Annual Report 2003-2004

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

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Executive Summary

This Annual Report covers the seventh year of operation of the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) – the second year of the five-year renewal (September 2002 through September 2007). The non-competitive base funding for the seventh year was $2,280,000.

The JIFSAN Advisory Council met in October 2003. The meeting involves updates from JIFSAN, CFSAN, and CVM and discussions with Council members concerning issues of current interest. A day-long symposium involving presentations by principle investigators of projects funded by JIFSAN in the Internal Competitive Grants Program was held the day preceding the Council meeting. New JIFSAN staff employees during the year were Ms. Stephanie Swartz (part-time account clerk) and Ms. Sharahn Boykin (half-time Editorial Assistant). A Steering Committee was named to oversee and guide activities of the Food Safety Risk Analysis Clearinghouse.

Research plays an important role in JIFSAN's programs and assists in strengthening the knowledge base for public health policy. The Internal Competitive Grants Program funded 15 projects, with one of these being a new project initiated in July 2004 and five projects scheduled for completion during the year. Only one new project was awarded because of a severe reduction in JIFSAN's funding. Due to the accumulation of a significant amount of carry-forward funding over a period of more than six years before approval for is use was received, it was possible for JIFSAN to fund a significant amount of short-term (one or two years) research. In collaboration with the Food Industry Alliance (an informal designation for food companies that have contributed funding for research) and the International Life Sciences Institute (ILSI) North America (NA), JIFSAN provided funding for five projects dealing with issues raised from finding acrylamide in foods. Five additional projects were funded in areas addressing needs identified by CFSAN. One new project was funded to support the JIFSAN portion of a collaborative research effort being developed with the Department of Natural Resources and Environment, Victoria, Australia with whom JIFSAN has a cooperative agreement.

Three new postdoctoral associates were hired in the Postdoctoral Program. In this program, research scientists work in FDA laboratories for one or two years. These three positions replace individuals who had completed their programs.

A senior research scientist, split-funded with the Department of Chemistry and Biochemistry, continued a highly productive research program in carotenoids. The individual has established an international reputation for work in this area.

The Food Safety Risk Analysis Clearinghouse added several new sections (food allergy, bioterrorism, perchlorate) to the website and expanded others. A discussion forum was added to provide an open platform for addressing food safety issues. Several additions were made to the Clearinghouse's exclusive holdings, such as the Animal-derived Cosmetic Ingredient Database and the Food Handling Practices Model (FHPM). The Clearinghouse website received an average of 85,000 hits per month from over 120 different countries. Operation of the

Acrylamide Infonet (the FAO/WHO Acrylamide in Food Research Network) continued. The Infonet has approximately 200 research projects listed and over 110 research publications. The website received about 80,000 hits between July 2003 and January 2004.

The establishment of education and outreach programs, in areas within JIFSAN's responsibilities, is of vital importance. These programs are both domestic and international in scope. JIFSAN was involved as sponsor or co-sponsor in 10 different meetings (workshops, conferences, etc) this past year. After its successful Acrylamide in Food Workshop, organized with the food industry and NCFST, in 2002, JIFSAN organized and hosted a second workshop "Update – Scientific Issues, Uncertainties, and Research Strategies on Acrylamide in Food" in April 2002 in Chicago. More than 125 participants were involved from several countries, particularly the EU. The workshop focused on scientific progress made since 2002 and identification of remaining critical gaps. Information from the workshop is posted on the JIFSAN website.

JIFSAN joined with the International Life Sciences Institute (ILSI) North America (NA) in organizing and co-sponsoring a symposium and workshop on "Biotechnology-derived Nutritious Foods: Challenges and Opportunities in Asia." This was held in Nusa Dua, Bali, Indonesia in February/March 2004 just prior to the 4th Asian Conference on Food and Nutrition Safety. Approximately 55-60 individuals were involved in the symposium (first day) and workshop (second day). The focus of the workshop was the questions: Does biotechnology have a role in assisting in solutions to the nutrition problems encountered in Asia? If so, what is that role?

Continuing its interest and participation in the important issues surrounding transmissible spongiform encephalopathies (TSEs), JIFSAN co-sponsored two meetings dealing with this area. One of these was a Ceres® Forum on "Transmissible Spongiform Encephalopathies in Animal and Human Health: The Science and the Policy" in March 2004 (80-85 participants). The second was a symposium on "Prion Inactivation: Current Technology and Future Research Needs Symposium" in April 2004 (about 100 participants).

The Food Safety Risk Analysis Professional Development Program continued its development and offering of face-to-face courses. During the past year, efforts have been expanded with introduction of two courses that have been converted to distance delivery/learning technology. Conversion of a third course is being completed and a fourth will be done before funds from the grant supporting this effort are expended. The face-to-face courses were condensed and offered in a three-week Summer Integrated Program (SIP) in July 2004. This facilitates participation of individuals for whom attending individual face-to-face courses is not possible or inconvenient. The SIP was well-received, attracting 84 participants from 7 countries. It will be offered again in July 2005.

A strong program of international cooperation and training programs is being developed and offered. One of JIFSAN's major programs is the Good Agricultural Practices (GAPs) International Training Program for the production of fresh produce with reduced microbiological contamination. The five-day program was offered in Antigua, Guatemala; Tegucigalpa, Honduras; and Suwon, Korea. Approximately 50 individuals participated in each training program. Discussions have been initiated with a private sector firm to expand this type of program to additional commodity areas and countries.

As part of a cooperative agreement with the Central Science Laboratory (CSL) in the U.K., the fifth annual Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Analytical Innovations in Food Safety was held in York, England in June/July 2004. Approximately 100 individuals participated in the four sessions of the symposium.

Development of the Center for Risk Communication Research (CRCR), led by the Department of Communication (UM), is continuing with seed money being furnished by JIFSAN for a three-year period. An Advisory Board is being organized, two distinguished speakers gave invited seminars, and two research projects are currently in progress. The CRCR will serve as a focal point for scholarly activity and discussion related to risk communication.

The JIFSAN Student Internship Program continues to increase in importance and participation. UM undergraduate students are offered an opportunity to work with FDA scientists in their laboratories on specific projects identified by the FDA scientist involved. During 2003-2004, 44 different University of Maryland students volunteered for a total of 106 semesters in FDA laboratories. UM Students were co-authors on 20 posters presented at the FDA Science Forum

Background

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 multidisciplinary research, education and outreach 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, and Dr. Elizabeth Calvey (CFSAN, FDA) is the Deputy Associate Director. 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. 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 will help ensure that federal regulatory and scientific personnel remain in the forefront of food safety issues. This also will provide 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 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 through operation of 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. 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 competitive research grant 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 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.

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 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 supports and utilizes major instrumentation facilities (electron microscopy and nuclear magnetic resonance spectroscopy) on the campus and those facilities house University of Maryland and FDA personnel. These and other synergistic relationships outlined in the MOU 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.

FDA and the University provided financial support for the operation of JIFSAN. FDA provided a cooperative agreement for $6.5M for five years starting on September 30, 1997, subsequently supplemented to a total amount of $11,450,053. The cooperative agreement was renewed for an additional five-year period (September 2002 through September 2007) for up to $3,000,000 per year. 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 began 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 seventh 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 sixth year was $2,280,000.

Administrative Structure

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

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

- Ms. Stephanie Swartz began work in October 2003 as a part-time account clerk to assist the JIFSAN Budget Officer. This half-time position was established on the basis of a recommendation following an FDA site visit.

- Ms. Jennifer Hinton, previously a part-time employee in the JIFSAN Risk Analysis Clearinghouse, was hired as the Clearinghouse Web Librarian. Unfortunately, she resigned in May 2004 due to a change in her spouse’s employment location. She is currently working part-time from her new home location.

- Ms. Sharahn Boykin was hired in May 2004 to fill the half-time position as Editorial Assistant established to assist the JIFSAN Coordinator of Conferences and Communications.
- A Research Symposium highlighting the projects funded through the JIFSAN Internal Competitive Grants program was held October 29, 2003. The program involved Principle Investigators giving brief reports of the status of their research investigations. Several members of the Advisory Council attended the Symposium.

- The fourth meeting of the JIFSAN Advisory Council was held October 29-30, 2003. The Advisory Council is composed of representatives from 15 private sector corporations, six academic institutions, three from the consumer community, and one from a federal (UK) laboratory.

- The search to fill the position of Coordinator (Director) of the Food Safety Risk Analysis Clearinghouse was terminated without success. A Steering Committee has been named to oversee and guide activities of the Clearinghouse.

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 October 29-30. 2003. The next meeting has been scheduled for November 18-19, 2004. These meetings involve updates from JIFSAN, CFSAN and CVM and brief presentations on selected research and education/outreach programs. Also, discussions are held concerning potential industry needs that could influence future programs of JIFSAN.

The Director maintains contact with several Advisory Council representatives throughout the year.

Members of the Advisory Council include:

- Private sector industry

  Cargill, Inc (Dr. Jeanne McCaherty).
  Coca-Cola Company (Dr. Michael Carakostas)
  Campbell Soup Company (Dr. George Evancho)
  Frito-Lay (Dr. Steve Saunders)
  General Mills (Ms. Sarah Geisert)
  Gerber Products Company (Dr. Nicholas Hether)
  Kellogg Company (Dr. Mark Moorman)
  Kraft Foods (Mr. Ron Triani)
  McCormick and Company (Dr. Hamed Faridi)
  McNeil Specialty Products Company (Dr. Leslie Goldsmith)
  Masterfoods USA (Dr. Steven Rizk)
  Mead Johnson Nutritionals (Dr. Craig Hadley)
  Monsanto Company (Dr. Jerry Hjelle)
  Procter and Gamble Company (Dr. Keith Triebwasser)
  Unilever Bestfoods NA (Dr. Richard Lane)

- Representatives of Consumers' Interests

  Ms. Carol Tucker Foreman (Consumer Federation of America)
  Ms. Mary Heersink (Safe Tables Our Priority)
  Ms. Linda Golodner (National Consumers League)

- 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

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 Internal Competitive Research Program:

First initiated in 1998, 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. Additional collaborators may be from other institutions, if the PI so desires. These projects contribute to the science for current and future regulatory issues and activities that impact on public health policies.

Effective July 2002, projects are funded at $30,000 per year to be used for partial support of either a graduate research assistant or a postdoctoral associate and some operational support. 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 for continuation of the research, and availability of funding.

Four projects funded in July 2001 were completed in July 2004 or extended without addition funding to allow completion. On the basis of satisfactory progress towards meeting objectives and annual reports, five projects funded in July 2002 were continued for a third year and four projects in July 2003 were continued for a second year. One new project, proposed for a three-year period, was funded in July 2004.

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

Projects funded in July 2001 and due for completion in 2004:

- The use of tissue fluid correlations to predict drug residue levels in edible tissues, Natalie Eddington (Pharmaceutical Sciences, UMAB), James Peggins, Keesla Moulton, Jurgen von Bredow, and Pamela Chamberlain (FDA)
- Comparison of the effects of curcumin supplements in young and aged rats, Bernadene Magnuson, Monica Giusti (UM), Fred Hines, Sabine Franke and Hamida Alam (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 (Leah Waks following move of K. McComas) (UM) and Linda Sherman (FDA, originally Linda Suydam)
- 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)

Third-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) (funded for one year; extended for one year without additional funding)
- 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 (UM), Renate Reimschuessel and Badar Sheikh (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 Song (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).

Second-year projects (funded July 2003):

- Molecular mechanisms of fluoroquinolone and erythromycin resistance in *Campylobacter jejuni/coli*, Jianghong Meng (UM), Patrick McDermott and David White (FDA)
- *Campylobacter jejuni*-host interaction on the intestinal mucosal surface, Wenxia Song (UM), Shaohua Zhao and Ruby Singh (FDA)
- The impact of risk messages about bioterrorism on the U.S. food supply on audience attitudes and behaviors, Linda Aldoory (UM), Marjorie Davidson, Brenda Derby, Laura Fox, and Alan Levy (FDA)

- An integrated approach for identifying phototoxic cosmetic ingredients, Daniel Falvey (UM), Wayne Wamer and Patty Fu (FDA)

First-year project (funded July 2004):

- Predicting exposure estimates: Experimental food additive partitioning studies and model development, Robert Walker (UM), Timothy Begley and William Limm (FDA).

Collaborative/Cooperative Research Projects:

An important function of JIFSAN is establishing research efforts involving collaboration/cooperation with other organizations addressing current issues of mutual interest. The extent of this effort is dependent upon the availability of funding. During the past year, the availability of carry-forward funding has enabled JIFSAN to participate in developing several projects. Most of these projects are of one-year's duration and some have involved additional funding from external sources. Research projects have been funded to address issues involved with the finding of acrylamide in foods, to develop collaborative research with a partner in Victoria, Australia, and to address issues of specific interest to CFSAN. These research projects are described in more detail in Appendix B.

1. In collaboration with the Industry Acrylamide Alliance, an informal designation for food industries that have donated financial support for research, JIFSAN funded the following research projects. Financial support from JIFSAN involved the use of carry-forward funds.

