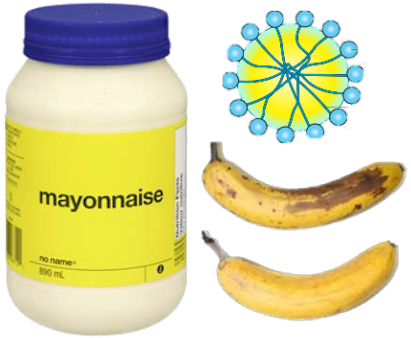
U.S. Endowment for Forestry and Communities

USDA Forest Service
U.S. DEPARTMENT OF AGRICULTURE

Safety and Regulatory Aspects of Novel Biobased Materials: Challenges and Unusual Issues

Jo Anne Shatkin, Kimberly Ong, James Ede



14 JUNE 2023

Vireo Advisors, LLC
Raising the Bar on Sustainability in Innovation

TAPPI NANO DIVISION

AGENDA

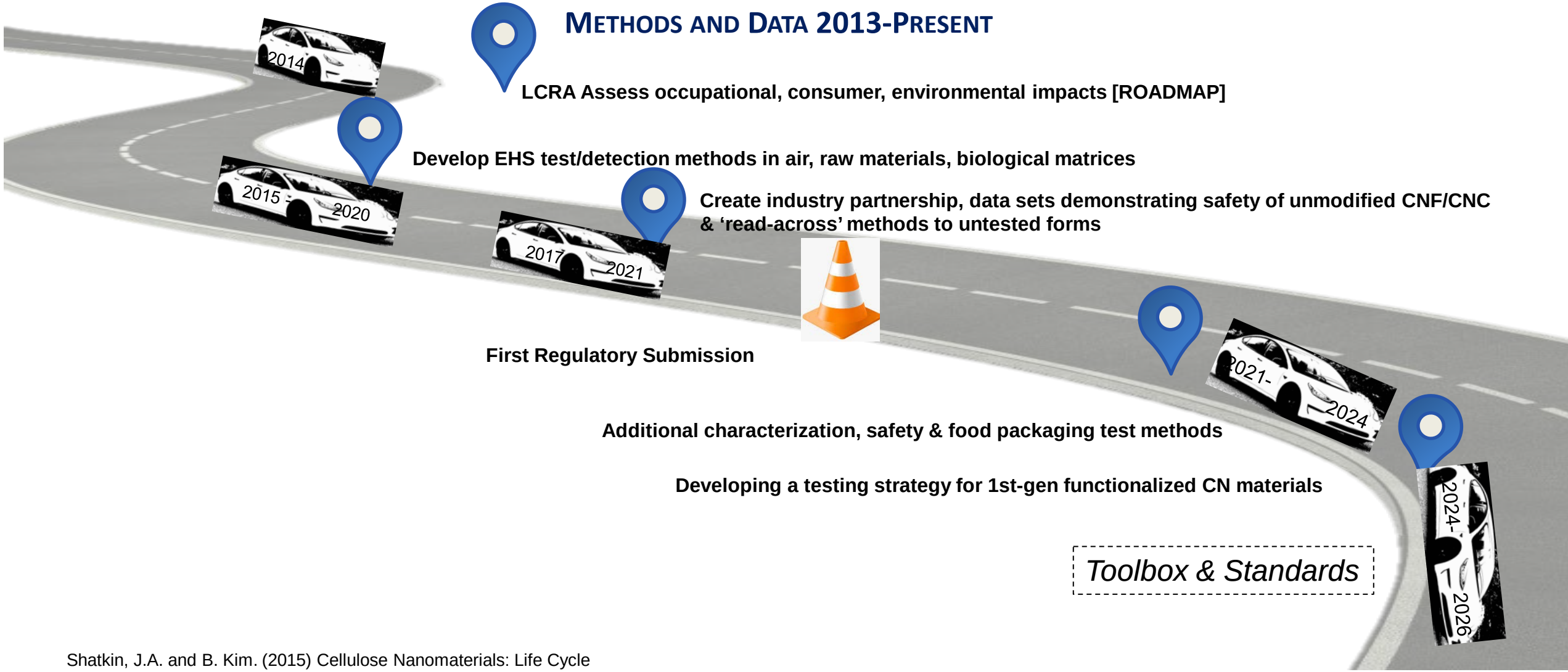
1. Update on Food Safety Study/Alliance
2. Functionalized CN synthesis, characterization and safety testing, Toolbox
3. Challenges
4. Unusual Issues

NANO COLLABORATORS

Environmental Health and Safety EHS ROADMAP – Cellulose Nanomaterials

Life-Cycle Risk Assessment (LCRA)

Methods and Data 2013-Present



Shatkin, J.A. and B. Kim. (2015) Cellulose Nanomaterials: Life Cycle Risk Assessment, and Environmental Health and Safety Roadmap

