



## **Annual Report 2004-2005**

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

**0220 Symons Hall  
University of Maryland  
College Park, Maryland 20742**

**David R. Lineback, Director**  
(prepared by Paul Mazzocchi, Associate Director)

## Index

	<u>Page</u>
Executive Summary	3
Background	6
Progress Report	9
Administrative Structure	9
JIFSAN Advisory Council	10
Research Initiatives	11
JIFSAN Internal Competitive Research Program	11
Collaborative/Cooperative Research Projects	12
JIFSAN Postdoctoral Research Associate Program	14
JIFSAN Senior Research Scientist	14
Leveraging	15
Education and Outreach Programs	15
JIFSAN Student Internship Program	15
Risk Analysis/JIFSAN Food Risk Analysis Clearinghouse	17
Acrylamide Infonet	17
JIFSAN Participation in Exhibitions	18
The JIFSAN newsletter, IMPACT	18
The JIFSAN International Training Center	18
Good Agricultural Practices Program	18
WTO/SPS Sanitary/Phytosanitary Leadership	
Development Program	19
The JohnsonDiversey International Training Initiative	19
Other International Cooperation and Training Opportunities	19
Other Activities	21
Establishment of the Center for Risk Communication Research	21
Future Plans (2005-2006)	23
Appendix A – Projects Funded through JIFSAN Competitive Internal Research Program	28
Appendix B - Collaborative/Cooperative Research Projects	48
Appendix C - JIFSAN Post-Doctoral Research Program	57
Appendix D – Research of Dr. Frederick Khachik	61

## **Executive Summary**

This Annual Report covers the eighth year of operation of the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) – the third year of the five-year renewal (September 2002 through September 2007). The non-competitive base funding for the eighth year was \$ 535,000 plus \$1,461,000 in carry-forward funds.

The JIFSAN Advisory Council met on November 18-19, 2004. The meeting involves updates from JIFSAN, CFSAN, and CVM and discussions with Council members concerning issues of current interest. A Research Grant Symposium featuring reports by Principle Investigators of projects funded through the JIFSAN Internal Competitive Grants Program was held on November 18. The meeting was well attended with representatives from the University, the FDA and the JIFSAN Advisory Council.

Although research has played an important role in JIFSAN's programs and has assisted in strengthening the knowledge base for public health policy, the JIFSAN Internal Competitive Grants Program is being phased out due to lack of funding. No new grants were made in this grant year and only four programs remain active. Due to the accumulation of a significant amount of carry-forward funding over a period of more than six years, it was possible for JIFSAN to fund a significant amount of short-term (one or two years) research. In collaboration with the Food Industry Alliance (an informal designation for food companies that have contributed funding for research) and the International Life Sciences Institute (ILSI) North America (NA), JIFSAN provided funding for five projects dealing with issues raised from finding acrylamide in foods in 2004. Those projects are complete or are nearing completion. Five additional projects were funded in areas addressing needs identified by CFSAN. One new project was funded to support the JIFSAN portion of a collaborative research effort being developed with the Department of Natural Resources and Environment, Victoria, Australia with whom JIFSAN has a cooperative agreement.

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

The Food Safety Risk Analysis Clearinghouse added several new sections (food allergy, bioterrorism, perchlorate) to the website and expanded others. A discussion forum was added to provide an open platform for addressing food safety issues. Several additions were made to the Clearinghouse's exclusive holdings, such as the Animal-derived Cosmetic Ingredient Database and the Food Handling Practices Model (FHPM). The Clearinghouse website received an average of 85,000 hits per month from over 120 different countries. Operation of the Acrylamide Infonet (the FAO/WHO Acrylamide in Food Research Network) continued. The Infonet has approximately 200 research projects listed and over 110 research publications.

The establishment of education and outreach programs, in areas within JIFSAN's responsibilities, is of vital importance. These programs are both domestic and

international in scope. JIFSAN was involved as sponsor or co-sponsor of a number of different meetings (workshops, conferences, etc.) this past year.

The Food Safety Risk Analysis Professional Development Program continued its development and offering of face-to-face courses. During the past year, efforts have been expanded with introduction of two courses that have been converted to distance delivery/learning technology. Conversion of a third course is being completed and a fourth will be done before funds from the grant supporting this effort are expended. The face-to-face courses were condensed and offered for a second time in a three-week Summer Integrated Program (SIP) in July 2005. This facilitates participation of individuals for whom attending individual face-to-face courses is not possible or inconvenient. The SIP was well received, attracting 84 participants from 7 countries. It will be offered again in July 2006. JIFSAN also developed what is called International Blended Learning in Food Safety Risk Analysis Program, a mix of face-to-face and on-line offerings that can be tailored to recipient needs.

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

As part of a cooperative agreement with the Central Science Laboratory (CSL) in the U.K. the Sixth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition was held at the University of Maryland on June 28-30, 2005. The topic was Bioactive Food Components. The University approved the formation of the JIFSAN International Training Center as a site for all JIFSAN international training programs. These include the World Trade Organization Sanitary/Phytosanitary (WTO SPS) Leadership Development Program, the JohnsonDiversey International Training Initiative which was funded this year, the Good Aquacultural Practices program presently being developed, the GAPs program and the International Blended Learning In Food Safety Risk Analysis Program. JIFSAN signed an agreement with the Korea Food and Drug Administration for risk analysis training and scientist exchange and is negotiating with the Thai Ministry of Science and Technology for a similar arrangement. JIFSAN is investigating the development of an international training program in low acid canned foods.

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

The JIFSAN Student Internship Program continues to increase in importance and participation. UM undergraduate students are offered an opportunity to work with FDA scientists in their laboratories on specific projects identified by the FDA scientist involved. During 2004-2005, 47 different University of Maryland students participated for a total of 104 semesters in FDA laboratories. UM Students were co-authors on 17 posters presented at the FDA Science Forum

## **Background**

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

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) is a multidisciplinary research, education and outreach program. Dr. David R. Lineback (University of Maryland) was appointed the Director until his retirement on September 30, 2005. Currently, Dr. Maureen L. Storey serves as Interim Director, JIFSAN; Dr. Paul Mazzocchi (University of Maryland) is Associate Director, and Dr. Elizabeth Calvey (CFSAN, FDA) is the Acting 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 and food safety, food safety risk analysis, and economics.

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

Risk analysis (risk assessment, management, and communication) is one focus of JIFSAN programs. This effort promotes the development of risk-based, scientifically supportable safety standards. These standards can deliver the intended degree of measurable public health protection and can be used to identify priorities to effectively

apply available resources. JIFSAN is developing new approaches to information management related to risk analysis through operation of a web-based Food Safety Risk Analysis Clearinghouse. This provides a mechanism to collect and disseminate available data and methodologies from government, academia, and industry. The intent of the Clearinghouse is to provide a centralized information source in areas of risk analysis related to food safety. The unique feature of this clearinghouse model resides in the examination and documentation of state-of-the-art methods, data sources, and current results of on-going risk assessments so that a more complete and up-to-date picture of risk assessment is assembled.

An internal competitive research grant program provided 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. This program is being discontinued due to lack of funds.

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

Trade initiatives have put food safety high on the international agenda. JIFSAN is actively involved in developing collaborations with international organizations to facilitate cooperative research and education programs and the exchange of scientists.

The MOU established a set of relationships that closely link the University with CFSAN and CVM by committing to the sharing of facilities, personnel, and intellectual resources when appropriate. Thus, FDA personnel have access to University facilities such as libraries and may be appointed as adjunct 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. He retired on September 30, 2005. Dr. Maureen Storey was appointed Interim Director on October 1, 2005. A search for Director will commence when a new 5-year cooperative agreement with FDA is completed.



## **Progress Report**

During the eighth 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 eighth year was \$535,000 plus \$1,461,000 in carry-forward funds.

### **Administrative Structure**

A unique administrative structure was established for JIFSAN in planning, organizing, and implementing multidisciplinary, multi-institutional programs in research, education, and outreach. 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:

- Dr. David Lineback retired as Director on September 30, 2005.
- Dr. Maureen Storey was appointed Deputy Director on August 1 and will become Interim Director of JIFSAN on October 1, 2005.
- Mr. Kenneth Hunter joined JIFSAN on a part-time basis as International Training Coordinator. He will be involved in coordinating new international training programs funded from external sources.
- A Research Symposium highlighting the projects funded through the JIFSAN Internal Competitive Grants program was held November 18, 2004. The program involved Principle Investigators giving brief reports of the status of their research investigations. Several members of the Advisory Council attended the Symposium. This has become a standard preliminary event to the annual Advisory Council meeting.
- The fourth meeting of the JIFSAN Advisory Council was held November 18-19, 2004. The Advisory Council is composed of representatives from 14 private sector corporations, five academic institutions, two from the consumer community, and one from a federal (UK) laboratory.
- A Steering Committee continues to oversee and guide activities of the Food Safety Risk Analysis Clearinghouse

## The JIFSAN Advisory Council

Central to the operation of JIFSAN is an Advisory Council composed of members from private sector business, government agencies, academia, and representatives of consumers' interests. This group provides guidance to JIFSAN in developing research, education, and outreach programs to address problems in food safety, nutrition, animal health sciences, and risk analysis.

The Advisory Council met on November 18-19, 2004. These meetings involve updates from JIFSAN, CFSAN and CVM and brief presentations on selected research and education/outreach programs. Also, discussions are held concerning potential industry needs that could influence future programs of JIFSAN.

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

Members of the Advisory Council include:

- Private sector industry

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

- Representatives of Consumers' Interests

- Ms. Mary Heersink (Safe Tables Our Priority)
- Ms. Linda Golodner (National Consumers League)

- Academia

- Dr. Michael Doyle (University of Georgia)
- Dr. Julie Miller Jones (College of St. Catherines)
- Dr. Sanford Miller (Center for Food Nutrition and Agriculture Policy,

University of Maryland)  
Dr. Michael Pariza (University of Wisconsin)  
Dr. Stephen Taylor (University of Nebraska)

- Government

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

- Individuals

Dr. David Lineback (Director-Retired, JIFSAN)  
Dr. Gilbert Leveille

## **Research Initiatives**

Research has been a major focus of JIFSAN, however, reduced funding has led to the elimination of the research program. Collaborative research has supported the goal to develop a strong science base to address ongoing and increasingly complex public food safety issues.

### **JIFSAN Internal Competitive Research Program:**

First initiated in 1998, each project required collaboration between at least one University of Maryland faculty member as Principal Investigator (PI) and one or more FDA collaborators. The latter help provide additional scientific expertise and insight into public health impacts of the research. Additional collaborators may be from other institutions, if the PI so desires. These projects contribute to the science for current and future regulatory issues and activities that impact public health.

