



Annual Report 2002-2003

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

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Executive Summary

This Annual Report covers the sixth year of operation of the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) - the first year of the five-year renewal (September 2002 through September 2007). The non-competitive base funding for the sixth year was \$3,000,000.

The Food Safety Risk Analysis Clearinghouse relocated in February 2002 to facilities adjacent to the JIFSAN offices. The JIFSAN Advisory Council met in November 2002. The meeting involved updates from JIFSAN, CFSAN and CVM and discussions with Council members concerning issues of current interest. A new Executive Administrative Assistant began work in July 2003 following the retirement of Ms. Judy Dillon.

Research plays an important role in JIFSAN's and assists in strengthening the knowledge base for public health policy. The Internal Competitive Research Program funded 16 projects, with four of these being new projects initiated in July 2003. While most of the projects involved laboratories on the College Park campus, one was at the University of Maryland at Baltimore and one at University of Maryland Eastern Shore. Each project had a University of Maryland faculty member as the Principal Investigator and one or more FDA collaborators from CFSAN, CVM, or the National Center for Food Safety and Technology (NCFST).

JIFSAN has a Postdoctoral Research Associate Program in which research scientists work in FDA laboratories for one or two years. Six individuals had previously been hired for this program. During the current reporting period, three new positions were advertised to fill vacancies. One individual was hired and one was offered a position. The third position will be re-advertised.

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

The Food Safety Risk Analysis Clearinghouse redesigned and improved the functionality of its website, added several new sections and participated, with its interactive display, at three meetings involving risk analysis. The Risk Analyst and Research database, with over 200 members, averages over 5000 hits per month. The Clearinghouse is also operating the Acrylamide Infonet, the WHO/FAO Acrylamide in Food Network, established at the request of the FAO and WHO. The Infonet has several information bases (database) averaging almost 12,000 hits per month. The network functions as a global resource and inventory of research on acrylamide in food.

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. Four meetings were co-sponsored by JIFSAN including an acrylamide workshop in Chicago that was organized by JIFSAN and NCFST. The workshop was attended by 170 invited experts and resulted in the development of four

research projects for which funding is being sought. Information from the workshop is posted on the JIFSAN website.

A Food Safety Risk Analysis Professional Development Program has been developed with eight courses ranging from one to three days in length. In 2002, 10 classes were offered to a total of 275 individuals and 323 participated in 17 classes in 2003.

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 training program was offered in Culiacan, Mexico; Veracruz, Mexico; and Lima, Peru. Approximately 50 individuals participated in each training program.

As part of a cooperative agreement with the Central Science Laboratory (CSL) in the U.K., the fourth annual Joint CSL/JIFSAN Symposium on Food Safety and Nutrition was held on the University of Maryland campus. The theme was Risk Analysis and approximately 50 individuals participated in each of the four sessions.

A joint symposium on "Risk Communication: The Mode and Message" was held at the University of Guelph as part of a memorandum of agreement between JIFSAN and the Canadian Institute for Food Inspection and Regulation (CIFIR). This relationship will not be developed further since CIFIR was not renewed at the end of its three-year trial period.

JIFSAN provided organizational support for a workshop "Science and Technology Based Countermeasures to Foodborne Terrorism" in which over 100 individuals participated. The meeting was sponsored through a BARD (US-Israel Binational Agricultural Research and Development) Fund grant. Publication of the proceedings is planned.

As part of JIFSAN's emphasis on risk analysis, "seed money" is being furnished for three years to initiate establishment of a Center for Risk Communication Research (CRCR) led by the Department of Communication, UM. The CRCR will serve as a focal point for scholarly activity and discussion related to risk communication.

One of the original programs that has steadily increased in importance and participation is the JIFSAN Student Internship Program. UM undergraduate students are offered an opportunity to work with FDA scientists in their laboratories on specific projects identified by the FDA scientist involved and related to the JIFSAN mission. Non-laboratory opportunities are being developed. During 2002-2003, 69 University of Maryland students (45 different individuals) were involved as student interns in CFSAN laboratories. One student intern was listed as a co-author on a scientific publication resulting from the work in which the individual was involved.

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 jointly administered 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 help ensure that federal regulatory and scientific personnel remain in the forefront of food safety issues. This also provides visiting scientists, faculty and staff insight into regulatory processes. Opportunities for undergraduate and graduate students to work with FDA scientists as interns enhance students' understanding of regulatory processes and 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 collaborative research program provides seed funding to University of Maryland faculty to support research projects that are closely aligned with FDA's research needs. FDA collaborators on each project help provide additional scientific expertise and insight into public health impacts of the research.

The Harvey W. Wiley Federal Building, CFSAN's office and laboratory facility, is located adjacent to the University of Maryland in College Park enabling FDA and the University to share many resources, such as major instrumentation and library facilities. Programs initiated by JIFSAN have demonstrated that the benefits to be achieved by this partnership are substantial.

Trade initiatives have put food safety high on the international agenda. JIFSAN is actively involved in developing collaborations with international organizations to facilitate cooperative research and education programs and the exchange of scientists. In addition, JIFSAN has been designated a Pan American Health Organization/World Health Organization Food Safety Collaborating Center that focuses on risk analysis and food contamination monitoring. This designation is in the process of being renewed. Designation of JIFSAN as a member of the FAO Network of Excellence on Food Quality, Safety, and Nutrition (F.S.Q.N.) is in progress.

The MOU established a set of relationships that closely link the University with CFSAN and CVM by committing to the sharing of facilities, personnel, and intellectual resources when appropriate. Thus, FDA personnel 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 sixth 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 \$3,000,000.

Administrative Structure

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

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

- Ms. Judy Dillon, Administrative Assistant, retired in February 2003. Ms. Nora Petty was hired as Executive Administrative Assistant, starting work on July 22, 2003. The position was upgraded due to not being able to find qualified candidates at the Administrative Assistant I level.
- The Food Safety Risk Analysis Clearinghouse moved to facilities in Symons Hall adjacent to the JIFSAN offices in February 2003. JIFSAN obtained two additional rooms to house the Clearinghouse.

- FDA conducted a site visit in July 2003 and a report was received in November 2003. The site visit involved Peggy Jones, Cynthia Polit, Elizabeth Calvey, Christine Hileman, Juanita Pointer, Cassandra Jackson, and James Schuchardt from FDA. University of Maryland participants included Edward Waskiewicz, Helena Moynahan, Monique Anderson, Margarita Morales, Mary Grimley and Frank DeGeorge.
- A search is in progress to fill the position of Coordinator (Director) of the Food Safety Risk Analysis Clearinghouse. This position became vacant when the previous Coordinator accepted another position.

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 November 7-8, 2002. The next meeting has been scheduled for October 29-30, 2003. These meetings involve updates from JIFSAN, CFSAN and CFM 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
 - Unilever Bestfoods NA (Dr. Richard Lane)
 - Coca-Cola Company (Dr. Michael Carakostas)
 - Campbell Soup Company (Dr. George Evancho)
 - Frito-Lay (Dr. Steve Saunders)
 - General Mills (Mr. Frederick Hegele)
 - Gerber Products Company (Dr. Nicholas Hether)
 - Kellogg Company (Dr. Tracie Sheehan)
 - Kraft Foods (Mr. Ron Triani)
 - McCormick and Company (Dr. Hamed Faridi)
 - McNeil Specialty Products Company (Dr. Leslie Goldsmith)
 - M&M/Mars (Dr. Steven Rizk)
 - Mead Johnson Nutritionals (Ms. Susan Waltman)
 - Monsanto Company (Dr. Jerry Hjelle)
 - Procter and Gamble Company (Dr. Keith Triebwasser)

- Representatives of Consumers' Interests

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

- Academia

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

- Government

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

- Individuals

Dr. Gilbert Leveille (Cargill, Inc.)

Research Initiatives

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

JIFSAN is involved in research in a number of ways:

- By supporting core facilities that benefit FDA and University scientists and their collaborators,
- By funding a competitive internal research program,
- By developing cooperative programs with external constituencies,
- By facilitating programs funded from multiple institutions and other granting sources, and
- By supporting scientists working on JIFSAN programs.

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

JIFSAN Internal Competitive Research Program:

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

Effective July 2002, projects are funded at \$30,000 per year to be used for 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.

On the basis of satisfactory progress towards meeting objectives and annual reports, three projects funded in January 2000 were continued and due for completion in January 2003. Four projects funded in July 2001 were continued for a third year and one, originally a one-year project, was extended without additional funding. Six projects were funded in July 2002 and five were continued for a second year with one project (originally for one year) being extended for a year without additional funding. Four new projects were funded in July 2003, all being for three years.

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

Third-year projects (funded in January 2000):

- Using a probabilistic risk assessment model to study risk of *E. coli* O157:H7 contamination in hard cheeses, Mohammad Modarres (UM) and Joseph Schlessler (NCFST, FDA)
- Antibiotic resistance integrons in Shiga toxin-producing *Escherichia coli* and *Campylobacter jejuni/coli*, Jianghong Meng (UM), David White, Shaohua Zhao, and David Wagner (FDA)
- The evaluation and removal of bacterial biofilms from food and food processing materials. Paul Schreuders (UM) and Leila Ali (FDA)

Third-year projects (funded in July 2001):

- 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)
- 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)
- Monitoring and compliance under seafood HACCP, Anna Alberini, Erik Lichtenberg, Dominic Mancini (UM) and Robert Scharff (FDA) (Originally a one-year project, extended one year without additional funding):

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

First-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)

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. Prior to the period for this proposal, 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.

