the past decade, but disparities in knowledge and practices vary considerably according to age, gender, ethnicity, and education level. Furthermore, despite several well-publicized foodborne disease outbreaks, many Americans fail to practice proper food handling and preparation habits, and major misconceptions exist regarding the causes, symptoms, and risks of microbiological foodborne diseases. Risky practices such as consuming raw or undercooked foods probably contribute to the burden of foodborne disease in the U.S.

The fact that many Americans follow unsafe food-related practices has been documented recently through telephone surveys and direct observations in households. When observed at home, consumers in a majority of households made fundamental food safety mistakes, with such violations as improper handwashing, cross-contamination, and insufficient thermometer use occurring in 57%, 76%, and 92% of observed households, respectively.

Similarly, although many Americans receive information about nutrition through the media, confusion and misinformation about a proper diet are common. Increasingly, researchers and policy makers are recognizing that to be effective, even simple messages about food safety must be unambiguous and specifically tailored to the intended recipients. In order to develop effective educational programs, it is important first to identify

and understand where there are gaps in knowledge, confusion, or misconceptions among the targeted audiences.

This one-year project is designed to investigate the reasons that many Americans fail to adopt safe food handling practices. A telephone survey will be conducted to study the knowledge, attitudes, and behavior of three groups of individuals who differ in their prior exposure to food safety information: (1) School cafeteria workers and supervisors (more exposure/training); (2) Adult and child daycare providers (less exposure/training); (3) Consumers who subscribe to a Maryland Cooperative Extension (MCE) health newsletter (least exposure/training - controls). The telephone survey will be developed based on a prior poll that will be administered through the internet, in which the expert opinion of food and nutrition professionals who subscribe to several food-related listservers will be solicited. The purpose of the initial poll is to identify issues that professionals believe are causing concern or confusion among consumers, where they perceive the major gaps in knowledge to be, and what they feel are the chief barriers or communication problems that prevent people from making positive behavioral changes. Results of the telephone survey will be used to formulate health messages pertaining to food safety that subsequently will be tested in focus groups comprised of representatives from the three experimental groups. Although the major focus of this proposal is on microbiological food safety issues, inquiries will also be made about the subjects' knowledge and use of dietary supplements. The data collected may be useful in designing effective food safety education programs and interventions tailored to specific groups.

An additional four or five new projects will be funded in January 2000.

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 scientist 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:

- To improve exposure assessments on food consumption to assist in microbial and chemical risk assessments.
- Development of sampling and detection methods for the identification of pathogens and toxins.
- Foster the national capabilities needed to respond to potential chemical and biological threats to the food supply from bioterrorism.

- **The Seychelles Child Development Study.**

Through JIFSAN, scientists at the University of Maryland are cooperating with colleagues from the University of Rochester in conducting an 18-month pilot study on human neurobehavioral outcomes of children at age 11 who participated in the Seychelles Child Development Study “Years Following Prenatal and Postnatal Exposure to Methylmercury from a Fish Diet.” JIFSAN played a critical role in bringing together the resources to continue this long-term study on the developmental effects of mercury. Funding for the project was provided by the FDA (through a supplement to the JIFSAN Cooperative Agreement), the Electric Power Research Institute (present funding \$486,000), the National Tuna Foundation (\$10,000), and the National Fisheries Institute (\$5,000).

The purpose of the study was to develop a battery of neurodevelopmental measures capable of detecting subtle CNS dysfunction in children. A sensitive and specific test battery will be designed and administered to 73 of the children to measure subtle changes in the children’s motor, sensory, cognitive, and neurophysiological functions based on tests that may be affected by methylmercury exposure in children and test the feasibility of using the battery in the Seychelles.

All tests originally proposed have been developed and field-tested on 15 children in Rochester. They were successfully transferred to the Seychelles and field-tested there. In November 1998, 61 children from the Seychelles Child Development Study pilot cohort were administered one half of the test battery. The subject’s age range was 10.5 to 11 with a mean age of 10.6 years. The remaining tasks were field tested in March 1999 in the Seychelles on 58 of the 61 children tested in November.