Effective July 2002, projects were funded at \$30,000 per year to be used for partial support of either a graduate research assistant or a postdoctoral associate and some operational support. Proposals were for as many as three years, but were funded for only one year at a time with continuation 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, four projects in July 2003 were continued for a third year. One project funded in July 2004 was continued for a second year. Unfortunately the JIFSAN Internal Competitive Research Program is being discontinued due to lack of funding. Only four projects remain.

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

### **Third-year projects (funded July 2002, end July 2005):**

- 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).

### **Third-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)

### **Second-year project (funded July 2004):**

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

### **Collaborative/Cooperative Research Projects:**

An important function of JIFSAN is establishing research efforts involving collaboration/cooperation with other organizations addressing current issues of mutual interest. The extent of this effort is dependent upon the availability of funding. The availability of carry-forward funding has enabled JIFSAN to participate in developing several projects. Most of these projects are of one-year's duration and some have involved additional funding from external sources. Research projects have been funded

to address issues involved with the finding of acrylamide in foods, to initiate collaborative research with a partner in Victoria, Australia, and to address issues of specific interest to CFSAN. Those continuing in the period covered by this report include the following, which are described in more detail in Appendix B.

1. In collaboration with the Industry Acrylamide Alliance, an informal designation for food industries that have donated financial support for research, JIFSAN funded the following research projects. Financial support from JIFSAN involved the use of carry-forward funds.
  - Effects of consumer food preparation on acrylamide formation, George Sadler/Lauren Jackson, Illinois Institute of Technology (IIT)/National Center for Food Safety and Technology (NCFST)
  - Acrylamide content of home-prepared surface-browned foods, George Sadler/Lauren Jackson, IIT/NCFST (a requested expansion of the project listed above)
2. As the JIFSAN portion of a cooperative research program being developed with the Department of Natural Resources and Environment, Melbourne, Victoria, Australia with whom JIFSAN has a Memorandum of Cooperation, the following three-year research project is in progress.
  - Rapid assay for detecting human enteric viruses and viral survival dynamics on fresh fruits and vegetables, Jianghong Meng, Department of Nutrition and Food Science, University of Maryland.

#### **Additional Research Projects Funded**

These projects are funded from JIFSAN carry-forward funds and address issues of specific interest to CFSAN/FDA.

- Analysis of sera from previous Norwalk-like virus human exposure study, Mark Sobsey, Department of Environmental Sciences and Engineering, University of North Carolina.
- Development of molecularly-imprinted polymers (MIPs) for selective detection of marine biotoxins, Kenneth J. Shea, Department of Chemistry, University of California, Irvine.
- Enzymatic Degradation of Prion Surrogate Proteins, Jason C. H. Shih, Department of Poultry Science, North Carolina State University.

- Analysis of data collected in epidemiologic and microbiologic field studies of domestic and imported produce, Christine Moe, Department of International Health, Emory University.
- Public perceptions of conflicting information about safety guidelines for consumption of fish, Linda Aldoory, Department of Communications (CRCR), University of Maryland. Collaborator: Marjorie Davidson, CFSAN, FDA (funded with JIFSAN DRIF)

**JIFSAN Postdoctoral Research Associate Program:**

This program strengthened the science base for public health policy by providing short-term research scientists to work in FDA laboratories. Originally, six postdoctoral research associates were recruited to work in FDA laboratories in areas in which significant knowledge gaps or the lack of appropriate scientific data, methods, or models exist.

Not only did this program generate significant amounts of new knowledge applicable to needs of the FDA, it allowed 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.

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

- Aquaculture drugs: LC/MS protocols for marker determination, Robert Dickey (FDA), Ann Abraham (JIFSAN Research Associate)
- Molecular phylogenetic identification of potential foodborne agents of bio-terrorism, Eric Brown (FDA), Alice Heyford (JIFSAN Research Associate)
- Development of a specific monoclonal antibody for *Enterobacter sakazakii*: Identification and an immunoassay using color-coded bio-nanotubes, Kun-Ho Seo (FDA), Sang-Bok Lee (UM), Kwang-Young Song (JIFSAN Research Associate)

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

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

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

## **Leveraging:**

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

### **Development of Core Facilities:**

The development of core facilities that will benefit FDA and University scientists and their collaborators is a cornerstone of JIFSAN's cooperative programs and objective to leverage resources. The effective use of the arrangements for shared facilities (electron microscopy and nuclear magnetic resonance) is increasing since CFSAN has relocated to its facilities in College Park.

## **Education and Outreach Programs**

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

### **JIFSAN Student Internship Program:**

The JIFSAN Student Internship program is designed to provide University of Maryland undergraduate students with an opportunity to collaborate with FDA scientists on specific projects related to the JIFSAN mission. This program was implemented as part of the agreement between the University and FDA to cooperate in educational efforts. These opportunities for students enhance their knowledge of and experience in science, particularly in a regulatory environment, and familiarize them with career opportunities in the regulatory sector of public service.

Students' participation in the program requires that they have completed two college semesters, be willing to commit to volunteering 100 hours during the first term as an intern, and submit a completed application form, current transcript and resume. The positions may be part-time during the semester and full-time during the summer. Undergraduate students volunteer to work on a project, listed by an FDA staff member. Upon successful completion of the initial 100-hour period, students can be converted to paid internships. During all phases of the internship, the students have a variety of ways to obtain academic credit for their internship experience.

Forty-seven different University of Maryland students participated as interns for a total of 104 semesters in FDA laboratories and supporting offices during 2004-2005. Efforts are

being made to extend opportunities for internships beyond laboratory experiences. Seven new Food Defense projects were initiated in June 2005 as a special program with projects of one-year's duration. UM students were co-authors on 17 posters presented at the FDA Science Forum

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

### **Food Safety Risk Analysis Clearinghouse**

The Food Safety Risk Analysis Clearinghouse was 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.

Development of the Risk Analysis Clearinghouse continued under the guidance of the recently appointed Steering Committee. The latter was organized due to funding constraints and an unsuccessful search for a Coordinator. A major emphasis is being placed on acquisition of data and material beyond those sought initially from microbiological risk assessments. Modernization and improvement of the Clearinghouse continues.

Progress for the Food Safety Risk Analysis Clearinghouse includes:

- Information structure updates include division of information heavy pages into easy-to-navigate smaller subsections.
- A shorter and easy to remember domain address, <http://www.FoodRisk.org>, has been acquired.
- Several new sections have been added to the Clearinghouse to keep pace with recent trends, such as sections on food allergy, bioterrorism, and perchlorate. In addition, some of the existing sections have been significantly expanded, such as risk profiles, weight management, and acrylamide.
- Digitization of 1993 Federal Register, Volume 58, Part IV is near completion. This volume, which is not available on the U.S. GPO web site, provides information on nutrition and labeling requirements.
- Development of a discussion forum with the aim of providing an open platform to discuss food safety-related issues. This was initiated with a QRA-Analytica listserv to discuss quantitative risk assessment issues related with the use of Analytica software.



- Several additions to its exclusive holdings.
- The Clearinghouse continues to host the Acrylamide Infonet

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

Issues related to the Clearinghouse include:

- Funding- there is no funding in the JIFSAN Cooperative agreement beyond September 2005. Efforts are being made to obtain replacement funding.

### **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](http://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](http://www.acrylamide-food.org) which contains a database of researchers/data providers; references for research published elsewhere; studies in development database; listing of acrylamide websites; acrylamide documentation (general information); events and activities; Infonet updates; and call for data (a call by WHO for analytical data for use in the 2005 JECFA risk assessment of acrylamide in food and subsequent risk assessments).

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.

Coordination is occurring with the European Food Standards Agency (EFSA Parma, Italy) 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. Approximately 200 projects have been entered.

## **JIFSAN Participation in Exhibitions**

JIFSAN has featured a booth (display) at the:

- Society for Risk Analysis Annual Meeting, Palm Springs, CA, December 5-8, 2004
- 7<sup>th</sup> Annual Food Safety Summit, Washington, D.C., March 17-18, 2005
- 11<sup>th</sup> Annual FDA Science Forum, Washington, D.C., April 27-28, 2005
- IAFP Annual Meeting, Baltimore, MD, August 14-17, 2005.

The Risk Analysis Clearinghouse participated in these displays.

## **JIFSAN Newsletter**

The first issue of the JIFSAN newsletter IMPACT occurred in February, 2005. The July issue can be viewed at <http://www.jifsan.umd.edu/>

## **JIFSAN International Training Center**

- JIFSAN has received approval from the University to formally establish the JIFSAN International Training Center. The Center combines all of the JIFSAN training programs in one site and provides visibility and some operational efficiencies.

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

- Good Agricultural Practices (GAPs) Train-the Trainer International Training Programs were offered in Korea, Nov. 8 - 12, 2004; Thailand, March 21-25, 2005; Brazil; September 13, 2005 and Mexico, September 19-23, 2005. Approximately 50 individuals attended each session. JIFSAN is making an effort to have the host countries share more of the cost of these training sessions so that we can continue to offer training with our reduced funding.
- The instructional team for the training programs is composed of faculty/staff from Clemson University, Mississippi State University, University of Maryland, Cornell University, and the FDA. A core group of instructors are used for each of the training programs that are offered in English or Spanish with text materials in either language.
- A comprehensive review of the GAPs Training Program was conducted by an external Review Team consisting of three experienced individuals. The review included site visits to Mayaguez, Puerto Rico (May 2004); Port of Spain, Trinidad (May 2004); Petrolina, Brazil (August 2004), and Puebla, Mexico (August 2004).

Additionally, one of the instructors from Clemson University participated in each of the site visits. A final report was presented and is attached as Appendix E. The report made several recommendations, but was generally quite favorable. Based on some of the recommendations of that report JIFSAN has contracted to have a minor revision of the GAPs manual.

### **World Trade Organization Sanitary/Phytosanitary (WTO SPS) Leadership Development Program**

- JIFSAN was instrumental in offering the World Trade Organization Sanitary/Phytosanitary (WTO SPS) Leadership Development Program for the People's Republic of China in cooperation with the UM's Institute for Global Chinese Affairs (IGCA). JIFSAN linked the program with the IGCA, which handled logistics. The Foreign Agricultural Service (FAS) of the USDA organized the program with assistance from the FDA, USTR, FSIS, APHIS, GIPSA, and EPA. From 6 September through 20 November, 2004, 15 Chinese officials were engaged in training at UM with site visits across the country. Funding was provided through the American Soybean Association. JIFSAN has been awarded \$295,000 to offer the second World Trade Organization Sanitary/Phytosanitary (WTO SPS) Leadership Development Program for 2006.