    - Effects of consumer food preparation on acrylamide formation, George Sadler/Lauren Jackson, Illinois Institute of Technology (IIT)/National Center for Food Safety and Technology (NCFST)

    - Acrylamide content of home-prepared surface-browned foods, George Sadler/Lauren Jackson, IIT/NCFST (a requested expansion of the project listed above)

    - The kinetics of acrylamide inhibition/destruction/scavenging under various reaction/process conditions, Bryan Hanley, Leatherhead International, Leatherhead, UK (partially funded)

    - Proposed Consumer Attitude / Communications Research on Acrylamide, David Schmidt, International Food Information Council (IFIC) (partially funded, project complete, final report available from JIFSAN)

    - Development of a PBPK/PD model for acrylamide. Daniel Doerge and John Young, National Center for Toxicological Research. (This project supplements current research in progress and is funded through a Cooperative Research and Development Agreement (CRADA) being established with D. R. Lineback (JIFSAN) as co-PI. The International Life Sciences Institute (ILSI) North America (NA) contributed to the funding for this project.)

2. As the JIFSAN portion of a cooperative research program being developed with the Department of Natural Resources and Environment, Melbourne, Victoria, Australia with whom JIFSAN has a Memorandum of Cooperation, the following three-year research project was funded.

    - Rapid assay for detecting human enteric viruses and viral survival dynamics on fresh fruits and vegetables, Jianghong Meng, Department of Nutrition and Food Science, University of Maryland.

3. Additional Research Projects Funded

    These projects are funded from JIFSAN carry-forward funds.

    - Analysis of sera from previous Norwalk-like virus human exposure study, Mark Sobsey, Department of Environmental Sciences and Engineering, University of North Carolina.

    - Development of molecularly-imprinted polymers (MIPs) for selective detection of marine biotoxins, Kenneth J. Shea, Department of Chemistry, University of California, Irvine.

    - Enzymatic Degradation of Prion Surrogate Proteins, Jason C. H. Shih, Department of Poultry Science, North Carolina State University.

    - Analysis of data collected in epidemiologic and microbiologic field studies of domestic and imported produce, Christine Moe, Department of International Health, Emory University.

    - Public perceptions of conflicting information about safety guidelines for consumption of fish, Linda Aldoory, Department of Communications (CRCR), University of Maryland. Collaborator: Marjorie Davidson, CFSAN, FDA (funded with JIFSAN DRIF)

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. Originally, six postdoctoral research associates were recruited to work in FDA laboratories in areas in which significant knowledge gaps or the lack of appropriate scientific data, methods, or models exist.

Not only does this program generate significant amounts of new knowledge applicable to needs of the FDA, it allows the postdoctoral research associates the opportunity to work in a regulatory environment. The FDA also has the important opportunity of evaluating the potential of these individuals to become productive staff members when vacancies exist.

Following response to the posting of position announcements to replace individuals who had completed their appointments, three new postdoctoral research associates have been hired. The search for one additional position was terminated in May 2004 due to budget reductions.

The following projects, identified by CFSAN and funded through this program, are currently in progress.

- Rapid determination of food integrity and identification of food borne bacteria using Fourier transform near infrared (FT-NIR) spectroscopy and pattern recognition techniques, Frederick Fry (FDA), Janie Dubois (JIFSAN Research Associate)
- Aquaculture drugs: LC/MS protocols for marker determination, Robert Dickey (FDA), Ann Abraham (JIFSAN Research Associate)
- Molecular phylogenetic identification of potential foodborne agents of bio-terrorism, Eric Brown (FDA), Alice Heyford (JIFSAN Research Associate)
- Development of a specific monoclonal antibody for *Enterobacter* sakazakii: Identification and an immunoassay using color-coded bio-nanotubes, Kun-Ho Seo (FDA), Sang-Bok Lee (UM), Kwang-Young Song (JIFSAN Research Associate)

Senior Research Scientist (split funded with Department of Chemistry and Biochemistry):

Dr. Frederick Khachik is a senior research scientist and adjunct professor with an appointment in the Department of Chemistry and Biochemistry. 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.

Additional information on progress in this research program is in Appendix C.

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.

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. The effective use of the arrangements for shared facilities (electron microscopy and nuclear magnetic resonance) is increasing since CFSAN has relocated to its facilities in College Park.

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 Student Internship Program:

The JIFSAN Student Internship program is designed to provide University of Maryland undergraduate 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.

Students' participation in the program requires that they have completed two college semesters, be willing to commit to volunteering 100 hours during the first term as an intern, and submit a completed application form, current transcript and resume. The positions may be part-time during the semester and full-time during the summer. Undergraduate students volunteer to work on a project, listed by an FDA staff member. Upon successful completion of the initial 100-hour 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.

Forty-four different University of Maryland students volunteered as interns for a total of 106 semesters in FDA laboratories and supporting offices during 2003-2004. Efforts are being made to extend opportunities for internships beyond laboratory experiences. The addition of new project descriptions enabled five students to work in non-lab related positions. UM Students were co-authors on 20 posters presented at the FDA Science Forum

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 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.

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 focus in its programs.

Progress for the Food Safety Risk Analysis Clearinghouse includes:

- Information structure updates include division of information heavy pages into easy-to-navigate smaller subsections. The regular search and advanced search functionalities have also been improved for more efficient information access.

- A shorter and easy to remember domain address, http://www.FoodRisk.org, has been acquired.

- Several new sections have been added to the Clearinghouse to keep pace with recent trends, such as sections on food allergy, bioterrorism, and perchlorate. In addition, some of the existing sections have been significantly expanded, such as risk profiles, weight management, and acrylamide.

- The digitization of 1993 Federal Register, Volume 58, Part IV is near completion. This volume, which is not available on the U.S. GPO web site, provides information on nutrition and labeling requirements.

- A discussion forum has been developed with the aim of providing an open platform to discuss food safety-related issues. In addition, QRA-Analytica listserv has been initiated to discuss quantitative risk assessment issues related with the use of Analytica software.

- The Clearinghouse web site received on average 85,000 hits per month from over 120 different countries.

- The Clearinghouse made several additions to its exclusive holdings. Examples of new additions include:

  - Animal-Derived Cosmetic Ingredient Database

    RTI, under contract with FDA, constructed this database on cosmetic ingredients that could potentially be derived from animals. These ingredients are of interest because of the potential for transmission of Bovine Spongiform Encephalopathy (BSE) or other Transmissible Spongiform Encephalopathies (TSEs).

  - The Food Handling Practices Model (FHPM)

    Each year millions of cases of foodborne illness occur in the United States. Preceding most cases of foodborne illness is contamination by pathogens and failure to destroy or sufficiently control pathogens in retail and foodservice establishments or households. FDA under contract with RTI International developed a quantitative simulation model of the effects of contributing factors on the incidence of foodborne illness. The food handling practices model is a computer simulation model that allows users to estimate how one or more changes in food handling practices at the retail, foodservice, or household level may affect the annual number of food-related illnesses in the US.

  - Current State of Food Product Open Dates in the U.S.

    Eastern Research Group, under contract with FDA, conducted this study of food product open dates. The information in this study was sought through literature reviews, multiple in-store surveys of food products, and discussions with industry personnel and trade organizations. The survey universe consists of 149 product

categories covering baked, dairy, frozen, refrigerated, shelf-stable, and chilled ready-to-eat food products.

The Clearinghouse participated, with its interactive display, in the following meetings:

- Society of Risk Analysis Annual Meeting, Baltimore, December 8-10, 2003
- 10th Annual FDA Science Forum, Washington DC, May 18-19, 2004
- IAFP Annual Meeting, Phoenix, August 08-11, 2004.

Acrylamide Infonet

At the request of the United Nations' World Health Organization (WHO) and Food and Agricultural Organization (FAO), JIFSAN operates the Acrylamide Infonet (www.acrylamide-food.org), the WHO/FAO Acrylamide in Food Network, through the Risk Analysis Clearinghouse. The focal point for the network is the website which contains a database of researchers/data providers; references to research publications; studies in development database; listing of acrylamide websites; acrylamide documentation (general information); events and activities; Infonet updates; and call for data (a call by WHO for analytical data). The Infonet has approximately 200 research projects listed (135 ongoing, 30 presented, 24 published, 9 in development) and about 110 publications listed. The website received close to 80,000 hits between July 2003 and January 2004.

The Infonet is intended to be a worldwide resource for the issues of acrylamide in food. It includes formal research, surveillance/monitoring and industry investigations, etc. Any interested party may submit information, and it is hoped that government agencies, research institutions, industry and others will share information via the network.

Coordination is occurring with the European Food Standards Agency (EFSA) and their information database and the Joint Research Centre IRMM and their acrylamide content in foods analytical database to ensure information and data sharing.

MEETINGS CO-SPONSORED BY JIFSAN

Workshop on Peer Review of Risk Assessment and Related Activities

JIFSAN, the Risk Assessment Consortium (RAC), and the Society for Risk Analysis (SRA) jointly sponsored a Workshop on Peer Review of Risk Assessments and Related Activities, September 30, 2003. The workshop included a discussion of the elements of a successful program for peer review of risk assessments. Its focus was on identifying key issues to be considered when implementing a peer review process. The role of data quality guidelines in peer review was also an element of the workshop. The objective of the workshop was to share information, thoughts, and opinions on all aspects of peer review of risk assessment and related activities among academia, industry, consumers and government. A concept paper will be developed on recommended guidelines for conducting peer review that can be used by any organization. This paper is to be posted on the JIFSAN Risk Analysis Clearinghouse.

First Annual Symposium on World Hunger

JIFSAN co-sponsored the First Annual Symposium on World Hunger at the University of Maryland at College Park on October 14, 2003. The keynote speaker was Dr. Per-Pinstrup Andersen, 2001 World Food Prize Laureate. More than 80 individuals participated in the symposium.

Expert Working Group on the "Role of Diet in Blood Glucose Response and Resulting Health Outcomes"

In collaboration with the International Life Sciences Institute North America (ILSI NA), JIFSAN co-sponsored an Expert Working Group discussion of the "Role of Diet in Blood Glucose Response and Resulting Health Outcomes." The meeting was held at ILSI headquarters in Washington D.C., October 12-13, 2003. A group of 10 experts, selected from around the world, discussed key issues involving the use of glycemic response to food as a basis for food selection in prevention of chronic diseases. They identified areas where there was scientific agreement within the group, where it is lacking, and where there is uncertainty. Recommendations were suggested for research needed to resolve the scientific debate on this topic. A summary is being prepared for submission for publication.

Workshop on Collection Food Attribution Data

In cooperation with the Food Safety Research Consortium (FSRC), JIFSAN co-sponsored a workshop concerning the collection of food attribution data to support risk ranking. The workshop was targeted toward understanding the strengths and weaknesses of approaches currently available to collect this data, such as outbreak data, and to develop consensus on how food attribution data can be obtained best. The workshop, in which approximately 30 people participated, was held in Atlanta, Georgia, October 31, 2003. A written report will be issued summarizing the deliberations and any data collection ideas and recommendations produced at the workshop.

Symposium and Workshop on "Biotechnology-derived Nutritious Foods: Challenges and Opportunities in Asia

In cooperation with the International Life Sciences Institute (ILSI), ILSI Human Nutrition Institute, ILSI Southeast Region, and Institut Pertainian Bogor (Bogor Agricultural University, Indonesia), JIFSAN organized and co-sponsored a Symposium and Workshop on "Biotechnology-derived Nutritious Foods: Challenges and Opportunities in Asia," February 29-March 1, Nusa Dua, Bali, Indonesia. The first day was a symposium and the second day an invitation-only workshop. Approximately 55-60 individuals were involved in the symposium and workshop together. The general purpose was to ask and discuss the questions: Does biotechnology have a role in assisting in solutions to the nutrition problems encountered in Asia? If so, what is that role? A publication should result from this symposium/workshop.

The Symposium and Workshop were held two days prior to the 4th Asian Conference on Food and Nutrition Safety held at the same location, March 2-5. JIFSAN was listed among the organizations whose support and co-sponsorship made the conference possible.

Ceres® Forum on "Transmissible Spongiform Encephalopathies in Animal and Human Health: The Science and the Policy"

In cooperation with the Center for Food and Nutrition Policy (CFNP), Virginia Tech – Alexandria, JIFSAN cosponsored the Ceres® Forum on "Transmissible Spongiform Encephalopathies in Animal and Human Health: The Science and the Policy. This forum, with an attendance of 80-85, was held March 8-9, 2004 in Washington, DC. It brought together scientists, policymakers, and other stakeholders to discuss a breadth of issues in this important area. Potential impacts of prion diseases on public health and the agricultural economy served as a background for identifying gaps in disease research and public policy. A copy of the proceedings is available on the CFNP website (http://www.cfnp.vt.edu/images/Misc/CFNP_TSE_Proceedings.pdf)

"Prion Inactivation: Current Technology and Future Research Needs Symposium"

In cooperation with the National Food Processors Association (NFPA), JIFSAN was one of the co-sponsors for a symposium on "Prion Inactivation: Current Technology and Future Research Needs." The symposium was held in Washington, D.C., April 1, 2004 with about 100 in attendance. The focus of the symposium was on the regulatory and technological approaches to minimizing exposure of human food and animal by-products to Transmissible Spongiform Encephalopathy prions. This included work that is in progress on decontaminating high-risk materials for alternative uses and future resesearch needs on prion inactivation. A CDROM of the proceedings is available on the NFPA website (http://www.nfpa-food.org/upload/pdfs/Publications_N-P.pdf).

Workshop "Update: Scientific Issues, Uncertainties, and Research Strategies on Acrylamide in Food"

JIFSAN has been involved in a coordination role, domestically and internationally, in the acrylamide in food issues essentially since April 2002 and, with the National Center for Food Safety and Technology (NCFST), organized a Workshop "Acrylamide in Food: Scientific Issues, Uncertainties, and Research Strategies" held in Chicago, IL, October 28-30, 2002.

