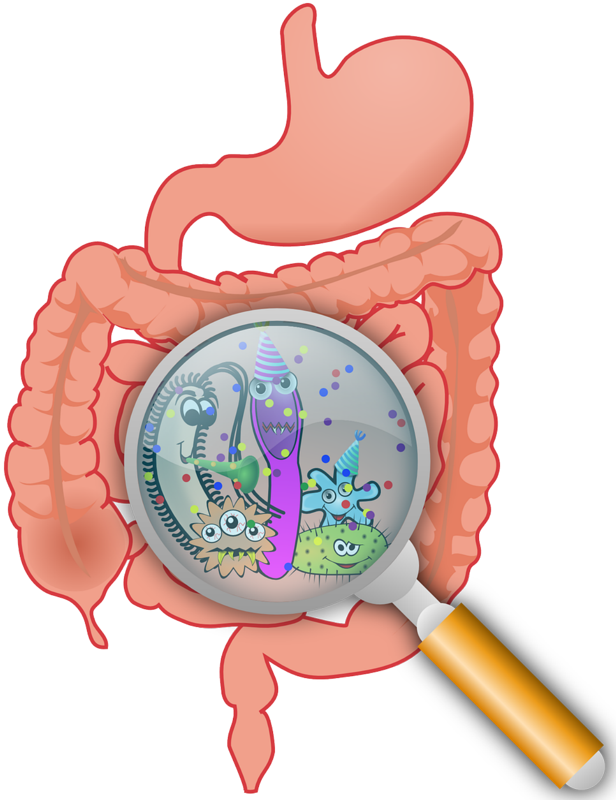
October 11, 2019

From Microbiome to Microbiome: How Environmental Microbes Are Protecting Our Health

microbialinsights

Human Health and the Gut Microbiome

- Estimated 10 - 100 trillion microbial cells in the human gut microbiota
- Potentially exceeding the estimated 10 trillion cells that comprise the human body
- 3.3 million nonredundant microbial genes
- 150x greater than the total number of genes encoded in the human genome



Relationship to health

The gut microbiota composition can be an indicator of:

- BMI
- blood glucose levels
- cholesterol levels
- cardiac health

Our microbiota is not only our first line of defense against disease but can also be used as an indicator of **health status** and **disease susceptibility**.

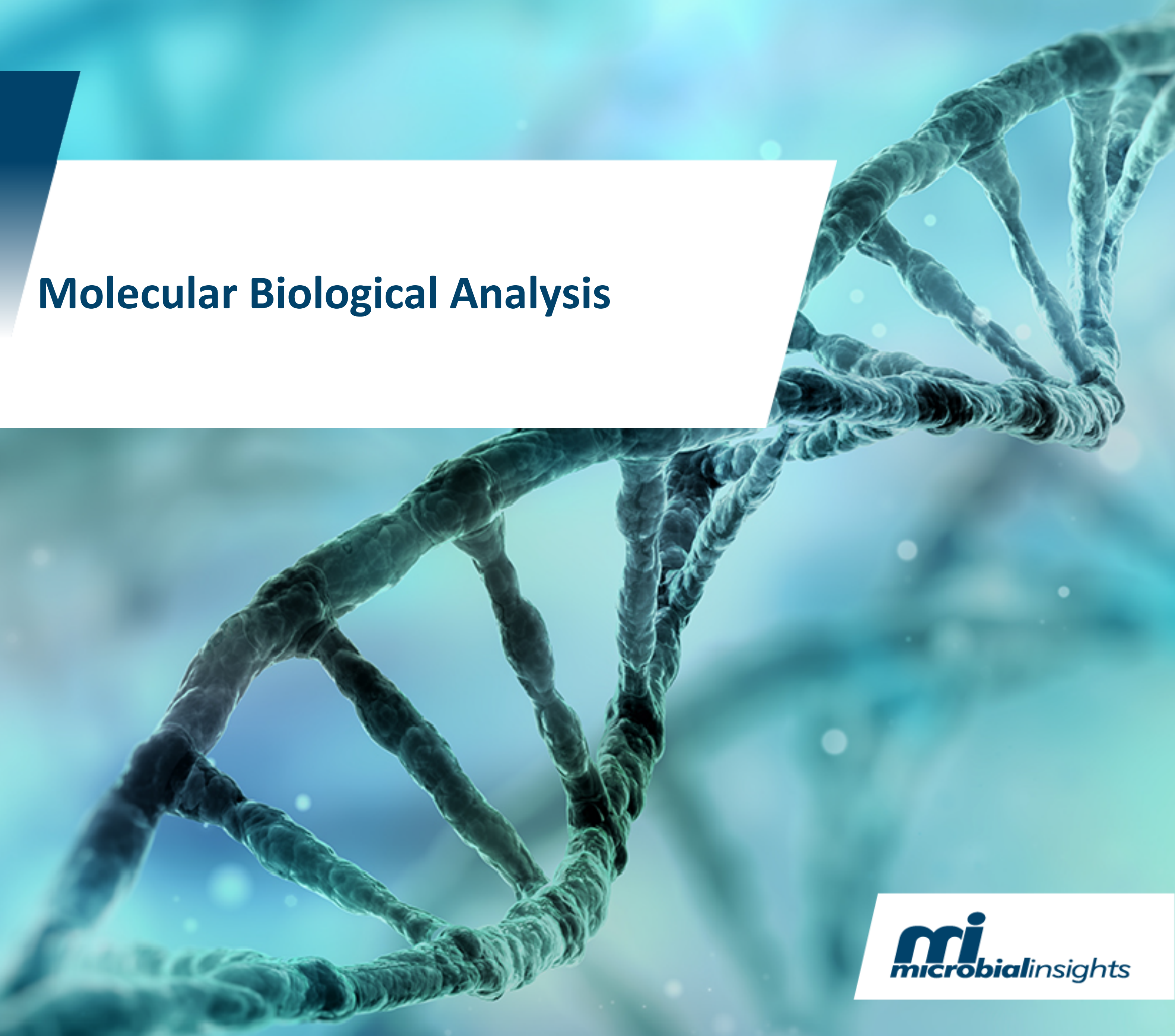
Microbial Insights

- Environmental Biotechnology laboratory
- Founded in 1992 as a technology transfer
- Specialize in molecular biological tools (MBTs)
- >27 Years Experience
- Accuracy & Precision
- QAQC
- Continuous Innovation

Dr. David C. White
University of Tennessee

That’s why we care.