### **The JohnsonDiversey International Training Initiative**

- JIFSAN was successful in finalizing the JohnsonDiversey International Training Initiative to conduct additional international training programs directed towards specific commodities such as poultry, seafood, and meats in identified countries and regions. JohnsonDiversey (JD) has made a three year commitment funded at \$600,000.
- The overall objective of the Initiative is to develop and training programs to appropriate international officials and representatives on food safety issues, thereby enabling them to develop more effective strategies and policies to promote food safety. The first JD training program will be on seafood food safety with a focus on shrimp aquaculture. Thailand will be the initial site. A group of faculty (UM and VPI) and an FDA representative are presently developing a manual and teaching tools.

### **Other International Training Opportunities**

- JIFSAN signed an MOU with the Korea Food and Drug Administration (KFDA) in August 2005. Expectations are that JIFSAN will participate by delivering training programs and facilitating scientist exchanges with KFDA.

- JIFSAN is negotiating an MOU with the Ministry of Science and Technology (MOST) of Thailand to offer risk analysis training in Thailand. MOST is working to obtain funding for this project

### **Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Bioactive Substances**

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 Sixth Joint CSL/JIFSAN Symposium on Food Safety and Nutrition: Bioactive Food Components was held June 28-30, 2005 at the Inn and Conference Center, University of Maryland University College. Speakers from Europe and the U.S. were involved. Material presented at the Symposium is posted on the JIFSAN website ([www.jifsan.umd.edu](http://www.jifsan.umd.edu)).

### **Food Safety Risk Analysis Professional Development Training Program**

The Professional Development Training Program in Food Safety Risk Analysis provides training in the key components of risk analysis. The program is developed and taught by FDA staff, UM faculty, and private consultants. Courses in the program are available in face-to-face (scheduled on demand and a summer integrated program) and distance delivery formats. Two face-to-face offerings of individual were scheduled during the past year. Individual courses will be offered only on demand in the future. The second offering of the summer integrated program, offered on July 19- August 6, 2005, placed courses back-to back in a three-week format. This was successfully offered for the first time last year and is developed for individuals who wish to take multiple courses at one time. It is particularly advantageous to international participants. Three courses are currently available and being scheduled in distance delivery format and a fourth one will be piloted in early fall. JIFSAN now offers the International Blended Learning in Food Safety Risk Analysis program. This is a mix of face-to-face and on-line courses that can be customized for individual foreign audiences

### **Global Good Agricultural Practices Research and Extension Conference**

In cooperation with Cornell University, JIFSAN assisted in the organization and co-sponsored a “Global Good Agricultural Practices Research and Extension Conference, in Orlando, FL, January 11-12, 2005. The conference involved more than 100 participants, domestic and international. The conference focused on new research findings in all aspects of good agricultural practices such as bacteria, viruses, parasites, water, manure, biosolids, animals, workers health and hygiene, field sanitation, transportation, and packing house sanitation. The objectives of the conference were to (a) convene a diverse international group of key industry,

academic and government leaders to network, share information and convey recommendations to their constituencies, (b) share current scientific and educational information as well as industry practices that can further enhance the microbiological safety of fresh fruits and vegetables, (c) review and evaluate current scientific and educational information to determine data gaps and identify areas for future research activities, and (d) develop new GAPs recommendations based on up-to-date research results that will help to focus future extension programs. Proceedings of the conference are to be published and presenters will be encouraged to submit their papers to peer-reviewed food safety journals.

Financial support was provided to Cornell University to assist in distribution of a farm worker video/DVD in English and Spanish to subscribers of Growers magazine. This was done as an adjunct to the GAPs and GMPs International Training Program. JIFSAN will use this video/DVD in its training programs.

### **Second Annual Symposium on World Hunger**

JIFSAN co-sponsored the Second Annual Symposium on World Hunger at the University of Maryland on December 2, 2004. The symposium highlighted the role of the land-grant system of universities in dealing with the issues of world hunger. Leaders from governmental and non-governmental organizations and academia addressed those topics. Approximately 100 participants were involved.

### **Seminar on the Role of Carbohydrates in Human Health and Disease: Evaluating Scientific Evidence for Dietary Guidance**

In cooperation with the International Life Sciences Institute (ILSI) Southeast Asia Region and the Nutrition Society of Malaysia, JIFSAN co-sponsored and assisted in the organization of a “Seminar on the Role of Carbohydrates in Human Health and Disease: Evaluating Scientific Evidence for Dietary Guidance.” The seminar was held July 26-27, 2005 in Kuala Lumpur, Malaysia. With the current discussions and debates on nutrition and diets, including the role of macronutrients such as carbohydrates, and changing eating patterns of populations, this symposium recognizes the continuing critical need for dietary guidance and advice for individuals. The meeting involved nutrition scientists, academics, health care providers, and regulators needing to evaluate the latest science of carbohydrates to formulate dietary guidance that will promote health, prevent disease and be culturally appropriate and economically viable. Proceedings from the seminar will be published.

### **Other Activities**

#### **Establishment of a Center for Risk Communication Research**

JIFSAN furnished “seed money” to initiate establishment of a Center for Risk Communication Research (CRCR) led by the Department of Communication,

University of Maryland. JIFSAN has pledged support for three years. The Center's mission is to advance understanding about how communication helps control risk, about how the public perceives risk communication, and about the political, economic and social contexts for risk communication. The Center's goals include helping to establish public and scholarly agendas for risk communication research, collaborating with other institutions and individuals to secure funding for research projects, and providing support for research and fellowships.

On October 29, 2004, the Center had its first annual meeting at the University of Maryland's Inn & Conference Center. During the meeting, the National Advisory Board discussed research trends regarding food safety, security, environment, and health and created a research agenda for the Center. Specifically, the board suggested moving towards developing a model for industry and government to use when needing to communicate risk to the public. The report of the Annual meeting can be found on the Center website. (<http://www.comm.riskcenter.umd.edu>) The second meeting of its Advisory Committee is scheduled for December 2, 2005. Members include a number of nationally recognized communicators. The Center will participate in the Center of Excellence for Behavioral and Social Research on Terrorism and Counter-Terrorism, recently awarded to the University of Maryland by the Department of Homeland Security.

## Future Plans (2005-2006)

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

### 1. Administrative

- David Lineback will retire as Director on September 30, 2005. Maureen Storey will be appointed as Interim Director as of October 1, 2005.
- JIFSAN reports to the VP for Research at UM but is administratively located in an academic college, presently the College of Chemical and Life Sciences. As of October 1, 2005 it will administratively report to the Dean of the College of Agriculture and Natural Resources.
- A Research Grant Symposium featuring reports by Principle Investigators of projects funded through the JIFSAN Internal Competitive Grants Program is scheduled for October 27, 2005. Since only five such projects remain, Principle Investigators from some of the Collaborative/Cooperative Research Projects may be included.
- A meeting of the JIFSAN Advisory Committee will be held on October 27-28, 2005. Advisory Committee members will participate in a comprehensive strategic planning exercise.
- Development of the Center for Risk Communications Research (CRCR) will continue.
- The JIFSAN newsletter, IMPACT, will continue to be published electronically.
- JIFSAN plans to participate with its display at the Society for Risk Analysis Annual Meeting, Orlando, FL, December 4-7, 2005; the 12<sup>th</sup> Annual FDA Science Forum, Washington, D.C.; and the IAFP Annual Meeting, Calgary, Alberta, Canada, August 13-16, 2006. The Risk Analysis Clearinghouse will participate in these displays.

### 2. Research Initiatives

- Four projects funded in July 2003 will be continued for their third and final year and the project funded in July 2004 will be continued for its second year, provided sufficient progress is made.
- No new proposals will be funded. The JIFSAN Internal Competitive Grants Program will be discontinued upon completion of the projects currently funded.
- The Collaborative/Cooperative research projects will be monitored and completed.

- Funding will be continued for the research project “Rapid assay for detecting human enteric viruses and viral survival dynamics on fresh fruits and vegetables,” J. Meng, Principal Investigator being done in collaboration with DNRE, Victoria, Australia.
- Funding will continue for the three postdoctoral research associates completing their programs, the last of which will be completed in April 2006. The JIFSAN Postdoctoral Research Associate Program then will be discontinued.

### **3. Education and Outreach Efforts**

- Operation of the Risk Analysis Clearinghouse will be further developed under the guidance of the recently appointed Steering Committee. A major emphasis will be acquisition of data beyond those sought from microbiological risk assessments. Operation of the Acrylamide Infonet, including acquisition of data on the occurrence of acrylamide in foods in countries around the world for later use in risk assessments, will continue. A critical issue for the Clearinghouse is funding. There will be no Clearinghouse support available from the cooperative agreement in the coming year.
- Operation of the JIFSAN Internship Program will continue.
- Train-the-trainer programs in minimizing food safety hazards in production of fresh fruits and vegetables (GAPs Training Programs) will be offered in Ecuador (late 2005), El Salvador/Honduras (2006), and possibly Morocco (2006).

An advanced GAPs course is under development and will be offered in 2006 in Mexico, El Salvador/Honduras and possibly Morocco. These will involve approximately 50 individuals at each location.

The final report from the comprehensive review of the GAPs International Training Program has been received. Based on some of its recommendations the GAPs manual is being revised.

- JIFSAN will initiate the first JohnsonDiversey Training Initiative training program in seafood aquaculture in Thailand. A consultant group is presently developing a training manual and materials.
- The 2005-2006 schedule of courses in the Risk Analysis Professional Development Program has been developed and appears at: <http://www.jifsan.umd.edu/pd2006/schedule.cfm>. This will include the Summer Integrated Program and offerings of the courses available in distance delivery format. Offerings of individual face-to-face courses will occur only if the demand warrants. A special effort was made to reduce operating costs to



put this program on a pay-as-you-go basis. Accordingly the SIP will be offered in a commercial location in Washington DC at lower cost.

Conversion of the Food Safety Risk Analysis Courses into on-line distance learning versions has continued through a no-cost extension of a USDA grant obtained by the College of Agriculture and Natural Resources. The Risk Communications on-line course was piloted in late February 2005. The College of Agriculture and Natural Resources is now committed to developing an on-line masters program in risk analysis and will utilize much of the material developed in the professional development program in this effort.

Risk analysis training is now also offered through the International Blended Learning In Food Safety Risk Analysis program. This is a program that comprises a mix of face- to- face and on-line courses that can be customized to fit recipient needs.