With the continuing emphasis on this issue globally, a second Workshop "Update: Scientific Issues, Uncertainties, and Research Strategies on Acrylamide in Food" was organized in collaboration with the food industry and held April 13-15, 2004 in Chicago. The goal of this workshop was to provide a venue for sharing the status and results of research conducted globally on acrylamide in food since the 2002 Workshop. The Workshop focused on science and identification of remaining critical data gaps for evaluating the impact of acrylamide in food. A limited number of high-priority needs for further action, project development, and potential funding were identified. Approximately 120-125 from North America and Europe participated in the Workshop.

The following publications result from JIFSAN and the JIFSAN Director's involvement in the acrylamide in foods issues and the facilitation/coordination role in which they have been involved domestically and internationally.

- M. A. Slayne and D. R. Lineback. 2005. Acrylamide: Considerations for risk management. J. AOAC Internatl. 88 (1): in press.
- D. R. Lineback, T. Wenzl, O.-P. Ostermann, B. De La Calle, and E. Anklam. 2005. Overview of acrylamide monitoring databases. J. AOAC Internatl. 88 (1): in press.

Ceres® Workshop on Highs and Lows of High-Fructose Corn Syrup (HFCS)

In cooperation with the Center for Food and Nutrition Policy (CFNP), Virginia Tech – Alexandria, JIFSAN co-sponsored a workshop on "The Highs and Lows of High-Fructose Corn Syrup" held May 10, 2004. A small group of experts in the field were invited to address issues being raised about the consumption of high-fructose corn syrup and its role in nutrition and obesity. Goals were to more clearly identify the issues, identify strengths and weaknesses in the scientific base underpinning the disagreements in this area, where there is uncertainty, and what needs to be done to gain clarity in this increasingly important issue. Minutes of the workshop and an Executive Summary are to distributed and posted on the CFNP website.

First International Workshop on Antioxidant Methods

In cooperation with the Division of Agricultural and Food Chemistry, American Chemical Society, JIFSAN served as a co-sponsor of the "First International Workshop on Antioxidant Methods" in Orlando, FL, June 16-18, 2004. The workshop brought together experts to discuss and determine consensus on analytical methods that will be validated by collaborative studies to establish benchmark methods to eventually support health claims.

TRAINING PROGRAMS AND COURSES

Food Safety Risk Analysis Professional Development Training Program

The Professional Development Training Program in Food Safety Risk Analysis is being developed to provide training to national and international audiences that target the key components of risk analysis. The program is developed and taught by FDA staff, UM faculty, and private consultants. It is intended to be self-funding.

This past year, domestic and international participation was significantly expanded with development and introduction of (a) an on-line distance version of a portion of the courses in the program and (b) a three-week Summer Integrated Program (SIP) held in July 2004. In the SIP, courses in the program were condensed and all were offered during the three-week period. This recognized the need and increased convenience for those who could not come to the University of Maryland campus for the individually-scheduled face-to-face offerings of the courses. The SIP involved 84 participants from 7 countries.

INTERNATIONAL COOPERATION AND TRAINING PROGRAMS

International Training Programs in Minimizing Food Safety Hazards for Fresh Fruits and Vegetables

- Good Agricultural Practices (GAPs) Train-the Trainer International Training Programs were offered in Antigua, Guatemala (March 2004) and Tegucigalpa, Honduras (June 2004). A third program planned for the period covered by this report will be offered in Suwon, Korea in November 2004. Approximately 50 individuals attended each of the five-day training programs.

- The instructional team for the training programs is composed of faculty/staff from Clemson University, Mississippi State University, University of Maryland, Cornell University, and the FDA. A core group of instructors are used for each of the training programs that are offered in English or Spanish with text materials in either language.

- A comprehensive review of the GAPs Training Program is in progress. The review was planned jointly by JIFSAN and FDA/CFSAN staff involved with the program. An external Review Team of three experienced individuals is conducting the review with site visits to Mayaguez, Puerto Rico (May 2004); Port of Spain, Trinidad (May 2004); Petrolina, Brazil (August 2004), and Puebla, Mexico (August 2004). Additionally, one of the instructors from Clemson University participated in each of the site visits. A final report will be presented following completion of the site visits.

Fifth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Analytical Innovations 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 symposium on themes relating to food safety and nutrition is included. These alternate between York, UK and College Park, MD. The Fifth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Analytical Innovations in Food Safety was held June 30th – July 2, 2004 at the Central Science Laboratory, Sand Hutton (York), UK. Approximately 100 individuals participated in the event. The announcement and program for the Symposium are available on the JIFSAN website (http://www.jifsan.umd.edu/csl2004.htm).

OTHER ACTIVITIES

Risk Analysis Seminar Series

JIFSAN and the CFSAN Staff College are co-sponsoring a CFSAN-wide education seminar series of risk analysis. The eight seminars in the series highlight several topic areas of current interest. Speakers are internationally-recognized authorities from within CFSAN and from other organizations in the U.S. and Europe. Topics include:

- The expanding role of “Risk Sciences” in food safety policy
- The Precautionary Principle and the risk assessment-risk management interface

- Analyzing risk from food allergens
- Hot topics in risk analysis at CFSAN – A panel discussion
- Evaluating BSE risks
- Risk analysis activities at CFSAN
- Benefits of the risk analysis approach: Perspectives from the food industry
- Assessing risks – handling uncertainty from analytical results

JIFSAN Participation in Exhibitions

JIFSAN staffed a booth (display) at

- IFT's International Food Safety and Quality Conference and Expo, Orlando, FL (November 2003)

- Society of Risk Analysis Annual Meeting, Baltimore, MD (December 2003)

- FDA Science Forum, Washington, D.C. (May 2004)

- International Association for Food Protection, Phoenix, AZ (August, 2004)

Establishment of a Center for Risk Communication Research

JIFSAN is furnishing "seed money" to initiate establishment of a Center for Risk Communication Research (CRCR) led by the Department of Communication, University of Maryland. JIFSAN has pledged support for three years. The Center's mission is to advance understanding about how communication helps control risk, about how the public perceives risk communication, and about the political, economic and social contexts for risk communication. The Center's goals include helping to establish public and scholarly agendas for risk communication research, collaborating with other institutions and individuals to secure funding for research projects, and providing support for research and fellowships.

The Center had a kick-off reception in December 2003 at the annual convention of the Society for Risk Analysis. The Center is in the process of securing funds to study public perceptions of conflicting risk information disseminated by the media. A website has been created (http://www.riskcenter.umd.edu). An Advisory Committee is being established. Members are being selected from a number of nationally recognized communicators who were hosted at a reception held at a national meeting. The CRCR also hosted a seminar in April 2003. The speaker was Dr. Sharon Dunwoody, University of Wisconsin, Madison, a national expert on risk communication.

Future Plans (2004-2005)

Due to severe budget reductions by the FDA, the budget allocated for this year will require the use of carry-forward funds. Reductions/eliminations in several JIFSAN programs are necessitated. Efforts will continue to obtain external funding for program support.

1. Administrative

- David Lineback will retire as Director on September 30, 2005.
- Develop a 2004-2005 work plan (Goals).
- A Research Grant Symposium featuring reports by Principle Investigators of projects funded through the JIFSAN Internal Competitive Grants Program is scheduled for November 18.
- A meeting of the JIFSAN Advisory Council is scheduled for November 18-19, 2004.
- Initiate a JIFSAN newsletter to be published electronically.
- JIFSAN plans to participate with its display at the Society for Risk Analysis Annual Meeting, December 5-8, Palm Springs, CA; the 11th Annual FDA Science Forum, April 27-28, Washington, D.C.; and the IAFP Annual Meeting, August 14-17, Baltimore, MD.

2. Research Initiatives

- JIFSAN Internal Competitive Grants Program
    - Five projects funded in July 2002 will be completed or extended without additional funding to allow completion.
    - Four projects funded in July 2003 will be continued.
    - One project funded in July 1, 2004 will be continued.
    - All of the above are dependent on filing an annual report indicating satisfactory progress in meeting project goals/objectives.
    - No new projects will be solicited or funded. Upon completion of these projects, the Internal Competitive Grants Program will be discontinued.
    - The JIFSAN Postdoctoral Research Associate program will be discontinued. Funding for a second year for those now employed in the program will be continued.
    - A Cooperative Research and Development Award (CRADA) will be initiated with the National Center for Toxicological Research (NCTR) to add funding to work already in progress to develop a PBPK/PD model for acrylamide.

3. Education and Outreach Efforts

- Operation of the Risk Analysis Clearinghouse will be further developed under the guidance of the recently appointed Steering Committee. A major emphasis will be acquisition of data beyond those sought from microbiological risk assessments. Operation of the Acrylamide Infonet, including acquisition of data on the occurrence of acrylamide in foods in countries around the world for later use in risk assessments, will continue.
- Operation of the JIFSAN Internship Program will continue.

- Train-the-trainer programs in minimizing food safety hazards in production of fresh fruits and vegetables (GAPs Training Programs) will be offered. Three locations have been identified: Mexico, Brazil, and Thailand. One offering planned for 2003-2004 in Korea is scheduled for November 2004.These will involve approximately 50 individuals at each location.

  The final report from the comprehensive review of the GAPs International Training Program is to be received in October 2004. It will be evaluated, discussed and the pertinent recommendations will be initiated.

- The Food Safety Risk Analysis Professional Development Program will be offered a second time as a Summer Integrated Program in July 2005. Conversion of the Food Safety Risk Analysis Courses into on-line distance learning versions will continue through continuation of a USDA grant obtained by the College of Agriculture and Natural Resources. The Risk Communications on-line course is scheduled to be piloted in late February.

- JIFSAN will co-sponsor the Second Annual Symposium on World Hunger at the University of Maryland at College Park, December 2, 2004.

- Support and development of the Center for Risk Communication Research will continue in collaboration with the Department of Communications (UM).

- One of the best means for obtaining visibility for JIFSAN and its education/outreach programs is through co-sponsorship of symposia, workshops, conferences and other types of meetings. This will continue in areas appropriate to JIFSAN's responsibilities. The extent will depend upon the availability of funding.

  - JIFSAN will-cosponsor a Short Course for the Food Sector: Foodbone & Waterborne Diseases in Kiev, Ukraine, December 13-15, 2004. This is being done in cooperation with the Coca Cola Company and FAO.

  - JIFSAN will co-sponsor a "Global Good Agricultural Practices Conference to Explore the Impact of Current Research and Extension Programs" in cooperation with Cornell University. Other sponsors are the FDA and the USDA. The conference will provide an update on new global GAPs research data and information for use by the teaching teams involved in GAPs training programs for Cornell University and JIFSAN. The conference is scheduled for February 2005 in Orlando, FL.

  - JIFSAN will co-sponsor a two-day "International Conference on Food Safety Priority Setting" that is planned for Europe in May 2005. This is being done with the Food Safety Research Consortium (FSRC), MED.VET.NET and WHO and is dependent upon obtaining the necessary funding from other co-sponsors.

- Initiation of an International Training Center will continue to develop and offer additional training programs internationally beyond the established Good Agricultural Practices Train-the-Trainer program. Negotiations are in the final stages with an industrial organization to develop and conduct training directed towards specific commodities in identified countries and regions.

4. International Collaboration

- Planning for the Sixth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition, to be held at the University of Maryland, June 28-30, 2005, is in progress. The topic will be Bioactive Food Components.

- Development of a cooperative research effort between JIFSAN and the Department of Natural Resources and Environment (DNRE), State of Victoria, Australia is being initiated. JIFSAN has a memorandum of understanding with DNRE for development of collaborative efforts in research. Efforts are being made to establish a research effort in the detection and measurement of microbial pathogens on fresh-cut produce. This will involve the development of rapid methods of detection/analysis, with an emphasis on viruses, by Dr. Jianghong Meng, Department of Nutrition and Food Science, College of Agriculture and Natural Resources, UM. This project was funded and initiated in 2004. Determination of microbial pathogens in/on freshly harvested produce will occur as part of the project to be funded in Victoria. Some analyses may be done with Maryland produce.

APPENDIX A

Projects Funded Through JIFSAN Competitive Internal Research Program

Projects funded in July 2001 (completion in 2004):

- **The use of tissue fluid correlations to predict drug residue levels in edible tissues.** Natalie Eddington (Pharmaceutical Sciences, UMAB), James Peggins, Keesla Moulton, Jurgen von Bredow, and Pamela Chamberlain (FDA)

  Final report from this project is pending.

- **Comparison of the effects of curcumin supplements in young and aged rats.** Bernadene Magnuson, Monica Giusti (UM), Fred Hines, Sabine Franke and Hamida Alam (FDA)

  It was demonstrated previously that aging had a significant effect on the chemopreventive properties of dietary curcumin. 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. In addition, all tissues from these rats were evaluated for pathological changes to determine whether there was any benefit or detriment to long-term dietary curcumin supplements in the different aged animals. The experiments were then conducted to elucidate a potential mechanism(s) for the age-related differences in response

  Summary (Final Report):

  It was demonstrated that the efficacy of curcumin inhibition of early stage of colon carcinogenesis varies with age, despite no age-related difference in the effect of curcumin on liver or serum parameters. Complete evaluation of histopathological changes in tissues from young, mature and old rats fed curcumin for 3 months did not find evidence for curcumin inhibition of common age-related pathological conditions, with the exception of neoplastic changes. The increased incidence of several pathological changes in curcumin-fed rats, such as myxomatous degeneration of heart valves, deserves further study. A reduction in malignant neoplasms in old rats fed curcumin compared to control diet, supports the hypothesis that curcumin is an effective chemopreventive agent in numerous tissues. Why curcumin is effective in chemoprevention in old, but not mature, rats remains unknown. The first experiment is being repeated to confirm that curcumin is ineffective in preventing colonic precancerous lesions in mature animals. If confirmed, it would be important to determine if curcumin would continue to be ineffective in adult rats through carcinogenesis to invasive tumor development. This observation has implications for the use of curcumin in colon cancer chemoprevention, and if the mechanisms were elucidated, could potentially facilitate improving design of preclinical studies for prevention of cancers that increase with age.