Environmental Science: Nano, (2):477-499. DOI: 10.1039/C5EN00059A

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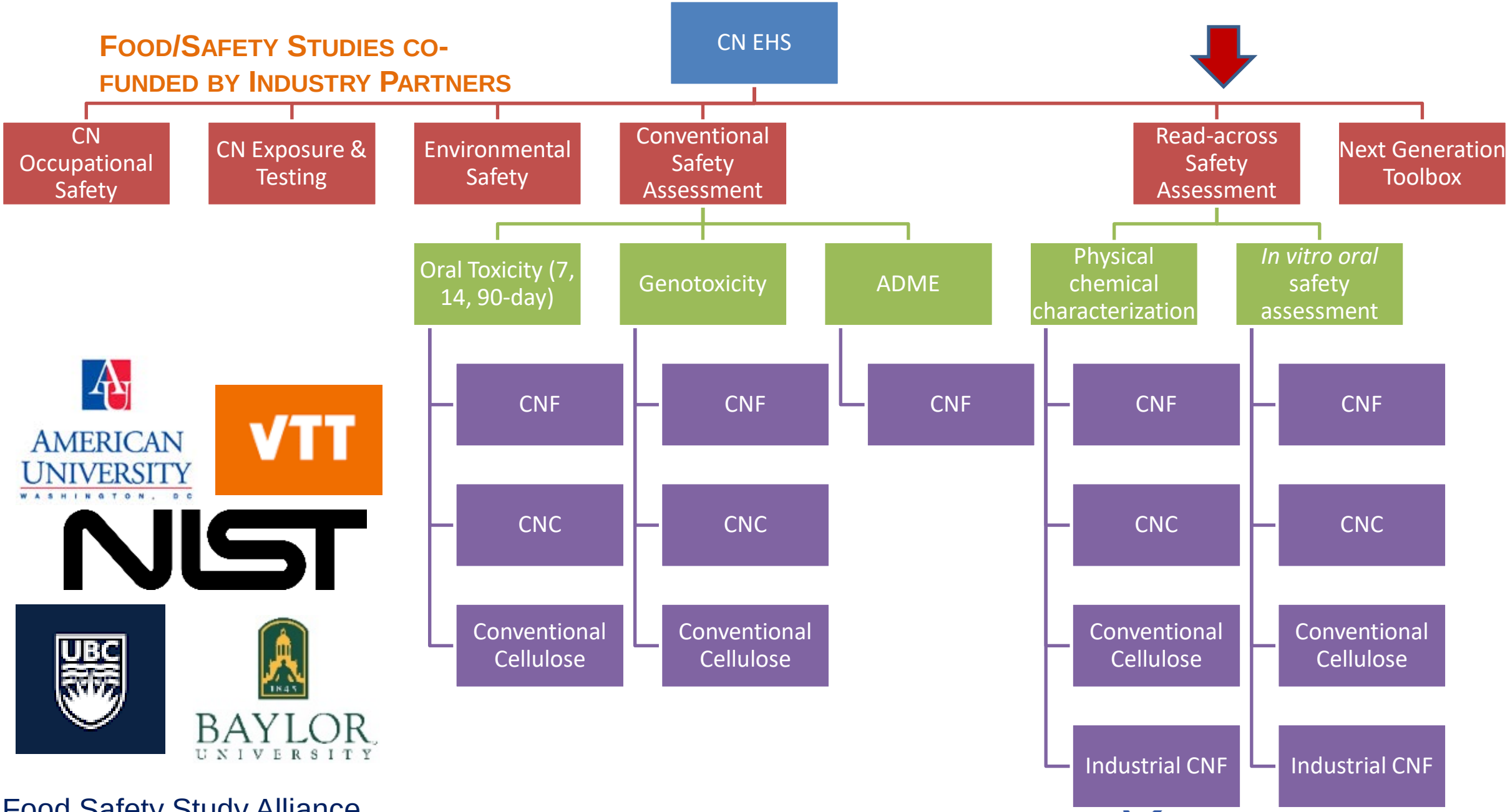
Food/Safety Studies co-funded by Industry Partners



Food Safety Study Alliance

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CELLULOSE NANOMATERIALS FOOD SAFETY STUDY

Animal Studies [1,3]		Cell-based Studies [2,3]	
Study	Result	Endpoint	Result
Acute Oral Rat Toxicity			
7-day Oral Toxicity (OECD TG 407)	NO ADVERSE EFFECTS	Cytotoxicity In Co-Culture Model	NO ADVERSE EFFECTS
14-day Oral Toxicity (OECD TG 407)	NO ADVERSE EFFECTS	Barrier Integrity Over 7-days	NO ADVERSE EFFECTS
Sub-chronic Oral Rat Toxicity		Oxidative Stress	NO ADVERSE EFFECTS
90-day Oral Toxicity (OECD TG 408)	NO ADVERSE EFFECTS	Inflammation	NO ADVERSE EFFECTS

- **CNC & CF behave similarly to conventional cellulose and raises no safety concerns when used as a food ingredient at 4% of diet;**
- **GRAS status (FDA) allows use in food and food contact applications.**

- **CNs behave similarly to conventional cellulose - supporting evidence for use in food;**
- **Baseline measurements for examining potential impact of future functionalizations on toxicity.**

[1] Ong, K.J et al. (2020)

[2] Pradhan et al. (2020)

[3] Ede et al. (2020)