Three new positions have been advertised with an active search in progress. The positions are in the following areas: (a) molecular phylogenetic identification of foodborne bacterial strains, (b) aquaculture drugs: LC/MS protocols for marker determination, and (c) rapid determination of food integrity and identification of foodborne bacteria using Fourier transform near infrared (FT-NIR) spectroscopy and pattern recognition techniques. Janet Dubois was hired beginning September 1, 2003, a second position has been offered and is awaiting FDA security clearance, and one position needs to be re-advertised. These positions will replace those who are completing their postdoctoral programs.

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.

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

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

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.

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.

JIFSAN has been charged with the responsibility of developing and operating a Risk Assessment Clearinghouse. In 1999, the name was changed to the JIFSAN Food Safety Risk Analysis Clearinghouse to more closely align with international nomenclature in which risk analysis is the umbrella term that includes risk assessment, risk communication, and risk management. The Clearinghouse is established to collect and disseminate available data and methodologies from government, academic, and industry sectors domestically and internationally. The Clearinghouse provides a centralized information source for risk analysis related to food safety. While initial emphasis was on microbial pathogens and their toxins, this is being expanded to other chemicals and toxins.

Progress for the Food Safety Risk Analysis Clearinghouse includes:

The Clearinghouse website has been redesigned and restructured to make it more user-friendly. The navigation and search engine functionalities have been improved. As a result of these changes, it is now easier to find information on the website.

The membership of the Risk Analyst and Researcher database is now over 200 and is by far the most popular section of the Clearinghouse website, attracting on average over 5000 hits per month. An online discussion forum is also being initiated to enable users to share ideas and ask questions.

Several new sections have been added to the Clearinghouse to keep pace with the recent trends, such as a section on weight management and a section devoted to information quality/peer review.

Recent additions to the Clearinghouse exclusive holdings include:

- AUV Tool – This software tool was developed by Junyu Zheng and H. Christopher Frey, for the Office of Research and Development, Environmental Protection Agency. It is for use in quantifying variability and uncertainty in quantitative analysis.
- Survey of *Listeria Monocytogenes* in Ready to Eat Foods - NFPA collected and tested about 31,700 samples in eight different categories of ready to eat foods from Maryland and California Food Net sites with the purpose of addressing uncertainties in risk assessment regarding the occurrence of *Listeria monocytogenes*.
- FDA Labeling Cost Model – FDA contracted with the Research Triangle Institute (RTI) to update RTI's 1990 labeling cost model to make the model more relevant for the types of analyses currently conducted by the FDA.

The Clearinghouse participated, with its interactive display, in the Society for Risk Analysis Annual Meeting, New Orleans, December 8-11, 2002; the 9th Annual FDA Science Forum, Washington, DC, April 24-25, 2003; and the Fourth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Risk Communication, Inn and Conference Center, University of Maryland University College, June 12, 2003.

A national search to fill position of Coordinator (Director) of the Food Safety Risk Analysis Clearinghouse is nearing completion. Interviews are being scheduled.

Acrylamide Infonet:

At the request of the United Nations' World Health Organization and Food and Agricultural Organization, JIFSAN is operating the *Acrylamide Infonet* (www.acrylamide-food.org), the WHO/FAO Acrylamide in Food Network, through the Risk Analysis Clearinghouse. This Network was established as a result of the June 2002 FAO/WHO Consultation on the Health Implications of Acrylamide in Food. The consultation recommended that an international network on acrylamide in food should be established inviting all interested parties to share relevant data as well as ongoing investigations.

The focal point for the network is the website www.acrylamide-food.org which contains an information base (database) of researchers/data providers; references for research published elsewhere; studies in development; listing of acrylamide websites; acrylamide documentation (general information); events and activities; Infonet updates; and call for

data (a call by WHO for analytical data). A discussion forum has been established, which provides space to share ideas related to acrylamide in food.

The Infonet website has received more than 107,000 hits between the period of January 2003 and September 2003, averaging almost 12,000 hits per month. Though most of the audience appears to be from North America, Europe, and Japan, the usage statistics data also suggest that the website is also accessed from governmental, educational, commercial, and international organizations around the world.

This network functions as a global resource and inventory of ongoing research on 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.

Hitesh Patel (JIFSAN Clearinghouse) and Mike Landavere (IT, College of Life Sciences, UM) spent three days at WHO (Geneva, Switzerland) in February/March to familiarize themselves with the GEMS format and SIGHT databases to be used to collect analytical data on the amount of acrylamide in different foods for future use in risk assessments.

Coordination is occurring with the Food Standards Agency (UK) and the European Commission (Brussels, Belgium) to ensure that pertinent entries into their databases are included in the Infonet. The Infonet is intended to be a worldwide resource for the issues of acrylamide in food. Currently, over 120 projects have been entered.

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.

MEETINGS CO-SPONSORED BY JIFSAN

Workshop "Acrylamide in Food: Scientific Issues, Uncertainties, and Research Strategies and subsequent activities.

JIFSAN has been involved in a coordination role, domestically and internationally, in the acrylamide in food issues essentially since the Swedish announcement (April 24, 2002) of its detection. This coordination role evolved from an ad hoc acrylamide working group with representatives from industry, government (FDA), academia, trade associations and others.

JIFSAN and the National Center for Food Safety and Technology (NCFST, Argo, IL) organized a Workshop “Acrylamide in Food: Scientific Issues, Uncertainties, and Research Strategies” held in Chicago, IL, October 28-30, 2002. An invited group of 170 experts participated in the workshop that was sponsored by all of the participants. The current status of issues concerning the occurrence of acrylamide in foods was discussed. Emphasis was placed on identifying needs and responses with respect to improving risk characterization of acrylamide in foods and in developing recommendations for a select number of high priority research needs. A goal of the workshop was to develop coordinated efforts between industry, government, and academia.

From the reports of the five Working Groups, including a limited number of recommendations concerning knowledge gaps, seven project proposals were generated and evaluated by a core group. These were reduced to four proposals for which funding is being sought from the food industry. A decision was made not to continue, at this time, with one of the projects (analytical proficiency). Funding the remaining three projects (two involving acrylamide formation and one involving risk communication) is being initiated with support from the Industry Acrylamide Coalition and JIFSAN.

Seminar on “Veterinary Drug Residues and the Global Food Supply”

JIFSAN co-sponsored the formal launch seminar for NFPA-Asia entitled “Veterinary Drug Residues and the Global Food Supply” held in Bangkok, Thailand, January 23, 2003. The seminar covered two issues of key interest to the global food industry. The primary focus was on veterinary drug residues in food, followed by a short overview on acrylamide in food. Representatives attended the meeting from key departments in the Thai government and from food industries.

WHO/FAO Workshop on General Principles and Methods for Risk Assessment of Chemicals in Food: Consumption and Exposure Analysis

JIFSAN co-sponsored the WHO/FAO Workshop on General Principles and Methods for Risk Assessment of Chemicals in Food: Consumption and Exposure Analysis held during the week of September 29th, 2003 at the Harvey W. Wiley Federal Building (FDA), College Park, MD. FAO/WHO are jointly updating and consolidating the principles and methods for the risk assessment of four areas: food additives, contaminants, pesticides and veterinary drugs. The goal of the workshop was to develop a document that provides a consensus framework for conducting risk assessments. This will be valuable internationally, especially in developing countries.

Symposium on Biosensor Technologies & Microbial Diagnostics

JIFSAN co-sponsored a Symposium on Biosensor Technologies & Microbial Diagnostics held at the Harvey W. Wiley Federal Building (FDA), College Park, MD on October 7, 2002. The Symposium brought together scientists from academia, government and industry, including researchers who lead developments in the biosensor field. The purpose was to foster collaborations between the developers and potential users of these

technologies and to help focus this emerging technology in order to enhance clinical, research, diagnostic and regulatory capabilities.

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.

During the past year, the following courses have been offered: Overview of Risk Analysis (1 day, offered 3 times), Introduction to Food Safety Risk Assessment (3 days, offered 3 times); Introduction to Food Safety Epidemiology (3 days, offered 3 times); Quantitative Risk Assessment Methods (4 days, offered 2 times); Introduction to Food Safety Risk Communication (3 days, offered 2 times); Introduction to Economics for Risk Analysis (2.5 days, offered 2 times); Food and Nutrition Toxicology (3 days, offered 2 times); and Introduction to Food Safety Risk Management (3 days, offered 2 times). Enrollments ranged from about 15 to 30 in each offering. A total of 275 (248 government/academic and 27 industry) individuals participated in 10 classes in 2002. In 2003, a total of 323 (271 government/academia and 52 industry) individuals participated in 17 classes. The majority of the government/academic participants have been from the FDA.

INTERNATIONAL COOPERATION AND TRAINING PROGRAMS

Good Agricultural Practices (GAPs) for the Safe Production of Fresh Fruits and Vegetables

The International Good Agricultural Practices (GAPs) Training Program emphasizes applying good agricultural practices and good management practices (GMPs) to the production of produce (raw fruits and vegetables) with reduced microbial and chemical loads. The week-long training program was offered in Culiacan, Mexico (March 2003); Veracruz, Mexico (July 2003); and Lima, Peru (September 2003). Approximately 50 individuals attended each of the five-day training programs. The program in Lima, Peru was organized for representatives from Andean Countries.