Publications:

1. Y. Kwon, M. Malik and B. A. Magnuson**.** 2004. Inhibition of colonic aberrant crypt foci by curcumin in rats is affected by age. Nutrition and Cancer. In press.
2. M. Malik and B. A. Magnuson, 2004. Rapid method for identification of chemopreventive compounds using multiplex RT-PCR for cyclooxygenase mRNA expression. Cancer Detection and Prevention. Accepted.

Abstracts:

1. M. Malik and B. Magnuson. 2002. *Semi-quantitative multiplex PCR using LabChip technology.* CE in Biotechnology and Pharmaceutical Industries. Practical Applications for Analysis of Proteins, Nucleotides and Small Molecules. Washington DC August 17-19.
2. S. Francke-Carroll, J. E. Montgomery, F. A. Hines, and B. A. Magnuson. 2002. 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.
3. Y. Kwon, J. Montgomery, M. Malik and B. Magnuson. 2002. Aging alters the inhibition of colonic aberrant crypt foci by curcumin. American Institute for Cancer Research Annual Research Conference, Washington DC. July 11-12.

- **Investigating the perceived credibility of FDA's Advisory Committee meetings as techniques for communicating about food, drug, biologics and medical device issues.** Katherine McComas (Leah Waks following move of K. McComas) (UM) and Linda Sherman (FDA) (Originally Linda Suydam, FDA)

  A final report from this project is pending. A summary report is available on the FDA website (http://www.fda.gov/oc/advisory/acstudy0904/JIFSANresearch.html).

- **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)

  Various primary models that predict the growth and survival of *L. monocytogenes* as a function of pH, temperature, and PURASAL P Opti. Form 4^{TM} (60% solution, 56% potassium lactate and 4% sodium diacetate) were compared in order to determine which model will best describe growth and survival kinetics of *L. monocytogenes* in BHI broth. The impact of pH, temperature, and concentrations of antimicrobial agent on the response of *L. monocytogenes* is the primary source of data for the development of primary and secondary models. A complete 6 × 4 × 4 factorial design arrangement of temperature (4, 10, 17, 24, 30 and 37ºC), pH (5.5, 6.0, 6.5 and 7.0) and concentration of PURASAL P Opti.Form 4^{TM} (0.0. 1.8, 3.0 and 4.5%) was used in which all combinations of different factors were investigated. This design allows a straight forward modeling of interactions between the environmental factors and growth and survival kinetics of *L. monocytogenes*. Growth curves were generated and iteratively fitted using non-linear regression analysis

to the Gompertz and Buchanan three-phase linear models using a GraphPad PRISM® as well as to Baranyi model using DMFit version 2.0, an Excel add-in for fitting sigmoid curves. Gomperz model has described the growth of *L. monocytogenes* efficiently at higher pH (6.5 and 7.0), however, the model was not able to describe the survival and death curves at pH 5.5 and 6.0 in the presence of PURASAL P Opti.Form 4™ excluding 1.8% at pH 6.0. On the other hand, the output results of Baranyi model showed no lag time values for faster growth rate at temperature, 30°C and 37°C, where the fitted data have only described the exponential and stationary phases. It was found that Buchanan three-phase linear equation has overcome the limitations of Gomperz and Baranyi models, as well as can be used to predict the death and survival of *L. monocytogenes*. Now, various secondary models for the lag time and growth and survival rates of *L. monocytogenes* are being compared as a function of environmental parameters including pH, temperatures, and concentrations of PURASAL P Opti.form 4.

Publications:

1. K. S. Yoon, C. N. Burnette, K. Abou Zeid, and R. C. Whiting. 2004. Control of growth and survival of *Listeria monocytogenes* on smoked salmon by combined potassium lactate and sodium diacetate and freezing stress during refrigeration and frozen storage. J. Food Prot. 67(11):2465-2471.
2. K. S. Yoon, C. N. Burnette, and R. C. Whiting 2003. 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 during storage at 4 or 10°C. J. Food Prot. 66(8):1469-1473.

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).

  This one-year study (extended for one year without additional funding) evaluated the transfer of soy isoflavones into the egg. The objective is 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 on the regular diet.

  Genistein and its metabolites were transferred into the egg yolks of birds receiving genistein supplements, with a double significance:
  - It demonstrates the potential for isoflavone exposure of embryo during development.
  - 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

1. Supplementation of encapsulated genistein and genistin

   Genistein and genistein metabolites were detected in the egg yolks of treated hens. Trace concentrations of genistein were detected in the control group, due to the presence of genistein derivatives in the diet. Neither genistein, nor its metabolites were found in egg white. Levels of genistein in the eggs increased significantly from the 3rd day of supplementation and reached the maximum about 2 days after the supplementation stopped. The higher dose of genistein supplementation resulted in a higher genistein concentrations in egg yolks. Glycosylation in genistin decreased the transfer and accumulation of genistein into the egg yolks.

2. Administering a variety of isoflavones in the diet

   Isoflavones in a typical bird diet:
   The analysis of a typical poultry diet, Purina diet, showed that about 5.5 mg/g total isoflavones (normalized to the aglycone form) were present, among which 51% was genistein derivatives, 36% was daidzein derivatives, 13% was glycitein derivatives. Hens fed with such diet may lay eggs containing isoflavones.

   Isoflavones mixture supplemented to quails
   Traces of glycitein were also found but were not quantified due to co-elution with daidzein. Therefore, the amount of daidzein may be slightly overestimated. The highest concentration (1.9 and 1.5 ug daidzein and genistein/g egg yolk, respectively) was reached on day 6, right after the last day of supplementation. No significant effect of isoflavones supplementation was found on cholesterol concentration in the eggs.

   Isoflavone content on commercial eggs
   About 5.5 mg/g total isoflavones (normalized to aglycone form) were found in the diet, among which 53% was genistein derivatives, 40% daidzein derivatives, 7% glycitein derivatives. Daidzein and genistein were found in all eggs evaluated, and the amounts of daidzein and genistein were highly correlated (r=0.866). However, the ratio of daidzein to genistein in the egg averaged 3.5:1, which was much higher than that in the diet (0.7:1). It is possible that more daidzein than genistein was transferred to the egg, or that genistein is easier to metabolize and the metabolites are present rather than the aglycone. No significant difference was found between isoflavone contents of regular and specialty eggs.

Our results showed that a mixture of isoflavones in the diet could be transferred into the egg yolks, and that commercially available isoflavone-enriched eggs could be developed by manipulating the diet of hens. This dietary source of phytoestrogens for humans would be complementary to a plethora of commercial nutritional supplements marketed primarily to women. It is therefore important to assess the impact of these compounds on health for both humans and for domestic species.

Publications:

1. F. Lin, J. Wu, M. A. Abdelnabi, M. A., Ottinger, and M. M. Giusti. 2004. Effects of dose and glycosylation on the transfer of genistein into the eggs of the Japanese quail (*Coturnix japonica*). J. Agric. Food Chem. 52 2397-2403.
2. M. A. Ottinger, F. Lin, J. M. Wu, M. J. Quinn, Jr., M. A. Abdelnabi, E. T. Lavoie, and M. M. Giusti. 2004. Isoflavones-enriched eggs: Transfer of isoflavones into eggs and health benefits. In The Amazing Egg: Nature's Perfect Functional Food for Health. The Post-Symposium Proceeding Book. The 3rd International Symposium on Egg Nutrition for Health. Banff, Alberta, Canada, April 18-21.

Presentations:

- F. Lin, and M. M. Giusti. 2004. Detection and quantitation of soy isoflavones in commercial eggs. Inst. Food Technol. Annual Meeting, Las Vegas, Nevada July 13-16.
- M. M. Giusti, F. Lin, J. Wu, M. A. Abdelnabi, and M. A. Ottinger. 2004. Effects of dose and glycosylation on the transfer of isoflavones into the eggs of the Japanese quail. 3rd International Symposium on Egg Nutrition for Health. Banff, Alberta, Canada, April 18-21.
- M. A. Ottinger, J. Wu, M. A. Abdelnabi, M. Quinn, and M. M. Giusti. 2002. Transfer and accumulation of genistein, a soybean isoflavone, into the eggs of the Japanese Quail (*Coturnix japonica*). Poultry Science Association (PSA) Annual Meeting, Newark, Delaware, August 11-14.

- **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).

  The purpose of this project is to optimize approaches for the protection of the United States food supply against bioterror agents such as anthrax and the botulinum toxin. Previous work in this laboratory has established that the antimicrobial peptides nisin and sublancin, which are of the lantibiotic family of antimicrobial peptides, are highly effective inhibitors of the outgrowth of spore pathogens such as *Bacillus anthracis* and *Clostridium botulinum*, especially when the nisin and sublancin peptides are employed as mixtures. In the current stage of the project, focus has been on a study of the target of nisin action. It is a premise of this project that the unusual dehydro residues of nisin exert an inhibitory effect by means of becoming covalently attached to a component or components of the bacterial spore/cell, and that this covalent modification of the sensitive target is important to the mechanism of inhibition. Identification and characterization of this sensitive target would be extremely useful for developing an understanding of how to optimize the inhibitory effect against spore pathogens.

  The strategy for identification of these targets is to label nisin in a variety of ways, and to treat spores/cells with this labeled nisin under conditions that should be

conducive to formation of covalent adducts between cell components and the labeled nisin. The cells are then disrupted, the lysates are boiled in SDS, and then electrophoresed on SDS gels. Since boiling in SDS generally disrupts non-covalent interactions, nisin-labeled bands that are substantially larger than nisin probably represent covalently-tagged cell proteins. Throughout this period of the project, nisin has been labeled with a variety of tags, including biotin and fluorescein, each of which has unique advantages. Biotin-labeled species on gels can be identified using a streptavidin probe, and biotin-labeled molecules can be isolated using a streptavidin affinity column. Fluorescein-tagged species can be directly visualized on gels using a fluorescence scanner (the Storm Imager). These labeled molecules can be recovered using an affinity column made with anti-fluorescein antibodies. Molecules recovered from the affinity columns can be identified by subjecting them to trypsin proteolysis, and then subjecting the tryptic peptides to analysis by mass spectroscopy. In the event that the labeled molecules are proteins, and a total genomic sequence for the organism is available, the mass spectral analysis of the tryptic peptides can identify the labeled proteins.

Good progress is being made in this strategy. Experiments in which spores/cells of *Bacillus cereus* were labeled with tagged nisin, about a half-dozen intensely-labeled bands were observed on SDS polyacrylamide gels. *Bacillus cereus* is an ideal non-pathogenic surrogate for *Bacillus anthracis*, and it would have been a straight-forward process to isolate these labeled molecules by affinity chromatography. However, at the time this was done, relatively little of the *B. cereus* genome had been sequenced, so it seemed unlikely that an identification of the proteins by means of mass spectroscopy would be successful. Instead, the labeling studies on *Bacillus subtilis* 168, whose genome has been completely sequenced, were repeated with the intention that these labeled proteins would be identified by affinity chromatography isolation followed by mass spectroscopy of tryptic digests. Unfortunately, a problem has been encountered in this alternate strategy. Although labeling of the *B. subtilis* proteins occurred, and the pattern of labeling resembled that obtained with *B. cereus*, the intensity of labeling of the *B. subtilis* was much less than the *B. cereus*. Although an attempt was made to isolate these labeled proteins by affinity chromatograpy, the quantities obtained were insufficient for mass spectral analysis. The investigators recently discovered that this is probably due to the ability of the cell wall of *B. subtilis* to sequester large quantities of nisin, which *B. cereus* does not do, and this sequestering of nisin prevented it from reaching its target in significant quantities. This is very likely the explanation of why *B. subtilis* is relatively resistant to nisin, whereas *B. cereus* is much more sensitive. Therefore, the decision was made to go back and work with the labeled *B. cereus* proteins instead. Fortunately, a complete genomic sequence of *B. cereus* has recently been completed, which can now be used to assist us in the identification of the tryptic digests of the nisin-labeled fragments.

- **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).

  Background and nature of the work; 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 '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. This study is investigating multiple aquacultured fish species to investigate drug metabolism *in vitro* 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 are being 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.

Accomplishments: Definitive, multispecies kinetic metabolism assays have been implemented. *In vitro* assays with Atlantic salmon, bluegill, channel catfish, hybrid striped bass, largemouth bass, and yellow perch have been completed and kinetic data for ethoxyresorufin O-deethylase, ethoxycoumarin O-deethylase, pentoxyresorufin O-deethylase, benzyloxyresorufin O-deethylase, glutathione S-transferase, sulfotransferase and glucuronosyltransferase activities have been amassed. Tilapia have recently been collected and the aforementioned kinetic data from that species have also been generated. Efforts with Atlantic salmon, tilapia, and rainbow trout have been conducted to evaluate *in vivo* metabolism and residue depletion profiles of the model drug Albendazole. Additional studies are being conducted to evaluate differences between farm-raised fish and laboratory-maintained specimens of the same species. These studies are warranted because there is evidence that environmental differences can alter metabolic profiles, and therefore may affect the validity of kinetic datasets. The *in vivo* and *in vitro* residue data and *in vitro* kinetic data are currently being prepared for final comparative analysis and metabolic profile determination.