Toxicokinetics rat study – Results (ADME) with U Maine PDC MFC in Commercial Lab

Mass balance

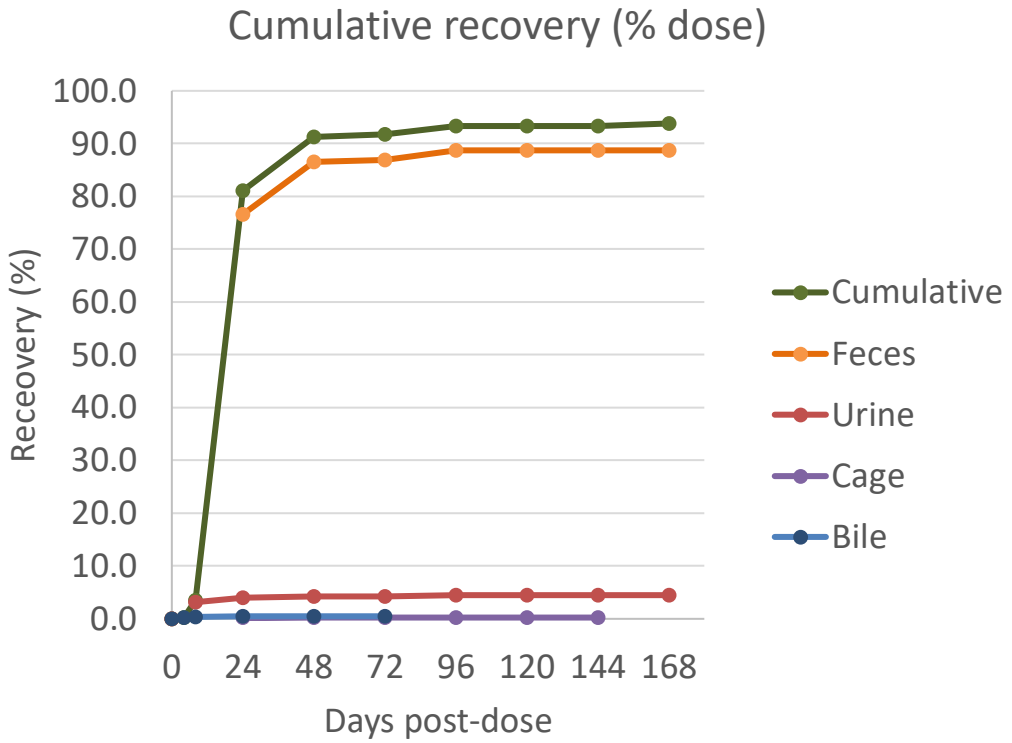
Cumulative recovery
= 93.5 ± 7.6%

Feces – 88.8%

Urine – 4.4%

Cage – 0.2%

Bile – 0.4%



Manuscript in Preparation

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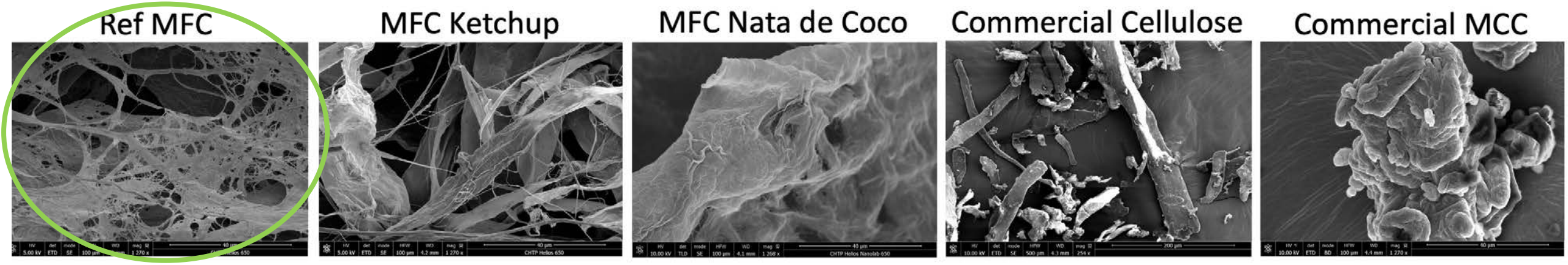
MFC is similar to cellulose used in food

2. Size/Morphology: Micro Scale SEMs



Ref MFC is from U Maine PDC.

MFC has a morphology composed of fibers with varying lengths and widths that form a complex webbed and entangled network.

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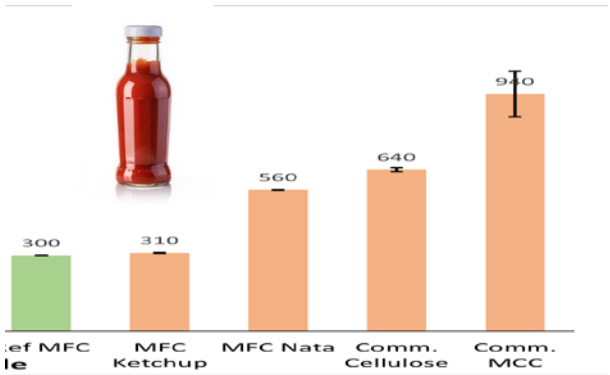
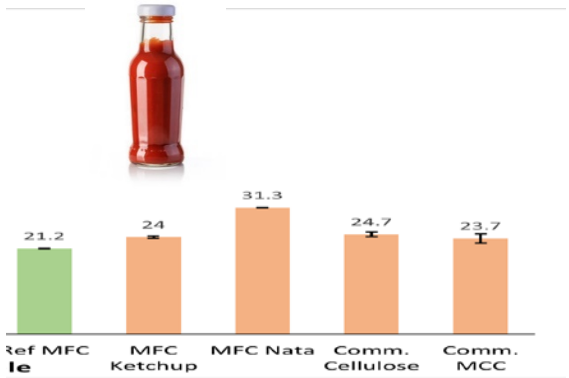
MFC is similar to celluloses long used in food

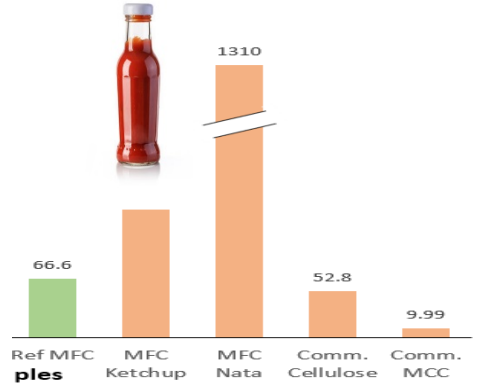
2. Size/Morphology: Micro Scale



Dynamic image analysis: Average fiber length (μm)



Dynamic image analysis: Average fiber width (μm)



Laser diffraction: Dv(50) (μm)

Ref MFC is from U Maine PDC, in **green**.

The average fiber lengths and widths of MFC are in the range of celluloses already used in food (**in orange**).

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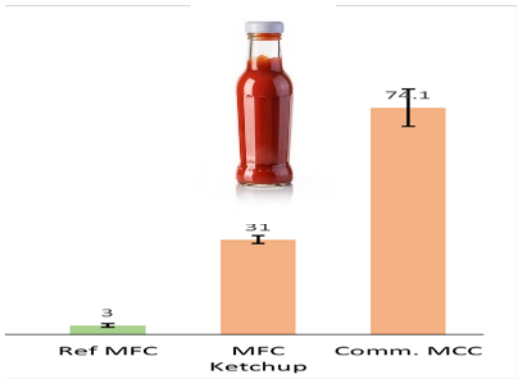
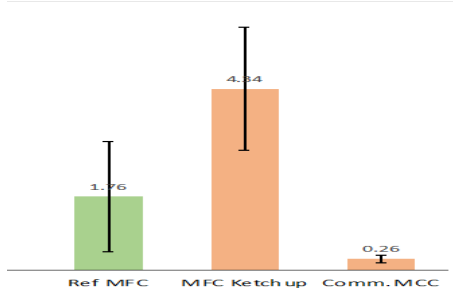
MFC is similar to cellulose in food

3. Size/Morphology: ***Finest Fraction***

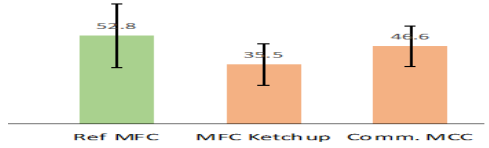
- Centrifugation protocol to isolate smallest fibers and fibrils (wt. %)
- Atomic force microscopy to characterize average fiber length and width in finest fraction
- MFC has a lower percentage of fine fibers than cellulose already used in/present in food.
- The finest fraction of MFC has fibers and fibrils with similar lengths and widths to cellulose already used in/present in food.