The project has successfully demonstrated that it is feasible to implement in the Seychelles a highly complex battery of tabletop and computer-based physiological and behavioral measures. Almost all tasks forming the battery were easily accepted by the Seychellois children and had performance characteristics similar to what would be expected if they were given to U.S. children. This would suggest that it is feasible to include nearly all tasks given in a final battery for use with re-evaluation of the Main Study cohort at 11 years of age. A decision has not yet been made on which tests and tasks should be included in a final battery. The Pilot Study tests will form only a part of the final battery. The analysis of Pilot Study data for methylmercury effects has not yet been accomplished.

- **Development and Validation of a New Technology for the Detection of *E. coli* O157:H7 and Other Enterohemorrhagic Serotypes.**

An important operational concept for JIFSAN is the establishment of effective collaborative research partnerships. An excellent example of this involves a University of Maryland faculty member, a FDA scientist, and an industrial partner (Diachemix Corp.) working on the identification of foodborne bacterial pathogens. Much of the recent work was accomplished by two JIFSAN interns working in the laboratories of the faculty member and the FDA scientist.

The goal of the research is the development of a new, simple pathogen detection system that can be used in a variety of public health and food safety settings involving a combination of core technologies licensed by the industrial partner. Preliminary data indicates that a high performance specification is available when these technologies are used together. The specificity was found to accommodate three important enterohemorrhagic *E. coli* (EHEC) serotypes of recent concern in foodborne disease, namely O157:H7, O111:NM, and O26:H11. The test also showed an excellent sensitivity of under ten organisms in the reaction tube, and the rapidity, greatly aided by the lack of need of a microbiological enrichment step, was 30-40 minutes from sample preparation to completion of the determination. Preliminary data demonstrated acceptable matrix compatibility with environmental materials obtained from a beef and a dairy herd.

This continuing collaborative research has fostered understanding between academic, industrial, and regulatory personnel in a multidisciplinary effort to maintain common food safety goals.

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

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

The FT-NIR instrument for this project was installed in December 1998 with subsequent training for Dr. Luis Rodriques-Saona, the postdoctoral associate. In addition, four FDA staff are involved with the development of the research - Dr. Elizabeth M. Calvey (project director), Dr. Fred S. Fry (chemometrics), Dr. Farukh M. Khambaty (microbiology) and Dr. Magdi M. Mossoba (IR spectroscopy). Three subprojects were initiated to fulfill the requirements of the task order contract: (1) Methodology was developed and evaluated for the rapid detection of threat agents (castor bean meal) in foods by using Fourier-transform near-infrared (FT-NIR) spectroscopy and multivariate data analysis techniques. Measurements were made on a FT-NIR system using a diffuse reflection-integrating sphere. Flours spiked with caffeine, crystalline sugar and corn meal, 1-20% w/w, were used as test articles to evaluate the methodologies. Food matrices (bleached flour, wheat flour and blueberry pancake mix) spiked with castor bean meal (CBM, 0.5-8% w/w) containing the toxic protein ricin were analyzed. Multiplicative scatter correction (MSC) transformed partial least squares regression (PLSR) models, using a specific NIR spectral region, predicted CBM contamination in foods with standard error of cross-validation (SECV) < 0.6% and coefficient of determination (R^2) > 94%. Models discriminated between flour samples contaminated with CBM and other protein sources (egg white, soybean meal, tofu, and infant formula). Castor bean meal had loading spectra with bands characteristic of amide groups (4880 and 4555 cm^{-1}) and lipids (5800 , 5685 , 4340 and 4261 cm^{-1}). Work is continuing on the affect of moisture on the model developed. (2) Several NIR detection devices and different sample preparation methods were tested to determine their analytical performance by using model solutions. Aqueous solutions of sugar mixtures (glucose, fructose and sucrose) at different levels (0-8% w/v) were prepared. Direct measurements were made by transfection using a reflectance accessory, by transmittance using a 0.5-mm cuvette, and by reflectance using a fiberglass filter paper containing the dried sugar extract. Partial least squares regression was used to create calibration models that were validated by using independent samples. The predictive ability of the models was evaluated on fruit juices. The affect of bacterial contamination on the predictive ability of the model will be evaluated. (3) Bacterial samples, (1) *E. coli* HB101 and (2) non-pathogenic *E. coli* O157:H7 ATCC43888, were evaluated in 10% dextrose solutions or colonies removed from the surface of LB agar. Dried extracts were used to avoid interference of the FT-NIR signal of water. The two strains of *E. coli* exhibited FT-NIR spectral differences and could be separated into different clusters by Hierarchical Cluster Analysis. Work is continuing on developing an appropriate sampling method for bacterial detection and model for bacterial differentiation.