Molecular Biological Analysis



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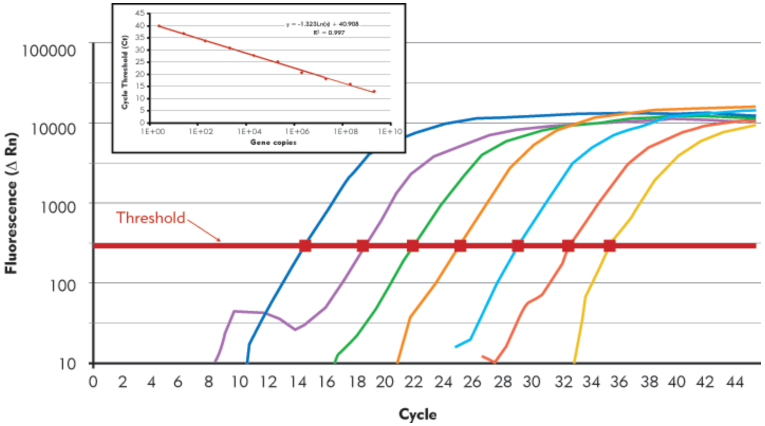
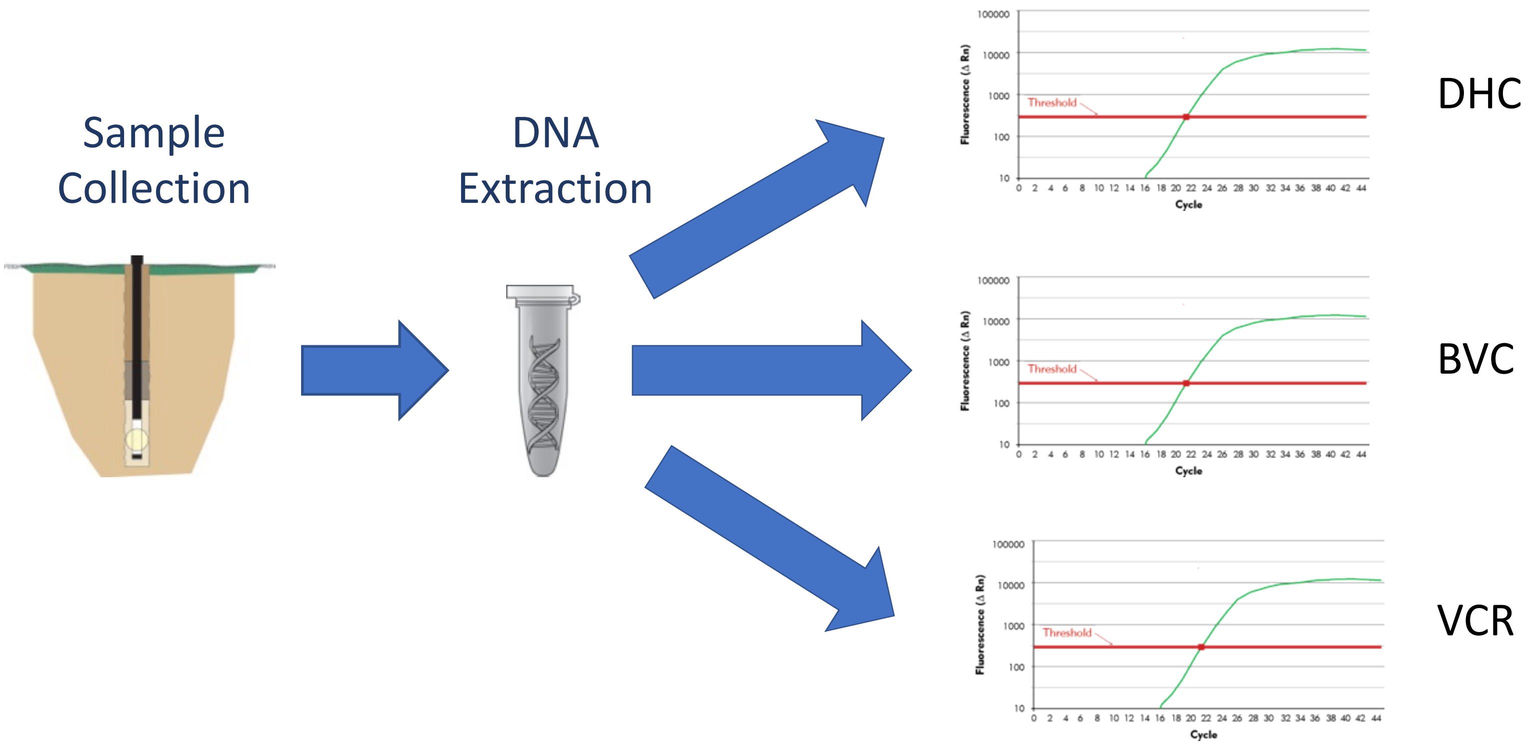
qPCR

Rapidly detect and quantify a target gene or microbial population

- qPCR Amplification
  - Primers & probe bind to target gene
  - Fluorescence signal increase proportional to concentration
- Two main types of target genes
  - Taxonomic (16S rRNA gene)
  - Functional (e.g. Benzene carboxylase)