Weight % finest fraction



Average fiber length (µm) finest fraction



Average fiber width (nm) finest fraction

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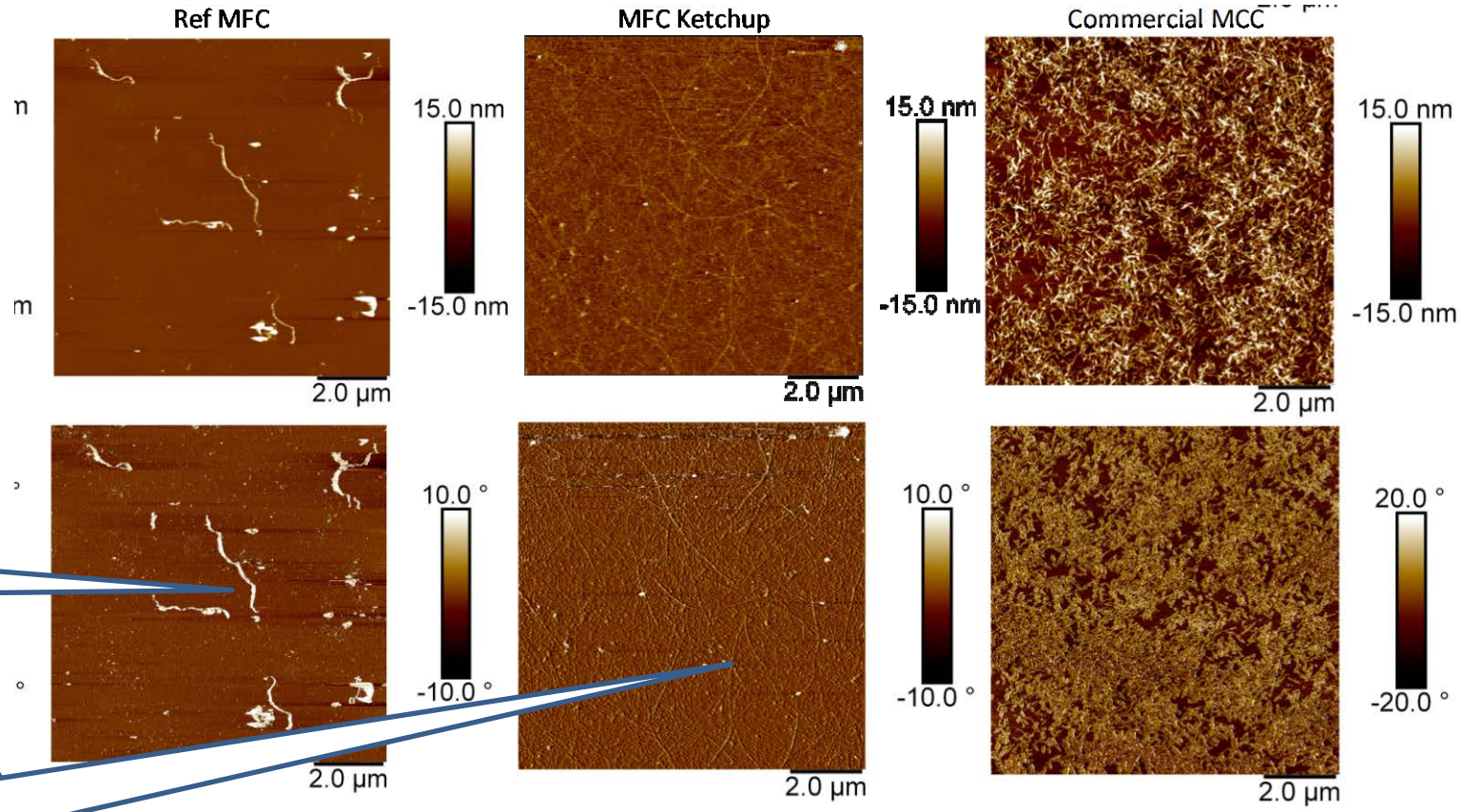
AFM Shows the isolated finest fraction similar to celluloses from fruit fiber long used in food

3. Size/Morphology: Finest Fraction

- Centrifugation protocol to isolate smallest fibers and fibrils
- Atomic force microscopy to characterize finest fraction
- The finest fraction of MFC has fibers with a similar morphology to celluloses already used in/present in food.



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Testing strategy

MFC paper and board films

Extraction testing – DIN EN standards

- Measure global migration/dry residue of extracts (gravimetric analysis)
- Characterize any discrete individual fibrils present in the extract using Atomic Force Microscopy (AFM)
- Cold and hot water, isooctane and 95% EtOH

MFC coatings on paper & plastic

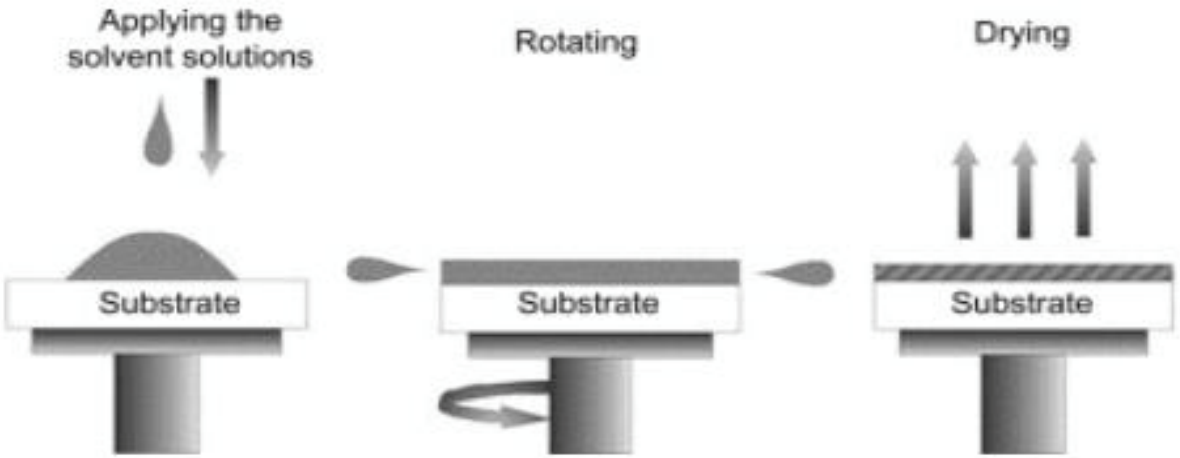
Migration testing – EFSA 10/2011

- Measure levels of MFC migration (with fluorescently labeled MFC)
- Characterize any discrete individual fibrils present in the migrants using Atomic Force Microscopy (AFM)
- 3% acetic acid, 10% EtOH, 95% EtOH
  - 60°C 10 days