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. FDA is supporting and using major instrumentation facilities (such as electron microscopy, nuclear magnetic resonance spectroscopy, and mass spectrometry) located in University of Maryland facilities on the College Park campus. FDA personnel will also be housed with University of Maryland colleagues in these facilities. The effective use of this arrangement will increase when CFSAN relocates to its new facilities in College Park in early 2002.

Education and Outreach Programs

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

JIFSAN Website: This comprehensive website lists JIFSAN activities in addition to a description of its program and mission. The address is <http://www.jifsan.umd.edu>. This website is now linked to that of the Risk Analysis Clearinghouse.

JIFSAN at IFT: For the third consecutive year, JIFSAN staffed booths at the Food Exposition at the Institute of Food Technologists' Annual Meeting. This meeting had an attendance of over 23,000. Many food scientists, nutritionists, and industry representatives had an opportunity to visit with personnel from JIFSAN and to become better acquainted with the JIFSAN programs and mission. Two adjoining booths were staffed in cooperation with personnel from the National Center for Food Science and Technology.

JIFSAN at Food Development and Processing Exposition in Baltimore: JIFSAN staffed a booth at the first Atlantic Food Development & Processing Exhibition (August 10-12). Three presentations were given (Dr. Fred Khachik, Dr. Jianghong Meng, and Dr. David Lineback) in the technical portion of the exposition. Personnel from food industries on the East Coast had an opportunity to learn about JIFSAN and its programs.

Non-credit course: "Identification of Microscopic Botanicals – Advanced Workshop" was offered in March 1999 to six participants at the Food and Drug Administration, 200 C Street, Washington, D.C.

Enhancing the Safety of Fresh Produce at the Source: Training Modalities and Methods, Needs and Opportunities: This three-day workshop (April 26-29, 1999) was cosponsored by JIFSAN and the FDA, working with its federal partners in the Interagency International Work Group. The workshop was held at the Inn and Conference Center at the University of Maryland at College Park. The purpose was to identify training needs for growers and producers who export

fresh produce to the United States. The meeting was an extension of a Presidential Initiative to ensure the safety of imported and domestic fruits and vegetables.

The workshop used the “Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables,” which had been published recently by FDA and USDA in consultation with industry, to begin a process for determining how to develop an education and outreach program for growers and producers that will benefit public health and the marketplace. Overall objectives were to identify common elements of a good training plan, create awareness of existing training programs, and identify potential partnerships for the development of practical training modules.

Domestic and international experts and leaders in food safety from government, industry, academia and the consumer community addressed the participants. The workshop drew 175 participants from 26 countries on four continents. Attendees included government experts, education and training counselors, scientists, farmers, producers, worker groups, academic institutions, and international organizations.

Enhancing the Safety of Fresh Produce at the Source: Training Modalities and Methods, Needs and Opportunities – The Next Step: This workshop, cosponsored by JIFSAN and the FDA, was held at the Inn and Conference Center at the University of Maryland at College Park, August 31, 1999. The 16 invited attendees represented FDA, USDA, international agencies, and academia. Representatives from Cornell University, University of Arkansas, University of Florida, and Clemson University presented and discussed training programs in fresh produce production in development or in progress at their institutions. Educational and country-specific aspects of such training programs were discussed. The participants developed a series of recommendations to be considered for the “next steps to be taken.” The results of this meeting will be used by JIFSAN to further develop training materials and programs for enhancing the safety of production of fresh produce. Part of this will be accomplished through subcontracts with domestic and international educational institutions.

International Food Safety Training Program: A three-week international training program was held for five individuals from the Ministry of Agriculture, Egypt and one person from Brazil, September 6-24, 1999. The first two weeks involved classroom presentations at the University of Maryland at College Park. The third week featured visits to appropriate laboratories of the FDA (CFSAN and CVM) and the USDA (Beltsville), and to commercial food production and processing facilities in areas of interest to participants in the training. Instruction was presented by individuals from federal agencies, the University of Maryland and other academic institutions.

The training program was designed to familiarize those having responsibility for regulatory aspects of food production and food safety in nations exporting food to the U.S. with the manner in which food safety is conceptualized and regulated in the U.S. Material presented in this training program will be revised and made available for other international groups with similar responsibilities.