- Upon the request of CFSAN, JIFSAN will continue to investigate the feasibility of organizing and providing an international training program in the area of low-acid canned foods over a five-year period. This will only be initiated if adequate funding beyond the Cooperative Agreement is available.
- JIFSAN was instrumental in offering the World Trade Organization Sanitary/Phytosanitary (WTO SPS) Leadership Development Program for the People's Republic of China in 2005 on behalf of the Foreign Agricultural Service (FAS) of the USDA with assistance from the FDA, USTR, FSIS, APHIS, GIPSA, and EPA. Funding was provided through the American Soybean Association. JIFSAN has been awarded \$295,000 from the FAS through the U.S. Wheat Associates to offer the second World Trade Organization Sanitary/Phytosanitary (WTO SPS) Leadership Development Program in 2006.
- JIFSAN was successful in finalizing the JohnsonDiversey International Training Initiative to conduct additional international training programs directed towards specific commodities such as poultry, seafood, and meats in selected countries and regions. The first JD training program will be on seafood food safety with a focus on shrimp aquaculture with Thailand as the site of the initial offering and late spring 2006 as the target. A group of faculty (UM and VPI) and an FDA representative are presently developing a manual and teaching tools.
- JIFSAN signed an MOU with the Korea Food and Drug Administration (KFDA) in August 2005. Expectations are that JIFSAN will participate by delivering training programs and facilitating scientist exchanges with KFDA.

- JIFSAN is developing an MOU with the Ministry of Science and Technology (MOST) of Thailand to offer risk analysis training in Thailand. MOST is working to obtain funding for this project
- JIFSAN has been invited by FAS to develop and deliver a comprehensive Good Agricultural Practices program to Afghani farmers and processors. The program that is being developed will have components in the US and Afghanistan.
- Support and development of the Center for Risk Communication Research will continue in collaboration with the Department of Communications (UM).
- One of the best means for obtaining visibility for JIFSAN and its education/outreach programs is through co-sponsorship of symposia, workshops, conferences and other types of meetings. This will continue in areas appropriate to JIFSAN's responsibilities. The extent will depend upon the availability of funding.
- JIFSAN will co-sponsor with the Coca-Cola Co. a Short Course for the Food Sector: Foodborne and Waterborne Diseases in Kiev, Ukraine the first week of October, 2005.
- JIFSAN will be a co-sponsor of an international Network of Excellence and the U.S. Food Safety Research Consortium. The conference is entitled "Priority Setting of Foodborne and Zoonotic Pathogens" and will be held at the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung), Berlin, Germany, July 19-21, 2006.
- JIFSAN will co-sponsor A Safe Meat Supply - From Farm To Table U.S.A. / Russian Federation Scientific Conference to Share Knowledge and Information Concerning the Safety of Animal Products (Moscow, Fall 2006)

#### **4. International Collaboration**

- Planning is in progress for the Seventh Joint CSL/JIFSAN Symposium on Food Safety and Nutrition, to be held at the Central Science laboratory, York, UK, June 6-8, 2006. The topic will be Quality Assurance in Food Safety - Networking of Laboratories.
- Development of a cooperative research effort between JIFSAN and the Department of Natural Resources and Environment (DNRE), State of Victoria, Australia was initiated based on a JIFSAN/DNRE memorandum of understanding for development of collaborative efforts in research. The research effort is in the detection and measurement of microbial pathogens on fresh-cut produce and involves the development of rapid methods of

detection/analysis, with an emphasis on viruses, by Dr. Jianghong Meng, Department of Nutrition and Food Science, College of Agriculture and Natural Resources, UM. This project was funded and initiated in 2004. Determination of microbial pathogens in/on freshly harvested produce will occur as part of the project to be funded in Victoria. Some analyses may be done with Maryland produce.

- JIFSAN will renew its designation as JIFSAN-PAHO/WHO Collaborating Center for Food Safety Risk Analysis
- JIFSAN will continue to develop its education/outreach efforts with Korea, Thailand, Afghanistan, and potentially other selected countries.

## APPENDIX A

### Projects Funded Through JIFSAN Competitive Internal Research Program

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

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

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

available, the mass spectral analysis of the tryptic peptides can identify the labeled proteins.

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

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

Background and nature of the work: There is a vital need to expand the repertoire of therapeutic drugs for veterinary use in aquaculture. However, very few drugs are currently approved by the FDA for use in aquaculture species, compared with traditional farm species. In order to facilitate the drug approval process for cultured fish species, it is desirable to establish species 'crop groupings' based on similar drug enzymatic metabolic profiles between species. The metabolic profiles are related to different species' drug excretion rates and tissue residues; these factors determine the appropriateness of the use of certain therapeutic drugs in aquacultured species destined for human consumption. This study is investigating multiple aquacultured fish species to investigate drug metabolism *in vitro* and establish relationships between

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

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

#### **Presentations:**

- Kinetics of phase I-II biotransformation kinetics in 8 species of aquacultured finfish, J. F. González, R. Reimschuessel, B. Shaikh and A. S. Kane, Society of Environmental Toxicology and Chemistry (SETAC) International Meeting, November, 2004 (accepted).
  - Kinetics of hepatic albendazole sulfoxidation in channel catfish, tilapia and rainbow trout, J. F. González, R. Reimschuessel, B. Shaikh and A. S. Kane, Society of Environmental Toxicology and Chemistry (SETAC) International Meeting, November, 2004 (accepted).
- **Moving whole-cell biosensing from a qualitative to quantitative tool: development of a dynamic cell immobilization mechanism.** Y. Martin Lo (UM) and Mahendra Kothary (FDA)

Objectives: The goal of this study is to enable quantitative whole-cell biosensing by developing a novel dynamic system for the immobilization of

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

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

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

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

Publications

Lo, Y.M., J. Wang, and G. Lala. 2006. Bioluminescence sensors in food processing. In: Encyclopedia of Agricultural, Food, and Biological Engineering. D.R. Heldman (ed.), Taylor & Frances, New York.

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

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

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



In addition, the data set was subjected to principal component analysis (PCA). This algorithm transforms the original data set into a set of new un-correlated linear combinations of the original variables, reducing the redundancy within the data by creating a new series of images. Results indicate that fluorescence images at 675 nm exhibited the greatest contrast between treated and untreated surfaces. Detection rates were improved using ratio images; in particular, higher detection rates were obtained for all volumes and concentrations using the 695/595, 675/555 and 555/665 nm image. Unsupervised classification images were more effective in allowing removal of unwanted areas, and isolating treated areas. PCA showed that the first six principal component images exhibited useful results for contamination detection. PC-2 and PC-5 displayed best contrast for contamination detection. False alarms presented a persistent problem when trying to identify fecal contamination, however, PC-5 provided contrast between them, creating ideal conditions for masking. Based on these findings, a systematic procedure will be developed for contamination detection. Simultaneously, strawberries' images will be subjected to similar algorithms. Further validations and extensive testing with statistics will be performed. Development of classification algorithms for detection of anomalies will continue and, finally, technology will be transferred.

#### **Publications:**

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

#### **Presentations:**

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

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

*Field sampling and laboratory measurements of apple fruit and cider–2003 and 2004.* During the 2003 growing season, a second longitudinal study of microbial flora was conducted. This focused solely on apple fruits. Fruits were sampled at seven dates during the growing season. Measurements were made at dormant, bloom, petal fall, and then at monthly intervals until harvest in late October. At each date, duplicate sets of apples were collected at each of four locations in every tree included in the study. Samples were again

taken from GoldRush and Enterprise trees. Insecticide, fungicide and streptomycin applications were used following recommendations for commercial orchard as in 2002. During this season, about 1500 plates from about 375 individual apple samples were enumerated.

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

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

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

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

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

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

#### **Projects funded in July 2003, To be completed in 2006:**

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

#### **Objective 1. To determine the role of point mutations in fluoroquinolones and erythromycin resistance**

Point mutations in the DNA gyrase gene, *gyrA*, have been shown to be associated with fluoroquinolone resistance in *Campylobacter*. In *C. jejuni*, point mutation of Thr-86-Ile in *gyrA*, which is homologous to Ser-83-Leu in *E. coli*, was predominantly observed in both clinical and laboratory-derived strains with high-level resistance to ciprofloxacin. Other reported mutations of *gyrA* in *C. jejuni* included Ala-70-Thr, Thr-86-Ala (low-level resistance to ciprofloxacin and high-level resistance to nalidixic acid), Thr-86-Lys, Asp-90-Asn, and Pro-104-Ser. Double mutations of *gyrA* combining Thr-86-Ile and Asp-85-Tyr, or Asp-90-Asn or Pro-104-Ser have been reported. The role of mutation in *gyrB* has also been examined, but not yet documented in *Campylobacter*. Mutation of Arg-139-Gln in *parC* has been reported in *C. jejuni*; however, subsequent studies reported by other investigators failed to confirm that *Campylobacter* possesses a *parC* gene. Despite all these observations, direct genetic evidence showing the cause-effect relationship between *gyrA* mutation and fluoroquinolone resistance in *Campylobacter* is lacking.

We adopted an insertional mutagenesis method and introduced a *Campylobacter* Cm<sup>r</sup> and point mutation of *gyrA* at the chromosomal level

to examine the effect of such alterations on the susceptibilities of *C. jejuni* to fluoroquinolones. It appears that the insertion of Cm<sup>r</sup> into *gyrA* upstream gene *Cj1028c* did not have any detectable effect on the susceptibility of *C. jejuni* strains to either ciprofloxacin or nalidixic acid. Point mutation at codon 86 of *gyrA* significantly increased *C. jejuni* mutants MICs to the drugs. In addition, when wild-type *gyrA* allele replaced the mutated copy in fluoroquinolone-resistance *C. jejuni* strains, the MICs showed significant decrease, although to a lesser extent to ciprofloxacin. This clearly demonstrated a direct causal effect between Thr-86-Ile point mutation in *gyrA* and fluoroquinolone resistance.

Objective 2: To identify putative efflux pumps associated with fluoroquinolones and erythromycin resistance

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

**PUBLICATIONS AND PRESENTATIONS:**

1. Meng, J., B. Ge, P. McDermatt, D.G. White, and S. Zhao. 2004. The Role of Efflux Pumps in Antimicrobial Resistance in *Campylobacter jejuni/coli*. The 5<sup>th</sup> World Congress Foodborne Infections and Intoxications, Berlin, Germany.
2. Ge, B., P. F. McDermott, D. G. White, and J. Meng. 2005. Role of Efflux Pumps and Topoisomerase Mutations in Fluoroquinolone Resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrob Agents

Chemother. 49: (will appear in August 2005 issue)

3. Cui, S., B. Ge, J. Zheng, and J. Meng. 2005. Prevalence and characterization of *Campylobacter* spp and *Salmonella* serovars in retail organic chickens of Maryland. *Appl. Environ. Microbil.* 71: 4108-4111
4. Williams, K., and J. Meng. 2005. Detection of noroviruses and indicator organisms on fresh produce. Abstract # P5-48, Annual Meeting of Intl. Assoc. Food Prot., Baltimore, MD., 2005

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

During the last grant year, we have made significant progress on Objectives 1 and 3 of the project. Our major accomplishments of the previous year are briefly summarized as follows:

1. We have completed the experiments proposed in Objective 1. The results of the experiments were presented in the Annual Meeting of American Society of Microbiology. A manuscript described these data has been submitted to *Journal of Food Protection*.

