U.S. FOOD & DRUG ADMINISTRATION

2022-2023

CENTER FOR FOOD SAFETY AND APPLIED NUTRITION

CENTERS OF EXCELLENCE ANNUAL REPORT

Western Center for Food Safety WCFS UCDAVIS

Joint Institute for Food Safety and Applied Nutrition UM FDA

THE UNIVERSITY OF MISSISSIPPI 1848 National Center for Natural Products Research

IFSH INSTITUTE FOR FOOD SAFETY AND HEALTH

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Introduction

The Center for Food Safety and Applied Nutrition, known as CFSAN, is one of six product-oriented centers, in addition to a nationwide field force, that carry out the mission of the Food and Drug Administration (FDA). FDA is a scientific regulatory agency responsible for the safety of the nation's domestically produced and imported foods, cosmetics, drugs, biologics, medical devices, and radiological products. CFSAN has the responsibility for ensuring that the United States food supply is safe, secure, sanitary, and properly labeled, as well as ensuring the safety and proper labeling of dietary supplements and cosmetic products. To help accomplish these goals, CFSAN recognizes the value of fostering collaborations with external partners to leverage research and regulatory resources in support of our science and capacity building activities. These partnerships assist the FDA in fulfilling its public health mission and in expanding the science base upon which future regulatory programs are developed.

CFSAN's Centers of Excellence (COE) program is one of several approaches CFSAN uses to collaborate with external partners to fulfill its public health mission. The COE program consists of formal partnerships with four academic institutions and provides opportunities to build diversified channels for infusing innovative ideas and knowledge, encourages dialogue among government, academia and industry, and develops novel approaches to solve complex food safety issues. COEs also partner and collaborate with other domestic and international organizations to conduct food safety research and capacity building. This collaboration leverages CFSAN's resources and enhances our ability to ensure public health. It also allows CFSAN to reach a larger portion of the global food safety community. CFSAN currently supports four COEs; 1) the National Center for Natural Products Research (NCNPR) at the University of Mississippi, Oxford, 2) the Joint Institute for Food Safety and Applied Nutrition (JIFSAN) at the University of Maryland, College Park, 3) the Institute for Food Safety and Health (IFSH)/National Center for Food Safety and Technology (NCFST) at the Illinois Institute of Technology, and 4) the Western Center for Food Safety (WCFS) at the University of California, Davis.

This report highlights selected research and capacity building efforts conducted by the COEs during the 2022-2023 Cooperative Agreement budget period.

National Center for Natural Products Research (NCNPR) - University of Mississippi, Oxford

The National Center for Natural Products Research (NCNPR) was established in 2001 at the University of Mississippi, Oxford, to assist the FDA with the regulatory framework that was created for dietary supplements under the Dietary Supplement Health & Education Act of 1994 (DSHEA). The cooperative research, education, and outreach programs developed by the NCNPR address scientific issues related to the safety of botanical dietary supplements (BDS) and botanical ingredients and complement the diverse activities of both the public and private sectors. Specifically, the NCNPR: 1) assists in the identification and development of a list of BDS and botanical ingredients, based on safety concerns, trends, and knowledge of botanicals being marketed in the U.S., to prioritize further research; 2) acquires, validates, and characterizes authenticated reference materials, including raw and processed plant materials and purified natural products of relevance to the FDA, for evaluation of their safety; 3) exchanges technical and scientific information, analytical methods, and reference material with the FDA scientists and other stakeholders; 4) collaborates with the FDA scientists in research areas of mutual interest; and, 5) coordinates scientific workshops and conferences on BDS-related topics of public health relevance to address high priority science and research needs.

NCNPR Director - Dr. Ikhlas A. Khan
NCNPR Assistant Director - Dr. Amar G. Chittiboyina
CFSAN Project Officer - Dr. Gregory O. Noonan

Analytical Investigations to Assure the Overall Quality of Botanical-Based Dietary Supplements (BDS)

The NCNPR has focused on developing several analytical methods to determine the authenticity of botanical-based dietary supplements (BDS) and foods. In association with American Herbal Pharmacopeia (AHP), the NCNPR is investigating the utility of high-performance thin-layer chromatography (HPTLC) and nuclear magnetic resonance (NMR)-metabolomic approaches to ensure the botanical authenticity of yerba santa (*Eriodictyon*) and its allied species. Yerba santa is commercially available as a raw dry herb for making tea, capsules, and liquid extracts; however, the safety and possible side effects are largely unknown. More than 145 samples from eight *Eriodictyon* species were investigated using NMR and HPTLC techniques to address these issues. Significant metabolite variations within and between the different species were observed and characterized. The species-specific fingerprint patterns for the different *Eriodictyon* species were determined. Moreover, as a part of a comprehensive investigation of phytochemicals present in *E. californicum,* a.k.a. California yerba santa, more than thirty compounds were isolated and identified from the leaves of *E. californicum*, of which ten were identified for the first time. In addition to these efforts, an ultra-high performance liquid chromatography with diode-array detection and quadrupole time-of-flight mass spectrometry (UHPLC/DAD/Q-ToF) analytical method was developed to gauge the chemical characterization and bioactive compound variation within yerba santa species. The developed method demonstrated excellent

linearity (R^2>0.99), sensitivity and could be applied to probe the quality of raw materials used in several yerba-based supplements in commerce.

In another example to address the potential food product safety concerns, morphine and codeine are the principal opiates in the opium poppy (*Papaver somniferum* L.), used for pain management, and seeds with low opiates are used for culinary purposes. Intentional adulteration of poppy seeds is common, often with immature or exhausted poppy seeds or substituted with morphologically similar seeds, such as amaranth, quinoa, and sesame. Moreover, the simultaneous analysis of *P. somniferum* and its adulterants is largely unknown. Pre- and post-processing further complicate the alkaloid content and may pose a significant health hazard. Two independent methods were investigated to address these issues, with eight botanically verified and fifteen commercial samples of poppy seeds. Microscopical features were established for the authenticity of raw poppy seeds from others. Morphine, codeine, and thebaine quantities ranged from 0.8–223, 0.2–386, and 0.1–176 mg/kg, respectively, using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QToF). In most cases, these three opiates were higher than papaverine and noscapine. The methodology provides anatomical and chemical profiles that can be effectively applied to distinguish poppy seeds from their adulterants and may serve as an effective tool to combat ongoing adulteration or could serve as a confirmatory and validation tool to substantiate the results obtained from handheld devices used at international mail facilities to examine seized drugs in mail parcels.

In another project, to investigate safety concerns of cannabidiol (CBD) and probe the quality of CBD in various personal care products (PCP), 233 products collected by CFSAN were analyzed for CBD, delta-9 THC, CBDA, THCAA, and other cannabinoids, namely CBDV, CBG, CBGA, THCV, CBN, delta-8 THC, and CBC. Even though all 233 products are touted to contain CBD, 3% were found to be completely devoid of cannabinoids, and *only* 12% were identified with only

CBD. The remaining products (84%) were identified as containing CBD and other cannabinoids at detectable levels, with 11% of those products identified to contain delta-9 THC, which is not claimed on the product's label. Further validation and reproducibility of inter- and intra-day recoveries and other analytical parameters are ongoing, and the results will be disseminated shortly. This research was funded through CFSAN's Cooperative Agreement with the NCNPR.