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. The minimum requirement for participation in the program is that a student is entering the sophomore year majoring in such disciplines as Biology, Microbiology, Biochemistry, Chemistry, Food Science, Entomology, and Animal Science. An undergraduate student must volunteer to work on a project for a semester. Upon successful completion of that initial period, students can be converted to paid internships. During all phases of the internship, the students have a variety of ways to obtain academic credit for their internship experience.

Thirty University of Maryland students volunteered as interns in CFSAN or CVM laboratories in the last year (October 1998 – September 1999). Students that volunteered for at least one semester were given the opportunity to apply for a paid JIFSAN student position for the summer. Thirteen students were paid to work at least thirty hours per week at CFSAN or CVM labs during the Summer 1999. The website listing internships is at <http://www.life.umd.edu/jifsan/internships.htm>.

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

JIFSAN Student Interns: Fall 1998 - Summer 1999

Rashi Agarwal (Health Education major, College of Health and Human Performance) is working with Dr. Kathy Cook on the analysis of vitamin K in Foods. She is obtaining knowledge of theory and mechanics of systems that separate vitamins from complex mixtures such as foods. She is quantifying the

vitamins and looking for patterns resulting from food processing methods. Started 1/99. *Paid Summer 99.

Michael Alessi (Chemistry/Chemical Engineering major, Colleges of Life Sciences and Engineering) worked with Dr. Kim Morehouse on Food Irradiation. The student used super critical fluid extraction for the isolation of radiolytically generated products formed during the irradiation of the lipid present in foods and utilized these radiolytic products as markers for irradiation treatment of food. The radiolytic products were monitored and identified using gas chromatography with either flame ionization detection or mass spectroscopic detection. Started 11/16/98. *Paid Spring '99

Rahim Curtis (Cell Molecular Biology and Genetics major, College of Life Sciences), is working with Dr Keith Lampel on the development of a universal extraction of foods for a Polymerase Chain Reaction (PCR) based assay for the rapid identification of food-borne pathogens. Rahim is using several representative food groups to develop washing and/or extraction methods that will efficiently isolate DNA templates from contaminating microbial pathogens and will then adapt these protocols to identify isolated organisms in the washes or extracts. Started 1/99. *Paid Summer '99.

Cassandra Grenade (Cell Molecular Biology and Genetics major, College of Life Sciences) is working with Dr. Mary Ann Principato on a project to Characterize the immunologic response of T lymphocyte populations in the mammalian gastrointestinal tract. Started 6/2/98. *Paid Fall '98, Spring '99, and Summer '99.

Joshua Hayes (Graduate Student in Microbiology, College of Life Sciences) works with Dr. David Wagner on his project, " Surveillance of Poultry and Other Stock for Carriage of Multi-resistant Enterococcus". Started 4/99. CVM

Laudan Izadi (Biology major, College of Life Sciences) is working with Dr Marianne Milliotis on identification and characterization of virulence determinants for *Vibrio parahaemolyticus*. The student will perform mutagenesis of a hemolysin-negative toxin producing strain of *V. parahaemolyticus* by conjugation of the vibrio with a transposon-containing *E. coli* strain. Started 1/99. *Paid Summer '99.

Christopher Larkin (Microbiology major, College of Life Sciences) worked with Dr. Robert Hall on the development of a detection method for enterohemorrhagic *Escherichia coli* O157:H7 and other serotypes using the polymerase chain reaction and a proprietary genetic amplification system. He received a summer stipend for continued development and validation of a new technology for the detection of this organism. Started 1/8/98. Finished 12/98. Paid summer and Fall 98.

Virginia Lau (Biology major, College of Life Sciences) worked with Dr. Marianne Milliotis on cloning and characterization of the *Aeromonas hydrophila* gene responsible for lipase activity. Started 6/25/97, left 2/16/98. Came back 1/99 and ended in 6/99.

Esther Lazar (Dietetics major, College of Agriculture and Natural Resources) works with Dr. Ken Ku studying issues related to Dr. Ku's project, "Dietary Fiber in Foods". She assists in routine techniques such as analysis of various food fiber types and is helping with the development of new methods for separation of specific fiber components. Started 6/99

John Lee (Microbiology major, College of Life Sciences) is working with Dr. Keith Lampel on the "Development of a Universal Extraction Method of Foods for a Polymerase Chain Reaction (PCR)-based Assay for the Rapid Identification of Food-borne Pathogens". Methods will be tested by spiking representative food samples with known pathogens currently associated with foodborne illness. Started 1/99. *Paid Summer '99.

