U.S. FOOD & DRUG ADMINISTRATION

Division of Microbiology

Carl E. Cerniglia, Ph.D., Director

Disclaimer: *The information in these materials is not a formal dissemination of information by FDA and does not represent agency position or policy.*

Division of Microbiology

Mission

To serve a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of FDA's responsibility in toxicology and regulatory science.

Vision

Strive to be a valued resource in advancing regulatory science research in microbiology for FDA.

Division Staff

- **Government Positions** —27 Full time employees (FTEs)
  - Research Scientists & Staff Fellows: 20 FTE
  - Support Scientists : 3 FTE
  - Administrative : 4 FTE
- **ORISE Post Docs, Graduate Students, etc.:** 7 FTE
- **Visiting Scientists:** 3 FTE
- **Total** = 37 FTE

Outreach

- Collaborations with:
  - All FDA Centers and NCTR Research Divisions
  - National Toxicology Program
  - USDA, CDC, Arkansas Health Department
  - Universities: Local, National and International
- Global/National Outreach:
  - WHO Committees: JECFA (food additives), JMPR (pesticide residues), VICH (veterinary drugs)
  - Science Advisory Boards
  - Journal Editorial Boards
  - U.S. Government Panels: USDA, EPA, NOAA, Microbiome Interagency Working Group on Federal Strategic Plan, GOMRI
  - Visiting Scientist Programs
  - FDA-wide Expert Committees, Working groups with FDA Centers

Microbiology Research Areas

- Evaluating the impact of antimicrobial agents, food contaminants, food additives, nanomaterials, and FDA-regulated products on the microbiome.
- Developing methods to detect and characterize microbial contaminants in FDA-regulated products.
- Determining antimicrobial resistance and virulence mechanisms of foodborne and other pathogens.
- Conducting research to aid FDA in the areas of women's health, tobacco products, and nanotechnology.
- Improving risk assessments of FDA-regulated products, including by integrating systems biology approaches.

Three Top Accomplishments

- Developed culture-based and molecular methods to detect and characterize microbial contaminants in tattoo inks and permanent makeup products. (CFSAN)

- Conducted host-microbiome assessments to evaluate the impact of xenobiotic compounds on the gastrointestinal microbiome and immune response and to establish a standardized approach within the NTP program for risk assessments. (NTP/NIEHS/NCTR)

- Evaluated the impact of antimicrobial veterinary drug residues in foods on the human intestinal microbiota and intestinal epithelial cells for safety assessments. (CVM)

Detection of Microbial Contaminants in Tattoo Inks

RESEARCH ISSUE:

- Approximately 25% of 18-50 year olds in the U.S. have at least one tattoo
- Multiple recent reports of outbreaks by pathogenic *Mycobacteria* following tattooing
- Tattoo inks were found to be contaminated with *Mycobacteria chelonae* and related species

OBJECTIVES

- Survey to determine whether microorganisms are present in the commercial tattoo and permanent makeup (PMU) inks available in the U.S.
- Develop culture-based and molecular methods for rapid detection of pathogenic mycobacteria, including *M. chelonae*, in tattoo inks

Detection of Microbial Contaminants in Tattoo Inks

ACCOMPLISHMENTS

- Surveyed 85 unopened, sealed tattoo and permanent makeup (PMU) inks, purchased from 13 companies available in the U.S., for microbial contamination
- 42 inks were contaminated with microorganisms (49%), such as bacteria and/or fungi
- Identified 83 bacterial isolates, including clinically relevant strains, such as *Bacillus pumilus*, *B. megaterium*, *B. cereus, Pseudomonas aeruginosa*, *Dermacoccus barathri*, and *Roseomonas mucosa.* Some of which have been previously associated with human skin infections.