CENSUS qPCR Approach



Sample Collection

DNA Extraction

DHC

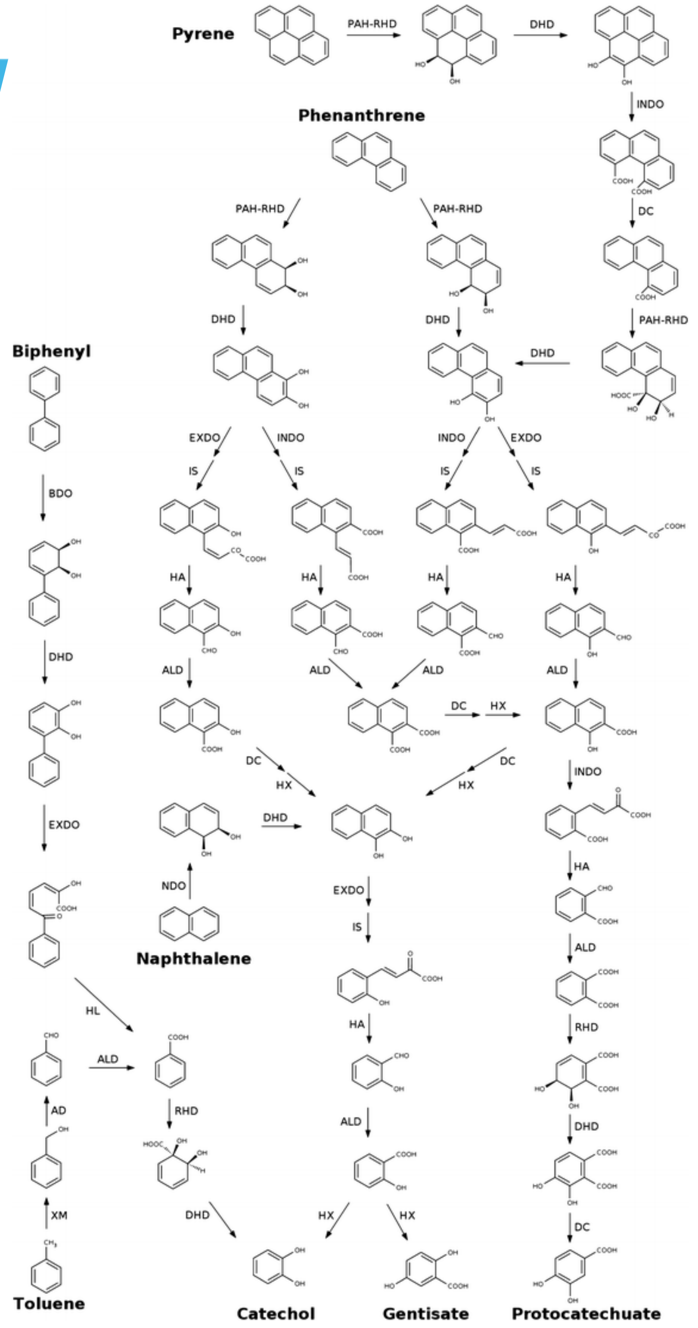
BVC

VCR

Petroleum Hydrocarbons

- Numerous pathways – <u>many are unknown</u>

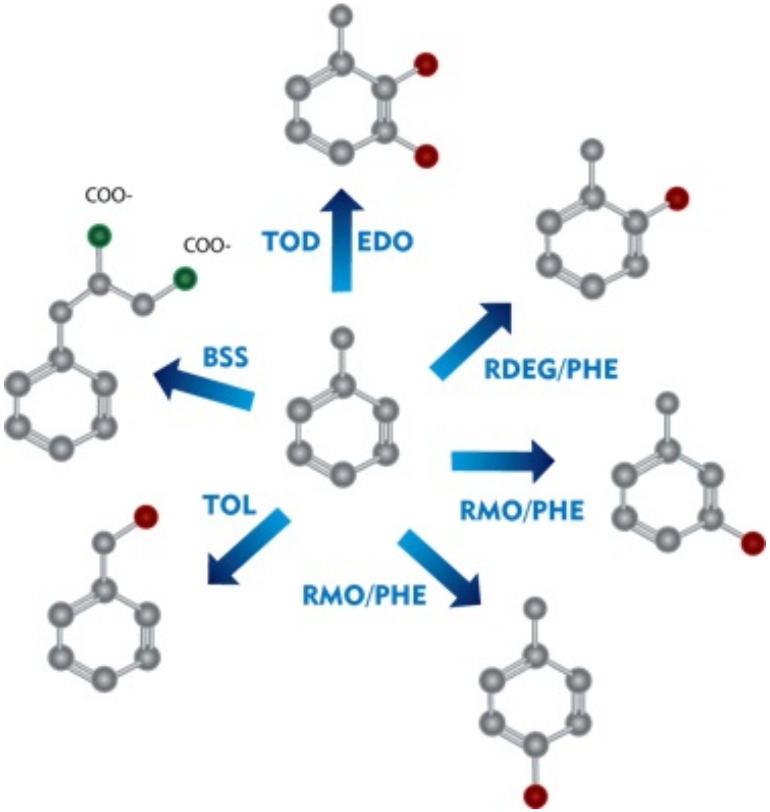
Toluene

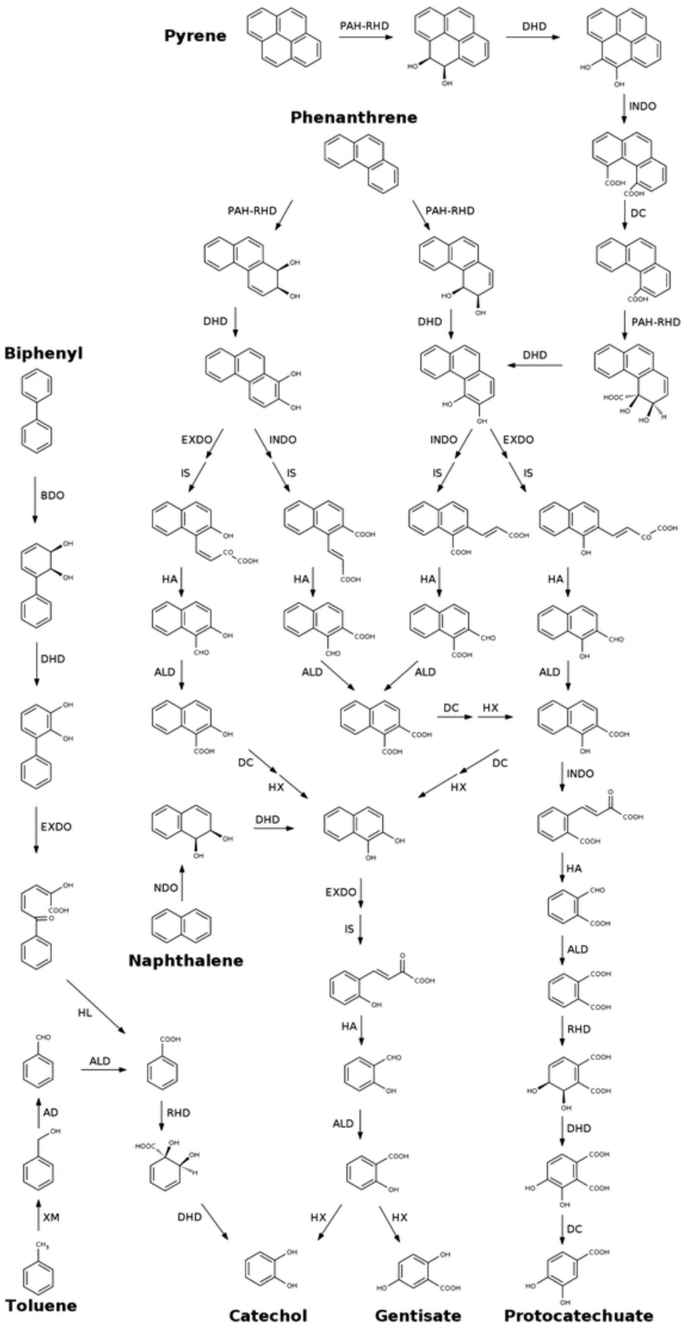


Fuentes, S. et al. Appl Microbiol Biotechnol (2014) 98:4781.

Petroleum Hydrocarbons

- More research is needed to identify key players





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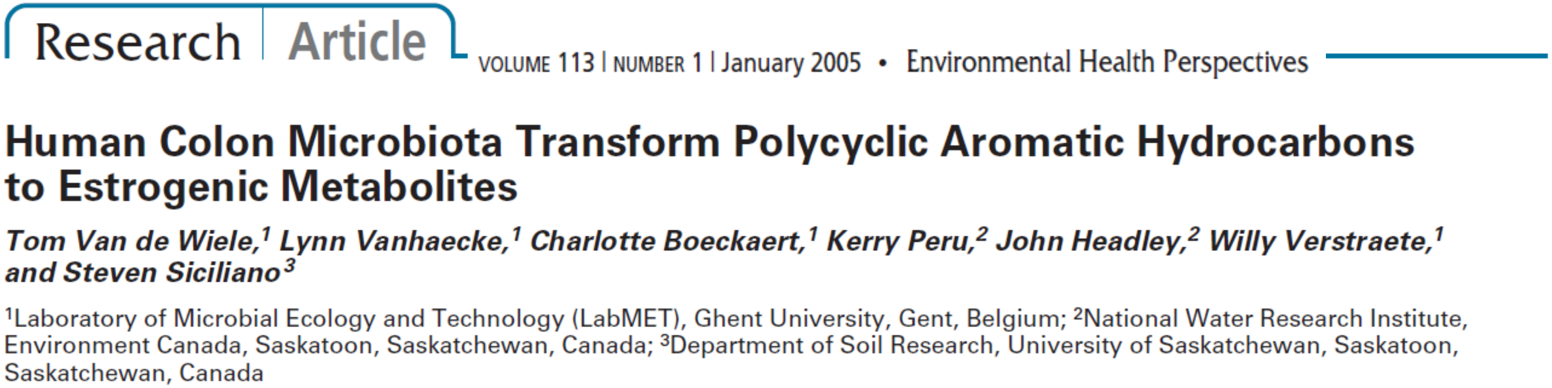
Effect of gut microbiome on toxins



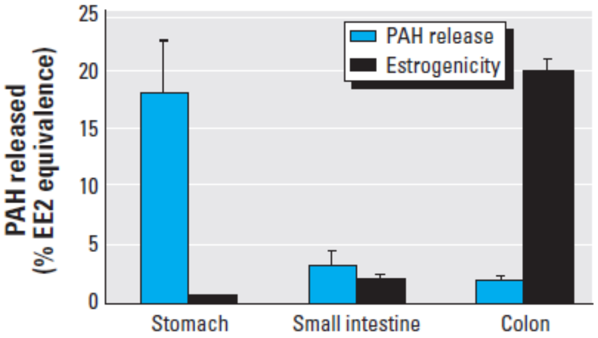
Research | Article VOLUME 113 | NUMBER 1 | January 2005 • Environmental Health Perspectives

Human Colon Microbiota Transform Polycyclic Aromatic Hydrocarbons to Estrogenic Metabolites

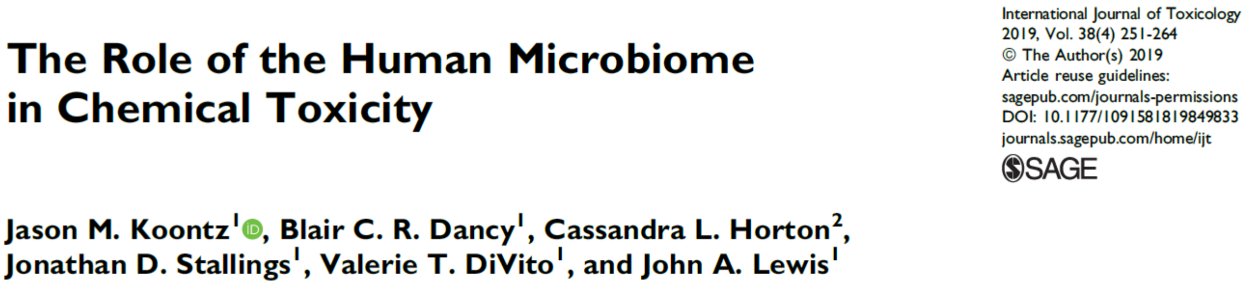
Tom Van de Wiele,[1] Lynn Vanhaecke,[1] Charlotte Boeckaert,[1] Kerry Peru,[2] John Headley,[2] Willy Verstraete,[1] and Steven Siciliano[3]

[1]Laboratory of Microbial Ecology and Technology (LabMET), Ghent University, Gent, Belgium; [2]National Water Research Institute, Environment Canada, Saskatoon, Saskatchewan, Canada; [3]Department of Soil Research, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

- Polyaromatic hydrocarbons converted to estrogenic metabolites in the colon:
  - Naphthalene
  - Phenanthrene
  - Pyrene
  - Benzo(a)pyrene



Effect of gut microbiome on toxins



The Role of the Human Microbiome in Chemical Toxicity

International Journal of Toxicology
2019, Vol. 38(4) 251-264
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DOI: 10.1177/1091581819849833
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Jason M. Koontz[1], Blair C. R. Dancy[1], Cassandra L. Horton[2], Jonathan D. Stallings[1], Valerie T. DiVito[1], and John A. Lewis[1]

