Assessing the Impact of Toxicants on the Microbiome

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Micro & Macro views

- Global Warming
- Greenhouse effect
- Environmental pollution
- Microbial World
- Heat interaction
- Microbiome

Images of Earth and industrial pollution.
What is the Human Microbiome?

**Ecological definition:**
the ecosystem made up of microbes within and on the human body—that is, the collection of microbes (bacteria, archaea, fungi, viruses and single-cell eukaryotes) that live in the human “habitat”.

**Genetic definition:**
the entire collection of genes found in all of the microbes associated with a particular host

Human microbiome: ~8,000,000 genes

Human genome: ~20,000 genes

Microbial cells: ~100 trillion

Human cells: ~10-37 trillion

Division of Microbiology NCTR/US FDA
Historical Perspective

- 1673: van Leeuwenhoek - First observation of live microorganisms
- 1857-1861: Pasteur - Fermentation and pasteurization
- 1876-1883: Koch-Germ theory of disease, Pure culture of *Mycobacterium tuberculosis*, *Vibrio cholerae*
- 1884: Metchnikoff - Probiotics
- 1886: Escherich - Enterobacter of infants
- 1928: Fleming - the first chemical compound with antibiotic properties, *Penicillin*
- 1946: Lederberg and Tatum - bacterial conjugation
- 2001: Joshua Lederberg first suggested the concept of the *Human microbiome*
- 1953: Watson and Crick - DNA structure
- 1977: Sanger - Sanger sequencing
- 1983: Mullis - PCR
- 1990-2003: Human Genome project
- 2007: Human Microbiome project
Microbiome studies

Microbiome research is a major growth area
- >23,400 new publications since 2007 (HMP).
- ~1,600 total prior to 2007.
- Applications for nutrition, drug and food safety, environmental health, precision medicine, etc.
Knowledge Gaps
Microbiome Assessment on Toxicology Studies

- Paucity of specific studies on the effects of the xenobiotics on the mammalian gut microbiota in mouse, rat or humans, i.e., lack of in vivo studies—most reports are in vitro tests.
- Insufficient data on effects of xenobiotics exposure on intestinal microbiome diversity, functions, and possible implications for human health risk.
- Limited studies that contain measurements of the amount of xenobiotics residues in the gastrointestinal tract.
- The effects of xenobiotics on the intestinal mucosa associated microbiota remains to be explored.
Assessment of the Role that the Microbiome May Play in the Toxicity of Xenobiotics
National Toxicology Program Capability Building for Microbiome Assessment on Toxicology Studies

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Co-PIs: Paul Howard, Ph.D. (Office of Scientific Coordination)
Sangeeta Khare, Ph.D. (Division of Microbiology)

Dr. Vicki Sutherland NIEHS/NTP
Project Number: E0220101 7/9/2015
Specific goals

- To conduct host-microbiome assessments of NCTR/NIEHS/NTP studies to evaluate the impact on the gastrointestinal microbiome and immunity
- To establish a standardized approach within the NTP program for
  1) Sample collection and methodologies for gastrointestinal analysis
  2) Standardized data analysis and approaches for data-repository and data presentation
  3) Establishing science-based standards for conducting hazard analysis of FDA-regulated products and improving the prediction of the safety assessment for such products.

FDA/NCTR NTP Significance

The results will be a step towards NCTR/FDA and NTP readiness to evaluate innovative emerging technologies for improving product assessment and quality, as well as, modernize toxicology.
Where is the human microbiome located?

- Our human body sites are colonized by an enormous number of microorganisms, of which the majority is bacterial species, and they form complex communities called the human microbiota.

- The total number of these bacterial cells is estimated to be more than $10^{14}$, accounting for 10 times more than the total number of eukaryotic cells that compose a human individual.

- Among them, the gut microbiota is the largest and most complex, and is composed of more than 1,000 different intestinal microbes.
Microbiome Analysis for Toxicology Risk Assessments

RNA, Protein, metabolite

Total DNA extraction

16S rRNA-based approach
- PCR amplification of 16S rDNA

Whole-genome shotgun approach
- NGS shotgun library preparation

NGS Sequencing

Phylogeny
- Bacterial composition & diversity

Metagenome
- High resolution microbiota profiling
- Gene contents from uncultivated microbes
- Genome sequences

Metagenomics
Genomics
Bioinformatics
Where does our microbiome come from? The first inoculum as an infant through continued change, modified by diet, genetics and the environment throughout life.