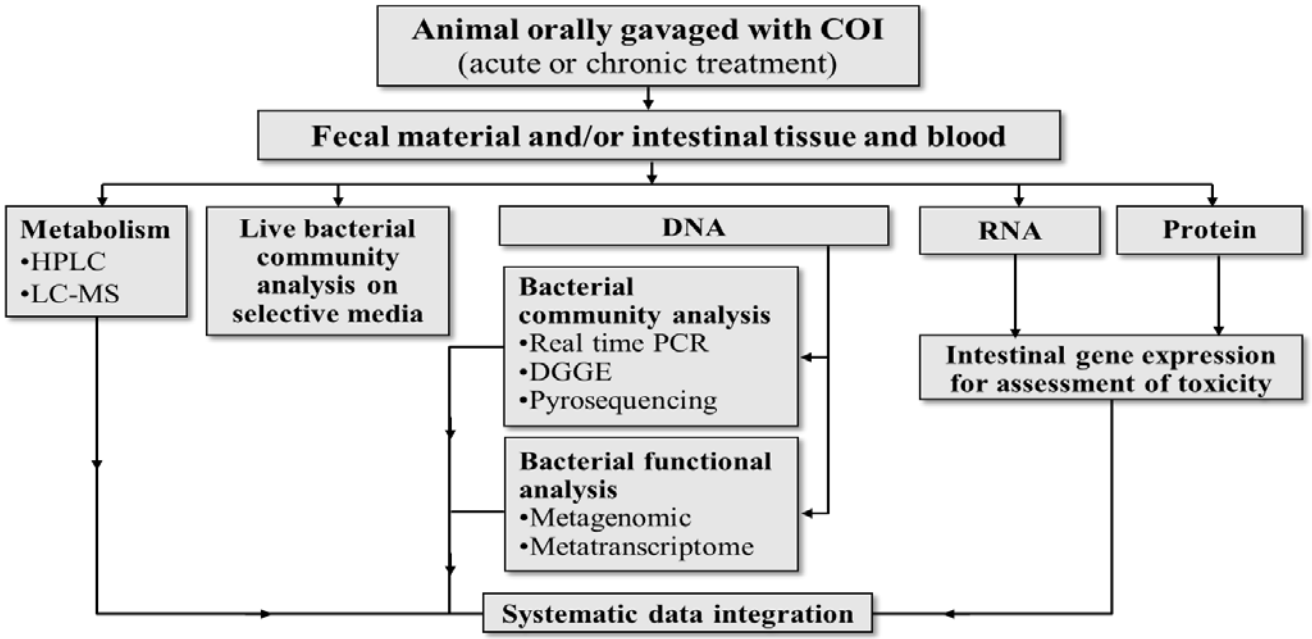
FUTURE PLANS

- Initiate a set of new experiments to confirm results of the survey study for the presence of microorganisms in tattoo and PMU inks
- Perform new test methodologies for detection of nontuberculous mycobacteria (NTM) and endotoxin test
- Provide FDA experimental data to help in the enforcement actions if necessary regarding tattoo and PMU ink products

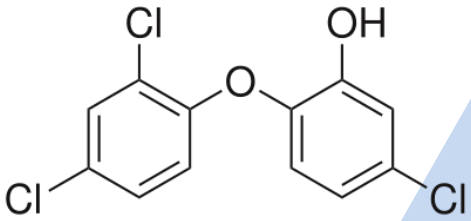
Assessment of the Role that the Microbiome May Play in the Toxicity of Xenobiotics

OBJECTIVES

- Capability-building for microbiome assessment on toxicology sudies
- Assessment of toxicity on the intestinal microbiota and gut-associated immune response using *in vivo* model system in rodent model



Tested Xenobiotics

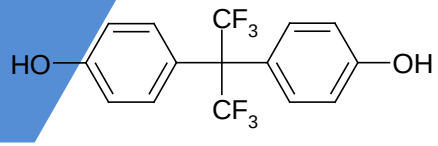


Triclosan

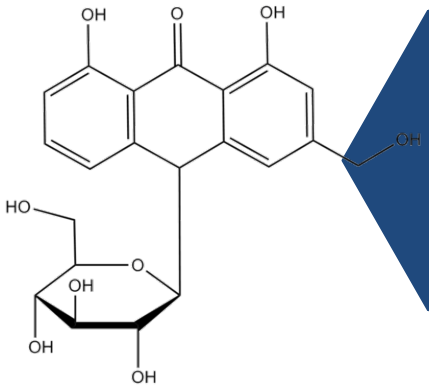
Progress of Project

Efficiency Testing

Bisphenol AF



GIT Homeostasis



Aloin/ Aloe vera

Arsenic

$_{33}As$

Nano particles

$_{47}Ag$

In collaboration with NTP/NIEHS

Assessment of the Role that the Microbiome May Play in the Toxicity of Xenobiotics

ACCOMPLISHMENTS

- Standardized the sample-collection methods for gastrointestinal microbiome analysis and gut mucosa-associated immune responses
- Conducted efficiency testing and comparative analysis of mouse vs. rat intestinal microbiome during vehicle vs. water gavage
- Preliminary findings showed that arsenic and BPAF had an adverse effect on intestinal microbiome and gut-associated immune responses during developmental stages of life in preliminary studies
- Triclosan had an adverse effect on the survival of gut bacteria as demonstrated by live bacteriology

FUTURE PLANS

- Continue data analysis for 16s sequencing and host gene expression for Arsenic and BPAF
- Conduct 16s sequencing and gut-associated immune response analysis for Triclosan

Evaluation of Potential Antimicrobial Resistance Selection in Human Intestinal Microbiota Following Long-Term Exposure to Residual Concentrations of Antimicrobial Drugs

OBJECTIVES

- Evaluating whether the ingestion of antimicrobial agents at residue-level concentrations impact the human GI tract microbiota
  - Are there shifts in the microbiota populations?
  - Is there selection of antimicrobial-resistant bacteria?
  - Do GI bacteria degrade or inactivate the drug?