2. We have finished Objective 2. The goal of Objective 2 is to determine *Campylobacter*-induced proinflammatory cytokine Interleukin (IL)-8 secretion by the polarized human colonic epithelial cells. IL-8 is an early proinflammatory cytokine that chemoattracts neutrophils and T lymphocytes to the infection sites, which initiates inflammation. We have compared the IL-8 secreting levels of polarized intestinal epithelial cells that were incubated with eight different *Campylobacter* retail meat isolates with different invasion and transcytosis abilities. We also compared the IL-8 secreting levels of the epithelial cells that were inoculated with *Campylobacter* either from the apical (the initial contact site of colonizing bacteria) or basolateral surface (bacteria can contact this surface after invasion and transcytosis). It was found that the polarized intestinal epithelial cells secreted a significant amount of IL-8 as early as 6 h post inoculation. Statistical analyses using linear regression suggested a correlation of the IL-8 secreting levels with the invasion, but not the transcytosis ability of *Campylobacter* retail meat isolates. Significantly, no matter whether the bacteria were inoculated from the apical or basolateral surface, the intestinal epithelial cells secreted IL-8 predominantly to the basolateral side where the epithelial cells contact with leukocytes. In addition, we found that treating the intestinal epithelial cells with the bacteria-free supernatant, which was generated from the apical or basolateral media of the epithelial cells that had incubated with *C. jejuni*, induced the IL-8 secretion to the level similar to the bacterial inoculation. This indicates that *Campylobacter*-secreted molecules are important for stimulating IL-8 production. Currently, we are examining the interrelationship between *Campylobacter*-induced IL-8 production and transcription factor NF- $\kappa$ B activation. The results of this objective will be presented in the Cold Spring Harbor Meet of Pathogenesis and Host Response. A manuscript describe these results is currently in preparation.

3. We have established *Campylobacter* strains that express the green fluorescence protein. This allows for study of *Campylobacter*-host interaction under a fluorescence microscopy using live cells. This will greatly facilitate the studies proposed in Objective 2.

4. We have established the method to enrich *Campylobacter* RNA from host cell-*Campylobacter* co-culture. This allows to identify *Campylobacter* genes that are induced to express upon *Campylobacter* interaction with the host epithelial cells. We have carried out preliminary study to identify host-induced *Campylobacter* genes using mRNA differential display. These studies and results from Objective 1 and 3 have provided the basis for a grant application that was submitted to USDA.

In the next grant year, besides to get the results of Objective 1 and 3 to be published, we will focus on Objective 2 that examines the cellular mechanism for *Campylobacter* invasion and transcytosis of the intestinal epithelial cells.

### **Publications**

Sheela, R. R., U. Babu, J. Mu, E. Subbiah, D. A. Bautista, R. B. Raybourne, R. A. Heckert, and W. Song. 2003. Immune responses against Salmonella enterica serovar enteritidis infection in virally immunosuppressed chickens. *Clinical and Diagnostic Laboratory Immunology* 10(4):670-9.

Zheng, J., J. Meng, S. Zhao, R. Singh, and W. Song. 2005. Adherence and invasion of *Campylobacter jejuni/coli* isolated from retail meats to human intestinal epithelial cells. *Journal of Food Protection*. (in press)

- **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)

### **Chapter 2 Summary**

The original proposal for the grant described a multi-methodological approach to studying how different news scenarios might influence public perceptions about a bioterrorist attack on a food supply. We recommended that pre-test discussion groups be conducted in the first year, in order to explore different dimensions of attitudes and behaviors in response to different hypothetical news scenarios. This was accomplished in the first year and a research paper was presented based on findings. In the second year, an experiment was proposed, using the concepts discovered in the pre-test groups, to measure effects of a news scenario on public perceptions of food bioterrorism and spokesperson credibility. Second year objectives were achieved: an experiment has been conducted, data have been collected, and data are being analyzed for a research paper to submit to a scholarly communication conference.

### **Chapter 3 Scholarly Presentations Since First Year**

Research paper based on discussion groups completed first year won a top research paper award by the Public Relations Division of the Association for

Education in Journalism and Mass Communication. This paper was presented at AEJMC's national conference in Toronto, Canada, August, 2004.

#### **Chapter 4 Completed Second-Year Method**

As proposed in the original Project Plan, the second year entailed the following activities:

- Securing IRB approval for research on human subjects;
- Experiment designed, based on findings from pre-test discussion groups;
- Pre-test conducted and analyzed to measure validity and reliability of measures;
- Experiment implemented, using students in communication classes; and
- Data analysis has begun.

#### Activities that will be Completed by July, 2005

Data analysis will be completed by July 1, 2005. A second research paper will be written based on experimental findings and will be submitted to an academic conference.

#### Variables Measured in Instrument

After signing a consent form, research participants were asked to complete a pen-and-paper questionnaire. First few questions were about eating, media use, and food shopping behaviors. Then participants were presented with a news story that simulated an actual report of a terrorist attack on a local food supply. The independent variables were: shared risk with victim of attack; and shared involvement by official spokesperson. Dependent variables were:

- Perceived threat/problem recognition
- Level of personal involvement
- Perceived barriers/constraint recognition
- Self-efficacy
- Source credibility
- Fear arousal

#### Preliminary Findings

Findings reveal that the greater the shared sense of involvement with a source of information about the terrorist risk, the greater the perceived problem recognition and threat. The effects were moderated by gender, where women perceived greater shared involvement than men did.

#### Third Year Plan for Research Project

It was originally proposed that the third year of the research project would focus on public presentations and writing of findings for conference papers and publications. This is still the plan for the third year. We also hope to do fine-tune findings by exploring more closely the gender differences, and combine pre test discussion group findings with experimental findings to pose a multi-methodological based model of risk communication in the face of food terrorism.



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

Outline of Projected investigations:

(1) **Phototoxicity of TiO<sub>2</sub> pigments.** In vitro phototoxicity assays at FDA have found that the white pigment, titanium dioxide (TiO<sub>2</sub>), shows significant cytotoxicity under exposure to UV-A light. This pigment is a very common component in both white and colored tattoo inks. Moreover, the photocatalytic activity of TiO<sub>2</sub> is well-established by many previous studies. Commercial TiO<sub>2</sub>, however, is sold as two different polymorphs, anatase and rutile. The former is widely considered to be more photoactive, although the latter also shows photoactivity, and in fact captures a greater portion of the UV spectrum. Some TiO<sub>2</sub> suppliers, in fact recommend passivating strategies to increase the photostability of polymer formulations containing TiO<sub>2</sub>. These considerations lead to the question: is the level of phototoxicity correlated with the polymorphic form of TiO<sub>2</sub> and/or any surface passivation.

To address the issue of anatase vs. rutile, we will purchase samples of tattoo pigments from various commercial vendors. These will be (a) analyzed at UMD using X-ray powder diffraction to determine whether they contain rutile or anatase TiO<sub>2</sub>, or a mixture of the two. The same samples will be screened for in vitro photocytotoxicity at FDA. A correlation of photocytotoxicity data with polymorphic form could ultimately provide guidance to the safe formulation of cosmetics using TiO<sub>2</sub>.

One TiO<sub>2</sub> manufacturer (Dupont Technical Publication: “TiO<sub>2</sub>: Photochemistry and Color Applications” Dwight A. Holtzen Dr. Michael Diebold Dr. Philipp M. Niedenzu, 2002) recommends suppressing photocatalytic activity of TiO<sub>2</sub> in polymer formations by passivating the surface of the pigment particles through encapsulation with amorphous silica or alumina. In fact the same manufacturer defines three grades of TiO<sub>2</sub>: “durable” (high degree of surface coverage and low photocatalytic activity) “non-durable” (little or no surface coverage and a high degree of photocatalytic activity), and “semi-durable” which is intermediate between the two. Thus, we hypothesize that variations in phototoxicity of TiO<sub>2</sub> containing tattoo inks may be correlated with the grade of TiO<sub>2</sub> that was used in the formulation. As with the question of rutile vs. anatase, we will compare photocytotoxicity data from commercially purchased tattoo inks with their degree of surface passivation. The latter will be determined at UMD using the solubility test recommended by the manufacturer (ibid).

**2. Phototoxicity of Organic Pigments.** Studies at FDA have identified two pigments used in tattoo inks, Pigment Red 122 and Pigment Red 170, as being photocytotoxic. These will be subjected to further photochemical and photophysical investigation at UMD.

(a) There is very little, if any, information concerning the photophysical properties (excited state lifetimes, intersystem crossing rates, etc) of these pigments. Thus, our initial studies will be to determine these properties of these materials. Thus, we will attempt to dissolve the compounds in DMSO or some other suitable solvent and then determine their fluorescence quantum yields, lifetimes, and rates of intersystem crossing using spectrofluorometry and pulsed laser measurements at UMD.

(b) We will carry out steady state photolysis experiments with two goals. First the photostability of the pigments will be assessed both as solutes in whatever organic solvent system they can be dissolved in and then as solid suspensions. A low degree of stability under UV-A irradiation, would suggest that the phototoxicity of these materials might be traced to the formation of harmful photoproducts.

(c) If the steady-state photolysis experiments reveal that either of the pigments do decompose upon photolysis, then the photoproduct mixtures will be tested for cytotoxicity at FDA. If the mixtures show any level of toxicity, then we will attempt to isolate the photoproduct(s) that is (are) responsible for cytotoxicity and determine its (their) structure. This will be accomplished by chromatographic separation of the reaction mixture and NMR and mass spectral analysis of the products.

(d) Laser flash photolysis experiments will also be carried out on the two pigments. These can be used to probe for two effects. First, we can determine the effective yield of singlet oxygen from these materials using our IR fluorescence detector. Second, assuming that the pigments are not photostable, transient absorption measurements may help us to determine whether photolysis generates harmful reactive intermediates such as free radicals or radical cations, capable of damaging vital biomolecules such as DNA or proteins.

### **Project funded in July 2004, To be completed in 2007:**

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

#### **1. Introduction**

The overall goal of this project is to characterize the physico-chemical behavior of antioxidant additives commonly used in food packaging in order to better predict exposure estimates.

Specific objectives include the following:

a. Measure partition coefficients for common anti-oxidant additives in food packaging polymers between representative polymer matrices and a wide range of food extractant simulants

- b. Determine the temperature dependence of partition coefficients in order to determine the thermodynamic parameters that control partitioning
- c. Integrate information gathered from these experiments into existing models designed to predict exposure estimates.

During the past year, one graduate student and two undergraduates have worked on this project and have made considerable progress in characterizing fundamental properties of representative antioxidant additives and establishing protocols for measuring temperature dependent partition coefficients. This progress report serves to highlight these accomplishments, discuss their relevance to partitioning behavior of target antioxidant additives and identify potential bottlenecks that may hamper the project's evolution.