Carl Lewin (Microbiology major, College of Life Sciences) worked with Dr. Mark Walderhaug on the electronic version of the Center for Food Safety's Bad Bug Book. Started 9/25/98. Finished 12/98. * Paid Fall '98

Ann McCarthy (Biology major, College of Life Sciences) is working with Dr. Richard Whiting, Ph.D. on modeling the thermal inactivation of *Listeria monocytogenes*. Started 1/99. *Paid Summer '99

Julia Mukhina (Biology major, College of Life Sciences) worked with Mr. John Gecan studying allergenic proteins in foods. This work requires the use of different types of laboratory equipment such as laboratory scale food processing equipment. Started 7/20/98. *Paid Fall 98. Continued in Spring 99 working one day per week.

Linh Nguyen (Biochemistry major, College of Life Sciences) is working with Dr. Rene Sotomayer using DNA purification, acid hydrolysis and High Pressure Liquid Chromatography to study DNA adduct formation in kidneys of rats fed aflatoxin B₁. Started 6/98. Finished 12/98. *Paid Fall 98. Came back and paid Summer '99.

Rahma Nyang'anyi (Biology major, College of Life Sciences) working with Dr. Rene Sotomayer to study the genotoxic effects of the food-born carcinogen aflatoxin B₁ (AFB₁) in rat kidneys. This work requires extraction, purification and quantification of RNA from treated and control rat kidneys, hydrolysis of RNA to bases and high pressure liquid chromatography. Started 6/98

Jennifer O'Driscoll (Non-degree seeking, College of Letters and Sciences) is working with Dr. Bader Shaikh on the establishment of withdrawal time for

furosemide, a diuretic drug used in dairy cows. Started 1/99. Volunteer Spring '99 and Summer '99.

Phares Okelo (Graduate Student in Biological Resources Engineering, College of Agriculture and Natural Resources) is working with Dr. David Wagner on his project, "Surveillance of Poultry and Other Stock for Carriage of Multi-resistant Enterococcus". Started 4/99. CVM

Depali Patel (Agriculture and Veterinary Medicine major, College of Agriculture and Natural Resources) is working with Dr. Mary Carson on antibiotic resistance transfer, nitrification, efficiency and establishment of bacterial pathogens in fluidized sand biofilters. She prepares samples for liquid chromatographic analysis, using processes such as filtering, liquid-liquid extraction, centrifugation, and solid phase extraction. During the course of the project, she is becoming familiar with the operation and maintenance of a liquid chromatograph. Started 1/99. Volunteer Summer '99.

Frank Ponce III (Biology major, College of Life Sciences) worked with Dr. June Bradlaw using isolated cells, tissues, or embryos to design models for safety assessment and biomedical devices. In Spring '99 started working with Dr. Jan Johannessen on his project to develop improved methods to detect agents of transmissible spongiform encephalopathies and to assess their risk. Started 5/18/98 *Paid Fall '98 and Spring '99.

Kristen Pulio (Biochemistry major, College of Life Sciences) is working with Dr. Kim Moorehouse on food irradiation safety issues. The student uses supercritical fluid extraction or accelerated solvent extraction for the isolation of radiolytically generated products formed during the irradiation of the lipid present in foods, and to utilize these radiolytic products as markers for irradiation treatment of food. The radiolytic products are monitored and identified using gas chromatography with either flame ionization detection or mass spectroscopic detection. Started 4/99. *Paid Summer '99.

Shahikala Ratnayake (Biochemistry and Microbiology major, College of Life Sciences) is working with Dr. Robert Hall on the purification and characterization of a novel cytotoxin of *Vibrio cholerae*. The student is developing skills and confidence in microbiological experimentation through safe handling of microorganisms. These skills contribute to an ongoing project to purify a virulence determinant from *Vibrio cholerae*. Started 2/12/99. *Paid Summer '99.

Jilla Shahnematollahi (Biochemistry and Neurophysiology major, College of Life Sciences) is working with Dr. Ian DeVeau on the predictability of the transfer of drugs and metabolites from plasma into milk of humans and animals. She assists in extractions and analysis of plasma and milk samples. She helps develop and validate analytical methods for the determination of enrofloxacin and fentanyl. Started 6/99.