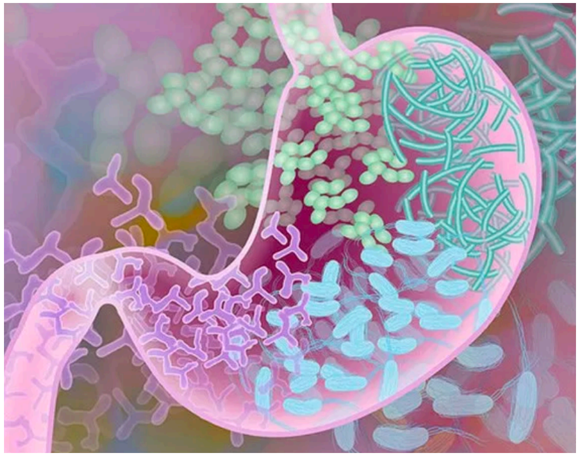
Sulfonation, methylation, oxidation, reduction:

The microbiota dictates how a toxin in modulated within the gut

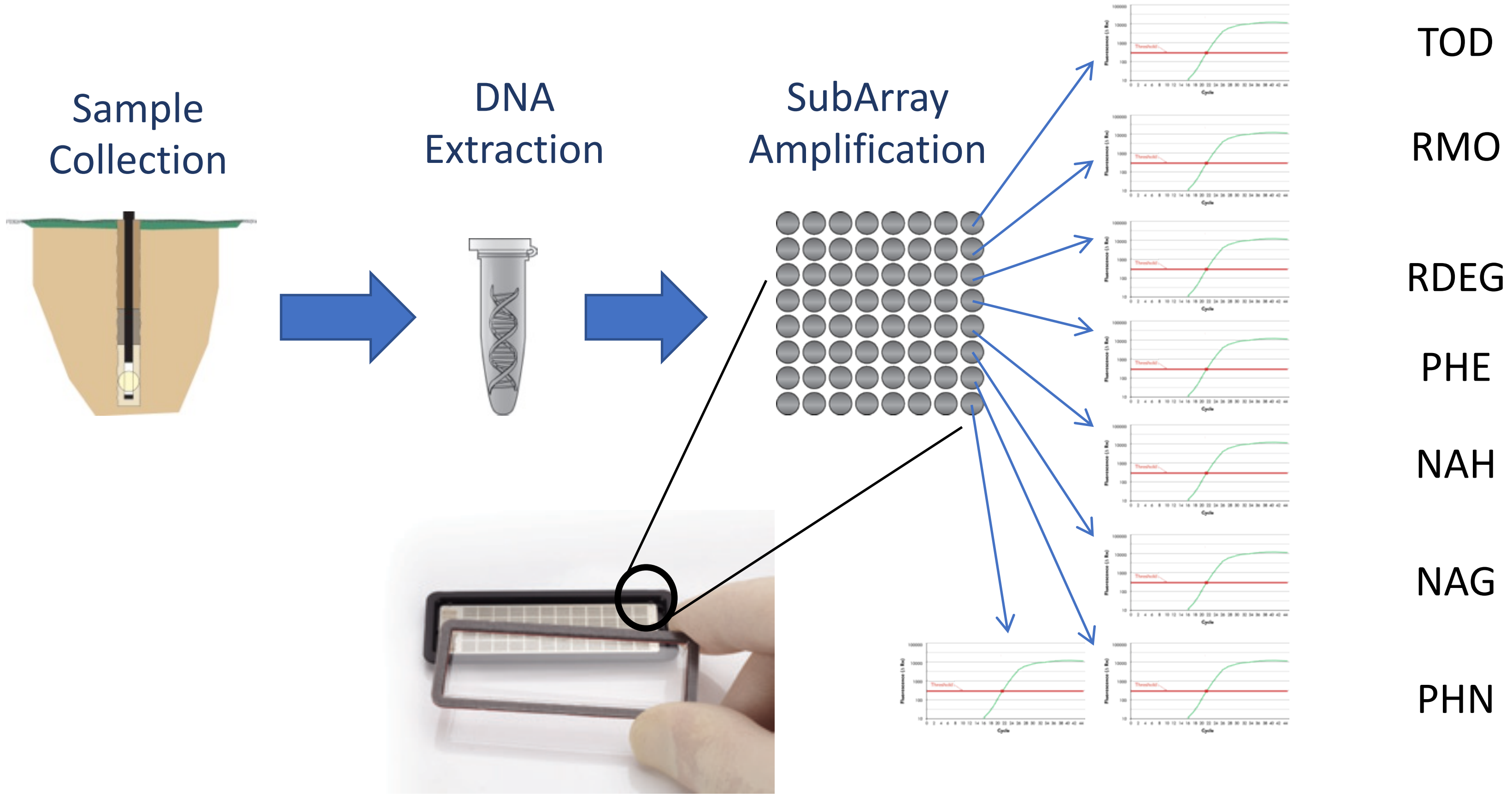
- Different people, different microbiota, different conversions of the toxin

Bioavailability changes

- Certain gut microbes can methylate mercury, making it more toxic and easier to absorb
- Some microbes can reduce the uptake of toxic elements