Detection of discrete fibrils in extract

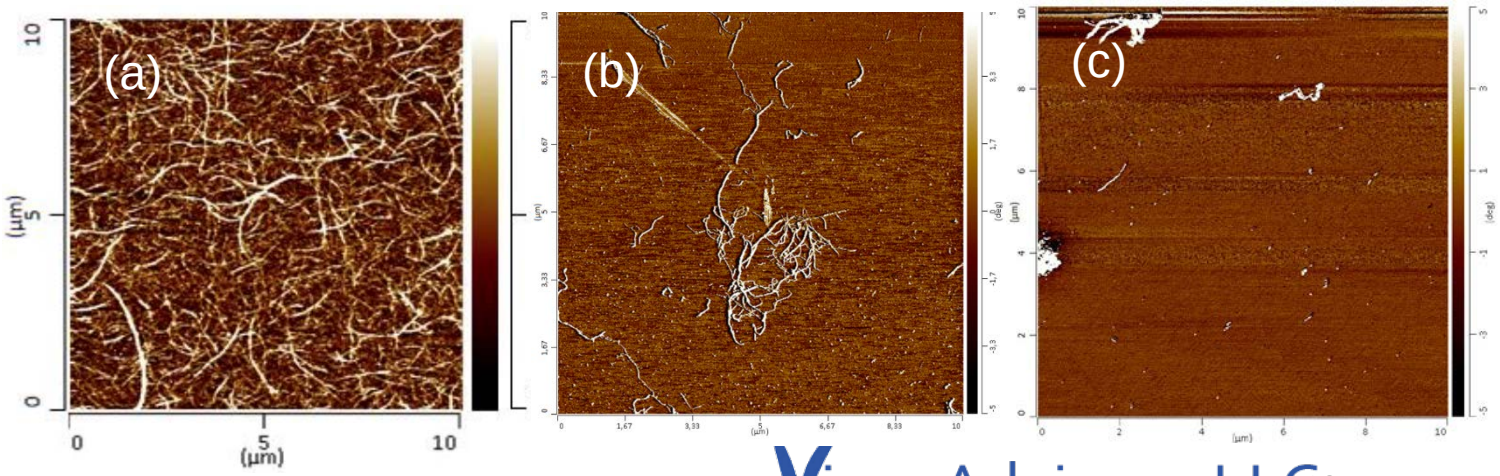
Atomic Force Microscopy (AFM)

- Centrifuge sample to separate smallest size fraction
- Spincoat
- Image with AFM
- Goal is to identify discrete fibrils in extract



Validation of method (representative MFC, these are not notified materials)

a) Representative image of MFC (finest fraction not separated)
b) Image of 0.15 wt% MFC, finest fraction separated – fibrillar material detected
c) Image of 1:5 dilution of 0.15 wt% MFC, finest fraction separated

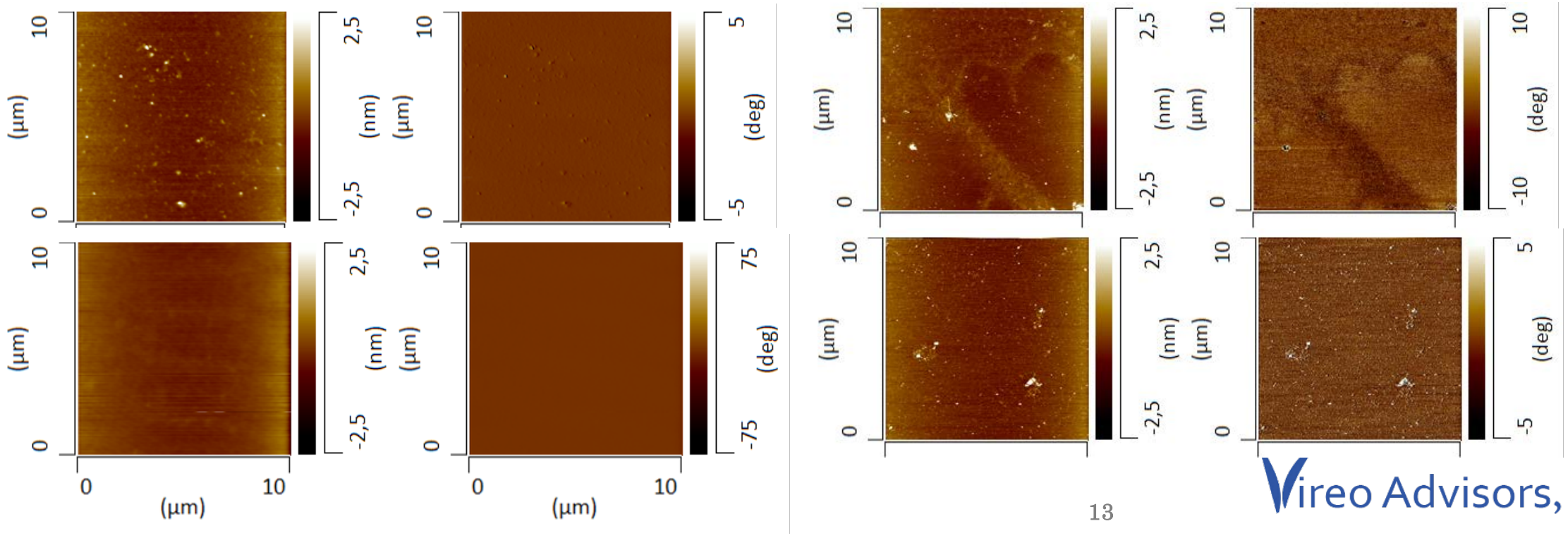


AFM Images of industrial material food contact extracts

Goal is to evaluate whether the extract contains any individual, discrete fibers

- ***Results***: None of the extracts contain discrete microfibrils. Extracts are composed of aggregated, tangled MFC, with no presence of discrete fibres or microfibrils in any of the extracts

(Representative images – no fibrillar material detected)