Alexander Shangraw (Microbiology major, College of Life Sciences) is working with Dr. Ben Tall on emerging pathogens in seafood. The student is involved in many aspects of the project that involves purification of fibrillae, production of monoclonal antibodies and invasion assays. Started 1/20/99. *Paid Summer '99.

Bhavana Sharma (Physiology and Neurobiology, College of Life Sciences) works with Dr. Dan Levy investigating the properties of *E. coli* and *Salmonella* strains and comparing them with the mutator phenotype. She is learning and using techniques of microbiology and molecular biology to characterize the way these bacterial strains acquire resistance to antibiotics used in agriculture and medicine. Her work centers on the response of mutator strains to clinical assays for antibiotic resistance, the genes responsible for anomalous responses to the field assays, and other investigations of the properties of these antibiotic resistant bacterial strains. Started 6/99.

Andrew Shifflet (Microbiology major, College of Life Sciences) is working with Dr. Mahendra Kothary on purification of a toxin from *Vibrio vulnificus*. The project involves techniques such as ammonium sulfate precipitation, gel filtration chromatography, hydrophobic interaction chromatography, ion-exchange chromatography, and isoelectric focusing. Started 8/31/98. *Paid Spring '99, Volunteer Summer '99.

Yildiz Suleman (Biology major, College of Life Sciences) worked with Dr. Ben Tall on identifying the mechanisms responsible for the persistence and emergence of marine vibrios from one seafood host to another, or to humans. She examined *Vibrio damsela* and *Vibrio alginolyticus* for hemagglutination activity against a variety of erythrocytes. She investigated the invasiveness of these pathogens in an invasion assay using Atlantic Menhaden liver cells. Started 1/99 and ended 6/99.

Michael Tims (Graduate Student in Plant Biology, College of Life Sciences) worked with Dr. Joseph Betz on the study of mistletoe lectins in the initiation of pathogenesis. He isolated and characterized biologically active compounds from American mistletoe using extraction, bioassay and analytical and semi-preparative High Pressure Liquid Chromatography of plant material. In Spring '99 he moved on to work with Dr. Mark Walderhaug on the Bad Bug Book. Started 6/25/97. *Paid as undergraduate Summer and Fall 98, paid as a graduate student Spring '99 and Summer '99, Risk Assessment.

Suraj Tuli (Microbiology major, College of Life Sciences) worked with Dr. Keith Lampel on antibiotic resistance in *Salmonella typhimurium* DT-104. The student learned plasmid isolation techniques, restriction endonuclease analysis polymerase chain reaction and nucleotide sequencing. Started 2/99 and ended 6/99.

Patrick Vorhees (Biology major, College of Life Sciences) is working with Dr. Robert Hall on the development of a detection system for *Escherichia coli* 0157:H7 and other enterohemorrhagic serotypes. He is learning safe handling of microorganisms, design of experiments and genetic amplification methods. Started 1/99. Volunteer Summer '99.

Alice Wang (Neurology and Physiology major, College of Life Sciences) is working with Dr. Jurgen Van Bredow and Dr. Chamberlain on the predictability of the transfer of drugs and metabolites from plasma into milk of humans and animals. The student has developed the analytical skills to be able to determine the concentration of specific antibodies in milk and in eggs. She performs the analysis of milk and egg samples to monitor for the presence of chemical residues of antibodies. Started 2/19/99. Volunteer Summer '99.

Future Plans (1999-2000):

Continue development of JIFSAN Advisory Council

Continue development of educational materials and training programs for enhancing the safety of fresh produce at the source in countries exporting to the U.S.

Continue internal grants program

Conduct a limited number of symposia and workshops on topics of importance to the food safety, applied nutrition, and animal health communities including:

Fumonisin Risk Assessment Workshop (scheduled for January 10-12, 2000)

Analytical Techniques for Allergenic Residues (scheduled for April 27-28, 2000)

First Annual CSL/JIFSAN Joint Symposium in Food Safety and Applied Nutrition: Risk Assessment and Communication in Food Safety (scheduled for June 20-22, 2000 in York, England)

Valuing Health Benefits of Food Safety (scheduled for September 13-15, 2000)

Schedule an additional offering of the International Food Safety Training Program

Expand operation of the Risk Analysis Clearinghouse

Develop strategic plan for JIFSAN

Continue development of international contacts and collaborative programs