QuantArray Approach



Sample Collection

DNA Extraction

SubArray Amplification

TOD

RMO

RDEG

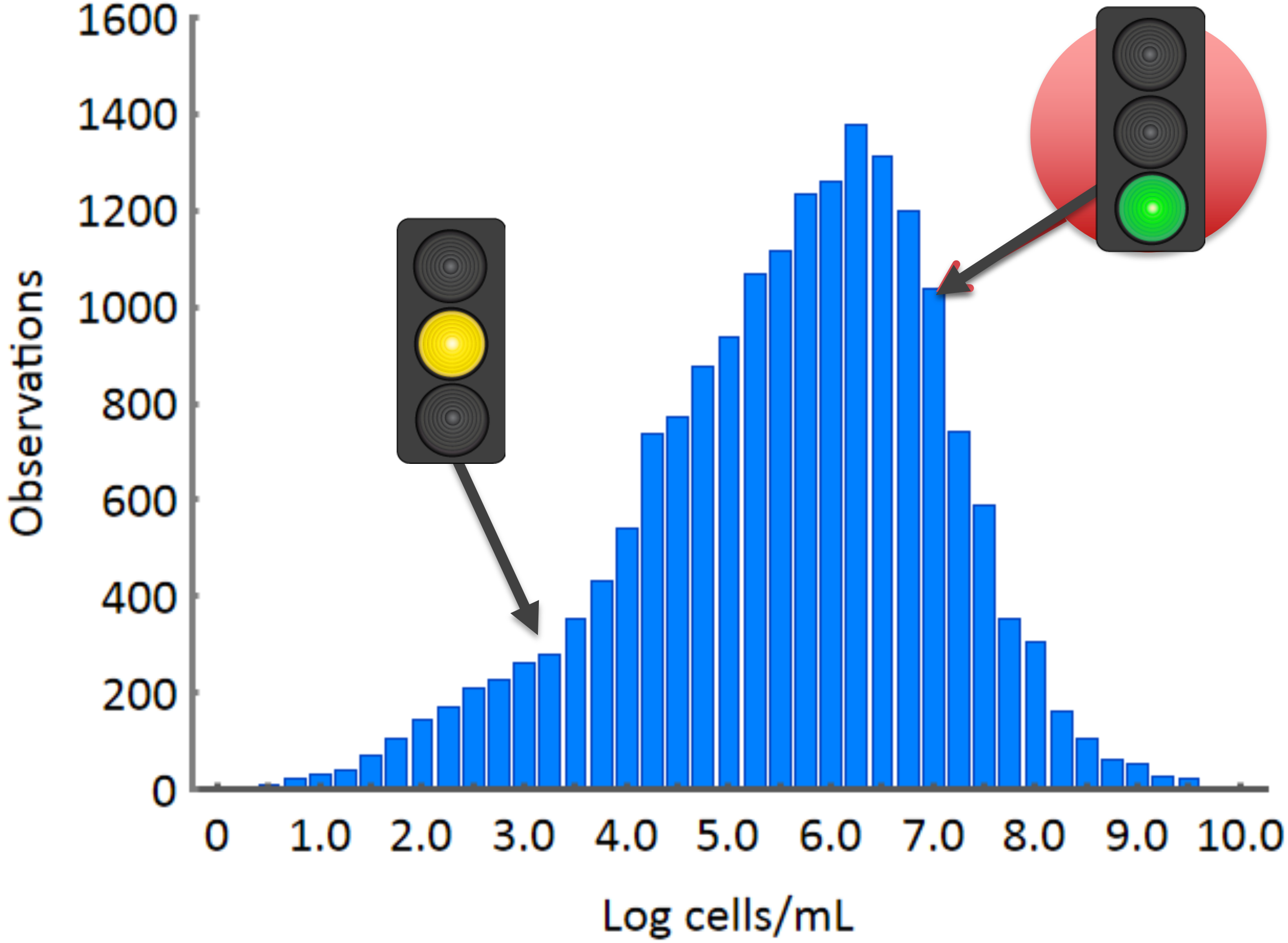
PHE

NAH

NAG

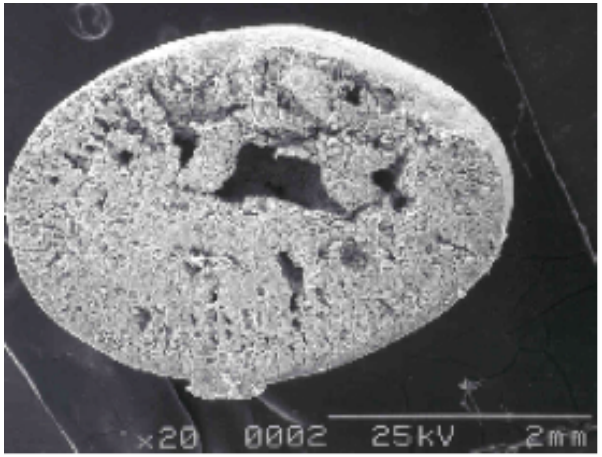
PHN

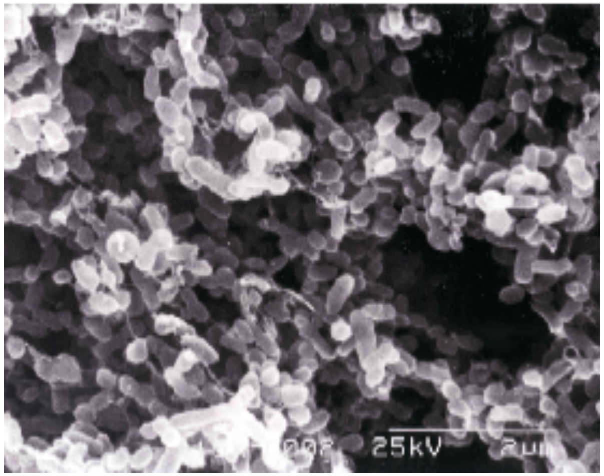
Microbial Insights Database – Adding Context



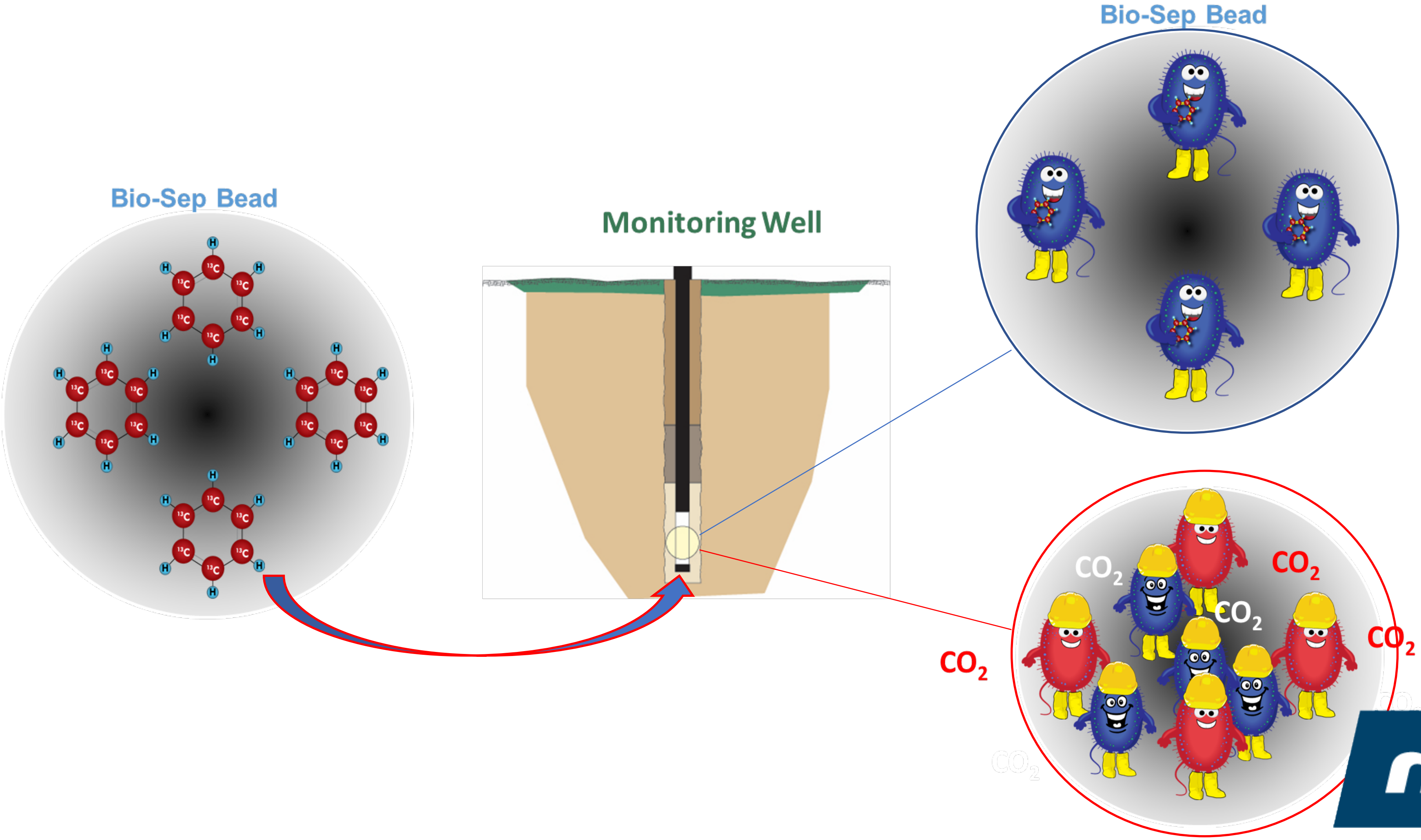
Bio-Traps

- 3-4 mm in diameter
- 25% Nomex and 75% PAC
- 74% porosity
- 600 m^2 of surface area/g
- Heat sterilized at 270°C
- Colonized by native microbes



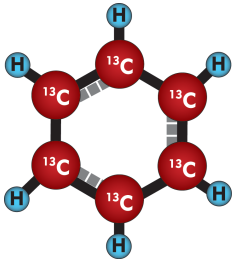


Stable Isotope Probing (SIP)



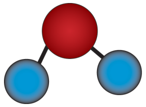
Stable Isotope Probing (SIP)

Residual ^{13}C-Compound



Local relative rate

^{13}C/^{12}C Dissolved Inorganic Carbon



Mineralization

(C for energy)

^{13}C/^{12}C of Biomarkers

PLFA

DNA

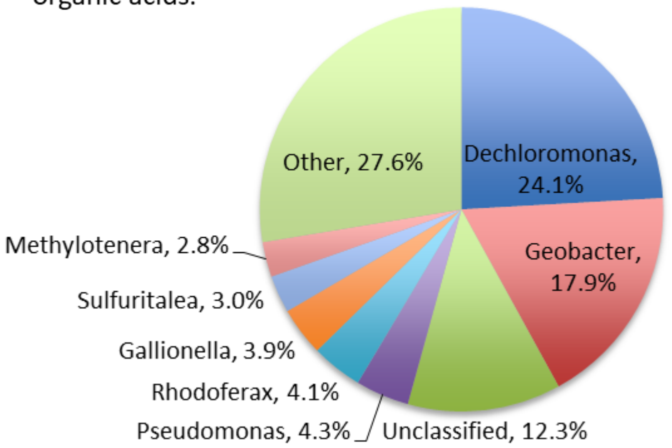
RNA

Metabolism

(C for growth)