## **2. Methods**

Given the lack of information about the photophysical properties of common antioxidant additives, our first task has been to evaluate different optical methods as means of quantifying antioxidant concentrations in different media including polymer matrices (representative of food packaging material) as well as liquids (that serve as food simulants). Optical spectroscopy has the advantage of being noninvasive and amenable to integration into applications requiring rapid screening. In contrast, more traditional methods of determining partition coefficients rely on mass spectrometry (LC-MS, GC-MS, Electrospray-MS), techniques that involves time consuming sample preparation. Of course, an advantage of MS over optical methods is that the former provides unambiguous, quantitative information about molecular identity and concentration. Optical spectroscopy can only identify properties associated with an active chromophore. If multiple analytes contain the same chromophore (such as different Irganox species) in a sample, additional methods must be employed to discriminate the different analyte concentrations. This project intends to quantitatively correlate optical data acquired at UMCP with LC-MS measurements performed in the CFSAN laboratories. Comparing data from two different types of techniques will allow us to critically evaluate lower limits to analyte detection – MS is more sensitive than steady-state optical techniques – and the viability of different optical methods for use in determining partition coefficients. Optical techniques currently being used in this work include UV-Vis absorbance and steady-state fluorescence excitation and emission. In addition, we will soon use time-correlated single photon counting to measure the fluorescence lifetimes of analytes in different media. This latter method promises to improve detection limits by several orders of magnitude.

In addition to characterizing the optical behavior of anti-oxidant additives in different media, we have also initiated a survey study of the two-dimensional surface properties of representative anti-oxidants at the air/liquid interface. Many additives have distinguishable amphiphilic character. This balance of hydrophobic and hydrophilic functionality makes these additives surface active and capable of establishing surface excess concentrations that are higher than the concentrations in either bulk phase. Consequently, knowing the interfacial behaviors of different additives as well as bulk solution properties is essential if one is to quantitatively account for all of the additive present in a given

polymer/simulant system. These surface studies have been carried out at the air/water interface using a recently acquired Langmuir trough/Wilhelmy plate tensiometer.

### **3. Accomplishments**

#### *3a. Optical calibration*

Accurate determination of analyte partitioning requires knowing how much of an analyte migrates from one phase to another. To this end, our work began with measuring the optical absorbance, excitation and emission spectra of different additive analytes in solutions of varying concentrations. Solvents were chosen to represent those liquids most frequently used as food mimics in previous partitioning studies and included isooctane, water and ethanol/water mixtures. Fluorescence emission consistently proved to be the most sensitive method for detecting small concentrations of analyte additives in different solutions. The data also show evidence aggregate formation at intermediate concentrations. Based on signal to noise considerations, we estimate that fluorescence emission allows us to detect concentrations of IRG1076 as low as 1-2  $\mu\text{gMolar}$ . However, the drawback to these data are that fluorescence emission – the most sensitive of the optical techniques developed thus far – requires a series of sample dependent corrections before data can be used to determine absolute concentrations in solution. (These corrections include accounting for a solute's quantum yield – a quantity that depends on concentration and solvent composition – as well as instrument collection efficiency.)

#### *3b. Initial partitioning measurements*

Having determined the sensitivity limits and reproducibility of our ability to quantify antioxidants in different solutions that serve as food simulants, we tested our ability to accurately measure partitioning coefficients using polymer standards having well defined quantities of different antioxidant additives. European collaborators of Tim Begley provided different polymer standards containing varying combinations of antioxidant additives. Since acquiring these samples, we have begun to measure the partitioning of antioxidant additives between both low density polyethylene (LDPE) and polypropylene (PP) matrices and isooctane, a common food simulant for highly hydrophobic media.

#### *3c. Temperature dependent partitioning*

In related work, we have developed an assembly that will enable us to make partitioning measurements over a wide temperature range, thus allowing us to determine the thermodynamic parameters ( $\Delta G^H$  and  $\Delta G^S$ ) that govern additive partitioning between a polymer matrix and different food simulants. The temperature-controlled apparatus was constructed specifically to examine solute partitioning between two immiscible liquids, but is readily adapted to accommodate the polymer/liquid systems. The circulating, constant temperature bath can control temperatures over a range from 0 degrees to 115 degrees Celsius. In the liquid/liquid experiments (carried out with different nitrophenol solutes), aliquots from the aqueous and organic phases in each system were taken without removing the systems from the bath. The absorbance of the solute in the aliquots was measured and converted to bulk solution concentration using Beer's Law

calibration curves. Next, the temperature of the bath was increased by approximately 3 degrees Celsius. After another 24 to 72 hours of equilibration, more aliquots were taken, and the partitioning coefficient was calculated for the new temperature. From the data the Gibbs free energy of partitioning could be determined at each temperature from the measured partition coefficient.

### *3d. Two-dimensional surface behavior of antioxidant additives*

Most antioxidants have characteristic functional groups that would classify them as surfactants. In particular, the alcohol functional group found on many Irganox and Tunisian additives as well as the phosphine groups of Irgafos additives are hydrophilic, while the alkyl and aryl pieces of these molecules are decidedly hydrophobic. Given their amphiphilic structure, these additives are likely to accumulate in excess at interfaces between media having differing polarities. These monolayers can serve as sinks for additives, meaning that adsorbed additives will not be detected by experiments designed to probe bulk solution concentrations. If the systems of interest have relatively large interfacial areas *or* if additive concentrations are sufficiently dilute, the relative amount of additive adsorbed to a surface can represent a non-zero fraction of the total amount of additive present in the entire system.

To better understand the surface behavior of different additives, we have begun a series of experiments designed to examine the two dimensional properties of IRG1076, IRG168 and

Tinuvin 238. Dilute monolayers of these additives are spread on the air/water interface and then the monolayers are compressed by the moveable arms of a Langmuir trough. A high precision tensiometer measures the surface tension of the system using a paper Wilhelmy plate. Isotherms are typically plotted in terms of surface pressure ( $\Delta S$ ) vs. surface coverage ( $\text{\AA}^2/\text{molecule}$ ), where the surface pressure describes the difference in surface tension between the tension of the neat interface (72 mN/m for H<sub>2</sub>O) and the tension of the system under study. Surfactants lower the interfacial tension of water leading to positive measured surface pressures.

## **4. Short term objectives and limitations**

Having fully explored the photophysical and surface properties of several important antioxidant additives, we are now well positioned to begin characterizing the thermodynamic and kinetic parameters that control migration of these additives into food simulants. At the same time, we will continue to develop more sensitive optical methods for detecting additives that have migrated from packaging to simulant as well as those that have assembled spontaneously at solid/liquid interfaces.

### *4a. Thermodynamic partitioning data*

The UV-vis absorbance data examining the partitioning of IRG1076/IRF168 between LDPE and isooctane as well as between PP and isooctane raise a number of interesting issues. The observation that more additive migrates from the LDPE than from the PP into the organic solvent implies that analytes are less tightly bound by the LDPE than by the PP. However, isooctane is known to be an aggressive simulant in terms of its ability to extract additives from solid matrices. As mentioned above, our first priority is to determine the relative

amounts of IRG1076 or IRF168 that actually migrate from the polymer into the isooctane solution using LC-MS instruments at CFSAN.

During the upcoming summer months, we will ascertain whether data presented above represent true equilibrium partitioning constants or whether the migration of additives from PP is simply much slower than from LDPE but the total amount of extracted analyte relative to what remains in the polymer is the same for both samples. These studies will require taking aliquots of solvent from a sample on successive days, measuring the aliquot's absorbance and emission properties and then repeating the process until the properties stop changing. After determining the timescales required to establish equilibria, these experiments will be repeated at different temperatures in order to calculate the enthalpies and entropies of partitioning in a manner similar to that described above.

The partitioning data described above suggests that the polymer matrix contributes to the amount of analyte that migrates into isooctane. One may also wonder if the identity of the food simulant also plays a role in the amount of material that migrates. To answer this question, we will begin a parallel series of experiments using a different mixtures of ethanol and water. Given the rather poor solubility of most additives in aqueous solvents, we expect that higher percentages of water will lead to less migration and a greater likelihood of monolayer formation at the packaging/simulant interface.

#### *4b. Additional characterization of antioxidant additives in solution and at surfaces*

A limitation to steady-state or static optical measurements is the inability to distinguish one species from another if both have a common, optically active chromophore. As mentioned above, this obstacle prevents us from using fluorescence emission and UV-vis absorbance to distinguish different species containing the same BHT-based, alkoxy-toluene functional group. However, there exist techniques that take advantage of differences in the dynamic behavior of different analytes *may* be able to distinguish IRG from IRF additives. We have recently finished constructing a femtosecond fluorometer capable of carrying out time-correlated single photon counting lifetime measurements. We will soon be using this new instrument to measure the fluorescence lifetimes of IRG1076, IRG1010, IRF168, T328 and BHT in different solvents. If the fluorescence lifetimes of different analytes are suitably different, then this technique may stand out as an accurate, convenient – albeit technically challenging – means of distinguishing relative concentrations of different analytes in the same solution. Antioxidants such as IRG, IRF, and Tinuvin derivatives are amphiphilic and exhibit distinctive behavior at hydrophilic/hydrophobic surfaces. Surface pressure isotherms such can reveal detailed information about the two dimensional thermodynamic behavior of these additives, but the data do not provide any information about the molecular orientation or interaction mechanisms of adsorbed species. Furthermore, optical methods such as fluorescence and absorbance are not well suited for studying molecules at surfaces because these techniques lack surface specificity – the ability to distinguish molecules at surfaces from those in bulk solution. For the past eighteen months, our laboratory has been able to acquire

vibrational spectra of molecules adsorbed to liquid surfaces using a nonlinear-optical technique known as vibrational sum frequency generation (VSFG). Spectra acquired by VSFG contain unprecedented detail about how structure and organization of molecules at surfaces. During the next year, we intend to apply this technique to better understand how additives adsorb to air/liquid and solid/liquid interfaces.

#### *4c. Limitations to progress*

Our ability to develop accurate, quantitative models that describe the migration and partitioning of antioxidant additives between polymer matrices and liquid food simulants depends in large part on our ability to work with materials having well controlled structural properties and well defined, homogeneously distributed additive concentrations. From collaborators in Europe, we have obtained additive containing polymer standards that have allowed us to develop the experimental procedures described above. However, our supply of these materials is limited and consequently constrain the number of experiments we can carry out. During the F'04 semester, we attempted to purchase (using different resources than those provided by JIFSAN) and refurbish a polymer extruder so that we could make our own materials for use in the planned partitioning studies. Our efforts were unsuccessful and the time lost set back our planned experimental agenda. Currently, Tim Begley is in contact with colleagues at the FDA Labs in Illinois in an attempt to borrow an extruder that has not been used for several years. Access to this instrument would greatly improve our ability to design experiments to examine specific aspects of additive migration and partitioning by allowing us to make polymer materials with just a single additive – the standards from Europe all have at least two additives present in each sample – at varying concentrations.