Presentations:

- Kinetics of phase I-II biotransformation kinetics in 8 species of aquacultured finfish, J. F. González, R. Reimschuessel, B. Shaikh and A. S. Kane, Society of Environmental Toxicology and Chemistry (SETAC) International Meeting, November, 2004 (accepted).
- Kinetics of hepatic albendazole sulfoxidation in channel catfish, tilapia and rainbow trout, J. F. González, R. Reimschuessel, B. Shaikh and A. S. Kane, Society of Environmental Toxicology and Chemistry (SETAC) International Meeting, November, 2004 (accepted).

- **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)

  Objectives: 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. The project has entered its final year with timely progress towards completing the proposed activities, while extra studies have shown promising data to apply for extramural funding.

  Results: A panel of six bioluminescent strains containing selected stress-responsive *E. coli* promoters fused to the *Photorhabdus luminesens lux*CDABE reporter is the focus of this study.

  - Biosensing of harmful algal blooms (HABs): Harmful algal blooms (HABs) pose serious public health and economic problems due to the production of biotoxins by a number of algae species. The biosensing panel was used to detect and characterize the response generated when encountering four critical harmful algae, *Karlodinium micrum, Pfiesteria piscicida, Chattonella marina,* and *Prorocentrum minimum*. At the concentration of 6,000 cells/ml, these algal species induced stress responses much higher than did the control, a non-toxic dinoflagellate *Akashiwo sanguinea*. The stress responses induced by the harmful species showed unique patterns as well as signal strength, suggesting that characteristic fingerprints could be generated based on such stress responses. Moreover, dose dependency was observed between the bioluminescence emitted by the sensing strains and the level of algae concentrations studied, enabling the quantification of harmfu algae based on specific stress responses.
  - Biosensing of ephedrine alkaloids: Ephedrine-type alkaloids (ETA) are major active ingredients of Ephedra, a traditional Chinese medicinal herb used to treat asthma and nasal congestion. Until recently, large amounts of ephedra were used in dietary supplements for weight loss and athletic performance enhancement. However, indiscriminate consumption of ETA-containing products has resulted in more than 1,000 reported cases of adverse effects. The toxicities of (-)-ephedrine and (+)-pseudoephedrine were measured using MTT assay on human neuroblastoma (SH-SY5Y) and rat myoblastoma (H9c (2-1)), while the stress responses of a panel of biosensing bioluminescent strains were analyzed. SH-SY5Y showed similar sensitivity to (-)-ephedrine and (+)-pseudoephedrine, while H9c2 (2-1) could differentiate the cytoxicity of (-)-ephedrine and (+)-pseudoephedrine. The biosensing of the *E. coli* strains was highly sensitive to the toxicity of ETA and could yield instantaneous response. The RLU ratios dependent on the construct of the strains gave unique fingerprinting pattern of ETA.

  In addition, a modular sensing device using focal lenses and fiber optics has been constructed with a signal transmission interface enabling recording and analyzing the bioluminescent data on a laptop computer. This device will be presented at the JIFSAN Research Symposium in November 2004.

- **Safety inspection of fresh cut fruits and vegetables using spectral sensing and machine vision techniques.** Yang Tao (UM), Robert Buchanan (FDA), Yoonseok Song (FDA, NCFST) and Yaguang Luo (USDA)

  Fecal contamination detection on produce has become increasingly important since the Food and Drug Administration (FDA) averred fecal contamination as a major source of human pathogens. In general, fruits and vegetables can become contaminated through contact with soil, animals, or humans during any stage of the food handling chain, including growing and harvesting operations as well as while in processing plants. The research is focused on the development of an automated system, based on visible and near-infrared spectroscopy, which can be used in fruit inspection procedures for detection of visible and invisible contaminations in fruit. This optical sensor technology will reduce microbial harborage and cross-contamination by detecting and removing potential food safety hazards from the packaging stream.

  Optical sensing technologies for detecting contamination and physical damage of fresh produce were evaluated. Lab-based assessment and verification of obtained information was conducted to fill in usable techniques. Particularly, hyperspectral-sensing technology was evaluated in both reflectance and fluorescence imaging for fecal contamination detection on cantaloupes and strawberries. Currently, efforts have been focused on classification algorithms for detection of anomalies on cantaloupes. Image fusion has been developed to identify targets, increase image interpretation reliability and improve classification. Its main purposes are to sharpen images, improve geometric correlation, and enhance certain features not visible in single images alone. Hence, fluorescent images taken after samples were treated with diluted cow feces at different concentrations and volumes were subjected to subtraction, addition, multiplication and division of the peaks and valleys selected from the natural spectral were calculated. By visual assessment, ratio images presented the most significant results for fecal contamination detection. All possible permutations of these wavelengths were generated; significant images were selected based on greatest contrast between treated and untreated regions. Other ratio images were ignored because the image did not display fecal spots or any relevant information. The selected ratio images were subjected to unsupervised classification. This technique, deals with clustering the pixels into a desired number of classes, without any *a priory* information on them. The classification is intended to identify treated areas, potential damaged regions, and color variation undetectable by visual inspection or band ratio techniques.

  In addition, the data set was subjected to principal component analysis (PCA). This algorithm transforms the original data set into a set of new un-correlated linear combinations of the original variables, reducing the redundancy within the data by creating a new series of images. Results indicate that fluorescence images at 675 nm exhibited the greatest contrast between treated and untreated surfaces. Detection rates were improved using ratio images; in particular, higher detection rates were obtained for all volumes and concentrations using the 695/595, 675/555 and 555/665 nm image. Unsupervised classification images were more effective in allowing removal of unwanted areas, and isolating treated areas. PCA showed that the first six principal

component images exhibited useful results for contamination detection. PC-2 and PC-5 displayed best contrast for contamination detection. False alarms presented a persistent problem when trying to identify fecal contamination, however, PC-5 provided contrast between them, creating ideal conditions for masking. Based on theses findings, a systematic procedure will be developed for contamination detection. Simultaneously, strawberries' images will be subjected to similar algorithms. Further validations and extensive testing with statistics will be performed. Development of classification algorithms for detection of anomalies will continue and, finally, technology will be transferred.

Publications:

M. S. Kim, A. M. Lefcourt, Y., R. Cheng and Y. Tao, K. 2004. Automated detection of fecal contamination of apples based on multispectral fluorescence image fusion. J. Agric. Engineering, in press.

Presentations:

A. M. Vargas, Y. Tao, M. Kim, A. M. Lefcourt, and Y. R. Chen. 2004. Safety inspection of fruit and vegetables using optical sensing and imaging techniques. ASAE Annual International Meeting, Ottawa, Ontario, Canada. August 31- July 4, 2004.

- **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).

Field sampling and laboratory measurements of apple fruit and cider–2003 and 2004. During the 2003 growing season, a second longitudinal study of microbial flora was conducted. This focused solely on apple fruits. Fruits were sampled at seven dates during the growing season. Measurements were made at dormant, bloom, petal fall, and then at monthly intervals until harvest in late October. At each date, duplicate sets of apples were collected at each of four locations in every tree included in the study. Samples were again taken from GoldRush and Enterprise trees. Insecticide, fungicide and streptomycin applications were used following recommendations for commercial orchard as in 2002. During this season, about 1500 plates from about 375 individual apple samples were enumerated.

The results of the 2003 field study validated the preliminary findings of 2002. On three of the four media tested, pesticide application significantly reduced the number of colony-forming units (CFU) per apple. This reduction was detectable in some early-season samples, but increased as the season progressed. By the end of the 2003 growing season, pesticide treatments had caused a half-log reduction of CFU on BHIA media, MacConkey media, and on the 3M Petri film used to measure total coliforms. This effect appeared to result from full-season pesticide treatments as well as early-season treatments. It is not known whether this is the result of a long-term response to the use of streptomycin in April and May, or if fungicide and insecticide applications decrease epiphyte populations indirectly. An indirect effect could occur

by reducing leaf and fruit damage, thereby reducing available carbohydrates on the surface of fruit. This in turn could suppress the growth of epiphytic bacteria.

All fruit were harvested in late-October, and representative samples stored for testing the effects of cultivar and pesticide treatment on fruit quality and marketability. As expected, pesticide application reduced the presence of fruit damage from insects and plant pathogens. From these fruit samples, replicated batches of apple cider were made in the laboratory using a fruit juicer. Consistent differences in bacterial counts were found in cider samples produced from apples harvested from sprayed and unsprayed trees. Ciders produced from fruit harvested from sprayed trees had one log increase in counts than ciders produced from sprayed (control) trees. No fecal coliforms were detected from any of these juice samples.

To verify these cider findings, trees at the Keedysville orchard were again treated with the same pesticide treatments in 2004 that were used in this study previously. Fruit were harvested in late-October and stored at 0C (32F). Cider production and bacterial enumeration will be completed with these fruit in December, 2004.

Controlled inoculation studies in the greenhouse-2004. A study testing the effect of prior pesticide treatment on the survival of non-pathogenic *E. coli* in controlled conditions in College Park was initiated in March, 2004. Sixty apple trees budded onto Malling 9 (dwarfing) rootstock were planted in 5-gallon nursery containers in a commercial 'soil-less mix'. Trees were planted in mid-March, prior to bud burst. GoldRush and Enterprise cultivars were again selected for study. Trees were allowed to leaf out, bloom and set fruit outdoors in a plant nursery.

In June, 2004 we began preparing a section in the University of Maryland's Research Greenhouse Complex for controlled inoculations of these apple trees. Initial work focused on developing adequate containment procedures, cleaning and sanitizing procedures, and validating these procedures with laboratory testing of samples taken from the greenhouse. Container-grown trees were moved inside the greenhouse in mid-summer. Using excised shoots and leaves, laboratory tests of viability of surrogate *E. coli* were conducted. By maintaining adequate humidity, bacteria were shown to persist for about a week under laboratory conditions.

Inside the greenhouse, trees were then sprayed with standare mixtures of either organic or conventional pesticides. In the next step of the study, *E. coli* modified to contain a green fluorescing protein is to be applied to individual treated leaves. These leaves will be harvested at regular intervals, and bacteria enumerated to study the effects of time and type of pesticide treatment on bacterial survival.

Projects funded in July 2003:

- **Molecular mechanisms of fluoroquinolone and erythromycin resistance in *Campylobacter jejuni/coli***, Jianghong Meng (UM), Patrick McDermott and David White (FDA)

The objective during this reporting period was to identify putative efflux pumps associated with fluoroquinolones and erythromycin resistance. The contribution of the *Campylobacter* multidrug efflux pump, *cmeABC*, and nine other putative efflux pump genes or operons to the antimicrobial susceptibilities of *Campylobacter* species were investigated. Mutant strains were constructed by inserting a *Campylobacter* chloramphenicol or kanamycin resistance cassette to target efflux genes, thus disrupting expression of the genes. When comparing the susceptibilities of the mutant and parent strains to four antimicrobial agents (chloramphenicol, ciprofloxacin, erythromycin, and tetracycline) by agar dilution, insertional mutations in *cmeB* resulted in 4- to 128-fold decreased minimum inhibitory concentrations (MICs) to chloramphenicol, ciprofloxacin, erythromycin and tetracycline, with erythromycin being the mostly affected. In addition, *cmeB* mutants completely changed the susceptibility category by reversing a resistance phenotype to a susceptible phenotype in two *C. coli* strains co-resistant to ciprofloxacin and erythromycin. In contrast, mutants of all other putative efflux pumps did not show decreased MIC to any of the four agents tested. This finding indicates CmeABC is the only efflux pump tested that is important to antimicrobial resistance in *Campylobacter* species, and further studies are under way to characterize the gene expression regulation of this efflux pump in *Campylobacter* strains.

Presentations:

1. J. Meng, B. Ge, P. McDermott, D. G. White, and S. Zhao. 2004. The role of efflux pumps in antimicrobial resistance in *Campylobacter jejuni/coli*. The 5th World Congress Foodborne Infections and Intoxications, Berlin, Germany.
2. J. Ge, P. McDermott, D.G. White, S. Zhao, and J. Meng. 2004. Efflux pumps and GyrA mutations on the resistance to Ciprofloxacin and Erythromycin in *Campylobacter jejuni/coli*. The 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC.

- ***Campylobacter jejuni*-host interaction on the intestinal mucosal surface**, Wenxia Song (UM), Shaohua Zhao and Ruby Singh (FDA)

  Major recent accomplishments include:

  - In addition to primers previously used, another set of PCR primers has been designed for the putative virulence genes of *Campylobacter*. The presence of the eight virulence genes was screened among 43 retail meat isolates using PCR. Putative virulence genes in some of the isolates were detected by one PCR primer, but not the other. This suggests that these genes are highly variable, and different variants may function differently.
  - Statistical analysis was carried out using linear regression to test the interrelationship between the invasion and adherence abilities of *Campylobacter* to human colonic epithelial cells. The correlation coefficiency between the invasion and adherence abilities of the 43 retail meat isolates is 0.574 with $P<0.01$, which indicates that there is a significant correlation between the invasion and adherence ability of *Campylobacter* and suggests

that the abilities of *Campylobacter* to invade human colonic epithelium is closely associated with its ability to adhere to the epithelium.