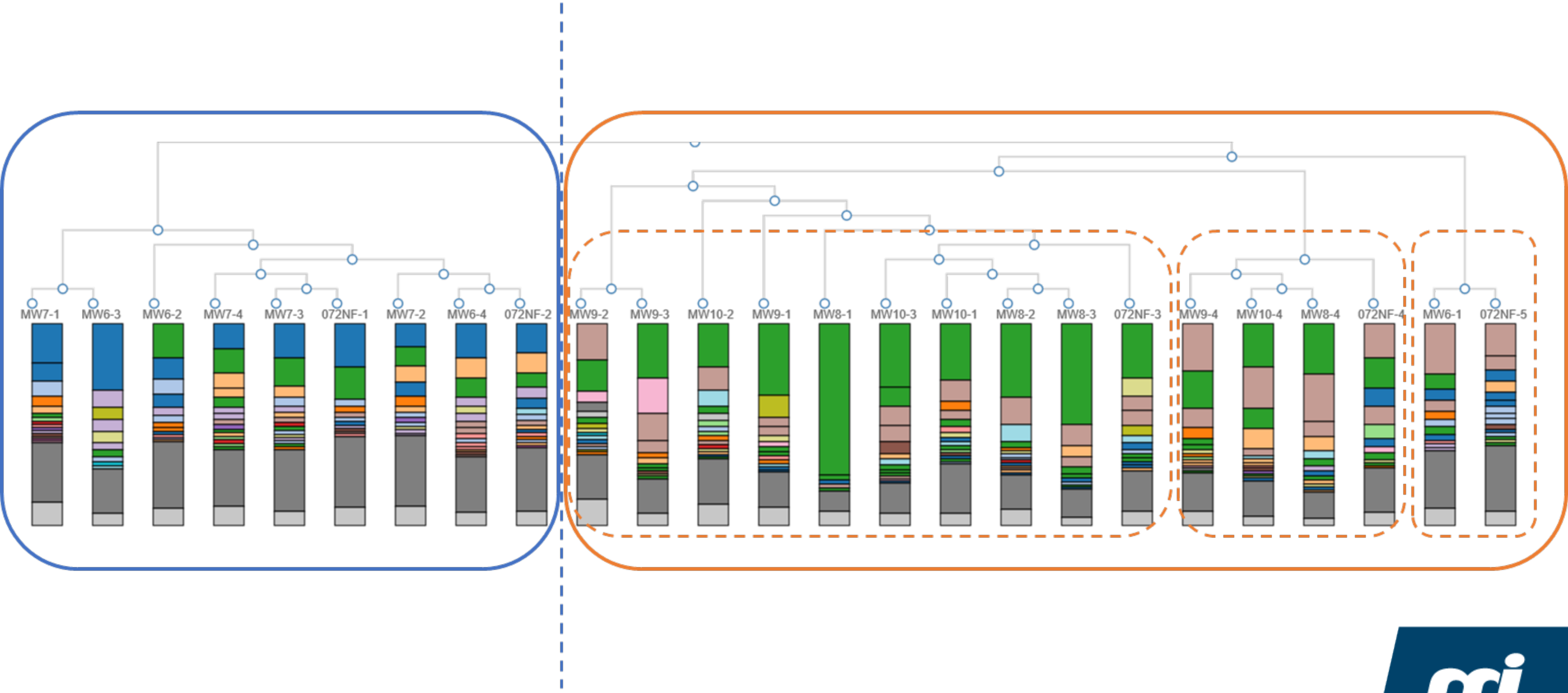
Next Generation Sequencing

Classification	Number of Reads	% Total Reads	Description
Dechloromonas	146,290	24.1%	Facultative anaerobic bacteria (uses oxygen as electron acceptor when available). Some strains utilize nitrate as an electron acceptor and some can reduce perchlorate and chlorate.
Geobacter	108,799	17.9%	Anaerobic, gram-negative, iron reducing bacteria. Some species can also reduce sulfur.
Unclassified at Genus Level	74,511	12.3%	
Pseudomonas	26,248	4.3%	*Pseudomonas* is a metabolically diverse genus of aerobic organisms. Some species can also denitrify. Some strains use common hydrocarbons as carbon sources.
Rhodoferax	25,011	4.1%	anaerobic genus that oxidizes acetate with the reduction of Fe (III).
Gallionella	23,727	3.9%	Aerobic, iron oxidizing bacteria
Sulfuritalea	18,234	3.0%	Genus of facultative anaerobes bacteria (uses oxygen as electron acceptor when available) that also reduce nitrate. Grows chemolithoautotrophically by oxidation of reduced sulfur compounds and hydrogen under anoxic conditions. Heterotrophic growth on organic acids.
Methylotenera	16,927		



MI NGS
MOLECULAR BIOLOGICAL TOOL

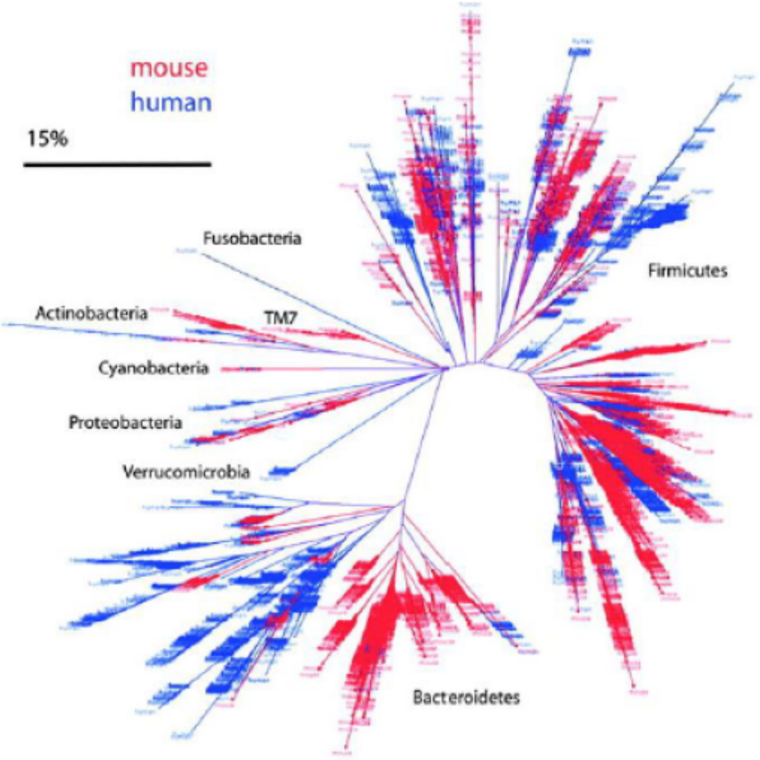
Hierarchical Clustering - Biodiversity



The effect of toxins on the gut microbiome

Changes in diversity matter

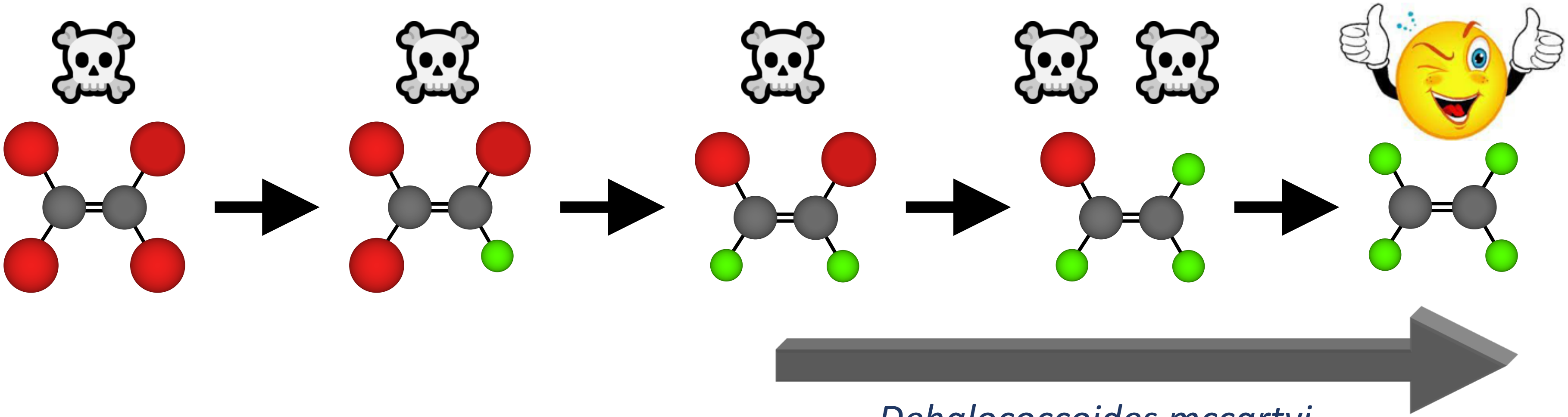
- Cadmium, arsenic and lead
    - Gut microbial diversity significantly changed
- Herbicides in mice
    - Depression and anxiety symptoms that correlate with changes in the gut microbiota



Human and Mouse Intestinal Microbiota

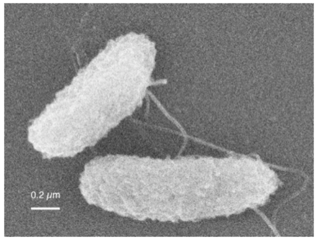
Chlorinated Solvents

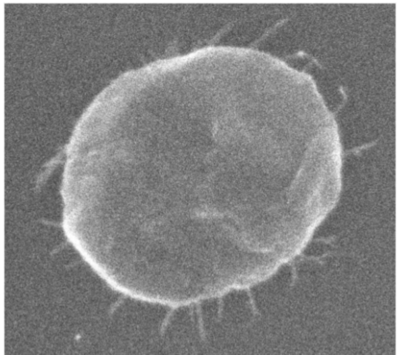
Stall? VC is more toxic!