**Newborn**
- Initial gut bacteria (founder species) depends upon delivery mode
  - Vaginal delivery: *Lactobacillus, Prevotella* spp.
  - C-section: *Staphylococcus, Corynebacterium, Propionibacterium* spp.
  - Higher susceptibility to certain pathogens
  - Higher risk of atopic diseases

**Early childhood**
- New strains (less certain in origin) outcompete old ones
  - Rapid increase in diversity
  - Early microbiota development = high instability
  - Shifts in response to diet, illness

**Adult**
- Highly distinct, differentiated microbiota
  - Microbial community may continue to change, but at a slower rate than in childhood

**Elderly**
- Substantially different gut communities than in younger adults
  - Larger inter-individual variability
  - Reduced biodiversity and stability

Age is an important determinant that impacts microbial composition of GI tract and also impacts toxicant absorption, bioavailability, and metabolism of xenobiotics.

Dominguez-Bello, M.G. *et al.*, Gastroenterology (2011)
Is everyone’s microbiome the same?

In adults, each part of the body supports a distinct microbial community

What is the relationship between the gut microbiota in health and intestinal disease?

- The gastrointestinal microbiota play a role in host physiology, metabolism and nutrition.
- An alteration in the gut microbial community is linked to a number of intestinal conditions, including cancer, obesity, autism, depression, asthma, and a variety of bowel disorders.
- The contribution of beneficial components of the gut microbiome to host physiology, metabolism and immune function has become the focus of ever more attention, and will undoubtedly lead to new therapeutic approaches.

Host Influences in Gut Microbial Ecology

- Age
- Genetics
- Diet, Drugs
- Environmental
- Stress responses
- Defense mechanism
- Health status
- Newborn delivery mode

Streptococci
Lactobacilli
Enterobacteriaceae
Bifidobacteria

Esophagus  Stomach  D  Jejunum  Ileum  Colon

$10^9$ bacteria/ml  $10^4$  $10^8$  $10^{11}$ - $10^{14}$
Which bacteria make up the gut microbiota?

The five dominant bacterial phyla (Firmicutes, Bacteriodes, Actinobacteria, Proteobacteria, Verrucomicrobia) and one archaea (Methanobrevibacter)

**Firmicutes**
- Clostridium
- Eubacterium
- Lactobacillus

**Bacteriodes**
- Bacteriodes
- Prevotella

**Actinobacteria**
- Bifidobacterium

**Proteobacteria**
- *E. coli*
- Desulfovibrio

**Verrucomicrobia**
- Akkermansia

**Archaea**
- Methanobrevibacter
What is the intestinal microbiota doing as an essential component of human physiology?

Intestinal mucosa is the largest surface area in the human body

Potential Microbiological Endpoints in Toxicology Assessments

**Structural Functions**
- Barriers
- Apical tightening of tight junction
- Development of immune system
- Control Intraepithelial cell differentiation and proliferation

**Defensive Functions**
- Pathogen displacement
- Nutrient competition
- Receptor competition
- Production of antimicrobial factors
- Induction of IgA

**Metabolic Functions**
- Metabolize dietary carcinogens
- Synthesize biotin and folate
- Ferment non-digestible dietary residue and mucus

**Immune Functions**
- Peyer’s patch-mucosal immunity hub
- Epithelial signaling
- Inflammatory responses
The metabolism of xenobiotics by human gut microbiota

Liver
- Oxidative metabolism
- Phase 1 metabolite
- Conjugation
- Glucuronide

Conjugated metabolites → Biliary secretion

Deconjugated metabolites

Ingested xenobiotic
- Portal vein

Absorption

Small intestine

Stomach

Large intestine

Human digestion
- The indigestibles
- The leftovers

Gut microbiota
- Deconjugation
- Reduction
- Hydrolysis
- Azoreduction
- Ring cleavage
- Thiazole ring cleavage
- Many other reactions

Bacterial metabolism

Urinary excretion

Kidney

Stool elimination

Division of Microbiology
NCTR/US FDA
The Microbiota-Gut-Brain Axis

ACTH, adrenocorticotropic hormone
CRH, corticotropin-releasing hormone
GABA, gamma-aminobutyric acid
HPA, hypothalamic-pituitary-adrenal
SFCAs, short-chain fatty acids

Kennedy, PJ (2016) TFT
Exposure of Intestinal Bacteria to the Ingested Chemical Residues Under Different Scenarios

- After oral ingestion, chemical residues in food can reach the colon due to incomplete absorption, enterohepatic circulation, or secretion across the intestinal epithelial mucosa.

- The fraction of the chemical residue (oral dose) available to the microbiota can be greatly affected by dose and dosing frequency as well as the extent of binding to intestinal contents and metabolism.