The instructional team for the training programs was composed of faculty/staff from Clemson University, Mississippi State University, University of Maryland, 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 member of the staff from the Department of Natural Resources and Environment (DNRE), State of Victoria, Australia participated in the GAPs and GMPs Training

Program in Lima, Peru and will file an evaluation report on his observations. DNRE has a training program, similar to JIFSAN's, which they offer in Victoria and in Indonesia.

Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Risk Analysis

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 Fourth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Risk Analysis was held at the Inn and Conference Center, University of Maryland University College, June 11-13, 2003. Speakers were from the UK and U.S. Approximately 50 attended the meeting and participated in discussions in each of the four sessions. Material presented at the Symposium is posted on the JIFSAN website (www.jifsan.umd.edu).

Symposium on "Risk Communication: The Mode and the Message"

JIFSAN co-sponsored a joint symposium "Risk Communication: The Mode and the Message" at the University of Guelph, Guelph, Ontario, Canada on September 17, 2003. This was done with the Canadian Institute for Food Inspection and Regulation (CIFIR) with whom JIFSAN has a memorandum of agreement for development of cooperative efforts. This symposium was to initiate this collaboration. However, the three-year trial period involving the CFIA and the University of Guelph has concluded and CIFIR was not continued.

OTHER ACTIVITIES

Workshop on "Science and Technology Based Countermeasures to Foodborne Terrorism"

JIFSAN provided organizational support for meeting arrangements, registration, local transportation, housing and meals and contributed to program development for the Workshop on "Science and Technology Based Countermeasures to Foodborne Terrorism" held at the U.S. Fish and Wildlife National Training Center, Shepherdstown, WV, June 29-July 2. The meeting was sponsored through a BARD (U.S.-Israel Binational Agricultural Research and Development Fund) grant and financially supported by the grant and registration fees. The conference, involving a combination of lectures, question and answer periods, and ample discussion time, focused on technology-based efforts that can be employed to prevent, detect, and minimize health effects of terrorist attacks throughout the food chain, from production to consumption. Sessions evaluated state-of-the-art science and technology for: (1) assessment of threats; (2) detection of chemical and biological threat agents; (3) trace, track, authentication, and anti-tampering technologies, and (4) hazard mitigation. World experts were assembled in this forum in which more than 100 individuals participated. Publication of the proceedings is planned.

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 with the funding coming from its DRIF funds. The CRCR will serve as a focal point for scholarly activity and discussion related to risk communication. Activities may involve bringing scholars and scientists together to do basic and applied research, sponsoring risk communication symposia and workshops, funding postdoctoral fellowships or research assistantships, serving as an information clearinghouse, providing educational services and training to the scientific community and others, and advancing awareness of risk communication among constituent communities. The CRCR will devote significant resources to understanding and improving communication related to food safety and applied nutrition. However, the CRCR will consider the scope of its mission to include other important areas of risk communication as well.

JIFSAN Participation in Exhibitions

JIFSAN has staffed a booth (display) at

- Society of Risk Analysis Annual Meeting, New Orleans, LA (December 2002)
- IFT International Food Safety and Quality Conference and Expo, Atlanta, GA (February 2002)
- FDA Science Forum, Washington, D.C. (April 2003)
- International Association for Food Protection, New Orleans, LA (August 2003)

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.

During 2002-2003, 69 University of Maryland students (45 different individuals) volunteered as interns in CFSAN laboratories 2002-2003. Efforts are being made to extend opportunities for internships beyond laboratory experiences. The limitations on this, so far, have been an absence of projects listed in such areas by FDA staff.

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

Publication by an intern:

S.F.Hinkley, J.A. Moore, J. Squillari, H. Tak, R. Oleszewski, E.P. Mazzola, and B.B. Jarvis, "New Atranones from the Fungus *Stachybotrys chartarum*," *Magnetic Resonance in Chemistry* 41:337-343 (2003).

Future Plans (2003-2004):

1. Administrative

- With the continuing development of JIFSAN, including the complexity of the budgetary issues involved, the increase in meetings in which the Institute is involved, and the need for a communications effort, it has become quite clear that additional support is required in the areas of finance and communications. Hiring the Executive Administrative Assistant should alleviate a portion of this need, but certainly not all.
- In response to a recommendation from an FDA Site Visit, a half-time position is being added to the seventh-year budget to assist the JIFSAN Financial Officer.
- The JIFSAN Conference and Communications Coordinator is so fully committed to the conferences aspects of the position responsibilities, due to the number of conferences, workshops, and training programs being organized and scheduled, that insufficient time is available to initiate the communications component of the responsibilities. A half-time position is being added to the seventh-year budget to assist in initiating a communications effort for JIFSAN, including a newsletter.

2. Research Initiatives

- The four projects currently funded for the third year, and the two projects extended without additional funding, will be completed. The five projects funded in July 2002 and the four projects funded in July 2003 will be continued provided sufficient progress is made and funding is available. A Call for Proposals will be issued and up to five proposals will be selected for funding beginning in July 2004.

- Research projects addressing issues (mechanisms of formation, analytical methodology, exposure/biomarkers, toxicology/metabolic consequences) raised by finding acrylamide in foods will be supported using funding obtained externally. Currently funding is being obtained for four projects in cooperation with the Industry Acrylamide Coalition and supplemented by JIFSAN through carry-forward funds. Additional projects may be considered, if funding can be obtained through the Coalition or upon approval of carry-forward funds by the FDA. JIFSAN will administer the funding and monitor progress of the research.
- Efforts will continue to develop collaborations with industry, consumer organizations and trade groups to address critical issues in food safety.
- Opportunities will be sought to obtain additional external research funding to expand the resources available to JIFSAN and faculty/staff associated with its programs.
- Efforts to develop collaborative programs and external funding from industry, trade associations, and other sources will continue. FDA (CFSAN) will need to develop guidelines for development of such partnerships and acquisition of funding before this can occur.
- Operation of the Risk Analysis Clearinghouse will be further developed. A new Coordinator should be in place. A major emphasis will be 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 a risk assessment by the Codex Joint Expert Committee on Food Additives (JECFA).
- The JIFSAN Postdoctoral Research Associate program will continue with three new postdoctoral research associates hired. Tentative plans are to advertise positions for three more positions in areas of need for the FDA.

3. Education and Outreach Efforts

- JIFSAN will staff a booth at the following meetings: IFT's International Food Safety and Quality Conference and Expo, Orlando, FL, November, 2003 and FDA Science Forum, Washington, DC, May 2004.
- Efforts to expand the JIFSAN internship program to include non-laboratory experience will continue. This will be dependent upon FDA listing such positions. The effects of increased security measures upon student access to laboratories may adversely affect this program and will need to be evaluated.
- Train-the-trainer programs in minimizing food safety hazards in production of fresh fruits and vegetables (GAPs and GMPs Training Programs) will be offered in Guatemala, Honduras and Korea. These will involve approximately 50 individuals in each offering. If approval for the use of carry-forward funds is approved, a teaching tool kit will be developed under subcontract (\$150,000 including indirect costs). This teaching tool kit will include educational materials (in English and Spanish) to be used by trainers in offering the training to workers involved in fresh produce production and packing (for export). The kit will include printed material, appropriate visuals and materials that can be tailored to the needs at each location where the training is presented. A comprehensive evaluation of the program, planned jointly by JIFSAN and FDA/CFSAN, will be

- accomplished under subcontract. The Review Panel will consist of Dr. Edward (Ted) Wilson (retired), former Deputy Administrator at CSREES; Dr. Keith Roache, former International Programs Director, Lincoln University; and Dr. Trevor Suslow, Extension Research Specialist, Postharvest Quality and Safety from Seed to Shelf, Department of Vegetable Crops, University of California, Davis.
- The 2004 schedule of courses in the Risk Analysis Professional Development Program is being developed. Multiple offerings of the courses offered in 2003 are anticipated. Plans call for including the distance learning courses being developed through a USDA grant to the College of Agriculture and Natural Resources.
 - JIFSAN will co-sponsor the First Annual Symposium on World Hunger at the University of Maryland at College Park on October 14, 2003. The keynote speaker will be Dr. Per Pinstrup-Andersen, Cornell University and formerly Director, International Food Policy Research Institute (IFPRI), Washington, DC. Dr. Pinstrup-Andersen is the 2001 World Food Prize Laureate.
 - Discussions are being initiated to organize and co-sponsor a second Acrylamide in Food Workshop to review progress being made in the U.S. and other countries on research initiated during the past year. This will be organized in collaboration with government, academia, industry, and international colleagues. The meeting is scheduled for April 13-15, 2004. It will be a meeting to address scientific issues and identify gaps that may still exist in knowledge/evidence concerning the issues of acrylamide in foods.
 - JIFSAN will co-sponsor a symposium at the Society for Risk Analysis Annual Conference (Baltimore, MD, December, 2003) entitled “The State of Risk Analysis Education and Training, Are we Meeting the Needs?” This symposium seeks to initiate a dialogue across academic, government, and private sectors about training opportunities, challenges, and future needs for training and education in risk analysis. The symposium will be in a roundtable format, with approximately eight posters.
 - JIFSAN will co-sponsor an E-learning workshop at the Society for Risk Analysis (SRA) Annual Conference (Baltimore, MD, December 2003). The workshop “Overview of Food Safety Risk Analysis” will be conducted entirely over the Internet prior to the SRA annual conference.
 - JIFSAN will co-sponsor an Expert Working Group on the Role of Diet in Blood Glucose Control. This will be done in collaboration with the International Life Sciences Institute North America (ILSI NA) and other ILSI international branches. The Working Group will identify key areas of controversy and uncertainty surrounding the use of information on the glycemic response to food as a basis for food selection in prevention and management of chronic diseases, and make recommendations on research required to resolve the scientific debate on this topic. A summary will be submitted to an appropriate peer-reviewed journal. The two-day meeting will be held October 12-13, 2003 at ILSI headquarters in Washington, D.C.
 - In cooperation with the Food Safety Research Consortium, JIFSAN will co-sponsor a workshop regarding Data Collection to Support Food Safety Risk

- Ranking. The expert workshop will (a) develop ideas and recommendations for how food attribution data might be collected to produce the most useful results in an efficient manner, and (2) further define and prioritize other data collection needs to support food safety risk ranking as a tool for priority setting. To be held in Atlanta, Georgia. October 31, 2003, the workshop will convene invited experts from federal food safety agencies, CDC, the academic research community, the food industry, and consumer organizations. Preparation for the workshop will include a background paper that describes currently available food attribution data and data collection challenges and proposed issues for discussion at the workshop.
- 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 the co-sponsorship of symposia, workshops, conferences and others types of meetings. Several of these are indicated in the Progress Report section of this proposal. Future plans are for an item in the annual budget to be directed to these important efforts.