ACCOMPLISHMENTS

- Determined if tetracycline, at low residue concentrations, could impact the human intestinal microbiome structure and the resistance-gene profile, following acute and subchronic exposure.
- The evaluation of bacterial community changes at the genus level, from control to tetracycline-treated fecal samples, suggested that tetracycline under the conditions of this study could lead to slight differences in the composition of intestinal microbiota.
  - *Firmicutes* and *Bacteroidetes* were the predominant phyla in the three fecal samples.
  - The genera *Bacteroides* and *Clostridium* family XI was slightly increased
- There was person-to-person variability for the presence of bacteria conferring antibiotic resistance
- Among the 23 tetracycline resistance genes (TRGs) screened, four (*tetO*, *Q*, *W*, and *X*) were major TRGs in control and tetracycline-dosed fecal samples.
  - An increase of resistance genes appeared to be related to tetracycline exposure.
- Chronic exposure of antibiotic may affect the bacteria carrying the antibiotic resistance genes for other classes of antibiotics

FUTURE PLANS

- Single and repetitive exposure of tetracycline and erythromycin
- Assess the antibiotic resistance in the mucosa associated bacteria

Effects of Residual Levels of Tetracycline on the Barrier Functions of Human Intestinal Epithelial Cells

OBJECTIVES

- To develop an experimental model to assess the barrier function of the intestinal epithelial layer
- To evaluate the effects of residual levels of tetracycline on intestinal barrier permeability by changes in resistance of epithelial cells and translocation of bacteria
- To analyze the gene expression of permeability-related genes

ACCOMPLISHMENTS

- A single-dose exposure to tetracycline concentrations to intestinal epithelial cells above the ADI value had a negative impact on intestinal epithelial permeability
- Tetracycline caused decrease in the permeability and facilitated the translocation of GFP labeled bacteria from apical to basal compartment
- The permeability study revealed that exposure of tetracycline above the ADI value (15 µg/ml and 150 µg/ml) causes barrier disruption, down regulation of gap junctional genes which resulted in compromised barrier function

FUTURE PLANS

Continue studies on the effect of tetracycline and erythromycin on intestinal epithelial cells.

Additional Representative Projects

Comparative methods study for the detection of *Burkholderia cepacia* complex (BCC) from pharmaceutical products (CDER)

ACCOMPLISHMENTS

- Developed resuscitative steps and enrichment techniques for BCC recovery
- Evaluated the United States Pharmacopeia (USP) <61> and USP <62> methods used for BCC enumeration
  - Compared the diluted media method to the current USP <61> and USP <62> methods used for BCC enumeration
- Developed a rapid PCR based detection method for BCC from water and other pharmaceutical manufacturing raw materials

FUTURE PLANS

- Further validate enrichment methodology to show its utility for improved recovery of BCC organisms present in pharmaceutical water

Additional Representative Projects

Microbial populations and the development of tobacco specific nitrosamines in moist snuff products (CTP)

ACCOMPLISHMENTS

- Each of the products contained viable populations of bacteria and/or fungi
  - Products from the same manufacturer tended to have more similar characteristics (both microbial and physiochemical)
- Storage conditions of products impacted several characteristics of the products
  - Products stored under refrigeration generally had higher numbers of bacteria and fungi than those at room or elevated temperatures
  - TSNA changes were less consistent across different storage conditions, with some product having lower levels at lower temperatures

FUTURE PLANS

- Mine study data to develop future studies to specifically address how different organisms impact TSNA formation

Future Direction of the Division

STRATEGIES

- Continued emphasis on research studies to better understand the impact of FDA-regulated products on the microbiome and host response to improve toxicology risk safety assessments
- Advance new scientific approaches to determine the impact of microbial contaminants in foods and other FDA-regulated products on human and veterinary health
- Continue to conduct research to provide data for the safety assessments of human and veterinary drugs through the integration of systems biology approaches

Future Direction of the Division

- Build on previously funded studies with CTP to pursue new projects to advance their mission critical research priorities on the regulation of tobacco products
- Continue to develop nanotechnology projects in collaboration with the NCTR/ORA NanoCore Facility and FDA regulatory Centers
- Continue to conduct research on funded studies in women's health and identify research gaps to address new research initiatives within the Office of Women's Health

Future Direction of the Division

- Identify improved ways to leverage opportunities with other federal, state and international regulatory and public health agencies, academia and industry
- Enhance mechanisms of communication to reach out to our stakeholders to develop research projects that help them address their needs to meet FDA's mission
- Prioritize our research efforts by moving away from areas that currently have less need to those more pressing to the Agency to meet FDA's mission

Feedback Requested

- Is the Division addressing the needs of the FDA Centers?
  - What emerging sciences/technologies can you advise the Division to pursue?
    - More work in the microbiome area as it relates to regulatory science?
- How can we do a better job of engaging the Centers to learn about the needs?
- What future directions do you recommend for this Division that would impact the FDA?

Thanks

- **Members of the Science Advisory Board**
- **Representatives of FDA Centers and Offices**
- **Dr. William Slikker. Jr., Director, NCTR**
- **Dr. Daniel Acosta, Deputy Director, NCTR**
- **Dr. Donna Mendrick, Assoc. Director for Regulatory Activities**
- **Division of Microbiology Staff**

Contact Information:

Carl E. Cerniglia, PhD.

Division of Microbiology
National Center for Toxicological Research
US Food and Drug Administration
3900 NCTR Road
Jefferson, AR 72079