- What is critical in delineating this comparison of residue “loading” is the observation that the components contained within a single meal do not enter the colon as a bolus dose.
Acute exposure:
Acute intake of xenobiotic residues would be a single exposure event wherein the dose is ingested as a one-meal time event and transits down the gastrointestinal tract into the colon that contains no comparable levels of ingested xenobiotic residue.

Chronic exposure:
In chronic exposure of xenobiotic residues, there is an assumption of daily ingestion of the xenobiotics that is each day the ingested meal enters into an intestinal tract that already has xenobiotics spanning the intestine due to ingestion from the day before over a lifetime.

- Collectively, studies show that ingested materials enter the colon in a continuum, not a single bolus, with colonic fill starting as early as 1 to 5h of oral dose leading to roughly 80 to 90% loading within 12h.
- Excretion also begins within 12h with mean total transit times in the order of 24 to 40h.
Toxicity Tests

- Acute and Chronic Systemic Toxicity
- Carcinogenicity
- Dermal Penetration
- Ecotoxicity
- Endocrine Disruptors
- Genotoxicity
- Neurotoxicity
- Pharmacokinetics & Metabolism
- Phototoxicity
- Repeated Dose/Organ Toxicity
- Reproductive & Developmental Toxicity
- Allergenicity/Skin Sensitization
- **Microbiome Toxicity and other Microbiological Effects**
Methods for Measuring the Effects of Xenobiotic Compounds on the Human Intestinal Microbiota

**In vitro**
- Shorten term anaerobic incubation of fecal suspensions
- Continuous and semi-continuous culture systems
- Simulated gut models
- Intestinal fed batch culture
- Gut-on-a-Chip

**In vivo**
- Conventional and germ-free laboratory animals
- Human flora associated animals
- Human volunteers

**Ex vivo**
- Explant cultures (tissue cultures) extracted from the colon or rectum
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Intestinal Microbiome Analysis Approaches

Toxicant → Fecal Pellet → Ileum Tissue → Facies from colon

GI Microbiome
- Selective culturing
- qPCR
- 16S sequencing

Metabolites

Live bacteria

Microbe DNA

Fecal Pellet

Immune-related mRNA Expression

Host mRNA

Immune-related Protein Expression (30-plex protein assay)

Host Protein

Systemic Data Integration
# Microbiome NTP Projects Overall Summary and Status

<table>
<thead>
<tr>
<th>Xenobiotic Compounds</th>
<th>Experimental Model</th>
<th>Experiments</th>
<th>Status</th>
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| Silver Nanoparticles       | Rat                | ➢ Host Microbiome  
➢ Immunotoxicity                                                               | ✓ Completed                 |
| Arsenic                    | Mouse              | ➢ Fecal aerobic and anaerobic intestinal culture  
➢ Intestinal Microbiome (16S)  
➢ Immunotoxicity                                                               | ✓ Completed; Data analysis ongoing  
○ In progress  
○ In progress |
|                            | Rat                | ➢ Fecal aerobic and anaerobic culture  
➢ Intestinal Microbiome (16S)  
➢ Immunotoxicity                                                               | ✓ Completed; Data analysis ongoing  
○ Samples Collected  
○ Samples Collected |
| Aloin                      | In vitro           | ➢ MIC on pure E.coli and Lactobacillus  
➢ Microbiome, SFCAs and Aloin metabolism in fecal content | ✓ Completed  
○ Ongoing |
| Bisphenol AF               | Rat                | ➢ Intestinal Microbiome (16S)  
➢ Immunotoxicity                                                               | ✓ Samples Collected  
○ Samples Collected |
| Triclosan/Triclocarban     | Rat                | ➢ Fecal aerobic and anaerobic intestinal culture  
➢ Intestinal Microbiome (16S)  
➢ Immunotoxicity                                                               | ✓ Planning |

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**Note:** The statuses are based on the information provided and may change as the projects progress.
Research Team and Acknowledgements

**NCTR**
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- Dr. Paul Howard (OSC)
- Ms Michelle Vanlandingham (DBT)
- Dr. Dan Doerge (DBT)
- Dr. Mary Boudreau (DBT)

**NTP/NIEHS**
- Dr. Vicky Sutherland
- Dr. Nigel Walker
Current Knowledge

- Screening for environment/diet/xenobiotics-microbiome interactions
- Building diagnostic tools, genomic databases, and predictive models for toxicological risk assessment
- Testing causality of human microbiome’s contribution to health and disease

Unexplored Knowledge

Translation into better risk assessment to determine the effects of xenobiotics on the microbiome
Thanks a lot!!!