4. International Collaboration

- Planning for the Fifth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition to be held in York, UK, June 30 – July 2, 2002 is in progress. It will involve a subject in the area of novel applications of analytical methodology. Dr. Michael Roberts, CEO, CSL plans to participate in the JIFSAN Advisory Council, of which he is a member, scheduled for October 29-30 at the Inn and Conference Center, University of Maryland University College.
- Development of a cooperative research effort between JIFSAN and the Department of Natural Resources and Environment (DNRE), State of Victoria, Australia will continue. 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 by a faculty member from the Department of Nutrition and Food Science, College of Agriculture and Natural Resources, UM and determination of microbial pathogens in/on freshly harvested produce in Victoria. Some analyses may be done with Maryland produce. This collaboration may involve a matching of funds through a program in the State of Victoria.

APPENDIX A

Projects Funded Through JIFSAN Competitive Internal Research Program

Projects funded in January 2000 (Completing January 2003):

- **Using a probabilistic risk assessment model to study risk of *E. coli* O157:H7 contamination in hard cheeses.** Mohammad Modarres (UM) and Joseph Schlessler (NCFST, FDA)
- **Antibiotic resistance integrons in Shiga toxin-producing *Escherichia coli* and *Campylobacter jejuni/coli*.** Jianhong Meng (UM), David White, S. Zhao, and David Wagner (FDA)
- **The evaluation and removal of bacterial biofilms from food and food processing materials.** Paul Schreuders, S. Joseph, A. Lomander (UM) and Leila Ali (FDA)

Final reports from these three projects are pending.

Projects funded July 2001:

- **Monitoring and compliance under seafood HACCP: An econometric investigation.** Anna Alberini, Erik Lichtenberg (UM), Dominic Mancini, and Robert Scharff (FDA). (Funded for one year, extended without additional costs for one year)

Final report from this project is pending.

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

Experiments were designed and carried out to evaluate the pharmacokinetics and tissue distribution of gentamicin in large (Holstein steers) and small (Sprague-Dawley rats) animal species with a specific objective to develop a physiologically based pharmacokinetic (PBPK) model as the predictive tool to draw tissue-fluid correlation with different dosage-regimen of gentamicin. Gentamicin pharmacokinetics and tissue distribution were evaluated after single and multiple doses via i.v. and i.m. routes in steers and via single dose urinary excretion and multiple dose study in rats.

The following conclusions could be drawn from the large animal (steers) studies:

- a) Gentamicin exhibits a multi-compartmental pharmacokinetics in steers when administered via i.v. as well as i.m. route.
- b) The accumulation of gentamicin by the kidney was remarkable. A slower release from the tissue sites may account for its longer depletion time in plasma.
- c) The tissue uptake could differ across different regimens and therefore the studies looking at tissue uptake and defining tissue-plasma relation needs careful planning and analysis.
- d) The long terminal phase observed in plasma after i.v. and i.m. administration does not contribute to appreciable accumulation in case of multiple dose pharmacokinetics of gentamicin and, thus, may be excluded during the design of such studies. Nevertheless it is indicative of the prolonged tissue washout phase associated with gentamicin use and may be of importance while deciding on withdrawal times in food producing animals.

The objective of the small animal studies was to develop and validate a sensitive and specific analytical method or simultaneous determination and quantitation of Gentamicin complex (with individual components) in rat urine, plasma and tissues.

A rapid and sensitive assay method utilizing fluorescent derivatization using FMOC1 (9-fluorenylmethylchloroformate) followed by elution on RP-HPLC system was developed, validated and later applied for urinary excretion and tissue distribution study of gentamicin in rats. Neomycin was used as internal standard.

The urinary excretion rate versus time plot for gentamicin in rats exhibited a tri-exponential decline pattern similar to that observed in steer plasma with long terminal half-life and almost 70-80% of the drug was recovered in urine. Gentamicin exhibited high accumulation in kidney tissue, lung, liver and spleen similar to that observed with steers.

Current status of physiological-based pharmacokinetic model in rats: With the available information from literature and experimental data, a preliminary PBPK model has been developed. The model specifically focuses on the events undergoing in kidney in terms of renal handling of gentamicin. Gentamicin is excreted via glomerular filtration and around 30% of filtered gentamicin is reabsorbed via a saturable uptake (Michelis-Menten kinetics). Since a nonlinear process is involved, the earlier attempt of constructing a model using matrix system did not give satisfactory output. The model currently under investigation is written using differential equation format and results from the preliminary modeling have been encouraging. Building-up of model with inclusion of all major tissue compartments is under progress.

Current status of physiological based pharmacokinetic model in steers: The model developed for rats was used with physiological parameters in steers and plasma profile was simulated for 4 mg/kg i.v. dose of gentamicin. The simulated plasma profile exhibited a tri-exponential decline pattern as observed in the in vivo experiments. The development of PBPK model with more accurate inputs for physiological parameters and steady state tissue partition coefficients is under progress.

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

This project investigates the extent to which conflict-of-interest considerations influence the perceived credibility of FDA advisory committees, as well as the credibility of ensuing FDA decisions. In addition, this project examines the degree to which knowledge of the waiver process influences perceptions of impartiality. Finally, this project examines the extent to which conflict-of-interest considerations influence satisfaction with advisory committee meetings as techniques for helping FDA obtain the best scientific advice on policy decisions. The research design includes participant-observation of advisory committee meetings and questionnaires and focus groups conducted with individuals who have attended advisory committee meetings. It also includes interviews with experts who have served on FDA Advisory Committees and FDA officials, as well as a content analysis of media coverage of FDA Advisory Committee meetings. This project's findings will comprise a scientific foundation on which to base future communication efforts aimed at improving the credibility of, and satisfaction with, FDA policy making.

During Year 2, 35 in-depth interviews were completed with FDA employees; members of patient advocacy and citizens groups; and industry representatives, bringing the total number of interviews over the course of the project to 62. Survey data was collected at 11 FDA Advisory Committee meetings held in the Washington D.C. metropolitan area from March to July 2003. Those surveyed included meeting attendees and advisory committee members and 273 questionnaires were received from meeting attendees and 91 questionnaires (with comments) from advisory committee members.

Presentations:

- McComas, K. (2003). Investigating the perceived credibility of FDA advisory committee meetings. *Presented at the JIFSAN Research Symposium*, College Park, MD, October 29.

- Simone, L., and McComas, K.A. (2003, August). Perceptions of Media Coverage of Conflicts of Interest within the U.S. Food and Drug Administration's Advisory Committees. *Paper presented at the 2003 Association for Education in Journalism and Mass Communication Annual Convention*, Kansas City, MO.
- McComas, K.A., and Simone, L.M. (2002, December). Conflict of Interest, Credibility, and Advisory Committee Meetings: A Pilot Study. *Paper presented at the 2002 Society for Risk Analysis Annual Meeting*, New Orleans, LA, December 8-11.
- McComas, K.A. (2002, December). Credibility, Conflict of Interest, and Satisfaction with Advisory Committees: Report on Research In-Progress. *Invited presentation to the Center for Food Safety and Applied Nutrition Staff College at the U.S. Food and Drug Administration*, College Park, MD.

Publications:

- McComas, K.A., and Simone, L. (2003). Media Coverage of Conflicts of Interest in Science. *Science Communication*, 24 (4), 395-419.
- **Comparison of the effects of curcumin supplements in young and aged rats.** Bernadene Magnuson, Monica Giusti (UM), Fred Hines, Sabine Francke, and Hamida Alam (FDA).

Previous results demonstrated 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. The experiments conducted over the past year were aimed at elucidating a potential mechanism(s) for this age-related difference in response. As curcumin affects arachidonic acid metabolism, liver COX-1 and -2 mRNA expression were determined to investigate age-related difference of curcumin effect in COX-2 reduction. Total serum cholesterol level was measured as it has been reported that curcumin lowers total plasma cholesterol. In contrast to the age-related difference in inhibition of colonic ACF by curcumin, liver COX-2 and total serum cholesterol levels were similarly affected by curcumin regardless of age. These data suggest that curcumin absorption and/or metabolism were not significantly different in the three age groups.