Safety of Botanical Ingredients in Cosmetics and Other Personal Care Products

Botanical extracts and single compounds derived from botanicals are increasingly used in cosmetics, fragrances, and other personal care products. These products have developed into a burgeoning commercial market by targeting consumers with the products' "natural" appeal. Allergic contact dermatitis (ACD), skin irritation, and phototoxicity are the most common adverse effects reported for such products. For these reasons, regulatory agencies face the challenge of the safety assurance of botanical ingredients in cosmetics, and the lack of suitable, validated, non-animal testing methods is a further complication. Under the current program, the long-term goals include applying a combination of non-animal alternative methods to identify potential skin sensitizers and applying analytical methods to authenticate, characterize, and quantify candidate compounds of concern within the botanical ingredients.

The continuing employment of two *in-chemico* methods involving depletion of dansyl cysteamine (HTS-DCYA and NMR-DCYA) to evaluate skin sensitization along with three regulated methods direct peptide reactivity assay (DPRA), KeratinoSens, and human cell line activation test (hCLAT) have been established as routine methods at the NCNPR as part of a long-term commitment to producing cohesive testing data on chemicals that are typically associated with fragrances, cosmetics, and other personal care products. For example, differential skin sensitization potentials of two structurally similar compounds, eugenol and isoeugenol, were addressed with *in-chemico* methods. Ambiguity in increased skin sensitivity of isoeugenol was effectively addressed by identifying oxidation metabolites and determining structural information on intermediate species through their role in depleting dansyl cysteamine. A plausible rationale for isoeugenol's strong skin sensitization was proposed based on a hydroxy quinone methide as a reactive intermediate rather than the previously assumed quinone methide.

In addition to botanicals, our research mission has expanded to analytical investigations of per- and polyfluoroalkyl substances (PFAS) and acetyl hexapeptides in various cosmetic products to ensure overall quality and safety. For example, a UHPLC-MS/MS method was developed to separate and determine 16 PFAS compounds that are integral to many cosmetics and personal care products. The test sample was fortified with isotopically labeled surrogates before the extraction; then, the PFAS compounds were extracted from the cosmetic samples and further enriched using solid-phase extraction. The resulting sample was filtered, spiked with the internal standard solution, and analyzed using LC-MS/MS. The PFAS compounds were identified by multiple reaction monitoring (MRM) transitions and retention times matching the calibration standards. Ion ratios, when available, were used to confirm their identity. A total of 176 cosmetic samples were tested. Out of 16 suspected PFAS compounds, 13 analytes were identified. Only three of the analytes were not found in any of these products. More than 70% of the samples contained 6:2 FTSA (1H,1H,2H,2H-perfluorooctanesulfonic acid). Confirmatory and validation experiments are ongoing.

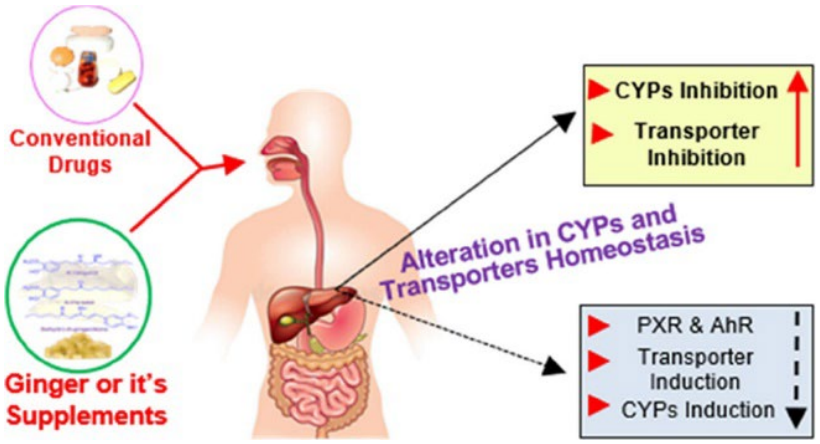
The NCNPR is also investigating the quality of six hexapeptide analytes within the cosmetic samples, analyzed using LC-MS/MS and identified by multiple reaction monitoring (MRM) transitions and retention time matching with calibration standards. Ion ratios were used to confirm their identity. Of the 77 cosmetic products tested with LC-MS/MS, 54.5% contained at least one of the six targeted peptide analytes. At the same time, all 77 cosmetic products listed acetyl hexapeptide-8 (AHP-8) as an ingredient on their label, but only 1/3 of the products contained detectable levels of AHP-8. This research was funded through CFSAN's Cooperative Agreement with the NCNPR.

Adverse Effects of BDS – Modulation of Drug-Metabolizing Enzymes and Transporters (DMET) and Implications to Herb-Drug Interactions

The last three decades have witnessed a surge in the consumption of herbal products. Chronic consumption of these products can often modulate the functions of various proteins and may, in turn, impose risks for herb-drug interactions (HDIs), leading to serious adverse health outcomes. The main goal of this work is to assess the two broad mechanisms underlying most pharmacokinetic herb-drug interactions – natural product-mediated xenobiotic-metabolizing enzyme/transporter inhibition or induction. Several botanical extracts, fractions, and principal components were evaluated for both herb-drug and herb-herb interactions for this reporting period.

The Pregnane X receptor (PXR) recognizes and detoxifies diverse xenobiotics encountered by humans. To comprehend the ability of PXR to bind various ligands and aid in dereplicating toxicological agents while mitigating the animal testing requirement, the NCNPR investigated the applicability of predictive machine learning quantitative structure–activity relationship (QSAR) models using 500 phytochemicals. QSAR data analysis revealed that machine-learning 3D-QSAR techniques were more accurate in predicting the activity of external terpenes (dietary PXR agonists) with a correlation coefficient (R^2) of 0.70 versus an R^2 of 0.52 in machine-learning 2D-QSAR. Robust QSAR models in this study provide a template for assessing PXR agonism from various chemical backbones to aid in the identification of potential causative agents in complex mixtures.

Herb-Drug Interactions: Several dietary supplements containing *Bulbine natalensis* are sold as "natural testosterone boosters." No scientifically valid clinical data (efficacy or safety) are available in the public domain. Continuing our earlier work on *B. natalensis*, both extract and its constituents were investigated for their ability to inhibit the P-glycoprotein (P-gp) transporter with the help of Rhodamine123 uptake assay. Interestingly, the extract and two of the seven constituents showed statistically significant, dose-dependent inhibition of P-gp in MDR1-MDCK and Caco-2 cells. Further functional analysis revealed that these two compounds lack P-gp induction even though these compounds marginally induced PXR activation, highlighting the clinical significance of selective inhibition of transporters. The harmonic relation between bulbine constituents in these supplements and their P-gp inhibitory profiles substantiated the potential adverse effects of such supplements if consumed concomitantly with pharmaceutical drugs.



Ginger (*Zingiber officinale*) is one of the most popular herbs commonly added to foods, beverages, and dietary supplements. The NCNPR evaluated the ability of a ginger extract and several of its phytoconstituents to modulate select nuclear receptors and cytochrome P450s, ATP-binding cassette (ABC) transporters. Our results revealed that ginger extract activated the aryl hydrocarbon receptor (AhR) in AhR-reporter cells and the pregnane X receptor (PXR) in intestinal and hepatic cells. Two ginger phytochemicals activated AhR, while two others activated PXR. Enzyme assays showed that ginger extract and its phytochemicals dramatically inhibited the catalytic activity of cytochrome P450 (CYP3A4, 2C9, 1A2, and 2B6), and efflux transport capabilities of P-gp and breast cancer resistance protein (BCRP). The phytochemical-mediated modulation of these proteins underlies many clinically relevant HDIs.