- Analysis has continued of *Campylobacter*-triggered cytokine secretion by human colonic epithelial cells. The time course and the polarity of *Campylobacter*-triggered IL-8 and the effect of *Campylobacter* dose on the secretion were determined. It was found that IL-8 became detectable 4 h after inoculation and reached a plateau by 6 h. Inoculation of *Campylobacter* from the apical surface, where the bacteria initiated contact with the host epithelium, induced the polarized secretion of IL-8 to the apical but not the basolateral side of human colonic epithelial cells. Increasing *Campylobacter* inoculation dose from 1:10 to 1:100 host cells to bacterial ratio did not increase the amount of IL-8 secreted by host epithelial cells.

- **The impact of risk messages about bioterrorism on the U.S. food supply on audience attitudes and behaviors**, Linda Aldoory (UM), Marjorie Davidson, Brenda Derby, Laura Fox, and Alan Levy (FDA)

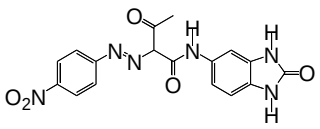
  The following activities were completed:

  - A conference paper, based on findings from the first phase of the research study, was accepted for presentation by the Public Relations Division of the Association for Education in Journalism and Mass Communication. The paper, titled, "Shared Involvement and Risk Perceptions in Responding to Bioterrorism: An Extension of the Situational Theory of Publics," won the division's Second Place Research Paper Award. It was presented in Toronto, Ontario, on August 6, 2004, and is co-authored by Linda Aldoory, Ph.D., and Mark Van Dyke, doctoral candidate, from the Department of Communication. The paper was based on findings from focus groups that explored how individuals reacted to simulated news coverage of a bioterrorist attack on a U.S. food supply.
  - Development of the second phase of the study has begun and has included the following tasks:
    - Selection of and meeting with new graduate research assistant, Natalie Tindall, Ph.D. student in the department of communication;
    - Literature and previous research gathered on theoretical perspectives informing public attitudes and perceptions about bioterrorism and food safety; and
    - Research design of experiment to test effects of messages about bioterrorism on U.S. food supply.

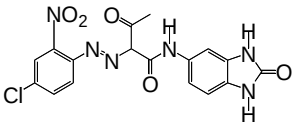
- **An integrated approach for identifying phototoxic cosmetic ingredients**, Daniel Falvey (UM), Wayne Wamer and Patty Fu (FDA)

  The long term objective of this research is development of non-animal assays that predict the risks associated with the use of cosmetic ingredients on sun-exposed skin. The investigators hypothesize that photophysical measurements, characterizing the formation and decay of a potential photosensitizer's excited states under biologically

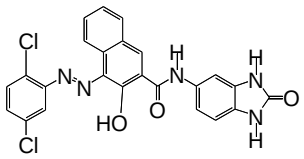
relevant conditions, and in vitro photobiological measurements, characterizing a potential photosensitizer's cytotoxicity and cellular targets, provide the mechanistic information needed for estimating acute and chronic phototoxic risks. A series of photophysical and photobiological studies, aimed at examining potential harmful effects of cosmetic ingredients that are commonly applied to sun-exposed skin, have been carried out. Work to date has focused on organic components of aloe vera gel and extracts: aloe emodin and rhein as well as retinol (vitamin-A). More recently attention has been turned to tattoo pigments that have been implicated in several adverse reaction reports.



Orange 62



Orange 36



Brown 25

The low solubility of these pigments creates some interesting technical challenges to the study of these pigments. Preliminary solution photophysical characterization of Pigments Brown 25, Orange 36, and Orange 62 has begun. Since these materials are sparingly in aqueous solution, their behavior has been explored in the organic solvents: dimethylsulfoxide (DMSO) and N,N-dimethylformamide (DMF). The preliminary objective is to identify any relevant excited states and/or intermediate species that are generated upon photolysis of these compounds. Current results show that

1. Each of these dyes is non-fluorescent, implying a very short singlet excited state lifetime. This in turn would suggest that direct interactions of this excited state with biomolecular targets is likely to be negligible.
2. Preliminary laser flash photolysis experiments on Orange 36 and Orange 62 show negligible signals attributable to the excited triplets states of these species. This suggests that photosensitization by the triplet state is unlikely.
3. In contrast, laser flash photolysis experiments on Brown 25 shows a significant, albeit short lived signal that is tentatively attributed to the excited triplet state of this molecule. The competence of this state at initiating cytotoxic processes remains to be demonstrated.
4. Solutions of the three pigments were photolyzed with a Xe arc lamp for several minutes. Each of the pigments was rapidly and irreversibly decomposed under these conditions. This high degree of photolability implies that direct photochemical decomposition of the pigments may be important in their phototoxicity. Current experiments are aimed at characterizing the stable solution photoproducts of these materials.

Project funded in July 2004:

Predicting exposure estimates: Experimental food additive partitioning studies and model development, Robert Walker (UM), Timothy Begley and William Limm (FDA)

Despite the importance of understanding how foods absorb different analytes (i.e. food packaging additives, chemical and/or biological pathogens, etc.), very little is known about the fundamental principles governing analyte partitioning. Here, partitioning describes an analyte's equilibrium distribution between a given food and an adjacent material. Several isolated studies have suggested that partitioning depends sensitively on analyte identity as well as the properties of the two adjacent phases. Furthermore, partitioning is known to change significantly with temperature although the origins of this behavior are still unresolved. The proposed research will examine systematically the phenomenon of analyte partitioning between typical food packaging materials and a wide variety of food simulants. Of particular interest is how partitioning changes with analyte identity, food stimulant identity and temperature. Analytes will be chosen to represent broad categories of antioxidant additives commonly found in food packaging materials. Food simulants will span the range from extremely hydrophobic (e.g. corn oil) to extremely hydrophilic (water). These results will be used to develop the quantitative models of analyte partitioning necessary for accurately predicting exposure estimates. These models would be directly applicable to the review of food contact notifications (FCNs) and letters of inquiry by the Office of Food Additive Safety.

APPENDIX B

Collaborative/Cooperative Research Projects

1. Industry Acrylamide Alliance/JIFSAN Project Funding

In collaboration with the Industry Acrylamide Alliance, an informal designation for food companies that have donated financial support for research, JIFSAN funded the following research projects as part of its efforts in monitoring and coordinating (domestically and globally) activities concerning the issues raised the occurrence of acrylamide in foods.

- **Effects of consumer food preparation on acrylamide formation**, George Sadler (IIT) and Lauren Jackson (FDA), National Center for Food Safety and Technology (NCFST) Argo-Summit, IL (Funded October 2003; nearing completion)

  This study is investigating the effects of processing (temperature, time, pH, moisture levels) and cooking (time/temperature) conditions on acrylamide formation in food. An aqueous model system is being used to determine in a carefully controlled environment the effects of time, temperature and pH on acrylamide formation and to determine the temperature at which acrylamide begins to form. Potato chips and bread (or other bakery products) were selected as the foods to be studied. The chips (before and after frying) are being analyzed for acrylamide, asparagine, and glucose levels. Acrylamide contents of chips cooked to the same level of "doneness" (i.e. brown color) are being compared to provide information on the combination of time/temperature on acrylamide formation. . Bread or rolls are being baked in a convection oven using different baking conditions. Thermocouples and an IR camera/gun are used to determine surface temperature while thermouples are used to monitor the internal bread temperature. The bread (crust and center) will be analyzed for acrylamide, asparagine, and glucose levels. The extent of browning and moisture content of crust are being measured. The overall purpose of these sets of experiments is to understand the processing conditions that influence acrylamide formation in food. With an understanding of processing conditions (in particular, time/temperature), it may be possible to reduce acrylamide levels in food.

  An important aspect of this investigation is the formation of acrylamide during home preparation of food. Very little data is available in this area. Effects of cooking time and temperature and degree of browning on acrylamide levels in bread (toast) and baked French fries will be studied. Bread slices are toasted in a toaster oven and French fries (frozen) are cooked in an oven for different times and temperatures. Temperature in the bread/french fry surface will be monitored with thermocouples and an IR camera/gun. Degree of browning, moisture content, the amounts of acrylamide, asparagine, and glucose are being measured in the processed food. The purpose of this experiment is to determine how consumer preparation affects acrylamide levels in food. In addition, this experiment provides information on the effects of time and temperature (oven and product) on acrylamide formation in food.

- **Acrylamide content of home-prepared surface-browned foods**, George Sadler (IIT) and Lauren Jackson (FDA), NCFST, Argo-Summit, IL (a requested expansion of the project listed above; funded March 2004, nearing completion)

  This project expands the scope of the Jackson/Sadler project "Effects of consumer food preparation on acrylamide formation" and was requested by the Industry Acrylamide Alliance/JIFSAN . The objective is to quantify acrylamide content in foods heated to surface brownness during in-home preparation. This project supplements the project described above to include a larger number of foods and a greater variety of home cooking appliances.

  The analyses examine foods where final preparation occurs at home and which are at times eaten even when slightly burnt. Foods conforming to this pattern include: Oven Baking (piecrust; pizza; oven breads such as garlic bread, biscuits, cookies, and rolls; oven entrees (e.g., Hot Pockets, Bagel Bites); and baked pasta such as lasagna), Skillet Frying (fried breaded meats (e.g. fish, shrimp, chicken); fried breaded cheeses; grilled cheese sandwiches; fried breads such as pancakes, hushpuppies, scones; pan-fried vegetables; fried rice; fried cured meats including hot dogs, sliced and Canadian bacon, omelets), Deep Fat Frying (fried breaded vegetables (e.g. onion rings, okra, zucchini); potatoes; doughnuts) and Toaster Preparation (bagels; toaster pastries; toaster waffles; English muffins; toasts made from ethnic and varietal breads)

  NCFST's test kitchen is used for preparation of the food products. A domestic electric range is used for baking and frying (both skillet and deep frying). A standard 2-slot toaster is used for toasting. Issues being investigated include:

  - the affect of "doneness" on acrylamide, since acrylamide often increases exponentially as foods are taken past their prepared ideal to a point of over doneness. Home preparers exhibit a broad range of accomplishment in cooking skills. Multitasking in the preparation of home meals predisposes to over doneness of some courses. Since in-home preparation is more likely to produce over processing than commercial products; it is likely that home cooked, over-processed foods contribute to dietary acrylamide at levels which vastly over represent to the fraction of calories supplied from home-cooked foods.
  - the correlation between free asparagine and acrylamide, since any correlation between browning and acrylamide formation may require some understanding of initial free asparagine.
  - spatial distribution of acrylamide in bread, since it is well known that acrylamide is not uniformly distributed in baked and fried foods. Instead it appears disproportionately in the darkened outer surface that experiences higher temperatures than the interior food. However, at least one report suggests acrylamide distribution in bread is bimodal, with a second accumulation of acrylamide occurring some distance within the loaf. This observation should be substantiated or repudiated.

- **The kinetics of acrylamide inhibition-destruction-scavenging under various reaction/process conditions**, Bryan Hanley, Leatherhead International, Leatherhead, England (Funded February 2004; completed, report pending)

A number of factors have been suggested that could inhibit the formation of acrylamide or contribute to its degradation. It is important to realise that a key aspect of such investigations, whether they focus on prevention of acrylamide formation or its subsequent removal from the food, should be the maintenance of the organoleptic and sensory properties of the food. Secondly, there is such a vast range of potential treatments that it is important to prioritise them in a meaningful and sensible fashion. Finally, it is possible that treatment to eliminate acrylamide formation could result in the formation of more toxic entities and this would clearly be particularly undesirable. For example, the putative intermediate Strecker aldehyde from asparagine could react with a range of amines to give imines and related products.

Potential approaches:

- Prevention of the formation of the Strecker aldehyde: this approach would rely upon prevention of the proposed reaction between the carbonyl (or dicarbonyl) precursor and asparagine, hence eliminating the putative mechanistic pathway at an early stage.
- Removal of asparagine: the removal of free asparagine may offer a sensible alternative, if its presence is not necessary for processing, flavour or colour development in products.
- Processing to remove acrylamide: the removal of acrylamide after formation may be possible in some cases. However this is unlikely to be sufficiently selective to prevent a severe deleterious effect on the food product.

Experimental plan:

Potato was used as a model food system. The effects of various treatments of the potato on acrylamide formation are being investigated. Individual potatoes were cut into pieces. Half of the pieces are being subjected to a range of treatments, for example the following:

Pre-treatments for removal of asparagine/reducing sugars

- surface washing
- blanching
- treatment with dilute acid
- treatment with asparaginase

Treatments for inhibition of formation of acrylamide

- treatment with amino acids (particularly glutamine, aspartame and lysine)
- treatment with thiamine
- treatment with glutathione/cysteine
- treatment with rosemary, basil, thyme (based upon recent studies that suggest the former may inhibit acrylamide formation)
- addition of hydrolysed nucleic acids

All of the potato pieces are being cooked at the same time using predetermined conditions and analysed for acrylamide. Comparison of the acrylamide concentrations between the treated and untreated potato are being made to determine the results of the treatment.

From the results of these experiments the two most promising lines of treatment are being selected, based on the reduction of acrylamide formation and the appearance and odour of the cooked products.

The ultimate test of any system is its applicability to industrial application. It will be possible to determine if the treatments recommended are feasible in real life. This type of reality check will be carried out throughout the phases of the project.

- **Consumer attitude – communications research on acrylamide**, David Schmidt, International Food Information Council (IFIC), Washington, D.C. (Partial funding; funded November 2003; completed; report can be obtained from JIFSAN)

  This study explored the extent to which the issue of "acrylamide in food" had permeated the consciousness of the consumer, and the extent to which it had impacted dietary choices or concerns. In addition, this qualitative research attempted to identify potential triggers to behaviorial change and acceptable messages that explain and provide context to risk information provided to the consumer.