The investigators speculate that age-related differences are more likely due to differences in cellular events in the colon rather than in curcumin absorption or metabolism. One potential cellular event is induction of heat shock protein 70 (HSP70). HSP70 is a stress response protein that has been demonstrated to be altered during aging and by curcumin. The effects of age and curcumin supplementation on the expression of heat shock protein 70 (HSP70) were investigated in the livers from the curcumin study. In addition, the effect of

age on expression of HSP70 in colon was investigated in three age groups of rats that did not receive any treatment. Although HSP70 expression in liver was not affected by either age or curcumin, compared to young and old rats, mature untreated rats had the highest level of HSP70 expression in the colon. These results support the hypothesis that there are colon-specific changes occurring during the aging process that may be responsible for the differential effectiveness of chemoprevention by curcumin. Additional analyses will be conducted on the tissues that were collected from the first experiment to support this hypothesis. One additional short-term animal feeding experiment is required to test this hypothesis more conclusively. This experiment will be conducted in the upcoming year.

A small preliminary experiment has been conducted to assess the effect of age on the efficacy of soy isoflavones supplements to prevent colon cancer in female rats. Unexpected toxicity was observed in older female rats fed the isoflavones. Possible mechanisms for this apparent age-related reaction are being investigated.

Presentations:

Francke-Carroll, S; Daly, K; Wang, T; and Magnuson, B. Underlying age-related liver pathology increases azoxymethane toxicity in female F344 rats in an aging study on colon cancer chemoprevention. Submitted to the Society of Toxicology meeting, Baltimore, March 2004.

Publications:

Y. Kwon, M. Malik and B.A. Magnuson, Aging alters inhibition of colonic aberrant crypt foci by curcumin. *Nutrition and Cancer* (Accepted)

- **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)

During this period, the protocol was developed to determine the antimicrobial effect of acidic calcium sulfate, Safe₂O™-ACS (Mionix Corp., Napeville, IL), on the growth and survival of *L. monocytogenes* in broth and smoked salmon stored at 4° and 10°C. Safe₂O™ is a non-harmful FDA- and USDA-approved food additive composed of GRAS materials. Safe₂O™ kills bacteria on contact at levels nearly equal to irradiation. Unlike irradiation, it continues to inhibit microbial growth and recontamination long after treatment. Since the pH of Safe₂O™ is about 1.9, finding the effective dilution level of Safe₂O™ solution, without changing the quality of smoked salmon, was a challenge for practical usage of Safe₂O™.

Data indicated that Safe₂O™ RTE 01 (mixture of acidic calcium sulfate, lactic acid and sodium phosphate) solution at less than 5% level did not affect quality of smoked salmon. The pH of RTE 01 was also changed to pH 1.9 by dilution. Therefore, *L. monocytogenes* on smoked salmon was not killed by dipping in 2% and 4% Safe₂O™ solution for 1 min, but dipping smoked salmon with 2% Safe₂O™ RTE 01 significantly suppressed the growth of *L. monocytogenes* during 12 days of storage at both 4° and 10°C. The growth of *L. monocytogenes* on smoked salmon treated with 4% Safe₂O™ RTE 01 was completely controlled over the storage studied, indicating the residual protective effect of 4% Safe₂O™ RTE 01. Sublethal injury of *L. monocytogenes* was also noticed with 2% and 4% Safe₂O™ dip. The result of this experiment indicated that the growth of *L. monocytogenes* on smoked salmon can be completely controlled with 4% Safe₂O™ RTE 01 dip without affecting the qualities of smoked salmon. In addition, total elimination of *L. monocytogenes* was observed in broth containing Safe₂O™ RTE 01 at all tested dilution levels, regardless of storage temperature. Continuously, the antimicrobial effect of Safe₂O™ solution on the growth of *L. monocytogenes* will be tested at ambient temperatures.

Presentations

K.S. Yoon 2003. Control of *Listeria monocytogenes* on ready to eat foods. Department of Food and Nutrition, Yonsei University, Seoul, Korea.

K.S. Yoon 2003. New direction in foodborne disease prevention: the risk-based approach. Department of Food and Nutrition, Kyunghee University, Seoul, Korea.

K.S. Yoon 2003. Control of *Listeria monocytogenes* on cold-smoked salmon using a combination of potassium lactate and sodium diacetate. JIFSAN/CFSAN Staff College Seminar, Harvey Wiley Building (FDA), College Park, Maryland, April, 23

K.A. Abou Zeid and K.S. Yoon. 2003. Fate of *Listeria monocytogenes* in brain heart infusion broth containing combined potassium lactate and sodium diacetate at various temperatures. IFT Annual Meeting, Paper 29F-21, Chicago, IL. July 12-16

K.S. Yoon, C.N. Burnette, K.A. Abou Zeid, R.C. Whiting. 2003. Growth and survival of *Listeria monocytogenes* inoculated on smoked salmon treated with combined potassium lactate and sodium diacetate during refrigeration and extended frozen storage. IFT Annual Meeting, Paper 59-2, Chicago, IL. July 12-16

Barratt, R.A., K.S. Yoon and Whiting, R.C. 2002 The effect of pH and agitation on the growth of *Listeria monocytogenes* in brain heart infusion

(BHI) broth containing combined potassium lactate and sodium diacetate stored at 4°C and 10°C. International Association of Food Protection, 89th Annual Meeting. Technical program abstract pp: 54. San Diego, CA. June 30 -July 4.

Publication:

K.S. Yoon, C.N. Burnette, 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: 1469-1473

- **In vitro metabolic profiles to characterize and predict drug residues in aquacultured finfish.** Andrew Kane (UM), Badar Shaikh, and Renate Reimschuessel (FDA) (Funded for one-year. The partial support of JIFSAN for one year and results obtained were used as the basis for obtaining JIFSAN funding for a 3-year project effective July 1, 2002 (see next section).]

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 costs.)

Background: Preliminary experiments have shown that 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.

This one-year study was designed to evaluate the transfer of soy isoflavones into the egg. In this study, evidence for the deposition of genistein and its glucoside derivative into the egg yolk is to be strengthened and expanded to include other isoflavones, simulating the proportions found on the regular diet.

PROGRESS:

Supplementation of encapsulated genistein and genistin: Adult reproductive female Japanese quail were randomly assigned to treatment groups that received encapsulated 50mg, 100mg genistein or 80mg genistin per day (4

quail per treatment) for 5 days. A control group (2 quail) received placebo capsules. Eggs were collected prior to treatment and then daily for 15 days starting on the first day of treatment. The egg, separated into yolk and white, and pulverized quail diet were extracted and analyzed by HPLC. 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 third day of supplementation and reached the maximum about two 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.

Administering a variety of isoflavones in the diet: Previous experiments have shown that genistein and its metabolites were transferred into the egg yolks of Japanese quail receiving pure genistein supplements. Evaluation of how a mixture of isoflavones in the diet could be transferred into the egg yolks was initiated. 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. Commercial eggs of 12 different brands were analyzed in order to determine whether isoflavones could be transferred to the eggs from the diet and in what proportion different isoflavone derivatives would be transferred. 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. Also, it is possible that genistein is easier to metabolize and that their metabolites are present rather than the aglycone, which is currently undetermined.

Analysis of commercial isoflavone supplements is currently in progress in order to design an isoflavone-enriched poultry diet to produce isoflavone-enriched eggs. An experiment has been designed to try to determine the maximum isoflavone deposition into the eggs.

This grant has contributed to the support of the research of a graduate student: Fei Lin, MS Thesis: Evaluation of soy isoflavones and their transfer into the egg yolks of Japanese quail. Master of Science, 2003. University of Maryland, Nutrition and Food Science Department.

Presentations:

Lin, F; M.M. Giusti, J. Wu, and M. A. Ottinger, 2002. Effects of dose and glycosylation on the transfer of genistin into the eggs of the Japanese Quail (*Coturnix japonica*). Presented at:

- IFT annual Meeting, Anaheim, California, 2002.
- Biosciences Research & Technology Review Day, College Park, MD, 2002
- IFT Maryland Section Supplier's Night, Timonium, MD, 2003

Publications:

- This research was highlighted in the Maryland Research magazine: Value-Added Eggs. *Maryland Research*. Spring 2002, vol. 11, no. 2, p 11.
- **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 the project is to explore the use of the antimicrobial peptides nisin and sublancin as components of strategies to protect the United States food supply against the introduction of food pathogens such as *Bacillus anthracis* and *Clostridium botulinum* through bioterrorism. Nisin and sublancin are highly effective inhibitors of bacterial spore outgrowth and when utilized as mixtures, can interfere with bacterial spore germination. This project is exploring the mechanism by which these non-toxic antimicrobial peptides exert their inhibitory effects, in expectation that an understanding of the underlying mechanism of action will be important in our ability to optimize their effectiveness against these, and other, bioterrorism threats. Another aim of the project is to evaluate the inhibitory effectiveness of nisin and sublancin when used in actual food systems.

An important break-through in the study of the mechanism of nisin action.

The previous Progress Report reported the utilization of a novel probe of nisin action, which is a fluorescein derivative of nisin called FITC-nisin. For many years, a limitation in our ability to study the target of nisin action has been the unavailability of a form of nisin that contained a “tag” that could be used to identify the molecular target of action. Because of previous work in the Hansen laboratory, an underlying premise of the project is that the mechanism of nisin inhibition involved a covalent attachment of the nisin molecule to a sensitive target that is present in bacterial spores and/or cells. This covalent attachment was assumed to occur through the electrophilic attack of a target nucleophile by one or more of the novel dehydro residues of the nisin molecule. In order to pursue this idea, it is essential to have a form of nisin that can establish the covalent interaction, and then permit recovery of the

nisin-tagged target molecule, and to then proceed to the identification of the target molecule.