Herb-Herb Interactions: Tens of thousands of multi-botanical dietary supplements containing ingredients from several herbs are readily available. Consumption has often outpaced adequate scientific understanding of potential herb-herb and herb-drug interactions. The NCNPR is proactively investigating the most prevalent herbals in polyherbal dietary supplements to gauge herb-herb interactions. Over 120 plants were extracted with 95% ethanol and assayed for potential agonistic effects on nuclear receptors (PXR and AhR) and inhibitory effects on cytochrome P450 (CYP450) and p-glycoprotein. Out of 123 plants, 16 increased transcriptional activity of PXR, while 18 plants increased AhR activity. Thirteen plants inhibited the activity

of CYP 3A4, while 10 plants inhibited 1A2 activity. Additionally, other plants tested in this study could activate PXR, AhR, or both to lesser extents, and several inhibited the catalytic activity of CYPs at higher concentrations. The results indicate that herb-herb interactions resulting from prolonged or excessive consumption of herbal preparations rich in such plants may pose a risk for CYP- and P-gp-mediated HDIs, leading to unwanted side effects due to the altered pharmacokinetics of concomitantly ingested medications. This research was funded through CFSAN's Cooperative Agreement with the NCNPR.

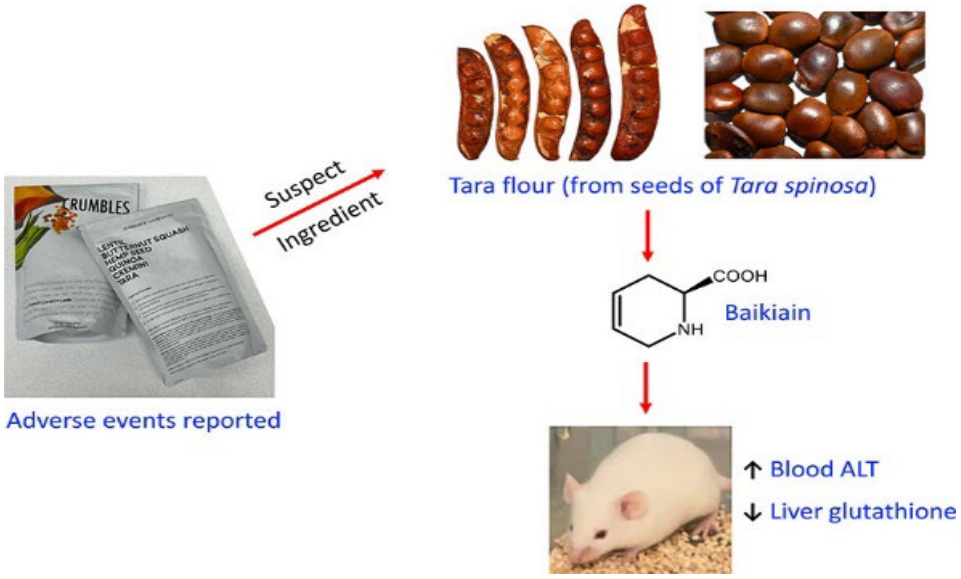
Public Awareness of Emerging Problems Associated with Botanicals

The NCNPR, in association with the Office of Regulatory Affairs (ORA), has provided hands-on training to FDA inspectors for current good manufacturing practice (cGMP) compliance issues associated with botanical ingredients (FD340). We conducted two training sessions in 2022-2023, adding 49 trained FDA inspectors as part of our long-term commitment. More than 1100 inspectors have been trained under these collaborative efforts to provide technical, educational, and hands-on experience for potential aspirants.

The NCNPR hosted the 21st Annual International Conference on the Science of Botanicals (ICSB) on April 24–27, 2023. Over 260 participants representing 20 countries heard informative and stimulating presentations from 66 speakers from academia, government, industry, and the media. Additionally, 85 poster presentations highlighted contemporary research from over 290 contributors. The ICSB is made possible through the support of our sponsors, including CFSAN, the involvement of national and international colleagues, and the dedication of our volunteers.

Global Impact of the Supplementary Role of Research Conducted by the NCNPR

The NCNPR was tasked to determine the potential causative agents associated with a recall of around 28,000 product units due to almost 400 reports of adverse illness events and 133 hospitalizations reported from the consumption of lentil crumbles. The NCNPR undertook a multipronged investigation and identified that tara flour manufactured from the *Tara spinosa* tree could be a potential source of causative agents.



Initial analytical confirmation indicated that the product contained no common food toxicants, adulterants, common toxic plant microbes, or heavy metals, corroborating the results published by CFSAN. NMR analysis established the identity of the flour, and chemical/histochemical analysis showed no contamination by other tara plant parts. A non-protein amino acid, baikiain, was identified as a compound of interest due to its abundance, possible metabolic fate, and close resemblance to irreversible inhibitors of *L*-pipecolate oxidase. Oral administration of baikiain in ND4 mice showed a statistically significant increase in blood ALT levels and a reduction in GSH levels. This work linked detailed chemical analyses and *in vivo* studies to address food product safety concerns. The NCNPR's resources, including infrastructure and technical personnel, were instrumental in addressing this issue in a timely manner.

Joint Institute for Food Safety and Applied Nutrition (JIFSAN) - University of Maryland, College Park

The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) was established in 1996 at the University of Maryland, College Park (UMD). The Institute is a jointly administered, multidisciplinary research, education, and outreach program. The research program includes genome sequencing and genomic analysis, bioinformatics, foodborne pathogens, development of training metrics, and risk assessment modeling. Additionally, JIFSAN's undergraduate internship program supports the science and research programs at CFSAN. JIFSAN's education and outreach programs serve the FDA internally, domestically, and internationally. The International Training Center is a train-the-trainer program and includes Food Safety Preventive Controls Alliance (FSPCA) and Produce Safety Alliance (PSA) training. It also provides training on Good Agricultural Practices (GAP), Good Aquacultural Practices (GAqP), Good Fishery Vessel Practices (GFvP), Commercially Sterile Packaged Food (CSPF), Food Inspector Training Course (FIT), and Collaborative Food Safety Training Centers. The Food Safety Risk Analysis Professional Development Program provides courses that focus on risk assessment methods and analysis to address food safety issues worldwide, and hosts FoodRisk.org that offers online resources for food safety risk analysis.

JIFSAN Director – Dr. Jianghong Meng
CFSAN Project Officer – Dr. Kelly M. Randolph

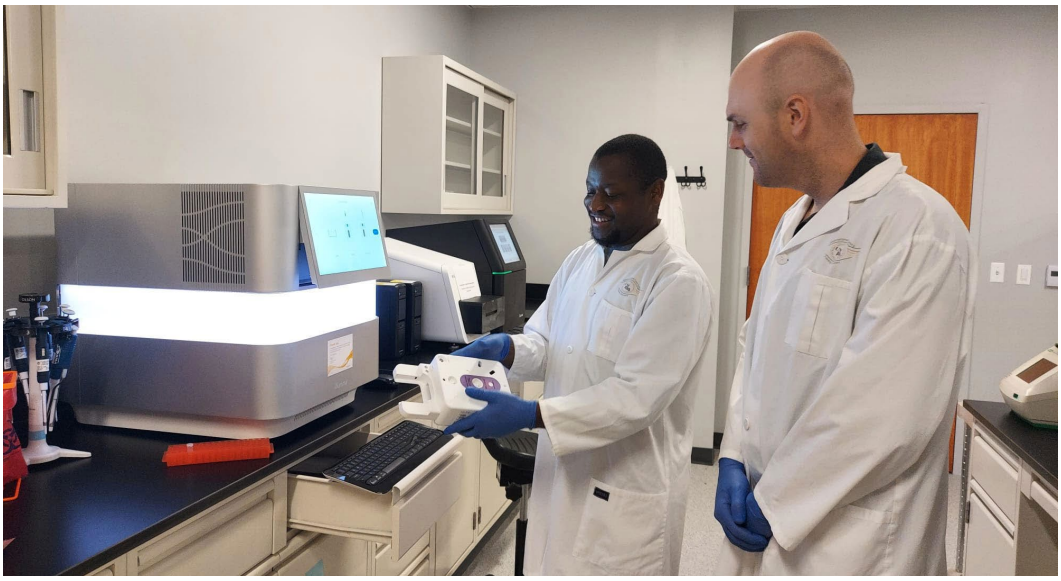
Salmonella enterica in Surface Water – JIFSAN and International Collaborators