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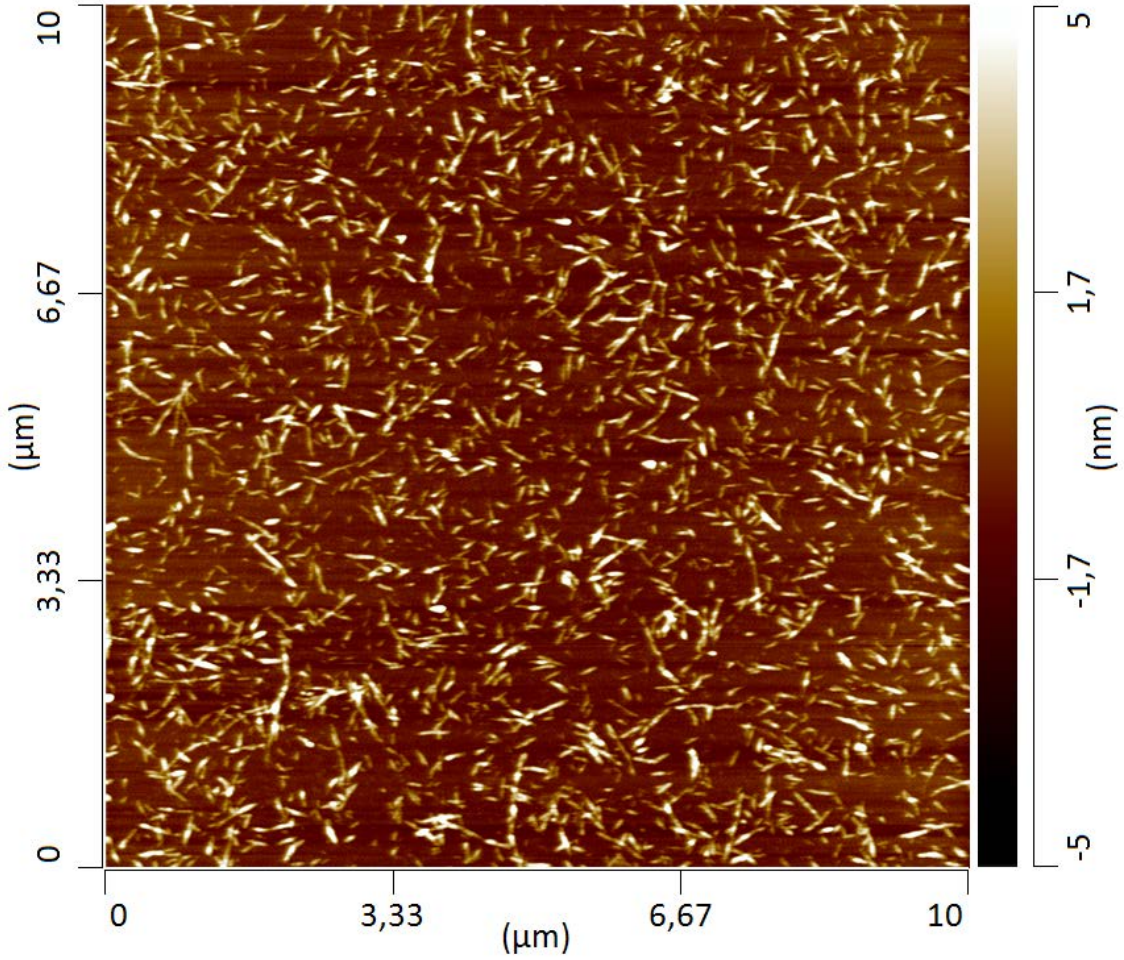
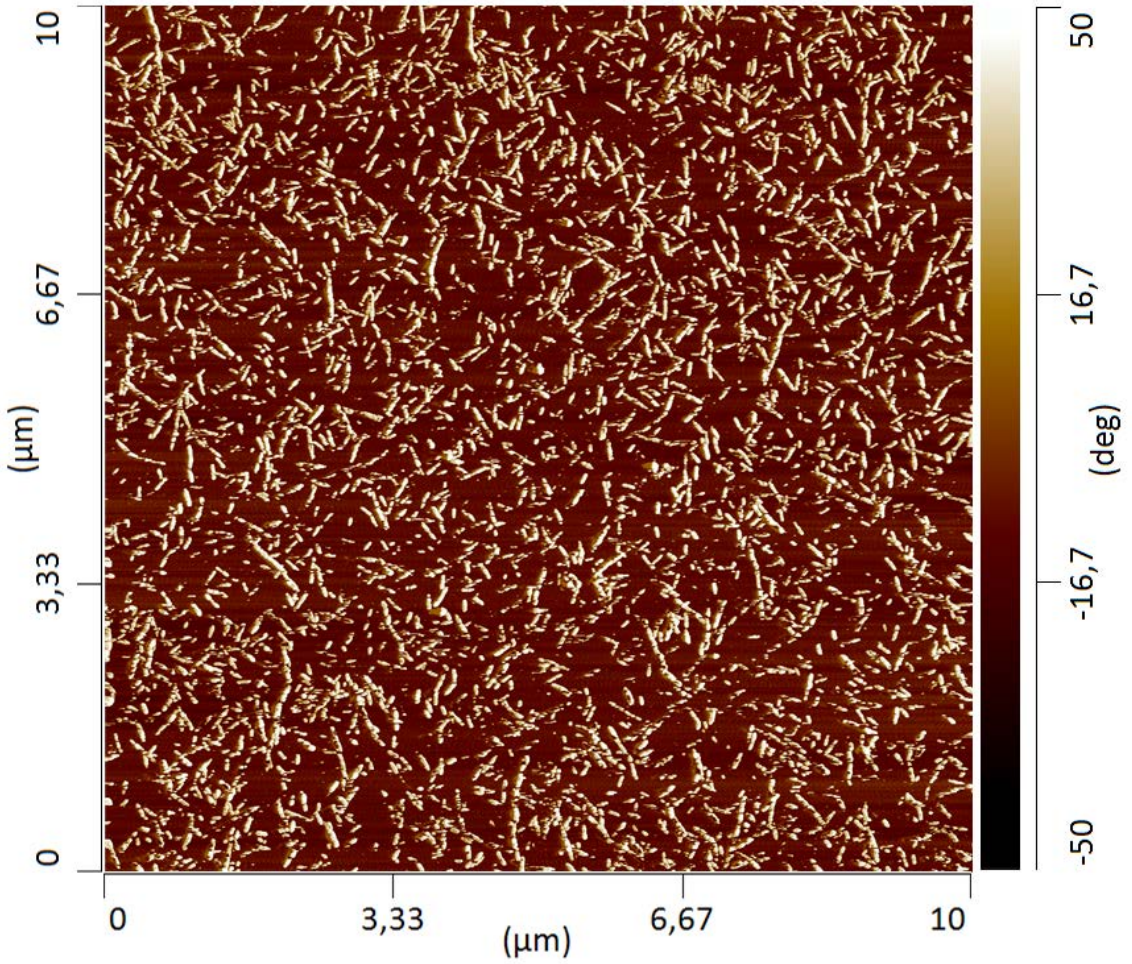
supernatant