In the previous report, the use of FITC-nisin, in which the fluoresce moiety is attached to one of the lysine residues in the nisin molecule, was introduced. Control experiments established that the modification did not affect nisin action, neither with respect to its ability to inhibit bacterial spore outgrowth, nor with its ability to lyse vegetative cells. Moreover, electron microscopy established that FITC-nisin could bind to highly-localized regions of ungerminated *B. cereus* spores, which are a surrogate for *B. anthracis* and *C. botulinum* spores. Also, FITC-nisin was reacted with lysates of *B. cereus* and *B. subtilis* cells, and showed that there were fluorescently-labeled adducts that were stable to boiling in SDS, which is strong evidence of covalent attachment to molecular components in these cells. SDS gels showed that there appeared to be several of these components.

Whereas the fluorescence of FITC-nisin shows that the kinds of covalent adducts, in which there is interest in studying, can form, the fluorescence alone provides no information about the identity of the molecular targets. The nisin-labeled targets have been isolated by utilizing antibodies that have been raised against the FITC epitope. Previous forms of labeled nisin, such as biotinylated nisin, have been plagued by serious background and non-specificity problems. FITC has the advantage of having no natural structural counterpart in cellular structures, so elimination of non-specificity and background problems is to be expected.

FITC-nisin was accordingly allowed to interact with *B. subtilis* cells. This leads to lysis of the cells, so that continued incubation of the lysate with the FITC-nisin should result in all accessible cellular components that can react with nisin, to do so. These lysates were centrifuged and concentrated with a Centricon reverse-osmosis filter, then resuspended in SDS buffer. The suspensions were boiled in the presence of SDS for 3 min, which is known to disrupt most known non-covalent interactions. The boiled lysates were then electrophoresed on SDS gels, and transblotted onto nitrocellulose membranes. When these membranes were scanned with the STORM fluorometer, fluorescent bands of a variety of molecular weights appeared, as we have seen before.

As an additional detection method, the membranes were subjected to Western analysis in which purified rabbit anti-FITC antibodies were used as an immuno-probe. Antibodies that were bound to the FITC-nisin were detected using a second antibody that was conjugated to alkaline phosphatase which forms a blue color in the presence of X-P substrate. The FITC-nisin lane was filled with bands, whereas the control lane with a lysate that was untreated with FITC nisin was totally blank.

Whereas a hasty initial interpretation of this result is that the FITC-nisin has reacted extensively with a multitude of components in the lysate, the true meaning is quite different. Many experiments have shown that if nisin is capable of forming covalent adducts, it is highly specific, and is simply incapable of forming adducts to a myriad of targets as the gel suggests. Instead, what must be happening is that the nisin molecules are either acting as cross-linking agents to link together multiple copies of a single (or very small number of) molecular species; or alternatively, the target of the nisin molecule has many attachment sites for nisin. The gel therefore consists of a “ladder” of these cross-linked or multiply-modified molecules. It is also possible that both cross-linking and multiple adducts are occurring. It is to be noted that the control lane is completely clean, so the signals that are shown in the sample lane are genuine FITC-nisin-labeled adducts.

This highly significant result is being investigated further.

Presentation:

JIFSAN Research Grant Symposium: “Study of Nisin and Sublancin in a Strategy for Protection of the United States Food Supply from Pathogenic Bacterial Spores Introduced through Bioterrorism” October 2003.

- **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: 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.

Progress:

In vitro efforts: Preliminary studies have been carried out relevant to obtaining *in vitro* metabolic kinetic data. These initial studies focused on determining the linearity of metabolic reactions with the different substrates (e.g., different alkoxyresorufins, 1-chloro-2,4-dinitrobenzene (CDNB), ethoxycoumarin) over time. These preliminary data were important for determining the length of time to run the definitive *in vitro* metabolic reactions, since data are expressed as product per minute per milligram protein.

Seven species of fish (Atlantic salmon, bluegill, channel catfish, hybrid striped bass, largemouth bass, and yellow perch), in groups of eight, were collected and their livers harvested. Livers were processed for obtaining cytosolic and microsomal fractions. Specimens of market size tilapia were not available during our “processing window” this last year, and will be collected early in the next project year.

Biotransformation reactions have been run for the aforementioned species using ethoxycoumarin, ethoxyresorufin for phase I reactions, and appropriate substrates to examine gluconyltransferase, sulfotransferase and glutathione-S-transferase-mediated phase II reactions. These metabolism data are being compiled to generate the species-specific baseline kinetic profiles, as proposed. Additional fish are being collected to add appropriate numbers to insure proper statistical comparisons

In vivo efforts: Three species of fish, Atlantic salmon, tilapia, and rainbow trout, were examined at the FDA for biotransformation *in vivo*. These species were able to biotransform albendazole into three of its major metabolites, albendazole sulfoxide, albendazole sulfone, and albendazole aminosulfone. While the parent drug albendazole was depleted within 24 hours in the muscle tissue of the three fish species, its pharmacologically active metabolite, albendazole sulfoxide, persisted to 96 hours in Atlantic salmon, and was depleted within 48 hours in rainbow trout and tilapia. One of its inactive metabolites, albendazole sulfone, depleted to <10 ppb in the three fish. However, the second inactive metabolite albendazole aminosulfone persisted longer and was depleted to <10 ppb in rainbow trout and Atlantic salmon at 96 hours, but continued to be present to <20 ppb in tilapia at 144 hours. The results from the *in vitro* studies of albendazole in fish, to be conducted in years 2-3 of this JIFSAN grant, will be correlated with the above *in vivo* metabolism and residue depletion profiles.

Presentations:

1. Gonzalez, JF, Reimschuessel, R, Shaikh, B, and Kane, AS. 2002. In vitro metabolic profiles to characterize and predict drug residues in aquacultured finfish. (Abstract) Presented at the Fourth International Symposium on Aquatic Animal Health, September 2-5, 2002, New Orleans, LA.
 2. Informal seminar at the US FDA, Center for Veterinary Medicine. Sponsorship for this research was credited to JIFSAN.
- **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 specific objective of the first two years is to establish the cell immobilization mechanism.

Results:

A panel of six bioluminescent strains containing selected stress-responsive *E. coli* promoters fused to the *Photorhabdus luminescens luxCDABE* reporter was used. These fusions are: *recA-lux* (in the SOS regulon), *grpE-lux* (in the heat shock regulon), *katG-lux* (in the OxyR regulon), *inaA-lux* (internal acidification responsive and in the Sox and Mar regulon), *yciG-lux* (in the σ^S -dependent stress response regulon), and *o513-lux* (in the σ^S -independent, stationary phase inducible stimulon). Bioluminescence was detected by a luminometer. The values of zeta potential of *E. coli* mm28, DPD-2224, and 2234 at exponential/stationary phase were -41.11/-45.08, -30.58/-35.69, and -32.54/-31.73, respectively. Both DPD-2224 and 2234 retained negative surface charges, yet at absolute values less than that of their parental strain. No significant difference between the two growth periods was observed, nor was it at environmental pH of 6, 7 and 8. The MATH experiments showed minimum degrees of hydrophobicity in all three strains, indicating that a mechanism stronger than adsorption (van der Waal force or hydrogen bond) is needed, such as positively charged crosslinking agents.

Without any chemical treatment, viscose fiber was found to possess the highest capacity to immobilize bioluminescent *E. coli* DPD2234 followed by silk, cotton, and polyester fiber. Cell adhesion originated mostly from van der

Waal force and electrostatic interactions. With chemical modification (0 to 1 % PEI) to the fiber surfaces providing sites for covalent bonding, more immobilization of cells could be observed for all four selected fibers. The increase of cell adhesion was verified microscopically by SEM. Among all conditions studied, viscose fiber with 0.667% PEI pre-treatment immobilized the most viable luminous *E. coli* DPD2234, followed by viscose with 0.33% PEI, viscose with 1% PEI, and cotton with 1% PEI treatment. Study of immobilized cells for an extended period was conducted using 0.667% PEI-treated viscose fiber as anchoring matrix owing to its aforementioned high capacity. Throughout 72 hours, induced light responses could be constantly and rapidly detected. These results suggested extended sustainability, viability, and reproducibility of the current experimental set-up. The initial signal emitted by the bioluminescent *E. coli* upon contact with specific stresses remained almost unchanged regardless the concentration of the stress present. The signal increased linearly ($R^2 > 0.95$) with increasing contacting time in all cases studied. The slopes of each of the linear regression were found polynomial functions of stress concentrations. All models carried excellent reproducibility with coefficient of variation values less than 5.0% for three replicates per sample.

Potential Value and Applicability:

Characterization of bioluminescence enables quantification of stress fingerprints specific to the types of damage sustained by the cell, leading to development of whole-cell biosensors capable of detecting the presence and severity of toxic compounds in food. The significance of the project is two-fold. First, of the various reporter systems available, bacterial bioluminescence has the unique advantage that gene expression can be monitored in real time without cell lysis. The stress-responsive luminous bacteria are capable of fingerprinting the specific stresses by responding with an SOS (real time) light signal. Second, the integrated cell immobilization mechanism enables rapid assembly of a biosensor for quantitative analysis of the light signals, which would have been greatly hindered in a suspension cell system. Should the signal reproducibility and stability be confirmed, it is expected that the results of the proposed research could establish procedures for rapid incorporation of similarly constructed biosensing strains.