Surface water is one of the primary sources of irrigation for agricultural production; as a result, contamination with infectious pathogens such as *Salmonella* can significantly impact food safety and public health. This project was launched to ascertain the presence of *Salmonella* in Latin American surface water and to collect whole genome sequencing (WGS) data to enrich the global genomic database of pathogens that cause foodborne illness. A total of 3,816 water samples have been obtained; Brazil showed the highest prevalence of *Salmonella* in the water samples (70.9%), followed by Mexico (63.4%) and Chile (33.2%). This resulted in over 7,000 *Salmonella* enterica isolates, including 4,035 isolates from Chile, 1,894 from Brazil, and 1,211 from Mexico. Among the isolates, 4,271 have been sequenced, including 1,999 from Chile, 1,313 from Brazil, and 959 from Mexico. Comparative genomic analyses of 1,541 *Salmonella* isolates collected from surface waters in the three countries showed highly diverse isolates with over 100 serotypes. The project was expanded to investigate the interactions among microbes and with the environment in 2022. A total of 404 samples for metagenomic study have been included. Bioinformatic analyses of these samples revealed that the genera *Salmonella* and *Listeria* were detected in over 50% of the samples. The two laboratories in Chile have also recovered *Listeria monocytogenes* from surface waters since 2019. A total of 1,042 isolates of *L. monocytogenes* have been obtained, of which 120 isolates have been sequenced. This study provides a comprehensive analysis of *Salmonella* in the surface waters of Latin America associated with the production of fruits and vegetables. The data will contribute to expanding the global WGS database, assess the distribution and subtypes of *Salmonella* in these waters, and

provide insight into the microbial interaction that affects *Salmonella*'s persistence in the water and environment. This research was funded through CFSAN's Cooperative Agreement with JIFSAN.

Food Safety Microbiology Lab

The JIFSAN Microbiology Lab offers a venue for fostering research collaborations among UMD faculty, students/postdocs, FDA researchers, and visiting scientists. It is also an important resource for training in cutting-edge technologies like WGS. In addition to supporting the *Salmonella* in Surface Water project, several faculty members, along with their students and postdoctoral researchers from the Department of Nutrition and Food Science and the Department of Plant Science and Landscape Architecture, have engaged in WGS-related collaborative research in the lab. Their work spans a wide spectrum, including a water metagenomics study, the exploration of intricate ecological factors contributing to disease development in grapevines, and the examination of dynamics between fungicide-resistant and wild type *Colletotrichum* in berries. An international visiting student from Brazil successfully conducted her WGS research on 140 *Salmonella* isolates linked to outbreaks in poultry. Furthermore, the lab has been actively engaged in planning WGS training sessions for the GenomeTrakr network and Indonesia. Beyond these efforts, it has played a crucial role in offering technical and logistical support to CFSAN's WGS training and capacity-building initiatives in India, Indonesia, and Mexico, further solidifying its commitment to advancing scientific knowledge and expertise in this field. This facility was funded through CFSAN's Cooperative Agreement with JIFSAN.



The United States Department of Agriculture (USDA) - FoodData Central

FoodData Central (FDC) is a modern website that grants users unprecedented access to food composition data (https://fdc.nal.usda.gov). FDC provides users that are browsing Foundation Foods with links and tabs showing measurements and metadata of individual samples contributing to each food that they select. Working with the data company, Inonde, JIFSAN helped ensure that FDC included the features that USDA desires. In 2022-2023, JIFSAN continued to work on changes to improve data presentation, user experience, and application performance. This included working with Health Canada to pilot the publication of their food items to FDC, data quality documentation to detail the way data is being handled in the JIFSAN systems, and populating foods in the Child Nutrition Food Programs trade channel to the FDC Application Programming Interfaces (API) for publication to the Child Nutrition Database. This work was funded through USDA and CFSAN Cooperative Agreements with JIFSAN.

Congressional Mandate for Imported Shrimp

Under the 2021 Appropriations Act, Congress mandated the FDA to explore measures aimed at enhancing the safety of imported shrimp, including the establishment of international seafood agreements with the three leading shrimp exporters to the United States. To fulfill this directive, JIFSAN took the initiative to administer comprehensive training programs in Seafood HACCP, Seafood Sanitation, and Good Aquaculture Practices (GAqP) in India, Indonesia, and Ecuador. In a collaborative partnership with the FDA, JIFSAN conducted thorough needs assessments in Ecuador, India, and Indonesia. These assessments served as the foundation for custom-tailored training programs, uniquely designed to address the specific requirements and challenges of each country. Through these programs, we provided training to in-country professionals, equipping them with the skills and knowledge needed to proactively prevent, manage, and mitigate food safety hazards. Notably, this initiative has not only strengthened the capabilities of these host

countries but has also served as an incentive for them to conduct their own training initiatives, with the goal of elevating their food safety practices. This collective effort can help significantly reduce food safety risks associated with imported shrimp to the United States. Over the course of the period from September 2022 to August 2023, a total of 13 training courses were successfully executed, with five taking place in Ecuador, three in India, and five in Indonesia. These courses attracted a diverse group of participants, including representatives from industry, government agencies, and academic institutions.

Good Aquaculture Practices (GAqP) Training: JIFSAN's GAqP program provides a technical approach to preventing disease and food safety risks as well as demonstrates how to apply GAqP concepts to developing an aquaculture food safety plan. The GAqP training for this initiative was updated to focus on aquaculture shrimp food safety and the training was conducted in India and Indonesia. JIFSAN also collaborated with in-country partners to provide an overview of each country's current aquaculture farm food safety program and current perspectives on shrimp diseases.

Seafood Sanitation Training: This program was developed to provide a critical overview on proper food processing sanitation methods and constructing sanitation standard operating procedures (SSOPs) to improve safety and increase production restrictions necessary to prevent and minimize the impact of disease on aquaculture seafood. JIFSAN provided two Seafood Sanitation courses in India and two in Indonesia. The purpose of the trainings were to improve compliance with U.S. laws and regulations related to seafood sanitation in processing facilities.