#### **Summary**

During the past year, we have successfully identified photophysical properties of a number of different antioxidant additives that can serve as diagnostic indicators in studies of analyte migration and partitioning. Given the relative concentrations of these materials in food packaging, simple UV-vis absorbance appears capable of monitoring the total amount of extracted material. Fluorescence emission is even more sensitive and will be used in the event that lower detection limits are needed. We have also developed techniques capable of examining temperature dependent partitioning thus enabling us to determine the thermodynamic properties associated with the partitioning behavior of different analytes. Monolayers of common antioxidant additives exhibit a strong tendency to aggregate irreversibly at the air/water interface, a finding that may have consequences in systems where these analytes may develop large surface excesses. Finally, preliminary partitioning measurements using well defined polymer/additive standards have provided strong evidence that additive migration and partitioning depends sensitively on additive identity as well as the material from which the additive is being extracted. We are excited to build upon the foundation established during the past year, and begin evaluating quantitatively the important properties that control additive partitioning and, ultimately, exposure of the population to these materials.

## APPENDIX B

### Collaborative/Cooperative Research Projects

#### 1. Industry Acrylamide Alliance/JIFSAN Project Funding

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

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

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

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



in the processed food. The purpose of this experiment is to determine how consumer preparation affects acrylamide levels in food. In addition, this experiment provides information on the effects of time and temperature (oven and product) on acrylamide formation in food.

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

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

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

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

- the affect of “doneness” on acrylamide, since acrylamide often increases exponentially as foods are taken past their prepared ideal to a point of over doneness. Home preparers exhibit a broad range of accomplishment in cooking skills. Multitasking in the preparation of home meals predisposes to over doneness of some courses. Since in-home preparation is more likely to produce over processing than commercial products; it is likely that home cooked, over-processed foods contribute to dietary acrylamide at levels which vastly over represent to the fraction of calories supplied from home-cooked foods.
- the correlation between free asparagine and acrylamide, since any correlation between browning and acrylamide formation may require some understanding of initial free asparagine.
- spatial distribution of acrylamide in bread, since it is well known that acrylamide is not uniformly distributed in baked and fried foods. Instead it appears disproportionately in the darkened outer surface that

experiences higher temperatures than the interior food. However, at least one report suggests acrylamide distribution in bread is bimodal, with a second accumulation of acrylamide occurring some distance within the loaf. This observation should be substantiated or repudiated.

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

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

The goal of this CRADA is to develop, using toxicokinetic and biomarker data collected in B6C3F1 mice and F344 rats, a physiologically based pharmacokinetic-pharmacodynamic (PBPK/PD) model for acrylamide and glycidamide from which tissue levels of parent compound, its genotoxic metabolite, and their disposition can be simulated across species, including the human. Biomarkers of exposure including hemoglobin adducts of acrylamide and glycidamide and glycidamide-derived DNA adducts will provide a pharmacodynamic link with measures of tissue damage. The ultimate goal is to predict concentrations of acrylamide and glycidamide in human tissues along with the resultant DNA damage for use in assessing toxic risks from acrylamide in the diet.

Specific Aims:

- Collect a full set of toxicokinetic and biomarker data, including serum and tissue measurements along with adducts of DNA and hemoglobin, that are needed to develop a PBPK model for acrylamide and glycidamide.
- Develop a PBPK-PD model that will simulate serum and tissue levels of acrylamide and glycidamide simultaneously with levels of tissue DNA adducts and circulating hemoglobin adducts for use in extrapolation to humans.

## 2. Cooperative Research with DNRE, Victoria, Australia

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

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

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

**3. Additional Research Projects Funded (addressing FDA needs)**

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

Despite their importance as foodborne disease agents and their apparently high infectivity at low doses, HAV and Noroviruses have not been subjected to rigorous human infectivity dose-response analysis or quantitative microbial risk assessment. This is because human infectivity data for these viruses is either limited, has not been compiled and examined in a manner suitable for such analyses or has not been made readily available for such analyses.

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

A human volunteer study of Norwalk Virus was done on a collaborative basis by this lab (Mark D. Sobsey), Prof. Christine Moe and other investigators at the University of North Carolina. In those studies, about 50 volunteers were given different doses of the so-called 8FIIa inoculum of Norwalk Virus, which has been extensively used to infect human volunteers since the early 1970s. The human infectivity dose-response analyses of these data have not been completed, and such analyses would be done as another objective of this study. Preliminary analyses of these data revealed that the dose-response relationship was not

uniformly sigmoidal if all volunteers were included in the analyses. However, when the analyses excluded volunteers who were negative for Norwalk Virus antibodies and who were later shown to be of a human blood group genotype that lacks the carbohydrate receptor for virus attachment and infection, a sigmoidal dose-response relationship was obtained. Furthermore, the 8FIIa inoculum with which these volunteers were challenged has not been carefully analyzed for Norwalk Virus concentration; only a rough estimate of virus concentration was made. The objectives of this study include a careful, quantitative titration of this inoculum and then a careful analysis of the dose-response relationship. Also, efforts will be made to obtain as many inocula as possible from previous studies and to subject them to molecular and possibly other analyses to obtain estimates of the HAV concentrations.

Objectives:

1. Identify and acquire human infectivity dose-response data for HAV and NV from studies done primarily in the USA from the published literature and other available data sources, such as reports and experimental records.
  2. Acquire as many of the HAV and NV inocula as possible that were used in these human volunteer dose response studies. Better quantify the virus titers of as many of the acquired inocula as possible using RT-PCR and possibly other analytical methods.
  3. Quantify dose-response relationships of HAV and NV using various estimates of virus titers in the inocula and the health effects responses of human volunteers (infection, illness and if available, mortality). Develop quantitative dose-response relationships using various dose-response models appropriate for human pathogens.
  4. Search the published literature, other published reports and other legitimate data sources for the measured concentrations of HAV and NVs in foods, including foods consumed in outbreaks of NV gastroenteritis and of Hepatitis A (infectious hepatitis).
  5. Use the dose-response relationships from human volunteer studies with NV and HAV and the data on NV and HAV levels in food to estimate the risks of NV gastroenteritis and infectious hepatitis (Hepatitis A) from ingestion of these foods. Compare the predicted risks of illness of NV gastroenteritis and Hepatitis A obtained by these analyses to the actual risks of NV gastroenteritis and infectious hepatitis observed in foodborne and waterborne disease outbreaks. This provides a basis to compare the risks predicted from the quantitative microbial risk assessment analyses to the actual risks observed in outbreaks.
- **Development of molecularly imprinted polymers (MIPs) for selective detection of marine biotoxins**, Kenneth Shea, Chemistry, University of California, Irvine (Funded July 2004; one-year project)

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

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

Sup35NM and Sup35NM-His6 are prion-like proteins derived from yeast that have physical-chemical properties similar to that of the prion proteins responsible for Transmissible Spongiform Encephalopathies (TSEs), including Creutzfeldt-Jakob disease in humans, Mad Cow Disease (BSE) in cattle, and scrapie in sheep. Though non-pathogenic, yeast prions behave the same way as mammalian prion protein in their ability to change conformation, form aggregates and replicate themselves. Yeast prion protein Sup35NM and its recombinant protein derivative, Sup35NM-His6, have been cloned for production, purified and evaluated for suitability as a prion surrogate protein in this laboratory. Recent work has demonstrated that under specific conditions, a feather-degrading keratinase is capable of degrading the prions present in the brain tissues of BSE in cattle and scrapie in sheep. Therefore, a process of enzymatic degradation of prions may be developed that renders animal products free of prions and prevents the transmission of TSE. In this project, Sup35NM and Sup35NM-His6 will be compared and the better candidate selected for development of a standard Prion Surrogate Protein (PSP). The PSP will be mixed with normal nervous tissue in a pilot-scale pressure cooker and serve as a marker for prion degradability to

enzymatic action under industrial rendering conditions. Brain and spinal cord tissues will be added to mimic the specified risk materials (SRM) for BSE. To improve the specificity and activity of the keratinase, several genetically-modified keratinases will be produced and tested for efficacy against the PSP. If proven effective, modified keratinase enzyme species that specifically attack BSE prion may ultimately be developed.

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

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

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

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

### **Final Report**

This study investigates the potential sources of microbial contamination of produce in 10 farms and 13 packing sheds in the southern US. Produce items studied include groups that are minimally processed and eaten raw (leaf lettuce/spinach, parsley/cilantro/basil, green onions, cabbage, melons/cantaloupe). The majority of produce samples was domestic (from US farms) but we also sampled produce from farms in Mexico that were repackaged in US sheds. Sources of microbial contamination were identified by conducting sanitary surveys and interviews of shed managers, measuring microbial quality of water used for produce irrigation and processing, measuring microbial quality of workers' hands from hand rinse samples, and measuring microbial quality of environmental surfaces through swabs. Produce microbial quality was assayed by measuring total aerobic plate count, total coliforms, *E. coli*, enterococci and selected pathogens on produce. Over the two growing seasons studied, a total of 923 produce samples, 499 water and ice samples, 490 surface swab samples and 177 hand rinse samples were collected and analyzed. Data collection ended in 2004, and data cleaning and analyses are in progress.

To date, the results from this study indicate that most produce show no change in microbial quality as they move through the processing shed. Microbial contamination of cantaloupes and herbs increase as they move from the farm through the different steps of the processing shed. To address the mechanisms of this contamination, we examined microbial contamination of water samples in farms and sheds. The microbial quality of water used in the sheds was excellent and had low levels of microbial indicators. Irrigation water overall had significantly higher levels of microbial indicators than shed water. We then investigated whether the microbial quality of the produce samples was associated with the microbial quality of the water samples. We found no significant correlations and our best-fit, step-wise, linear regression model did not show any significant associations between produce and water microbial quality. We then asked whether contamination of surface areas affects microbial quality of produce. We found that most swabs collected from various shed locations exhibited low levels of microbial indicators. Swabs collected from locations used for cantaloupes had significantly higher levels of microbial contamination. The study also found that domestic produce was equally or more contaminated than imported Mexican produce. No *E. coli* O157:H7, Salmonella or Shigella was detected in our produce samples. A total of 3 of 43 domestic cabbage samples tested positive for *L. monocytogenes*. The next steps in this study are to: 1) analyze the hand-rinse data and address whether contamination on workers' hands contributed to contamination on produce and 2) examine the relationships between farm and shed practices and the microbial quality of processing water and produce.