Presentations:

1. Lo, Y.M., M.H. Kothary, and J. Wang (2002), Quantitative whole cell biosensor. *Bioscience Research and Technology Review*, College Park, MD, Nov. 19.
2. Wang, J. and Y.M. Lo (2003), Enabling whole-cell biosensing: Quantification of stress-responsive bioluminescence from a five-gene *luxCDABE* reporter system. *IFT Annual Meeting*, Chicago, IL, Jul. 12-16.

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

The objective of this project was to develop optical sensing and imaging techniques to detect major visible and invisible contaminations and defects on read-to-eat fresh fruit and vegetables. This safety-oriented optical sensor research was intended to reduce microbial harborage and cross-contamination by detecting and removing potentially hazardous materials from the processing and packaging stream.

During this reporting period, the research focus was devoted to the recent outbreak of imported cantaloupes from Mexico, where U.S. banned the import of the fruit for three months because of disease-causing contaminations such as animal feces that harbored pathogens. To evaluate and simulate surface fecal contamination, fresh cow feces collected from the USDA dairy were diluted with H₂O to different concentrations by weight and applied to samples in a range of dilutions. Hyperspectral and fluorescence imaging techniques were used to detect the contaminations. In general, the hyperspectral imaging method can detect contaminated spots on fruit surface that are invisible to eyes, even with a dilution of 1:500. In this sense, the method has the potential to identify the previously contaminated spots after certain washing or non-thorough washing.

Specifically, it was found that that fluorescence images at 680 nm exhibited the greatest contrast between feces concentrations and volumes on cantaloupes. An alternative method to look for fecal contamination is to examine ratio images at two wavelengths. It was observed that fecal contamination is better distinguished by taking the ratio of fluorescence emissions at 660 nm and 550 nm. In addition, strawberry images showed the greatest contrast at 510 nm and 530 nm, for feces-contaminated regions and decayed tissue, respectively.

As indicated in the FDA Guidance for safe fruits and vegetables, this research aims to detect the potential sources of the contamination on fruit surface, where bacteria can be harbored.

Presentations:

1. Tao, Y., A.M.Vargas, M. Kim, A.M. Lefcourt, Y.R. Chen, Y. Luo, J. Kelly, D. Chan, R.L. Buchanan, Y. Song. 2003. Safety inspection of fruit and vegetables using optical sensing and imaging techniques. Annual Symposium of JIFSAN Food Safety Research. University of Maryland. October 2003.

2. Vargas, A.M., Y. Tao, M. Kim, A.M. Lefcourt, Y.R. Chen, J. Kelly. 2003. Safety inspection of fruit and vegetables using optical sensing and imaging techniques. Poster at Bioscience Day, University of Maryland, November, 2003

Publications:

2. Mehl, P.M., Chen, Y.R., Kim, M.S., and Chan. D. Development of hyperspectral imaging technique for the detection of apple surface defects and contaminations. *J. Food Engineering* 61(1):67-81. 2004.
 3. Chen, Y.R. and Kim, M.S. Visible/NIR imaging spectroscopy for assessing quality and safety of agro-foods. 11th International Conference on Near Infrared Spectroscopy, Proceedings of NIR-2003, In press, 2003.
- **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).

To test the effects of pesticide use on microflora, apple (Goldrush) trees were treated with one of three pesticide regimes during the season;

1. Conventionally-treated with insecticide, fungicide and bactericide,
2. Limited conventional spray treatment during early spring, and
3. No pesticides (used to simulate 'transitional organic' treatment).

In general, the results from leaves, stems and fruits followed a similar pattern during the initial sampling in summer 2002. In leaf and fruit samples, measurable differences in microflora were seen, depending on pesticide treatment. Trees receiving no pesticide typically had a log-greater microbial load than trees treated with pesticides. This was a statistically-significant difference measurable in both leaves and fruits, and was measured using a number of different selective media. In the shoot samples, microbial load showed the greatest variability between replicates. Differences in microflora isolated from shoot tissues did not appear to differ statistically, although that difference followed the same trend between pesticide treatments.

Based on the results of this preliminary work, we adjusted our sampling protocol for 2003. Only developing fruits were harvested, allowing an increase in the number of trees in the study. A second disease-resistant apple variety, Enterprise, was included.

The results of the 2003 field study validated the preliminary findings from samples taken in 2002. On three of four media tested, pesticide application significantly reduced the number of colony-forming units (CFU) measured per apple. This reduction in CFU was statistically significant in early-season tissue samples but became greater as the season progressed. By the end of the growing season, pesticide treatment had caused a half-log reduction in CFU on BHIA media, MaConkey media and on 3M film used for measuring total coliforms, when compared to unsprayed controls. It is not known whether this is the result of a long-term response to use of streptomycin in April and May to control the plant pathogen, fireblight, or if fungicide and insecticide applications have an indirect effect on epiphytes. One potential indirect effect could occur by reducing leaf and fruit damage, thereby reducing available carbohydrates on the surface of the fruit. This could suppress the growth of epiphytic bacteria.

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)

Campylobacter species, mainly *Campylobacter jejuni* and *Campylobacter coli*, are the most common cause of bacterial gastroenteritis in the United States and worldwide. *Campylobacter* enteritis is primarily a foodborne illness; poultry is the major source for human infections. Most *Campylobacter* infections need not be treated with antibiotics; however, fluoroquinolones or erythromycin has been commonly used to treat serious campylobacteriosis, and fluoroquinolones are also used as the empiric therapy for travelers' diarrhea.

Campylobacter resistant to fluoroquinolones and erythromycin were recognized during the late 1980s. Studies suggested that such resistance was due, in part, to acquisition of resistant strains from animal sources. Antimicrobials have been used to treat infections and to promote growth of food animals. Fluoroquinolones are used for disease treatment in poultry and cattle. Tylosin belonging to the same class (macrolide) as erythromycin can be used for growth promotion. The mechanisms of resistance to fluoroquinolones and erythromycin in *Campylobacter* are not fully elucidated. Point mutations in several codons of the *Campylobacter* gyrase subunit A are associated with high-level fluoroquinolone resistance; similarly, three major point mutations occurring in the peptidyl-encoding region in domain V of the 23S rRNA gene have been linked to erythromycin resistance. However, none of these mutations has been determined for their specific role in *Campylobacter* antimicrobial resistance. In addition, there is growing evidence that efflux pumps (membrane transport proteins) involved in the extrusion of toxic

substances may also play a role in fluoroquinolone and erythromycin resistance of *Campylobacter*.

This investigation will determine the specific role of each point mutation in the *Campylobacter* fluoroquinolone and erythromycin resistance, identify membrane transport proteins that are associated with fluoroquinolone and erythromycin resistance of *Campylobacter* using site-directed mutagenesis, and to identify potential novel genes involved in such resistance using transposon random mutagenesis.

The results from this study will advance our understanding of the emergence and evolution of antimicrobial resistance in *Campylobacter*, and provide useful information for developing strategies to curtail the trend of antimicrobial resistance in foodborne pathogens.

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

(Abstract) *Campylobacter jejuni* (*C. jejuni*) is one of the leading causes of bacterial gastroenteritis in the United States. Retail poultry products contaminated by *C. jejuni* have been recognized as a significant source of human gastrointestinal diseases. Studies have found as many as 40 to 70% of retail poultry products may be contaminated with *C. jejuni*. Campylobacteriosis in humans is usually a mild to moderate, self-limiting diarrheal disease, however, severe and prolonged cases of enteritis, bacteremia, septic arthritis, and other extra-intestinal infections may also occur. Some serotypes of *C. jejuni* have been strongly associated with Guillain-Barré syndrome. The development of new and effective preventive measurements and treatments of campylobacteriosis requires a better understanding of pathogenesis of *C. jejuni*. *C. jejuni* primarily infects the intestinal epithelium, particularly the colon. Epithelial cells form tight monolayers, providing the first-line protection against bacterial infection in the intestine. Epithelial cells interact with each other through tight junctions that control the paracellular permeability and separate the plasma membrane into two distinct surfaces, the apical and basolateral surfaces. *C. jejuni* initially adheres to the apical surface, and then invades and migrates across epithelial cells. The transmigration of *C. jejuni* across the epithelium induces inflammation and permits the organisms to disseminate throughout the host. The molecular mechanisms by which *C. jejuni* infects the intestine epithelium and induces diseases are largely unknown. The goal of this proposal is to examine the processes of *C. jejuni* adherence to, invasion into, and transmigration across the epithelial monolayer and analyze the cellular responses of epithelial cells induced by *C. jejuni* during infection. A series of experiments are proposed to compare the abilities of different isolates of *C. jejuni* to adhere, invade, and migrates across the polarized epithelial layer, to determine the effect of *C. jejuni* on the organization of the cytoskeleton and tight junction and the expression of the chemokine and the homing receptor for leukocytes. The proposed studies will

help us understand how *C. jejuni* elicits host epithelial cells to achieve adherence, invasion and transmigration, causing diseases and provide new strategies for the prevention and elimination of *C. jejuni* infection.