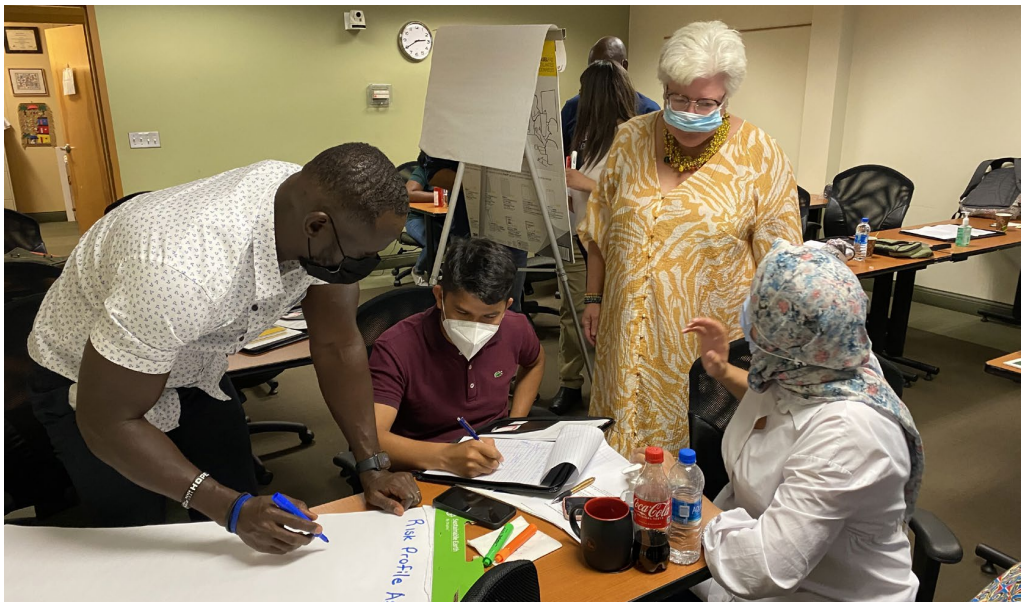
Seafood HACCP Train-the-Trainer (TTT) & Sanitation Control Procedures (SCP) Training: The Seafood HACCP TTT course was developed to prepare participants to become qualified Seafood HACCP Alliance instructors to then conduct their own in-country standard Seafood HACCP and accompanying SCP courses in accordance with their established protocol recognized by AFDO and the FDA. JIFSAN conducted a joint Seafood HACCP TTT and SCP training in Ecuador.

FDA Imports Operations Training Course: The FDA Office of Regulatory Affairs (ORA) Import Operations course was developed to provide a general overview of U.S. import operations to include the import process and FDA regulation requirements for imported products. FDA-ORA and JIFSAN jointly presented an in-country one-day ORA Import Operations training course in Indonesia.

In addition, JIFSAN provided logistical support for Sensory Decomposition and Environmental Water Sampling courses which were given by FDA personnel. Four Sensory Decomposition courses were conducted in Ecuador. This training provided knowledge on sensory analysis of seafood as a critical tool used to protect consumers from seafood that has become adulterated due to decomposition. The Environmental Water Sampling training was conducted in Indonesia and taught the latest techniques in the application of collecting water samples and processing these samples for the isolation of *Salmonella enterica*. These techniques are used to understand ecological relationships and environmental conditions on farms that impact food safety as well as surveillance of the geographical variation of foodborne pathogens in water for agricultural use. This training allows for planning the next steps, including sampling, sequencing, and participation in GenomeTrakr. This work was funded through CFSAN's Cooperative Agreement with JIFSAN.

Risk Analysis Trainings

Summer Integrated Program (SIP): The 2023 SIP Core classes were conducted online from June 1 to June 22, offering a comprehensive curriculum including Introduction to Risk Analysis in the Regulatory Process, Risk Management, Qualitative Risk Assessment, and Risk Communication. The advanced courses, namely Introduction to i-Risk® and Quantitative Risk Assessment, were rescheduled for September. The program registered a total of 26 participants with 40 class seats. The Introduction to Risk Analysis in the Regulatory Process and Risk Management classes each had six attendees, Qualitative Risk Assessment had nine participants, and the Risk Communication class drew 19 participants. The advanced courses have already secured the enrollment of 11 individuals to date. While most participants in the SIP Core classes were from the United States, it also attracted participants from various corners of the world, including Italy, Jamaica, Japan, Brazil, South Africa, Norway, and Taiwan.



JIFSAN-PolyU in Hong Kong: The Principles of Food Safety Risk Management course was taught to students from Hong Kong Polytechnic University on Friday and Saturday mornings during the months of September and October. The goal of the course was to teach students how risk analysis fits into food regulation and the basics of food safety risk management. In 2022, 27 students participated in this training, which has been provided to groups coordinated by Hong Kong Polytechnic University since 2019.

Regulatory Economics for Risk Management: In conjunction with the George Washington University Regulatory Studies Center, USDA Office of Risk Assessment and Cost-Benefit Analysis, and the Society for Benefit Cost Analysis, JIFSAN held a pilot course in "Regulatory Economics for Food, Health, and Environmental Risk Manager" via Zoom. The course was held Tuesday and Thursday afternoons the month of February with 17 participants representing U.S. government agencies.

Cost-Effective Risk Reduction Strategies for Foodborne Pathogens in Aquaculture/Risk Analysis and the Regulatory Process Trainings: "Foodborne Pathogens in Bangladeshi Aquaculture Value Chains and the Most Cost-Effective Risk Reduction Strategies" was presented in Bangladesh from December 11-20. This project pulled together a group of researchers from Washington State University; International Centre for Diarrheal Disease Research, Bangladesh; University of Maryland Eastern Shore; Centers for Disease Control and Prevention; Ministry of Fisheries and Livestock, Bangladesh; and Centre of Integrated Rural Development for Asia and the Pacific. In addition, a one-day training on Risk Analysis and the Regulatory Process was presented to 40 participants at the Bangladesh Food Safety Authority. The SIP program is tuition-based and fully funded by tuition. The other programs were funded by the sponsoring organizations.

Undergraduate Student Internship Program

JIFSAN provides a unique internship program designed to offer undergraduate students at UMD an opportunity to work with FDA scientists on specific projects related to food safety, applied nutrition, and public health. These research- and programmatic-related training opportunities enhance the students' knowledge of and experience in science, particularly in a regulatory environment, and familiarize them with career opportunities in the regulatory sector of public service. JIFSAN recruits students from across academic disciplines, who are full-time UMD undergraduates and have completed two college semesters. During the 2022-2023 funding period, 17 undergraduate students participated in the JIFSAN Internship Program during the fall and spring, logging in over 5,400 hours. In June 2023, a new cohort of 20 undergraduate students began their internship and have contributed over 4,500 hours thus far. Interns have produced posters, presentations, and contributions to peer-reviewed publications. This program was funded through CFSAN's Cooperative Agreement with JIFSAN.

Institute for Food Safety and Health (IFSH)/National Center for Food Safety and Technology (NCFST) - Illinois Institute of Technology

The National Center for Food Safety and Technology (NCFST) was established in 1988 at the Illinois Institute of Technology's (IIT) Moffett Campus in Bedford Park, IL, to bring together scientists from the FDA, academia, and industry to work collaboratively on food safety issues. The NCFST is a part of IIT's Institute for Food Safety and Health (IFSH) and is a unique food research consortium of IIT faculty and students, CFSAN's Division of Food Processing Science and Technology (DFPST), and food and food-related industries. NCFST's research addresses the safety of processed foods, food safety implications of emerging technologies in food processing and packaging, and laboratory method performance. In addition to the NCFST, other Centers within the IFSH structure include the Center for Processing Innovation, the Center for Nutrition Research, and the Center for Specialty Programs. IFSH also coordinates FSMA training programs through the IFSH-led Food Safety Preventive Controls Alliance (FSPCA) and Sprout Safety Alliance, including Preventive Controls for Human Food, Preventive Controls for Animal Food, Foreign Supplier Verification Programs (FSVP), Intentional Adulteration, and Sprout Safety. The FSPCA also provides a Technical Assistance Network (TAN) to industry on inquiries which are not related to FSMA rule interpretation.