Dehalococcoides mccartyi

Geobacter lovleyi, Dehalobacter, Sulfurospirillum, Desulfuromonas, Desulfitobacterium



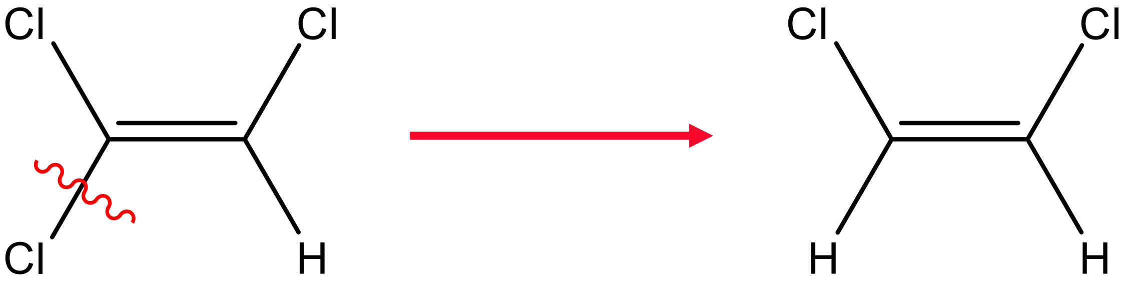


Löffler et al. 2013, IJSEM, 63:625

He et al. 2003, Nature, 424:62

Compound Specific Isotope Analysis

Contaminant degradation = breaking bonds

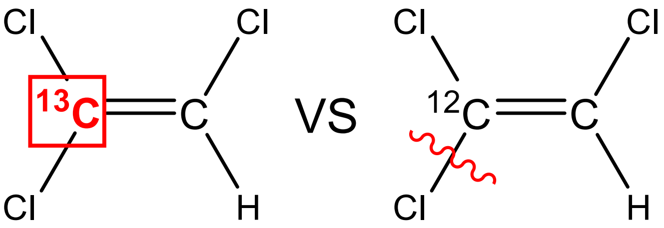


TCE

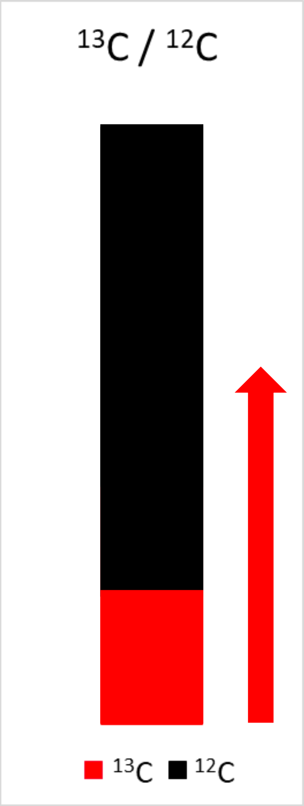
c-DCE

Isotope Fractionation

^{12}C bonds tend to break more readily than ^{13}C bonds. Throughout degradation, ^{13}C/ ^{12}C ratio increases.



- Proof of degradation
- Degradation mechanism information
- Source delineation



The future of MBTs

Data gaps – how can we do better?

- qPCR and QuantArray provide quantitative data for microbes and gene targets
  - What about activity?

- NGS can provide a bigger picture of the overall microbiome
  - What about the health of the microbiome?

- CSIA and SIP provide proof that a contaminant is being degraded
  - What about predicting future degradation trends?

Metabolomics

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-019-11311-9 OPEN

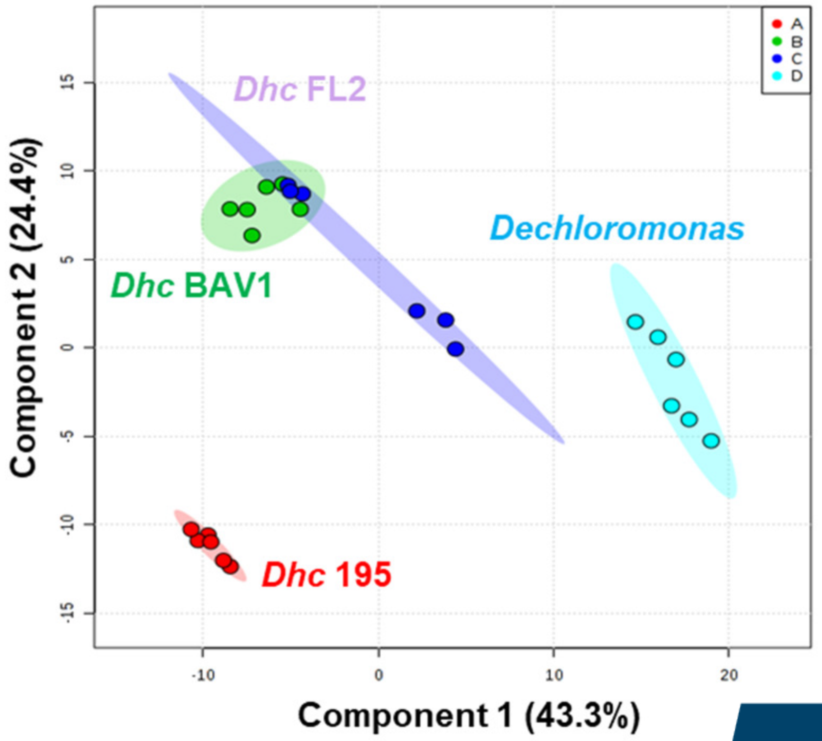
A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals

Joris Deelen et al.#

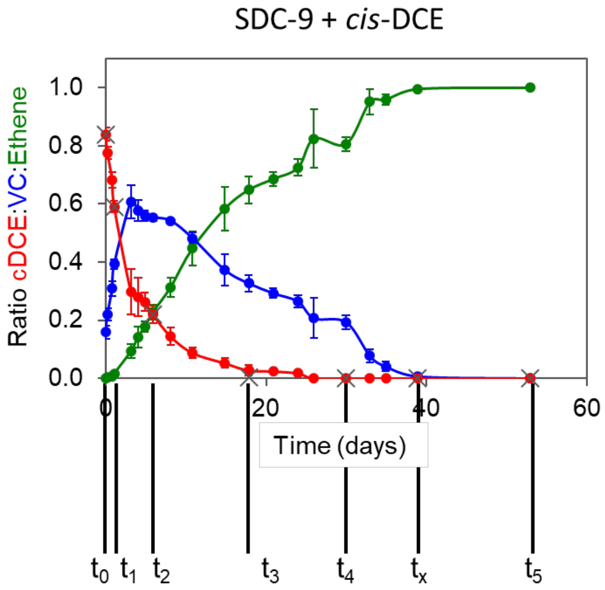
Metabolomics

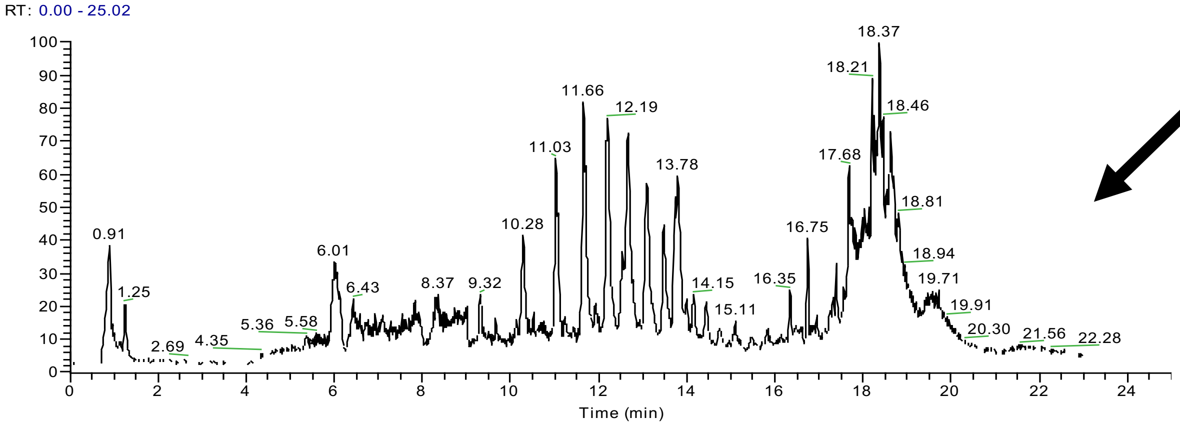
Analysis of all small molecules (MW <1100) within an environmental sample