AFM CNC_”Reference Material” Validation





Food Safety Study Materials

In Animal Tests

Novel forms

- UMaine CNF (cake)
- Hardwood CNC (powder)

Conventional/GRAS

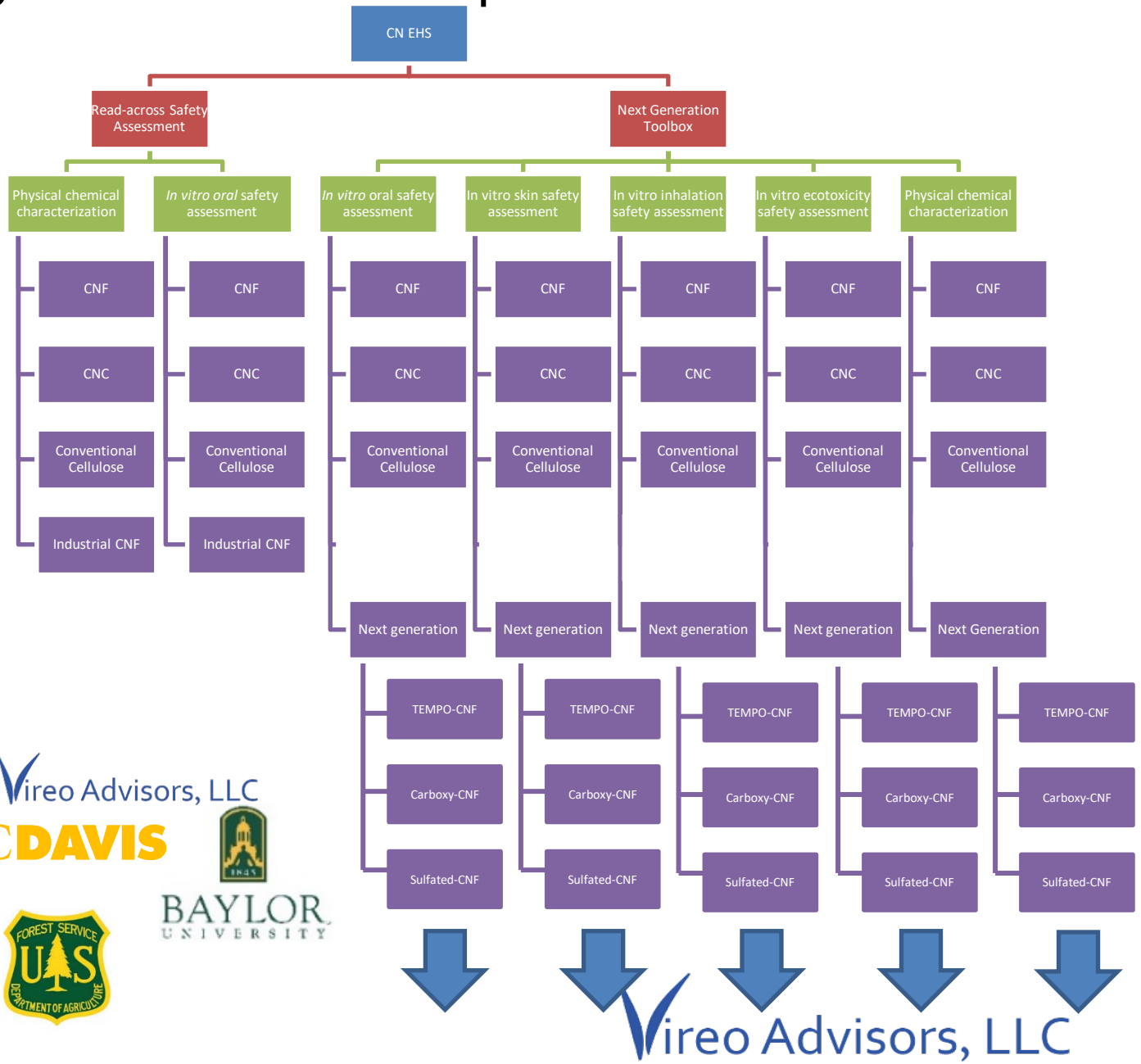
- Commercial Cellulose (SolkaFloc)

PChem/Alternative Tests

- UMaine CNF
- Hardwood CNC
- 6 industrial CMF/CNF
- Conventional cellulose

Testing strategy allows “read across” from animal to alternative testing

Safer by Design Toolbox Development



Functionalization Collaborators

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Christie Sayes, Amanda Zevcik, Clancy Collom
Nicole Stark, Robert Moon, Forest Products Lab

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UCDAVIS

BAYLOR UNIVERSITY

FOREST SERVICE U.S. DEPARTMENT OF AGRICULTURE

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Tool Box Development Goals

1. Generate standardized safety methods and data sets for CNs
   - Methods development
   - Working toward standard test methods & regulatory acceptance

2. ‘Read-across’ toxicity testing strategy for industrial and functionalized forms of CNs

1. Continue to develop ‘Safer-by-Design’ Toolbox for next generation CN materials
   - Commercially-relevant forms
   - Promote CN safety and regulatory acceptance for applications in food, food contact, cosmetics, *etc*.