IFSH Executive Director - Dr. Brian Schaneberg
IFSH Associate Director – Dr. Jason Wan
CFSAN Project Officer - Dr. Les Smoot

Evaluation of Foodborne Pathogen Survival on Dehydrated and Rehydrated Enoki and Wood Ear Mushrooms

Only sporadic foodborne outbreaks associated with mushrooms (fresh, dried, or unspecified) due to contamination with bacterial pathogens have occurred in the U.S. However, in 2020, a multistate outbreak due to *L. monocytogenes* contamination of enoki mushrooms occurred, which resulted in a total of 36 illnesses, 31 hospitalizations, and four deaths. The outbreak prompted an Import Alert and subsequent sampling in 2021 of imported mushrooms from South Korea and China. This was the first known outbreak due to *L. monocytogenes* contamination of a fungus. The lack of listeriosis outbreaks is interesting as studies have identified persistent strains of *L. monocytogenes* in mushroom production and processing facilities. Due to this fact and the recent enoki mushroom outbreak, this study aims to understand the survival of *L. monocytogenes* on different mushroom varieties. In addition, *S. enterica* was also evaluated due to the recent dried wood ear mushroom outbreak. Both foodborne pathogens were examined on enoki and wood ear mushrooms. Different preparations were also evaluated including fresh, fresh-cut, dehydrated, and rehydrated. Both mushroom types were inoculated at 3 log colony-forming unit (CFU)/g and stored for 7 d at 5, 10, or 25°C. For 5 and 10°C, both pathogens survived on whole and cut enoki and wood ear mushrooms with no significant change in population during storage. At 25°C, significant increases in populations were observed for both pathogens on both mushroom varieties. For whole and cut wood ear mushrooms, *L. monocytogenes* increased by

2.24 and 1.08 log CFU/g during storage at 25°C, respectively; *S. enterica* increased by 3.68 and 4.71 log CFU/g, respectively. The results of this study will aid in informing guidelines on proper time and temperature control for safety for mushrooms. This research was funded through CFSAN's Cooperative Agreement with IFSH and the CFSAN/DFPST operating budget.

Factors Affecting Growth and Survival of *Salmonella* on Packaged Fresh Peaches

Following the 2020 *Salmonella* Enteritidis outbreak linked to peaches, FDA issued an investigative report detailing several factors potentially contributing to contamination of the peaches at the farm and packinghouse levels. The outbreak strain of *Salmonella* was not found on fruit or leaves in the implicated grower's orchard. However, other *Salmonella* strains were found on the peach tree leaves; the strains identified were associated with nearby poultry farming operations. Together, analyses of the geographic surveys and WGS analysis of isolates informed the idea that airborne transmission of fugitive dust from these farms could be the source of the contamination. As *Salmonella* and peaches are considered a novel pathogen/commodity pair linked to this outbreak, an understanding of the ability of *Salmonella* to survive and potentially proliferate in pristine and blemished fresh peaches in package microenvironments at temperatures seen during post-packing and retail display is needed to better implement preventive controls and mitigate consumer risk. Additionally, assessment of the differences and similarities of the peach leaf and fruit microbiomes from various geographical locations may provide some indication that peach tree leaves could be used as a surrogate for peach fruit when determining the influence of nearby dust-generating farming operations. The influence of differences in peach tree leaves and fruit microbiomes during enrichment could also explain why the implicated outbreak strain was not found on the leaves. An examination of how these microbiomes influence *Salmonella* detection using the FDA Bacteriological Analytical Manual (BAM) enrichment techniques may provide insight into this problem and allow modification of the procedure to improve recovery. Thus far, examination of the growth and survival of dust-inoculated *Salmonella* on fresh peaches stored at different temperature and relative humidity (RH) combinations and in three different packaging types has been completed. Fresh peaches were either left intact or blemished and inoculated at 4-5 log CFU/peach using *Salmonella*-contaminated silica dust. Peaches were transferred to plastic clamshells, plastic produce bags, or cardboard trays and stored at 5°C/95% RH, 18°C/45% RH, or 25°C/45% RH. Peaches were stored for up to 28 d at 5°C and 14 d at 18 and 25°C. During 5 and 18°C storage, *Salmonella*

survived without significant decreases in population. During 25°C storage, the *Salmonella* populations decreased in peaches regardless of packaging container. Results from this study provide information on how *Salmonella* survives during both retail display and consumer storage of peaches. Data suggest that the temperature-humidity combination used for storage of fresh peaches plays a role in *Salmonella* survival more than the packaging container. Work is underway examining the population dynamics and relative abundance of *Salmonella* serovars during enrichment of peaches and leaves. Results from this study will aid in understanding the conditions that will allow survival or growth of *Salmonella* on packaged fresh peaches. In addition, metagenomics studies may be able to determine if peach tree leaves can act as a surrogate for the peach microbiome in instances where peaches are not available for analysis. This research was funded through CFSAN's Cooperative Agreement with IFSH and the CFSAN/DFPST operating budget.

Transfer of Seafood Allergens to Frying Oil and Subsequent Fried Products

Breaded and battered shrimp accounted for 21% of the major types of seafood purchased in the U.S. Batch and continuous fryers are commonly used to produce par-fried battered or breaded seafood, which is subsequently frozen. Some manufacturers of breaded and battered seafood also use the same fryers and oil to produce other par-fried foods such as French fries, raising concerns about potential allergen cross-contact due to oil reuse. The major goals for this project during the past year were to 1) develop shrimp protein extraction methods from used oil, 2) validate protein extraction efficacy, 3) investigate the transfer of shrimp proteins from reused oil to French fries cooked in a batch fryer, and 4) evaluate oil post-treatments on allergenic protein removal from frying oil. The results from this study demonstrated that the shrimp proteins can be transferred to frying oil and a subsequent prepared food. However, the amount of allergenic protein transferred were likely underestimated due to the decreased extractability and detectability of tropomyosin after frying. Future research will involve 1) improving recovery of shrimp proteins in reused frying oil, 2) performing shrimp tropomyosin quantification using alternative methods, 3) investigating the transfer of gluten from breaded shrimp into reused oil and a secondary food, and 4) determining the transfer of allergens and gluten to frying oil and another food product in a continuous fryer. This research was funded through CFSAN's Cooperative Agreement with IFSH and the CFSAN/DFPST operating budget.

Clostridium botulinum Challenge Study in Commercially Prepared Cold Brew Coffee

Several studies have demonstrated the antimicrobial properties of hot brew coffee and certain compounds have been identified as exerting an inhibitory effect on gram-positive and gram-negative organisms. Unlike traditional hot brew, cold brew is prepared by brewing the grounds at ≤25°C for approx. 8 to 36 hrs. Since the temperature greatly affects the aqueous solubility of compounds, the chemical composition and antimicrobial activity of cold brew extracts likely differ from that of hot brew. Although several studies have been conducted on the antimicrobial activity of hot brew, to our knowledge there are no reports on the inhibitory effect of cold brew on the growth of *C. botulinum*.