- Identification of 80 – 100 known compounds
- Thousands of unknown compounds
- Comparison of the overall metabolic profile
- Statistical Analysis and pattern recognition
    - Predictive capabilities
    - Activity of key degraders

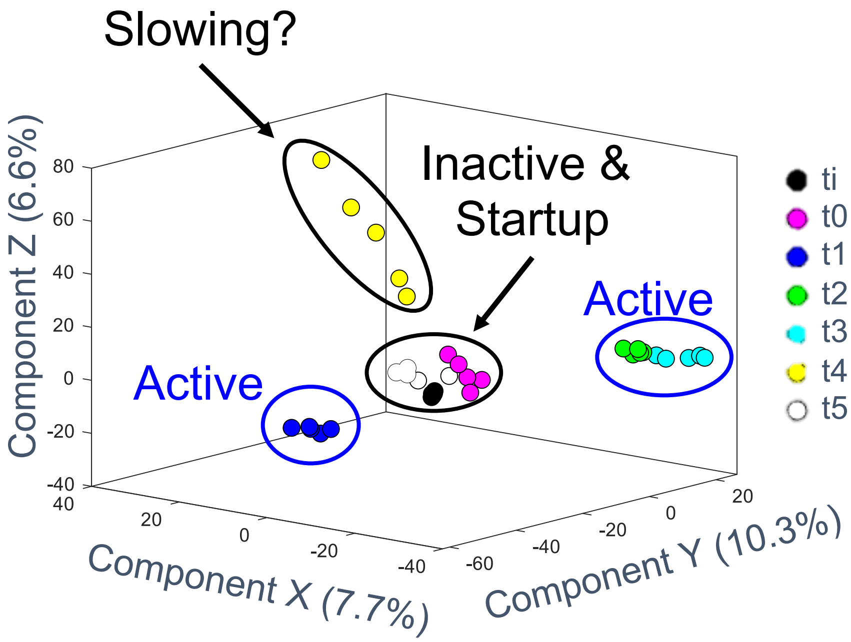


SBIR Metabolomics Study


SDC-9 + *cis*-DCE



SBIR Metabolomics Study

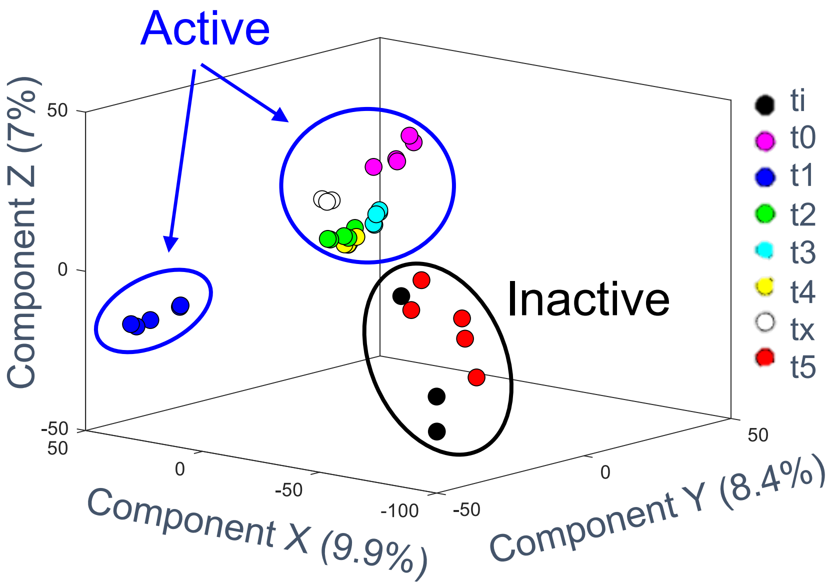

Slowing?

Inactive & Startup

Active

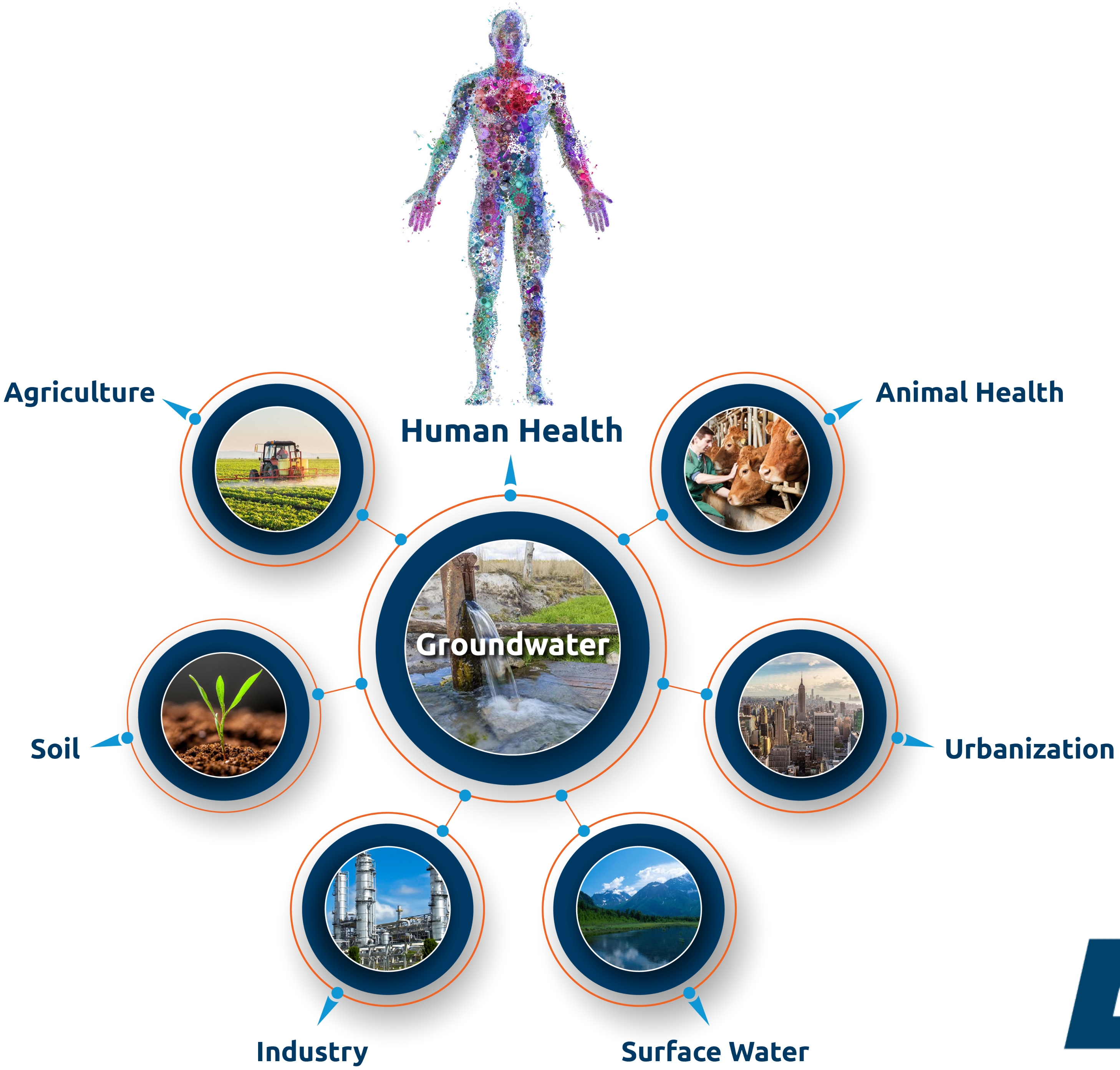
Active

Vinyl chloride


Active

Inactive

cis-DCE



Human Health

Agriculture

Animal Health

Groundwater

Soil

Urbanization

Industry

Surface Water

Where is bioremediation headed?

Thank you for your time

Are there any questions?