Safety testing methods for novel cellulose nanomaterials

KIMBERLY ONG, JAMES EDE, JO ANNE SHATKIN

VIREO ADVISORS, LLC

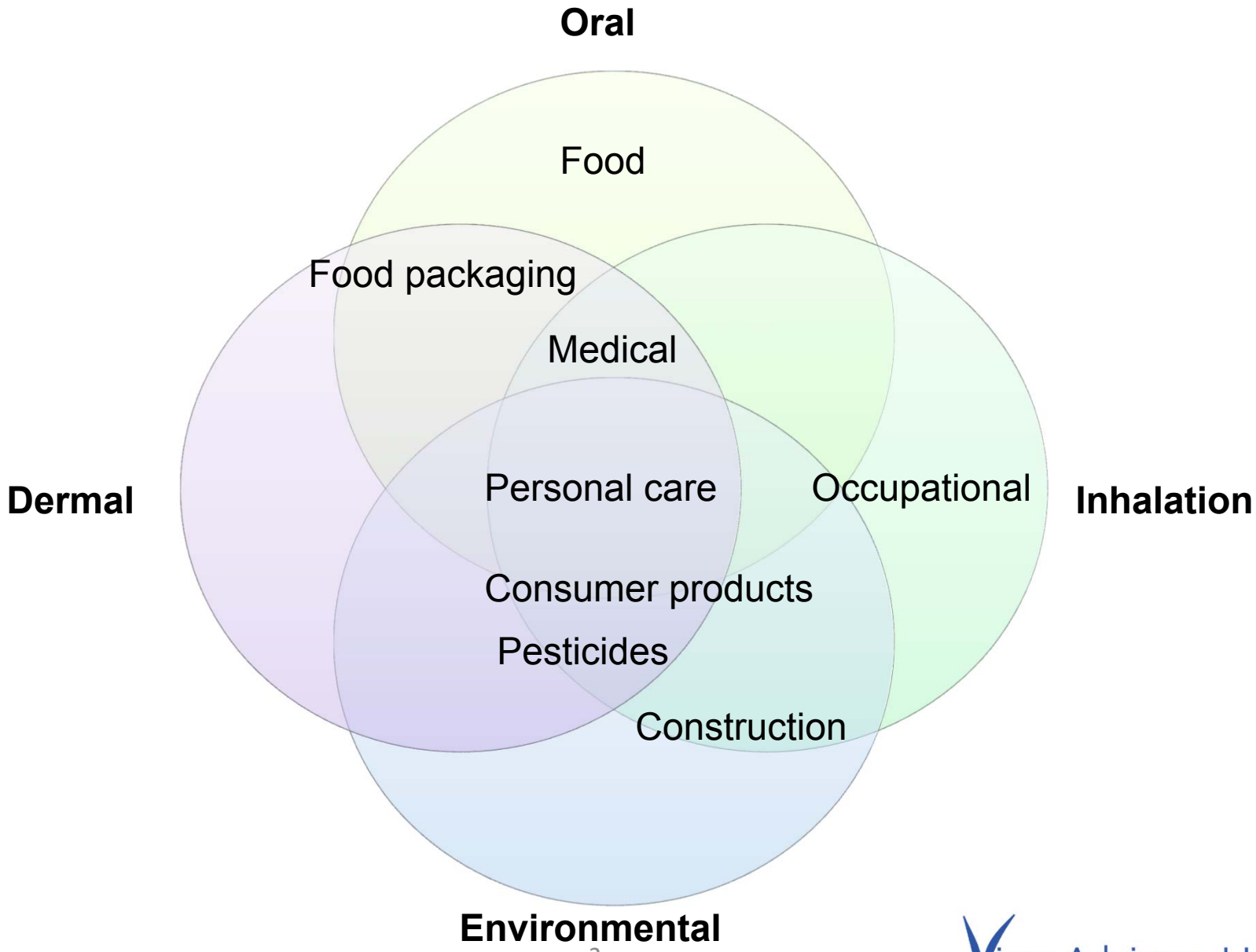
15 JUNE 2021

Vireo Advisors, LLC

TAPPI Nano 2021 Virtual Conference
15-16 June 2021

TAPPI NANO DIVISION

APPLICATIONS & TESTING



Vireo Advisors, LLC

Safety Testing Methods
Considerations

- Are they appropriate?
- Are they validated?
- Can they be used in multiple jurisdictions?
- Do they involve animal use?
- Do they need to be notified prior to start?
- Are there nanomaterial-specific considerations?