Five type A (69-A, CAM2-A, Clovis-A, Giorgio-A, CDC-CR1-A) and five proteolytic type B (Mush3-B, 6891-B, TJ-980B, NCA1-B, 7273-B) strains of *C. botulinum* were selected and enumerated for use in the 10-strain spore cocktail in the challenge study of cold brew coffee. A survey of 24 commercially available cold brew coffee products (black coffee) was conducted. These 24 products were evaluated for pH, water activity, Brix, titratable acidity, and total dissolved solids. Five products were chosen for a five-month challenge study based on high pH or low Brix (five additional products were made by diluting the five products with water, total dissolved solids between 0.48-0.69%). Coffee samples were inoculated with 3 log spores/ml with the 10-strain cocktail. The coffee products were sampled at time 0, one-month, three-months, and five-months. Water activity, pH, Brix, spore enumeration (MPN), aerobic plate count, anaerobic plate count, lactic acid bacteria, yeast and mold, and presence of botulinum toxin (Endopep-MS) were monitored for each sample time-point. One of the coffee products tested (pH 6.58, Brix 2.3, TDS 1.89%) produced *C. botulinum* toxin Type A and Type B at the three and five-month sampling points. The diluted version of this coffee (pH 6.71, Brix 0.8, TDS 0.64%) also produced Type A and Type B toxin at three and five-month sampling points. All other coffee tested did not produce toxin during the duration of the challenge study. The coffee that produced toxin is a shelf stable product that had been thermally processed and contained added potassium phosphates. The *C. botulinum* spore population increased from 3.2 log MPN/ml at Time 0 to 4.2, and 4.13 log MPN/ml for one-month and three-months, respectively, before dropping to 2.81 log MPN/ml at five-months. This suggests limited *C. botulinum* growth

produced toxin at three and five-months. Three additional lots of this coffee were purchased and inoculated with the 10-strain spore cocktail to confirm the initial results. All three lots tested produced botulinum toxin Type A and Type B at five and nine-months with no observed increase in the MPN/ml concentration of *C. botulinum*. Additional work will investigate the factors that led to this cold brew coffee product to support the growth and toxin production of *C. botulinum*. It is important to identify pH and Brix parameters, and chemical additives that could lead to a potentially hazardous product. This further research will provide the FDA Food Process Evaluation Team data to reference during the evaluation of cold brew coffee filings. Additionally, further research will use an untargeted approach using HPLC-Q-TOF-MS to identify differences in coffee stored under aerobic and anaerobic conditions to possibly identify compounds inactivating *C. botulinum* spores in coffee stored under aerobic conditions. This research was funded through CFSAN's Cooperative Agreement with IFSH and the CFSAN/DFPST operating budget.

Detection of *Salmonella* in Food Using FDA Real-Time qPCR Method and ABI 7500 Fast Real-Time PCR System: A Multi-Lab Validation Study

Since the FDA BAM *Salmonella* culture method takes at least three days for a presumptive positive result, the FDA developed a quantitative polymerase chain reaction (qPCR) method to detect *Salmonella* using the ABI 7500 PCR system. The multi-laboratory validation (MLV) studies in this case involved baby spinach and frozen fish. The study was aimed to measure the reproducibility of this qPCR method and compare its performance to the culture method.

The baby spinach MLV had sixteen participating laboratories with two rounds of study, and the frozen fish MLV had fourteen participating laboratories. For both MLVs, laboratories each analyzed twenty-four blind-coded test portions of either baby spinach or frozen fish by both the qPCR and culture methods in parallel (paired study). For the baby spinach study, the first round

of low-level test portions resulted in high positive rates that fell outside of the FDA's Microbiological Method Validation Guidelines of 25%-75%. Low-level test portions in the second round yielded ~68% and ~67% positive rates for qPCR and culture method, respectively. For the frozen fish MLV, low-level test portions resulted in a ~38% and ~40% positive rate for the qPCR and culture method, respectively. The relative level of detection (RLOD) was 0.969 for the second-round baby spinach study and 1.04 for the frozen fish study, both suggesting that qPCR and culture methods had statistically similar detection rates ($p > 0.05$). Reproducibility of the method was confirmed by both studies' standard deviation of laboratory effects ($\sigma \approx 0$) and inter-laboratory coefficients (ICC≈0). Overall, both studies demonstrated that the qPCR yields reproducible results and is sufficiently sensitive and specific for the detection of *Salmonella* in food. This research was funded through CFSAN's Cooperative Agreement with IFSH and the CFSAN/DFPST operating budget.

Western Center for Food Safety (WCFS) - University of California, Davis

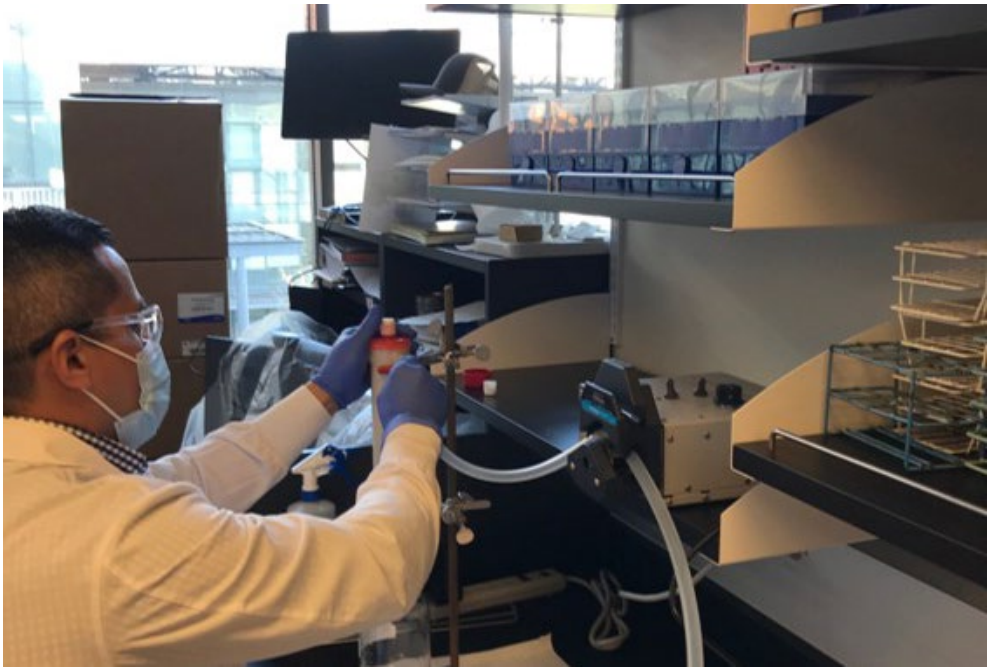
The Western Center for Food Safety (WCFS) was established in 2008 at the University of California, Davis, to address the development of approaches and data that are critical to understanding the risks associated with the interface between production agriculture and food protection. This information is used to develop scientifically validated "best practices" for mitigating risks at the production, harvest and postharvest (versus processing) level. In addition to research, the Center provides education, outreach, and technical assistance to food safety stakeholders. The WCFS' research portfolio includes projects related to exploring the sources of microbial contamination on fresh produce and nuts, including agricultural water and soil, as well as collaborations with other academic institutions to increase our understanding of best agricultural practices across varying agro-ecological landscapes. The WCFS' research and outreach efforts assist CFSAN and the food safety community in the implementation of FSMA provisions and regulations.

WCFS Principal Investigators – Dr. Robert Atwill and Dr. Linda J. Harris
WCFS Program Manager – Dr. Michele Jay-Russell
CFSAN Project Officers - Dr. Samir Assar and Heather Brown, Project Manager, GWCPM

Longitudinal Study to Investigate the Ecology and Epidemiology of Human Foodborne Pathogens in the California Central Coast

Between 2009 and 2018, FDA and CDC identified 40 foodborne outbreaks of Shiga toxin producing *E. coli* (STEC) infections in the U.S. with a confirmed or suspected link to leafy greens. In the winter of 2019, there were three *E. coli* O157:H7 foodborne illness outbreaks, A (167 illnesses), B (11 illnesses), and C (10 illnesses) associated with consumption of leafy greens from the Salinas Valley region of California. Of note, two unique *E. coli* O157:H7 outbreak strains have been detected repeatedly in distinct regions. The CDC has characterized these strains as reoccurring, emerging, and persisting (REP) strains, REPEXH01 and REPEXH02.