  Six focus groups were conducted in three markets in the United States. Focus group participants were drawn from a diverse set of consumers between 18 and 65 years old who acknowledged their role in making personal food choices or those for their family. All participants were required to meet specific screening criteria

  This research should yield a "Strategic Analysis" highlighting major findings and "Research Results," providing a descriptive analysis of participants' responses to all key discussion areas.

- **Development of a PBPK/PD model for acrylamide**, Daniel Doerge and John Young, National Center for Toxicological Research (NCTR), Jefferson, AR (Partial funding; pending)

  This project supplements current research being done by Dr. Doerge at NCTR and is being accomplished through a Cooperative Research and Development Agreement (CRADA) currently being established. The CRADA lists Dr. Doerge as PI and Dr. David Lineback (JIFSAN) as co-PI. Partial funding for this project came from the Internatonal Life Science Institute (ILSI) North America (NA).

  The goal of this CRADA is to develop, using toxicokinetic and biomarker data collected in B6C3F1 mice and F344 rats, a physiologically based pharmacokinetic-pharmacodynamic (PBPK/PD) model for acrylamide and glycidamide from which tissue levels of parent compound, its genotoxic metabolite, and their disposition can be simulated across species, including the human. Biomarkers of exposure including

hemoglobin adducts of acrylamide and glycidamide and glycidamide-derived DNA adducts will provide a pharmacodynamic link with measures of tissue damage. The ultimate goal is to predict concentrations of acrylamide and glycidamide in human tissues along with the resultant DNA damage for use in assessing toxic risks from acrylamide in the diet.

Specific Aims:

- Collect a full set of toxicokinetic and biomarker data, including serum and tissue measurements along with adducts of DNA and hemoglobin, that are needed to develop a PBPK model for acrylamide and glycidamide.

- Develop a PBPK-PD model that will simulate serum and tissue levels of acrylamide and glycidamide simultaneously with levels of tissue DNA adducts and circulating hemoglobin adducts for use in extrapolation to humans.

2. Cooperative Research with DNRE, Victoria, Australia

As the JIFSAN portion of a cooperative research program being developed with the Department of Natural Resources and Environment, Melbourne, Victoria, Australia with whom JIFSAN has a Memorandum of Cooperation, the following research project was funded for the period 1 July 2004 to 30 June 2007.

Rapid assay for detecting human enteric viruses and viral survival dynamics on fresh fruits and vegetables, Jianghong Meng, Nutrition & Food Science, University of Maryland (Funded April 2004; a three-year project)

Objective: There is a need to develop a more accurate indicator of the fecal viral risk associated with fresh fruits and vegetables. The aim of this project is to develop and evaluate a range of rapid assays based on virus-specific RNA to detect enteric viruses in both water supplies for irrigation and on the surface of vegetables. The assays will subsequently be utilized to study the prevalence of enteric viruses and die off of the viruses once they contaminate the surface of vegetables. The project will involve: (a) development of methods for detecting enteric viruses on fresh produce; (b) evaluation of fresh produce safety using *E. coli*, enterococci, and virus testing; (c) survival dynamics of the enteric viruses on fresh produce; and (d) screening and characterization of enteric viruses on retail fresh produce

3. Additional Research Projects Funded (addressing FDA needs)

- **Human infectivity dose-response analysis and qualitative risk assessment of Hepatitis A Virus (HAV) and Norovirus (Norwalk Virus; NV) in Foods**, Mark Sobsey, Environmental Sciences and Engineering, University of North Carolina, Chapel Hill (Funded August 2004; one-year project)

Despite their importance as foodborne disease agents and their apparently high infectivity at low doses, HAV and Noroviruses have not been subjected to rigorous human

infectivity dose-response analysis or quantitative microbial risk assessment. This is because human infectivity data for these viruses is either limited, has not been compiled and examined in a manner suitable for such analyses or has not been made readily available for such analyses.

Some data are available from human volunteer studies to conduct dose-response analyses for HAV and Noroviruses. Data from human volunteer studies on HAV go back to the time when the etiology of hepatitis A was not clearly known, and when it was not fully established that there are two viruses that cause infectious hepatitis, hepatitis A virus and hepatitis E virus. HAV is the more prevalent of these two viruses in the USA and was the one that was used in human volunteer studies in the USA.

A human volunteer study of Norwalk Virus was done on a collaborative basis by this lab (Mark D. Sobsey), Prof. Christine Moe and other investigators at the University of North Carolina. In those studies,about 50 volunteers were given different doses of the so-called 8FIIa inoculum of Norwalk Virus, which has been extensively used to infect human volunteers since the early 1970s. The human infectivity dose-response analyses of these data have not been completed, and such analyses would be done as another objective of this study. Preliminary analyses of these data revealed that the dose-response relationship was not uniformly sigmoidal if all volunteers were included in the analyses. However, when the analyses excluded volunteers who were negative for Norwalk Virus antibodies and who were later shown to be of a human blood group genotype that lacks the carbohydrate receptor for virus attachment and infection, a sigmoidal dose-response relationship was obtained. Furthermore, the 8FIIa inoculum with which these volunteers were challenged has not been carefully analyzed for Norwalk Virus concentration; only a rough estimate of virus concentration was made. The objectives of this study include a careful, quantitative titration of this inoculum and then a careful analysis of the dose-response relationship. Also, efforts will be made to obtain as many inocula as possible from previous studies and to subject them to molecular and possibly other analyses to obtain estimates of the HAV concentrations.

Objectives:

1. Identify and acquire human infectivity dose-response data for HAV and NV from studies done primarily in the USA from the published literature and other available data sources, such as reports and experimental records.
2. Acquire as many of the HAV and NV inocula as possible that were used in these human volunteer dose response studies. Better quantify the virus titers of as many of the acquired inocula as possible using RT-PCR and possibly other analytical methods.
3. Quantify dose-response relationships of HAV and NV using various estimates of virus titers in the inocula and the health effects responses of human volunteers (infection, illness and if available, mortality). Develop quantitative dose-response relationships using various dose-response models appropriate for human pathogens.
4. Search the published literature, other published reports and other legitimate data sources for the measured concentrations of HAV and NVs in foods, including foods consumed in outbreaks of NV gastroenteritis and of Hepatitis A (infectious hepatitis).

5. Use the dose-response relationships from human volunteer studies with NV and HAV and the data on NV and HAV levels in food to estimate the risks of NV gastroenteritis and infectious hepatitis (Hepatitis A) from ingestion of these foods. Compare the predicted risks of illness of NV gastroenteritis and Hepatitis A obtained by these analyses to the actual risks of NV gastroenteritis and infectious hepatitis observed in foodborne and waterborne disease outbreaks. This provides a basis to compare the risks predicted from the quantitative microbial risk assessment analyses to the actual risks observed in outbreaks.

- **Development of molecularly imprinted polymers (MIPs) for selective detection of marine biotoxins**, Kenneth Shea, Chemistry, University of Califorina, Irvine (Funded July 2004; one-year project)

  This project will develop molecularly imprinted polymers (synthetic polymer receptors) against the marine biotoxins domoic acid and microcystin-LR. The two water-soluble toxins are hazardous substances that are common contaminates in human drinking water and food supplies. The molecules possess chemical structures that present a challenge for developing robust, sensitive and selective materials for their detection. There are two facets of the proposed research. The first consists of the development of optimum conditions for synthesizing molecularly imprinted polymers (MIPs) against the two marine biotoxins, domoic acid and microcystin-LR. The PI will draw upon expertise from his laboratory and from the molecular imprinting literature to identify the most promising porogins, functional and cross-linking monomers for the MIP formulations. Small combinatorial arrays of functional monomers, cross linkers, will be prepared and evaluated semi-quantitatively to identify the best candidates. Larger scale synthesis will provide material for evaluation of affinity and selectively for domoic acid and Microcystin-LR. Analysis will include data from binding isotherms and where available, comparison with commercially available ELISA diagnostic kits. The second phase of this work will involve modifications of the imprinted receptor sites to incorporate a transducer for reporting the binding event. The PI will draw from his recent imprinting studies of functional, polymerizable monomers containing a florescent group. By analogy, binding of the biotoxins domoic acid and microcystin-LR would produce a detectable change in the fluorescence emission of the MIP. This approach will allow direct verification of the presence of the biotoxins in the field with a minimum requirement for peripheral instrumentation.

- **Enzymatic degradation of prion surrogate proteins**, Jason Shih, Poultry Science, North Carolina State University, Raleigh (Funded September 2004; two-year project)

  Sup35NM and Sup35NM-His6 are prion-like proteins derived from yeast that have physical-chemical properties similar to that of the prion proteins responsible for Transmissible Spongiform Encephalopathies (TSEs), including Creutzfeldt-Jakob disease in humans, Mad Cow Disease (BSE) in cattle, and scrapie in sheep. Though non-pathogenic, yeast prions behave the same way as mammalian prion protein in their ability to change conformation, form aggregates and replicate themselves. Yeast prion protein Sup35NM and its recombinant protein derivative, Sup35NM-His6, have been cloned for production, purified and evaluated for suitability as a prion surrogate protein in this

laboratory. Recent work has demonstrated that under specific conditions, a feather-degrading keratinase is capable of degrading the prions present in the brain tissues of BSE in cattle and scrapie in sheep. Therefore, a process of enzymatic degradation of prions may be developed that renders animal products free of prions and prevents the transmission of TSE. In this project, Sup35NM and Sup35NM-His6 will be compared and the better candidate selected for development of a standard Prion Surrogate Protein (PSP). The PSP will be mixed with normal nervous tissue in a pilot-scale pressure cooker and serve as a marker for prion degradability to enzymatic action under industrial rendering conditions. Brain and spinal cord tissues will be added to mimic the specified risk materials (SRM) for BSE. To improve the specificity and activity of the keratinase, several genetically-modified keratinases will be produced and tested for efficacy against the PSP. If proven effective, modified keratinase enzyme species that specifically attack BSE prion may ultimately be developed.

- **Analysis of data collected in epidemiological and microbiologic field studies of domestic and imported produce**, Christine Moe, International Health, Emory University (supplements existing research; funded June 2004; one-year project)

  Driving Force for Request: Data collection has recently been completed in a series of field studies over four growing seasons (Nov 2000 – Dec 2003) in the southwestern U. S. on agricultural practices and shed-processing practices for domestic and imported produce. These studies were supported by a grant from the USDA CREES NRI Epidemiologic Approaches for Food Safety program and are a collaboration between Emory University, the Health Studies Branch of the National Center for Environmental Health (CDC), North Carolina State University and the Texas Cooperative Extension Service of Texas A&M University. All the data has been double-entered into databases and the databases have been cross-checked and cleaned. However, resources to complete the analyses of this data are not available. Advice from CDC biostatisticians has been obtained, but support is needed for a doctoral level investigator to actually perform the data analyses under the supervision of the study investigators.

  Specific Outcomes: The data analyses will examine the associations between microbiological quality of produce and: agricultural and processing practices, environmental conditions, irrigation/packing shed water quality, farm and packing shed sanitation facilities and worker hygiene. The results of this study will be used to: (1) design effective intervention measures to reduce produce contamination during harvest, processing and packing and (2) compare foodborne disease risks associated with domestic versus imported produce.

- **Conflicting information about safety guidelines for consumption of fish**, Linda Aldoory, Communications/CRCR, University of Maryland (Funded July 2004, one-year project)

  This one-year project was awarded to the Center for Risk Communications Research and was funded through JIFSAN's DRIF funds. The funding supports a full-time graduate research assistant and a three-phase, multi-methodological study.

1. Phase 1 involves a content analysis of national daily newspapers in order to analyze how the media have framed the issue of safe fish consumption. The time period selected for the sample of newspapers was January 1, 2004 to September 30, 2004. This allowed for two months before the FDA released new guidelines about methyl mercury in fish, and then several months after the release to detect any changes in media coverage. The sample has been collected and includes 203 articles that refer to eating fish or the FDA guidelines. A coding scheme for the content analysis is in the process of being designed and pre-tested.
2. In Phase 2, a secondary analysis of research conducted in related theoretical and topical areas will be conducted. The review has already begun and will be an ongoing process.
3. During Phase 3, focus groups and individual interviews will be conducted to obtain in-depth and detailed understandings of how people perceive the conflicting information in media about the safety of eating fish. Research design has been drafted and potential participants have been targeted. Focus groups will likely be conducted in February and March 2005, and supplemental individual interviews will be conducted in April 2005.

APPENDIX C

JIFSAN Post-Doctoral Research Program

Rapid determination of food integrity and identification of food borne bacteria using Fourier transform near-infrared (FT-NIR) spectroscopy and pattern recognition techniques, Frederick Fry (FDA), Janie Dubois (JIFSAN Research Associate)

The results obtained during the first four months of this project suggested that the investigators had pinpointed the conditions for culture, sample preparation and spectral acquisition that would yield highly reproducible FT-NIR spectra. It is clear that careful sample preparation is necessary to obtain reproducible results and that no mathematical process can perform in a satisfactory manner when irreproducible results are used as input.

The next step was to tackle the issue of the database necessary for a bacterial identification system to be accurate and useful in the food microbiology environment. The dream of developing gigantic databases that would contain spectra from a very wide range or sources has been proven wrong in the mid-1990s and researchers involved in this field of work generally agree that dedicated databases perform better. The approach must be pragmatic and organized.