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Interdisciplinary approach to Safer by Design Toolbox

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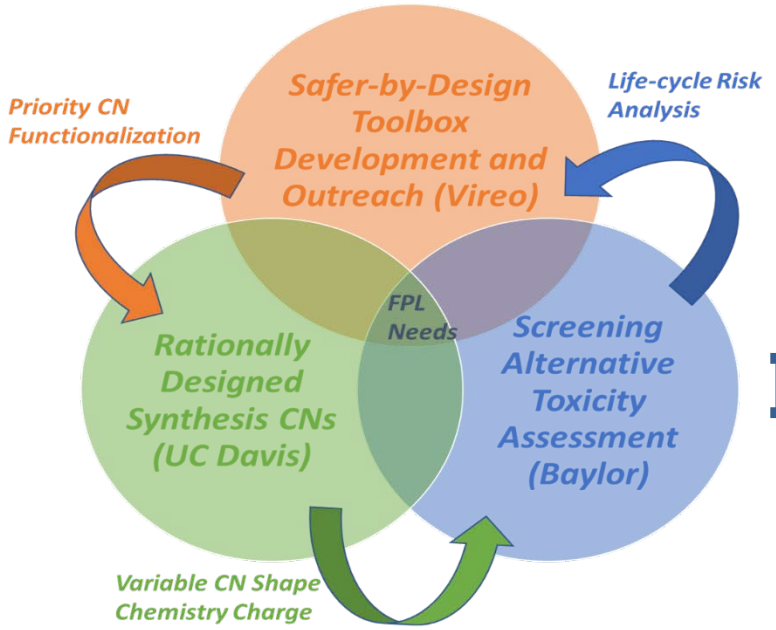

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Baylor University

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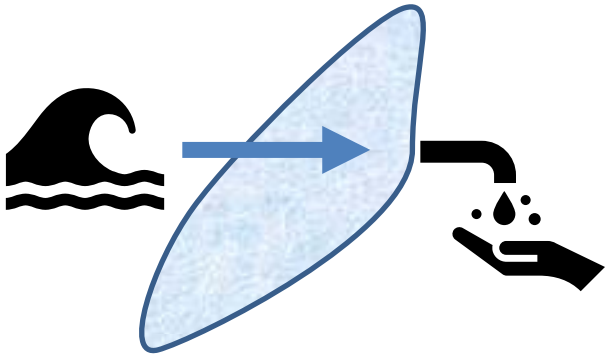
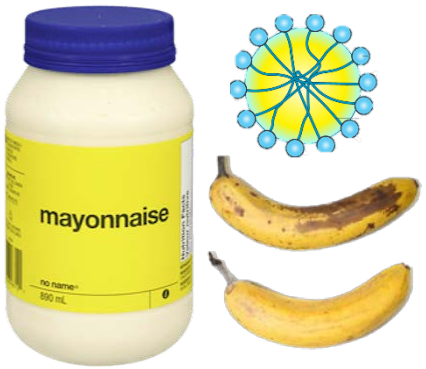
LCRA

Life Cycle Risk Analysis
Application of Toolbox to Demonstrate Safety of Priority Commercial CN Forms and Applications





CS1: Carboxylated CNF Water Filtration Membranes	**CS2.1**: Carboxylated CNF Food Packaging	**CS2.2**: Sulfated CNF Food Packaging	**CS3**: Carboxylated CNF Food Additive

Next Steps

Safety

1. Regulatory submissions
2. Publish ADME Study & PCHEM data
3. Standardize characterization methods

& Functionalization Work

1. Publish methods and data sets
2. Publish Safety findings
3. Data structure for toolbox
4. Additional synthesis methods & characterization
5. Standardize characterization methods

Technical Challenges

Technical

- Methods
  - Characterization
  - Labeling
  - Sample preparation
  - EHS Media assessments
    (air, biological media)

Regulatory

- Characterization
- Methods Validation
- Alternative testing strategies
  - Migration testing
  - Unique properties affect dosing
  - Particulate effects

Unusual Issues

- Methods
  - Free label
  - Measurement challenges
- Grouping and read-across
- Timing
  - Siloing, bureaucracy
  - COVID-19
  - Policy changes
  - Personnel changes

PUBLICATIONS TO DATE

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4. Ong, K.J, Ede, J.D., Pomeroy-Carter, C.A., Sayes, C.M., Mulenos, M.R., Shatkin, J.A. (2020) A 90-day dietary study with fibrillated cellulose in Sprague-Dawley rats. *Tox Rep* 7: 174-182. DOI: 10.1016/j.toxrep.2020.01.003 .

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8. Ede JD…Shatkin JA et al (2019) Risk Analysis of Cellulose Nanomaterials by Inhalation: Current State of Science, *Nanomaterials. 9(3):337. https://doi.org/10.3390/nano9030337*.

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10. Roberts R, R., Gettz, K., Stebounova, L.V., Peters, T., Shatkin, J.A., Foster, J.E (2018) Collection of airborne ultrafine cellulose nanocrystals by impinger with an efficiency mimicking deposition in the human respiratory system. *J Occup Environ Hygiene* DOI: 10.1080/15459624.2018.1540876

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The partners of the Alliance for the Food Safety Acceptance of Fibrillated and Crystalline Celluloses

The Vireo Team

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The Vireo Team

Dr. Jo Anne Shatkin is an expert in novel product safety and environmental and health policy issues, with over 20 years experience leading projects in risk analysis, safety and regulatory policy work including numerous publications.

She is founder and president of Vireo Advisors in Boston, Massachusetts.

Dr. James D. Ede is a toxicologist experienced in testing strategies for novel materials, including molecular, biochemical and cellular techniques, and is experienced in life cycle risk assessment.

Dr. Kimberly J. Ong is a biologist and environmental scientist. Dr. Ong is an expert in developing protocols specific for novel material testing to improve reliability for risk and exposure assessment and is experienced in regulatory analysis for novel products.

Dr. Shaun Clancy is a chemist with over 30 years experience in the chemicals industry, directing programs in health, safety, and regulatory affairs in major corporations. He is ANSI Co-Chair and participates in ISO TC229 and other international safety committees.

Fiona Case is a content writer with more than 20 years experience covering scientific innovations in foods, personal and home care products, sustainability, and computer-aided materials design.

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Leslie Hockman has industry experience working for a commercial manufacturer of cellulose nanomaterials and is Vireo's administrator.

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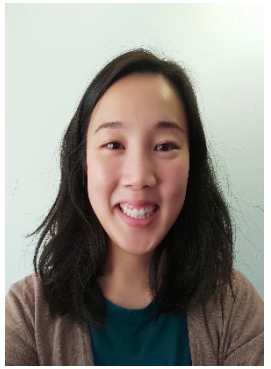

Wei Ng is an intern and is currently pursuing her PhD in Biological and Biomedical Science from Yale University.

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Kora Kukk is a fellow and a UMaine graduate with an M.S. in Biomedical Engineering.

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Tatiana von Rheinbaben is a fellow and a M.S. Environmental Engineering and Science graduate of Stanford University.

Thank you!

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