The California Longitudinal Study (CALS) was initiated in 2020, following publication of the Leafy Greens STEC Action Plan. CALS is a multi-year, "shoulder-to-shoulder" partnership between CFSAN, WCFS, the California Department of Food and Agriculture (CDFA), and numerous California agriculture industry partners. It is an environmental microbiology study that applies adaptive research strategies to address the recent outbreaks of *E. coli* O157:H7 associated with romaine lettuce and other leafy green crops grown in the central California coast. This new approach serves as a model to perform research in a large geographic area to better understand underlying causes of contamination in the production environment. The data generated by WCFS and CFSAN laboratories will help inform leafy green growers about the practices and mitigation strategies that are most effective to prevent foodborne illness. An important goal is to better define the importance of potential risks from adjacent farming operations (e.g., livestock operations, composters, viticulture), and characterize temporal and spatial trends of bacterial contaminants over time.



Metagenomic Analyses: Metagenomic analyses is a key tool being used in CALS. Unlike traditional bacterial culture methodology that detects one target, metagenomics involves broad analysis of DNA from a sample to detect membership in a whole microbial community. This technology may provide a more informed understanding of the water, sediment, soil, and air microbiomes, including how the microbiomes and *E. coli* populations compare across the region and temporally. The data may also provide clues to how factors like adjacent land use impacts the microbiomes and potentially STEC presence or persistence.

The CALS research team continued into the third year of the longitudinal study with successful sampling at a set of conventional and organic agricultural operations including multiple leafy green farms, composters, vineyards, and cattle ranches across the central coast. Additionally, approximately 30 public access sample sites along state or county roads were sampled in proximity these private agricultural operations. Research activities continued to implement the final *Standardized Methods and Sampling Plan* developed by FDA and WCFS scientists during the first years of CALS. The primary emphasis of CALS will continue to be on leafy green commodities grown and harvested during spring through fall in the California Central Coast, with a focus on the detection and molecular characterization of *E. coli* O157:H7 and other STEC from environmental samples. Based on initial findings, it was decided to discontinue *Salmonella* culture and focus primarily on STEC as CALS concludes. Analysis of a rich *Salmonella* dataset including metagenomics will be pursued by FDA and WCFS scientists.

2023 Major Flooding Events – Enhanced Sampling: Following record flooding in January and March 2023, WCFS collaborated with FDA to conduct additional sampling of flooded fields, pastures and watersheds to evaluate pathogen and metagenomic changes in environmental samples during active flooding These data will be compared with pathogen dynamics in pre-2023 and post-flood samples (through the 2023 growing season) to evaluate potential persistence (or die-off) of pathogens and impacts on the microbial communities. The enhanced flood sampling effort illustrates the "adaptive research" nature of CALS, and ability of the WCFS-FDA teams to respond rapidly to measure the potential impact on STEC occurrence in a major leafy green production area following a natural disaster.

From July 2020 to August 2023, approximately 3,600 samples were analyzed at WCFS laboratories to measure monthly to bi-monthly patterns of bacterial indicators, *Salmonella*, *E. coli* O157:H7, non-O157 STEC from environmental sources in the California Central Coast, especially the greater Salinas Valley region. Sample matrices included air (active and passive), water (river, creek, tailwater, reservoirs, and wells), sediment (usually paired with surface water samples), soil transects (fresh produce fields, vineyards), soil amendments (compost, manure and green waste feedstocks, heat treated poultry pellets), cattle and other livestock feces, wildlife feces (avian, mammal, fly pools), and other samples such as grape vine leaves (a proxy for dust). To date, approximately 1,000 STEC and *Salmonella* isolates have been recovered from CALS samples at WCFS laboratories and submitted to FDA's GenomeTrakr for WGS. Additionally, ~332 original samples (when available) were shared with FDA scientists for culture-independent metagenomics. Selected sample enrichments were also sent to FDA for culture-dependent metagenomics including 614 STEC enrichments and 1,299 *Salmonella* enrichments (modified buffered peptone water (mBPW), Rappaport–Vassiliadis (RV), and tetrathionate (TT)).

In summary, findings from the CALS project will support the development and improvement of environmental microbiology sampling and screening methods so that future studies related to produce safety can be supported by the most sensitive and reliable methodologies available. This longitudinal study will also generate new knowledge that is critical to addressing ongoing questions raised by recent *E. coli* O157 and other STEC outbreaks regarding the risk of microbial contamination from adjacent land uses, soil amendments, wildlife intrusion, irrigation water, and other key inputs into the produce production environment. Detailed WGS of hundreds of key bacterial isolates combined with metagenomic analyses of the associated microbiomes from key locations in this rich network of longitudinal environmental and biological samples will provide an unprecedented, detailed map of the fine-to-large scale mechanisms responsible for transporting these pathogenic strains of *E. coli* from key reservoirs into fields of produce in the Salinas Valley. We anticipate that results from this collaboration will lead to improved practices to prevent or mitigate food safety risks, and ultimately enhance the safety of the many millions of annual servings of leafy greens grown and shipped from California. The project addresses FDA's 2020 Leafy Greens STEC Action Plan (the Plan; https://www.fda.gov/food/foodborne-pathogens/2020-leafy-greens-stec-action-plan), and builds on current efforts in the Yuma, Arizona leafy greens growing region. This research was funded through CFSAN's Cooperative Agreement with WCFS.

San Joaquin-Sacramento Dairy Shiga Toxin-Producing *Escherichia coli* (STEC) Survey

Based on estimates from the Environmental Protection Agency (EPA), over 50 billion pounds of dairy manure are produced by California's dairy industry each year, with much of this organic waste used in crop agriculture and to produce livestock forages. The aim of this project is to generate a detailed profile of the major STEC genotypes circulating within this large concentration of California dairy cattle. Presence of STECs have been readily documented in dairy manure. This STEC reservoir may function as a biological source for central coastal California due to importation of dairy manure into the Salinas leafy green growing region, possibly contributing to the sporadic occurrence of STECs when dairy manure is used as a soil amendment in produce production.

To date, WCFS researchers have visited and collected samples from 18 dairy farms located in 13 cities throughout the Central Valley, California. During a 12-month period (September 2022 – August 2023), fresh manure and flush slurry samples were collected monthly from enrolled farms for testing *E. coli* O157 and non-O157 STEC. In total, 744 fresh manure and flush slurry samples were collected from enrolled farms. Additionally, 32 samples of pen floor soil, fresh manure and stacked manure were collected from four farms and sent to FDA for metagenomics. *E.coli* O157 and STEC isolates were sent to FDA bi-monthly for whole genome sequencing and other analysis. A database of information of farm enrollment, samples collection and testing and isolates of O157 and STEC was created and shared with FDA.

Despite the low occurrence of *E. coli* O157 in the current dairy STEC survey, phylogenetic analysis (performed by FDA) indicates potential links of dairy manure STEC with historical human clinical strains. The current survey provides preliminary data on the structure of STEC genomic diversity within this large biological reservoir in the largest dairy production region of the country. Continued sampling is proposed for one to two additional years in order to generate clearer insights into this large STEC reservoir, mechanisms of environmental dissemination (leakage) of STECs off the dairy, more detailed mapping of the genomic diversity of dairy-origin STEC, and better characterization of the seasonal dynamics of STEC occurrence in the nation's primary dairy production region. The project addresses FDA's 2020 Leafy Greens STEC Action Plan (the Plan; https://www.fda.gov/food/foodborne-pathogens/2020-leafy-greens-stec-action-plan), and complements the California Longitudinal Study in the Salinas Valley leafy green growing region. This research was funded through CFSAN's Cooperative Agreement with WCFS.

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