### **Progress Impact**

In 2004, we met with members of the agricultural community and the produce industry in the area where the study was performed to discuss preliminary findings and disseminate

the results of the study. They suggested we present our result to the local shed community. We presented our results to the shed managers as part of a one day training workshop. As a result of our study, several sheds improved their sanitary conditions and required their workers to wear gloves when handling produce. We will meet again with our community advisory committee, once we have additional results from hand rinse sample data.

In 2005, Dr. Leon was invited to present the results of this study as one of the symposium speakers at the International Association for Food Protection meeting in Baltimore, MD. Many industry, academia, and government representatives discussed the work with him after the talk.

#### Publications:

- Johnston, L.M., L. Jaykus, D. Moll, M. Martinez, J. Anciso, B. Mora, and C.L. Moe. A field study of the microbiological quality of fresh produce. (2005) *J. Food Prot.*, 68 (9): 1840-1847.
- Johnston, L.M., C.L. Moe, D. Moll, and L. Jaykus. The epidemiology of produce-associated outbreaks for foodborne disease. (2005) In: J.L. James (ed.), *Microbial Hazard Identification of Fresh Fruit and Vegetables*. John Wiley and Sons, Inc., Hoboken, NJ.,
- A field study of the microbiological quality of fresh produce of domestic and imported origin.(2005) Johnston, L.M, Jaykus L., Moll D. Martinez,C.M. Anciso J., Mora B, and Moe C.M. (submitted for publication to *Int. J. Food Micro.*)
- Relationship between fresh produce and processing waters in farms and packing sheds. (2005) Leon, J.S., Hall, R., Johnston, L.M, Jaykus, L., Moll, D., Martinez, C.M., Anciso, J., Mora, B., and Moe, C.M. (manuscript in preparation)
- Risk factors for contamination of fresh produce in farms and processing sheds: environmental and hygiene practices. (2005) Leon, J.S., Hall, R., Johnston, L.M, Jaykus, L., Moll, D., Martinez, C.M., Anciso, J., Mora, B., and Moe, C.M. (manuscript in preparation)



## APPENDIX C

### **JIFSAN Post-Doctoral Research Program**

**Aquaculture drugs: LC/MS protocols for marker determination,** Robert Dickey (FDA), Ann Abraham (JIFSAN Research Associate) (ends 2/28/06)

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

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

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

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

**Molecular phylogenetic identification of potential foodborne agents of bio-terrorism,** Eric Brown (FDA), Alice Heyford (JIFSAN Research Associate) (ends 1/12/06)

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

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

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

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

**Development of a specific monoclonal antibody for *Enterobacter sakazakii*: Identification and an immunoassay using color-coded bio-nanotubes**, Kun-Ho Seo (FDA), Sang-Bok Lee (UM), Kwang-Young Song (JIFSAN Research Associate) (ends 4/30/06)

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

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

80 times lower than that of the FITC-based method. The total detection time was within 2h.

The outer membrane proteins of *E. sakazakii* and non-*E. sakazakii* strains were prepared and analyzed using two-dimensional electrophoresis. The characterization of the proteins that expressed in *E. sakazakii* was performed using image analysis systems in collaboration with bioinformatics team (Instrumentation and Biophysics Branch, OSAS). Further analysis will be carried using N-terminal and internal amino acid sequences. A marker protein for *E. sakazakii* will be identified and purified and used for production of monoclonal antibodies.

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

**Publications and Poster Presentations – Kwang-Young Song, Ph.D. (details needed here)**

- 1. Rapid Detection of *Salmonella* Enteritidis by Quantum Dot Biolabeling Coupled with Immunomagnetic Separation**  
Kwang Y. Song, Kun H. Seo, Grace Thammasuvimol, and Robert E. Brackett
- 2. Optimization of Ferrioxamine E Concentration as Effective Supplementation for Selective Isolation of *Salmonella* Enteritidis in Egg White**  
Grace Thammasuvimol<sup>1</sup>, Kun H. Seo, and Kwang Y. Song
- 3. Poster Presentation**  
**Development of Selective Media for Detection of *Enterobacter sakazakii* by Using Various Iron Source and alpha-glucosidase Substrates**  
Kwang Y. Song, Kun H. Seo, Grace Thammasuvimol, and Robert E. Brackett
- 4. Poster Presentation**  
**Effectiveness of Commercial Disinfectants for Inactivating *Enterobacter sakazakii* in Water**  
Kwang Y. Song, Kun H. Seo, Scott Lee, Grace Thammasuvimol, and Robert E. Brackett
- 5. Poster Presentation**

**Evaluation of Selective Media for Detection of *Enterobacter sakazakii* by Using Various Iron Source and alpha-glucosidase Substrates**  
**Kwang Y. Song, Kun H. Seo, Grace Thammasuvimol, and Robert E. Brackett**

- 6. Detection of *Salmonella* Enteritidis in Incubated Pools of Shell Eggs Supplemented with Ferrioxamine E. by Lateral Flow Test Kit**  
**K. H. Seo, G. Thammasuvimol, and K.Y. Song**

## APPENDIX D

### Research of Dr. Frederick Khachik

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

#### Objective and Nature of Research

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

#### Accomplishments and Potential Value/Applicability

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

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

The second year study was similarly conducted with zeaxanthin (5 primates) at the same dose (10 mg/kg body weight) and also revealed a significant increase in the levels of this carotenoid and its metabolites in plasma and ocular tissues of the supplemented primates. The third year, which was completed in September of

2000, involved 5 primates that were supplemented with a 1/1 mixture of lutein and zeaxanthin each at the dose of 0.5 mg/Kg body weight for one year.

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

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

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

### 3. *Synthesis and Industrial Production of $\alpha$ -Cryptoxanthin and $\beta$ -Cryptoxanthin from Lutein*

$\alpha$ -Cryptoxanthin and  $\beta$ -cryptoxanthin, as measured in plasma of human subjects have been associated with reduction in blood pressure in an Oxford University large intervention trial. Healthy and diseased subjects have also been studied in a variety of prospective trials to correlate  $\beta$ -cryptoxanthin levels with cardiovascular parameters. Inflammatory markers such as C-reactive protein and fibrinogen have also been linked to low serum  $\beta$ -cryptoxanthin levels. There have also been some preliminary studies looking at the effect of  $\beta$ -cryptoxanthin on bone growth and the inhibition of bone re-absorption. *In vitro* studies have shown a positive effect of  $\beta$ -cryptoxanthin increasing bone calcium and enhancing bone alkaline phosphatase. The total synthesis of  $\alpha$ -cryptoxanthin and  $\beta$ -cryptoxanthin on laboratory scale has been proved to be extremely difficult and costly and consequently this approach cannot be applied to industrial production of these carotenoids.

Three industrially viable processes have been developed that convert commercially available lutein to  $\alpha$ -cryptoxanthin and  $\beta$ -cryptoxanthin (patents 1-3). These rare dietary carotenoids are not commercially available and their production allows investigators to conduct metabolic and clinical studies with these carotenoids. (3R)- $\beta$ -Cryptoxanthin and (3R,6'R)- $\alpha$ -cryptoxanthin can also be used as nutritional supplements or as a food coloring additives. The fore-mentioned patented processes have been licensed to Kemin Foods (Des Moines, Iowa) by the University of Maryland and are currently at the early stage of industrial production.

#### **Publications**

Sahin, K.; Ozercan, R.; Onderci, M.; Sahin, N.; Gursu, M.; Khachik, F.; Sarkar, H.; Munkarah, A.; Ali-Fehmi, R.; Kmak, D.; O. Kucuk, O. Lycopene supplementation prevents the development of spontaneous smooth muscle tumors of the oviduct in Japanese quail. *Nutrition & Cancer-an International Journal*, 50 (2): 181-189, 2004.

Khachik, F. Chemical and Metabolic Oxidation of Carotenoids. In: *Carotenoids and Retinoids, Molecular Aspects and Health Issues*, Packer, L.; Obermuller-Jevic, U.; Kraemer, K.; Sies, H.; Eds; AOCS Press, Champaign, Illinois, Chapter 4, pp 61-75, 2004.

#### **Presentations**

- Khachik, F. (Symposium organizer). Distribution, Metabolism, and the Role of Tomato Carotenoids in Disease Prevention”, Symposium entitled “Lycopene and

Other Carotenoids” 229th American Chemical Society National Meeting, San Diego, California, March 13-17, 2005.

- Khachik, F. Distribution and metabolism of dietary carotenoids in humans as a criterion for development of nutritional supplements. Plenary lecture, 14<sup>th</sup> International Carotenoid Symposium Edinburgh, Scotland, July 17-22, 2005.
- Khachik, F.; de Moura, F.F.; Chew, E.; Douglass, L.W.; Csaky, K.; Ferris III, F.; Dabas, K.; Sran, P. Dose ranging study of lutein supplementation in elderly with and without age related macular degeneration. 14<sup>th</sup> International Carotenoid Symposium Edinburgh, Scotland, July 17-22, 2005.
- Khachik, F.; London, E.; de Moura, F.F.; Johnson, M.; Steidl, S.; Detolla, L.; Shipley, S.; Sanchez, B.; Chen, X.Q.; Flaws, J.; Fowler, B.; Luty, G.; McLeod, S. Chronic ingestion of (3R,3'R,6'R)-lutein and (3R,3'R)-zeaxanthin in female rhesus macaque primates. 14<sup>th</sup> International Carotenoid Symposium Edinburgh, Scotland, July 17-22, 2005.
- Zhan, G.; Khachik, F.; Sulaiman, P.; Camras, C.B. Effect of lutein, zeaxanthin,  $\alpha$ - and  $\beta$ -cryptoxanthin, lycopene, and their mixture on oxidative stress in cultures of normal and glaucoma human ciliary muscle cells and rat retinal ganglion cells. 14<sup>th</sup> International Carotenoid Symposium Edinburgh, Scotland, July 17-22, 2005.
- Khachik, F.; Liu, Y.; Showalter, H. Industrial processes for partial synthesis of (3R)- $\beta$ -cryptoxanthin and (3R,6'R)- $\alpha$ -cryptoxanthin from (3R,3'R,6'R)-lutein. 14<sup>th</sup> International Carotenoid Symposium Edinburgh, Scotland, July 17-22, 2005.

## Patents

- F. Khachik, A novel method for production of rare carotenoids from commercially available lutein, U.S. patent 6,911,564 B2, June 28, 2005.
- Khachik, F. Process for Making a (3R,3'R)-Zeaxanthin Precursor, US Patent 6,818,798 B1, November 16, 2004.
- F. Khachik, Y. Liu, and H. Showalter, Process for preparation of alpha- and beta-cryptoxanthin, U.S. patent application disclosed October 15 2003; final application filed October 2004.



## **Awards**

- Winner of collaboration success award in March 2005 by the Council for Chemical Research.
- **President-elect International Carotenoid Society**