Two approaches were explored in this regard. First, the investigators developed a small expert system that interacts with the user to gather information about the culture conditions and observations in order to suggest a relevant infrared database to be used for identification. By using simple physical criteria, it is possible to greatly restrict the number of organisms that must be considered for identification. More traditional chemometrics were then applied for the classification and identification.

The second approach explored was to tackle the problem of gathering large databases by using the parallel spectral acquisition advantage offered by imaging techniques. This was looked at both in the mid- and near infrared regions, but the focus of the effort was kept on the near infrared region. The investigators were able to differentiate the organisms present in the sample, demonstrating that it may be possible to develop food-specific identification systems based on this technology. A patent application is being processed by the Office of Technology Commercialization, University of Maryland.

Publications:

L. E. Rodriguez-Saona, F. M. Khambaty, F. S. Fry, J. Dubois, and E. M. Calvey. (2004) Detection and identification of bacteria in a juice matrix using Fourier transform near-infrared spectroscopy and multivariate analysis. J. Food Protection 67: 2555-9

Presentations:

"Near-Infrared Imaging in Food Safety," FACSS 2004, Portland OR

"An Array-Based Approach for Bacteria Identification by Infrared Spectroscopy," Microscopy and Microanalysis 2004, Savannah GA

"Infrared Imaging in Disease Diagnosis: Because a Picture is Worth a Thousand Words." Microscopy Society of America, Northeastern Ohio Chapter, Akron OH, 2004.

"Rapid Bacterial Identification Using Infrared Spectroscopy: Meeting Real-World Challenges Using a Dedicated Expert System," FDA Science Forum, Washington, DC 2004

Aquaculture drugs: LC/MS protocols for marker determination, Robert Dickey (FDA), Ann Abraham (JIFSAN Research Associate)

The purpose of this research project is the identification of marker residues of difloxacin, select nitrofurans, and chloramphenicol in cultured fish and shrimp, and development of LC/MS protocols for marker determination in residue monitoring and surveillance programs.

The project objectives include 1) characterize the absorption, tissue distribution, metabolism, and elimination of unapproved aquaculture drugs in cultured catfish and shrimp; 2) identify a marker residue (e.g. parent drug or metabolic product) of drug exposure to catfish and shrimp; 3) develop protocols for the determination of the marker residues in catfish and shrimp using liquid chromatography – mass spectrometry; and 4) validate or peer verify the performance of the LC/MS method for acceptability in residue monitoring and surveillance programs.

The initial focus of this research was on nitrofurans including furazolidone, nitrofurazone, nitrofuratoin, and related compounds. Prior to 1991 nitrofurans were approved for use in food animals as a broad-spectrum antibiotic. The FDA prohibited systemic use of nitrofuran drugs in food-producing animals in 1991 because of evidence that the drugs may induce carcinogenic residues in animal tissues. Topical use of nitrofurans in food animals was banned in May of 2002. Nitrofurans were detected by the EU in aquaculture species (shrimp) produced in Southeast Asia. These compounds are of high regulatory concern because of toxicological issues, and analytically challenging because of their extensive metabolism and covalent binding in animal tissues.

Progress has been made on (1) improving existing methodology for extraction of bound residues of nitrofurans, representing the marker residues for regulatory monitoring and (2) initiating two studies of residue depletion and metabolism in aquaculture species.

Molecular phylogenetic identification of potential foodborne agents of bio-terrorism, Eric Brown (FDA), Alice Heyford (JIFSAN Research Associate)

This project has three experimental objectives. Each contributes to the rapid differentiation and identification of foodborne bacterial strains. These objectives are: (1) Cladistic analysis of DNA sequence diversity for the identification of suspect bio-terroristic microbial agents; (2) Identification of bio-terroristic strains using single-nucleotide signatures; and (3) Design and application of PCR-based markers for the differentiation of potential bio-terroristic strains of *E. coli* and *Shigella*

In order to differentiate closely related strains of a pathovar such as *Escherichia coli* O157:H7, it is useful to identify single nucleotide polymorphisms (SNPs) as molecular markers that discriminate members of the population. The sequencing of housekeeping genes has usually been inadequate, however, to differentiate strains of the O157:H7 serotype, making necessary a search for chromosomal sites with greater genetic variation.

The investigators compared the published sequences of the *E. coli* O157:H7 EDL933 and Sakai strains, along with *E. coli* K-12 (MG1655) and *E. coli* CFT073, in order to find such areas. A BLAST database was constructed for each gene from all four genomes and each gene was blasted against the database to find matches. Sets of matched genes were aligned and evaluated for informative SNP sites. A useful SNP site would show a difference between the two O157:H7 sequences and be flanked by conserved sequence suitable for primer binding, yet the region should not have paralogous matches elsewhere in the chromosome. Seventeen regions containing candidate SNPs were amplified and sequenced in a test set of 16 independent isolates of *E. coli* O157:H7 and ten other closely related serotypes. Five useful SNPs for distinguishing the test strains were identified. Pyrosequencing assays for each of the five SNPs were used to test a collection comprising 75 *E. coli* O157:H7 strains. The results divided the test population into four groups.

For further discrimination of O157:H7 strains, analysis of *roi*, a prophage gene encoding a DNA binding protein, established its usefulness as a molecular marker in three ways. First, it was not present in all strains. Second, in strains carrying the prophage gene, *roi* sequences fell into three diverged allele types. Finally, SNPs at two positions in the *roi* alignment further differentiated the groups of test strains. The discovery of informative sites by *in silico* genomic comparisons and assay of a diverse set of test strains is proving to be useful for the development of techniques that would aid strain attribution for significant human pathogens.

Development of a specific monoclonal antibody for *Enterobacter sakazakii*: Identification and an immunoassay using color-coded bio-nanotubes, Kun-Ho Seo (FDA), Sang-Bok Lee (UM), Kwang-Young Song (JIFSAN Research Associate)

This project has four experimental objectives: (1) To identify specific antigen of *E. sakazakii*, (2) To develop a specific monoclonal antibody for *E.* sakazakii, (3) To develop an immuno assay system using the monoclonal antibody and innovative fluorescence bio-nanotubes, and (4) To evaluate the immuno assay system for rapid, specific detection of *Enterobacter sakazakii* in infant formula and environmental samples

An experiment has been done using *Salmonella* antibodies to develop an immunoassay using nanoparticles (quantum dots). This experiment is a parallel experiment to show 'proof of concept' of color-coded bio-nanotube technology for *E. sakazakii*. The peak intensity of the fluorescence emission was proportional to the initial cell concentration of *Salmonella* Enteritidis in the range of 10^3 to 10^7 CFU/mL with a detection limit at least 80 times lower than that of the FITC-based method. The total detection time was within 2h.

The outer membrane proteins of *E. sakazakii* and non-*E. sakazakii* strains were prepared and analyzed using two-dimensional electrophoresis. The characterization of the proteins that expressed in *E. sakazakii* was performed using image analysis systems in collaboration with bioinformatics team (Instrumentation and Biophysics Branch, OSAS). Further analysis will be

carried using N-terminal and internal amino acid sequences. A maker protein for *E. sakazakii* will be identified and purified and used for production of monoclonal antibodies.

The effectiveness of ferrioxamines E as an iron source was evaluated to develop selective enrichment media for *E. sakazakii*. After 24 h of incubation at 37C, the *E. sakazakii* populations recovered from iron-free media supplemented with FAC, FS, and FE were log 6.2-6.3, 6.2-6.3 and 5.8-6.4 CFU/ml, respectively. However, only log 1.5-1.6 CFU/ml *E. sakazakii* were isolated from iron-free media without supplementation. Also, in egg white known as perfect iron-free media, the *E. sakazakii* populations from the media supplemented with FAC, FS, and FE were log 7.3-7.6, 5.8-6.2, and 6.8-6.9 CFU/ml, respectively, whereas no *E. sakazakii* was detected from the media without supplementation.

APPENDIX D

Research of Dr. Frederick Khachik

PROJECT TITLE: Dietary carotenoids and their metabolites in the prevention of chronic diseases in humans

Objective and Nature of Research

The research has focused on the nutritional prevention of chronic diseases such as cancer and age-related macular degeneration by dietary carotenoids. Theses studies are as follows: 1) bioavailability, toxicity and metabolic studies in primates supplemented with chronic doses of lutein and zeaxanthin, 2) the effect of lycopene supplementation in the prevention of spontaneous smooth muscle tumors in Japanese quails 3) synthesis and industrial production of α-cryptoxanthin and β-cryptoxanthin from lutein,

Accomplishments and Potential Value/Applicability

1. *Bioavailability, Toxicity and Metobolic Studies in Primates Supplemented with Chronic Doses of Lutein and Zeaxanthin*

   Lutein and zeaxanthin are two dietary carotenoids that accumulate in the human retina as well as other ocular tissues and have been implicated in the prevention of age-related macular degeneration (AMD). The objective of this study is to establish the bioavailability and ensure the safety of supplementation of these carotenoids in primates. This is a 3-year study that has been sponsored by the National Eye Insititute (NEI) [Fred Khachik, PI] and is conducted in collaboration with a number of investigators at the School of Ophthalmology and Veterinary Medicine (University of Maryland, Baltimore). In year one, 5 primates were daily supplemented with 10 mg/kg body weight of lutein for one year; there were also 3 control animals. Extensive toxicity data obtained by co-investigators in this study clearly indicate that there was no observed toxicity. This lutein dose is equivalent to 600 mg/day supplementation for a 60-kg human being. The results also revealed a dramatic increase in the lutein concentration in the plasma and ocular tissues (retina, ciliary body, iris, lens) of the supplemented animals compared to the controls. In addition, modest concentrations of lutein and its metabolites were also found in nearly all tissues and organs (e.g. liver, lung, colon, kidney, ovaries, breast, brain) of the supplemented primates.

   The second year study was similarly conducted with zeaxanthin (5 primates) at the same dose (10 mg/kg body weight) and also revealed a significant increase in the levels of this carotenoid and its metabolites in plasma and ocular tissues of the supplemented primates. The third year, which is currently in progress, involves 5 primates that are being fed a 1/1 mixture of lutein and zeaxanthin each at the dose of 0.5 mg/Kg body weight for one year. This will be completed in September of 2005.

The NEI is currently planning a nationwide multiclinical trial with a combination of lutein and zeaxanthin in a large number of age-related macular degeneration (AMD) patients in the U.S. to investigate the efficacy of these carotenoids in the prevention of AMD and cataracts. The established safety of lutein and zeaxanthin supplementation from the studies with primates allows the NEI investigators to proceed with clinical trials in humans.

2. *The Effect of Lycopene Supplementation in the Prevention of Spontaneous Smooth Muscle Tumors in Japanese Quails (Khachik, Co-PI)*

Leiomyomas (fibroids) are benign tumors of the uterus affecting millions of women. Spontaneous leiomyomas of the oviduct are common tumors of the Japanese quail (Coturnix coturnix japonica), which makes it a good animal model for screening potential agents for testing in the prevention and treatment of human myoma uteri. Since dietary intake of lycopene has been associated with a reduced risk of a variety of human cancers, in collaboration with Dr. Kucuk (PI) and his team (Wayne State University, Detroit, Michigan), Dr. Khachik investigated the effects of lycopene supplementation on the development of leiomyomas in the oviduct of Japanese quail. Serum levels of oxidative stress markers [malondialdehyde (MDA), homocysteine], lycopene, vitamins C, E, and A, and tissue biomarkers bcl-2 and bax expression were measured also. One hundred and twenty quails (6 m old) were assigned to 3 treatment groups consisting of 4 replicates of 10 birds in each group. Birds were fed either a basal diet (group C) or the basal diet supplemented with 100 mg (group L1) or 200 mg (group L2) of lycopene / kg of diet. The animals were sacrificed after 285 days and the tumors were identified. Lycopene supplementation significantly decreased the number of leiomyomas as compared to control subjects ($P = 0.056$). The tumors in lycopene-fed birds were smaller than those found in control birds ($P = 0.01$). There were no significant differences in the expression of tissue bcl-2 and bax among the study groups. Serum vitamins C, E, and A increased ($P = 0.01$), whereas MDA and homocysteine concentrations decreased ($P = 0.01$) with lycopene supplementation. No measurable lycopene could be detected in the serum of control birds while a dose-dependent increase was observed in the serum of lycopene-supplemented birds. The results indicate that dietary supplementation with lycopene reduces the incidence and size of spontaneously occurring leiomyoma of the oviduct in the Japanese quail. Clinical trials should be conducted to investigate the efficacy of lycopene supplementation in the prevention and treatment of uterine leiomyoma in humans.

3. *Synthesis and Industrial Production of α-Cryptoxanthin and β-Cryptoxanthin from Lutein*

α-Cryptoxanthin and β-cryptoxanthin, as measured in plasma of human subjects have been associated with reduction in blood pressure in an Oxford University large intervention trial. Healthy and diseased subjects have also been studied in a variety of prospective trials to correlate β-cryptoxanthin levels with cardiovascular.

Three industrially viable processes have been developed that convert commercially available lutein to α-cryptoxanthin and β-cryptoxanthin (patents 1-3). These rare dietary

carotenoids are not commercially available and their production allows investigators to conduct metabolic and clinical studies with these carotenodis. (3R)-β-Cryptoxanthin and (3R,6'R)-α-cryptoxanthin can also be used as nutritional supplements or as a food coloring additives. The fore-mentioned patented processes have been licensed to Kemin Foods (Des Moines, Iowa) by the University of Maryland and are currently at the early stage of industrial production.

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(15 December 2004)