- **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)

(Abstract) The overall purpose of the proposed study is to guide the Food and Drug Administration (FDA) in preparing contingency plans for risk communication to the public in the event of a bioterrorist attack involving food.

Different food bioterrorism scenarios, played out through simulated news stories over a period of days, will be tested to evaluate the effects of selected message characteristics on public responses. Exploratory research will first identify relevant dimensions of news stories and public responses to the news stories as they unfold.

Items to be tested include useful repeated measures of perceptions of the magnitude and personal relevance of the threat (related to the likelihood of panic and fear), appropriate vs. inappropriate coping behavior (e.g., inappropriate generalization of the threat to other kinds of food that may not be directly implicated), attitudes about the adequacy and helpfulness of the news coverage, and attitudes about the adequacy of government response to the threat. By varying the amount and timing of released information, the stated source of information, the nature of public spokespersons, recommendations for action, and messages of reassurance and vigilance, within the context of the simulated food bioterrorism scenarios, different approaches to communicating with the public will be compared. The research design is guided by risk communication theories that explain media effects on protective behaviors. The theories emphasize source credibility, uncertainty, severity of threat and personal relevance of threat as independent variables, and perceived self and response efficacy as mediating variables.

The proximity to the University of Maryland and Washington, DC, offers access to participants for pre-test groups and experiments. The results of the study will provide insights into how the FDA can communicate to the public to avoid unnecessary panic or overreaction in the event of a bioterrorist attack.

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

(Abstract) This proposal describes the use of mechanistically based photophysical measurements for identifying phototoxic cosmetic ingredients. The proposed photophysical measurements will greatly enhance the on-going

photobiological research at the FDA. Model photosensitizers, representative of colors, fragrances, vitamins and phytochemicals found in cosmetics, will be examined. Initially, retinol (i.e. vitamin A) and its esters will be studied. Additional compounds will be selected for later studies in collaboration with the FDA. Prominent candidates for future studies include tattoo pigments as well as phytochemicals such as berberine, hypericin and furocoumarins. Photophysical characterization of sensitizers will include measurement of steady state and transient spectra as well as quantum yields for the formation of excited states and reactive intermediates such as singlet oxygen. Photophysical studies will include measurements made on cells (i.e. human skin fibroblasts and keratinocytes) pre-incubated with sensitizers. These measurements will be performed in close collaboration with the FDA, allowing comparison of a sensitizer's photophysical and photochemical properties with the photobiological properties.

APPENDIX B

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. These efforts may be divided into six major research areas; these are: 1) bioavailability and metabolic studies with purified carotenoids in rodents, 2) bioavailability and metabolic studies with purified carotenoids in humans, 3) metabolism of carotenoids in human ocular tissues and appropriate non-primate animal models, 4) qualitative and quantitative analysis of carotenoids in foods, 5) development of industrial processes for synthesis of dietary carotenoids and their metabolites, 6) development of industrial processes for isolation and purification of dietary carotenoids from natural sources.

Accomplishments and Potential Value/Applicability

In collaboration with Dr. Shirley Blakely (FDA, CFSAN) and several other investigators, a supplementation study was conducted with lutein (a major dietary carotenoid) in combination with vitamins C and E in female lean and obese Zucker rats (publication 4). The results suggest that ingestion of lutein reduces the activity of superoxide dismutase (SOD) in female Zucker rats and alters more biomarkers of oxidative stress when its intake is combined with vitamin C and vitamin E. Further, lutein alone, or in combination with vitamins C and E played a role in reducing hyperinsulinemia in the obese rats. These results suggest that ingestion of moderate amounts of lutein, vitamins C and E effectively reduces the obesity-induced biomarkers.

In collaboration with Dr. Martin Lipkin and Dr. Nitin Telang at the Strang Cancer Research Laboratory at the Rockefeller University, a supplementation study was conducted with C57BL/6J strain of mouse that exhibits low incidence of spontaneous breast and colon cancer (Publication 3). Administration of Western-style diet (high fat, low calcium and low vitamin D) results in accelerated growth of precancerous lesions in colon and breast of these mice. In this study, the above colon model was investigated as well as cell abnormalities in other organs such as breast, brain, and liver in mice fed a Western diet with and without a multicarotenoid mixture (MCM) of nine dietary carotenoids. This study revealed that MCM supplemented group had fewer total number of dysplastic crypts in comparison to the control group and carotenoids were bioavailable in all of the tissues examined. Therefore, C57BL/6J female mice appear to serve as an appropriate model for investigating the efficacy of individual or purified mixtures of dietary carotenoids on cellular abnormalities relevant to target organ carcinogenesis.

Future studies with this animal model should provide mechanistic and phenomenological leads to understand the efficacy of multicarotenoid supplementation in cancer chemoprevention.

In collaboration with Dr. Emily Chew (National Eye Institute) and her colleagues, a supplementation study with lutein (presentation 2) was conducted. The goal of this study was to evaluate the association of varying doses of orally supplemented lutein with the resulting plasma levels of this carotenoid in human subjects over age 60 with and without age-related macular degeneration (AMD). Fifteen patients with small drusen or no AMD, 15 with large drusen, and 15 with end-stage AMD (neovascular or geographic atrophy) involving the center of the macula, were equally randomized to receive one of three oral doses of lutein, 2.5 mg, 5 mg, and 10 mg for 6 months with extended post-supplementation follow-up for 6 months. Serum levels of lutein, cholesterol and triglycerides were measured at baseline, and 1, 3, 6, 9, and 12 months. At each of these visits, eye exams including best-corrected visual acuity measurement and dilated funduscopy were conducted. Serum lutein levels increased at 1 month and peaked by 3 months of supplementation. No toxicity was found with any dose of lutein after monitoring laboratory values and ongoing adverse event assessments. This study has concluded that a dose response relationship exists between oral lutein supplementation and serum lutein level and dosing up to 10 mg/day for a period of 6 months appears to be safe. This study provides critical information for designing a large-scale multicenter clinical trial that NEI plans to conduct in the near future to evaluate the efficacy of lutein supplementation in the prevention of AMD.

In another study, the qualitative and quantitative distribution of carotenoids and their metabolites were determined in ocular tissues of human and several non-primate animal models (frogs and quails) (publication 7). This study has revealed the presence of a diverse range of dietary carotenoids such as lutein, zeaxanthin, lycopene, α -carotene, and β -carotene as well as a metabolite of lutein (*meso*-zeaxanthin) in nearly all structures of the human eye (RPE-Choroid, ciliary body, iris, lens). This study also provides further evidence for the metabolic pathways of lutein and zeaxanthin in human ocular tissues and demonstrates that quail is an appropriate non-primate animal model for metabolic studies with ocular carotenoids.

In view of the important role of lutein and zeaxanthin in the prevention of AMD, it is imperative that the dietary levels of these carotenoids are accurately measured in foods. In a recent study, fruits, vegetables, wheat, and pasta products, commonly consumed in the US, were analyzed and the concentration of these carotenoids determined (publication 2).

An industrially viable process has been developed that converts commercially available (3R,3'R,6'R)-lutein to its metabolite, 3'-epilutein, and further transforms the latter to (3R,3'R)-zeaxanthin (publication 1). These carotenoids are not commercially available and this process allows investigators to conduct metabolic and clinical studies with these carotenoids. Similarly, α -cryptoxanthin and β -cryptoxanthin have been synthesized from lutein in two-steps.

Several patented processes for the isolation of lutein, zeaxanthin, α -carotene, and β -carotene from various natural sources have been developed. These carotenoids serve as important nutritional supplement.

Presentations

2. Khachik, F. Distribution, Metabolism, and the Role of Carotenoids in Disease Prevention. An International Ceres® Forum, Examining the Health Benefits of Lycopene from Tomatoes, Organized by Center for Food and Nutrition Policy at Virginia Tech, Washington DC, April 1-2, 2003.
3. Chew, E. Y.; Ferris, III, F.L.; deMonasterio, F. M.; Thompson, D. J.; Kim, J.; Csaky, C.G.; Woods, M.; Khachik, F.; Bone, R.; Landrum, J. Dose Ranging Study of Lutein Supplementation in Persons Over Age 60, Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, Florida, May 4-9, 2003.
4. Khachik, F. Distribution, Metabolism, and Physiological Functions of Lutein and Zeaxanthin in Disease Prevention. A Conference on the role of lutein and zeaxanthin in the prevention of macular degeneration, organized by Agencia Nacional de Vigilancia Sanitaria (ANVISA), Government of Brazil, Brasilia, Brazil, August 21, 2003.

Publications

1. Khachik, F. An Efficient Conversion of (3R,3'R,6'R)-Lutein to (3R,3'S,6'R)-Lutein (3'-Epilutein) and (3R,3'R)-Zeaxanthin. *J. Nat. Prod.*, 66, 67-72, 2003.
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3. Khachik, F., Telang, Fumio Enjo, F., Lipkin, M. Development of a Mixture of Dietary Carotenoids as Cancer Chemopreventive Agents: C57BL/6J Mice as a Useful Animal Model for Efficacy Studies with Carotenoids. In: *Phytochemicals: Mechanisms of Action*, Meskin, M. (Ed), CRC Press, Boca Raton, FL, Chapter 10, pp 161-174, 2003.
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6. Khachik, F., Shahidi, F. Dietary Carotenoids and Their Potential Cancer Chemoprotective Effect. Proceedings of the International Conference and Exhibition on Nutraceuticals and Functional Foods, Houston, Texas, USA, Sept. 14-17, 2000 (In Press).