STANDARDS AND GUIDANCE

- Use standard testing methods: OECD, ISO Standards, ASTM
  - Modifications to standard test methods are allowed, but justification required
- Good Laboratory Practice (GLP)
  - Most commercial labs can do “GLP” testing, which ensures rigorous methodological standards and recordkeeping. This is a requirement for most regulatory submissions.
- Some guidance available
  - European Food Safety Authority,
  - For new substances, including nanomaterials, may require additional testing

SAFETY STUDIES

Human Health

Endpoint	
Genotoxicity	Read-across
Bacterial Assay	Cell viability, ROS production
In vitro Genotoxicity	*in vitro* immunotoxicity
In vivo Genotoxicity	Membrane/barrier integrity
Dermal/Eye	Oral
Skin Irritation/Corrosion	Toxicokinetics
Skin Sensitization	Simulated gut barrier
Eye Irritation	Simulated digestion
Inhalation/Dermal/Oral	Other
Acute toxicity	Reproductive/ Developmental
Sub-chronic toxicity	Multi-generational
Chronic toxicity	Carcinogenicity

Environment

Endpoint
Biodegradation
Aerobic degradation
Anaerobic degradation
Environmental Toxicity
Aquatic Plants
Bacteria
Aquatic Invertebrates (Acute, Chronic)
Fish (Acute, Chronic)
Bioaccumulation

Vireo Advisors, LLC

SAFETY STUDIES

Human Health

Endpoint	
Genotoxicity	Read-across
Bacterial Assay	Cell viability
In vitro Genotoxicity	ROS production
In vivo Genotoxicity	*in vitro* immunotoxicity
Dermal/Eye	Oral
Skin Irritation/Corrosion	Toxicokinetics
Skin Sensitization	Skin Sensitization
Eye Irritation	Eye Irritation
Inhalation/Dermal/Oral	Other
Acute toxicity	Reproductive/ Developmental
Sub-chronic toxicity	Multi-generational
Chronic toxicity	Carcinogenicity

Environment

Endpoint
Biodegradation
Aerobic degradation
Anaerobic degradation
Environmental Toxicity
Aquatic Plants
Bacteria
Aquatic Invertebrates (Acute, Chronic)
Fish (Acute, Chronic)
Bioaccumulation

Tests may not be valid for nanomaterials

Bacterial assay (Ames)

- Can't cross the membrane of the bacteria

Aquatic invertebrates

- CNs can bind to the organisms, preventing movement

Vireo Advisors, LLC

SAFETY STUDIES

METHODOLOGICAL CONSIDERATIONS

- Use appropriate concentrations
- Realistic delivery
- Control groups – other celluloses
  - There may be effects – but are they different/similar than other forms of cellulose?
- Sample preparation – consistent method
- Specifically for CN, viscosity can be a challenge
  - e.g. gavage
- Repeat-dose testing, must ensure that the CNs are uniformly dispersed throughout the food/media otherwise the organisms get different doses
  - Multiple batches must be produced, must be the same each time

DETECTION

- Important for many studies
  - To measure stability, presence, concentration
  - To track the CNs in the organism/media
- ”Conventional” methods not appropriate for CNs
- Detection/quantitative measurement
  - Electron microscopy
  - Mass balance
  - Biochemical assays
- Labeling of material
  - Radiolabel
  - Fluorescent label

DETECTION/QUANTITATIVE MEASUREMENT

- Electron microscopy
- Mass balance
- Biochemical assays
  - Standard methods of detection for fiber - AOAC 991.43 (dietary fiber), ANKOM, neutral/acid detergent fiber methods) have low detection limits
  - Glucose anthrone assay

Ong et al. 2020. A 90-day dietary study with fibrillated cellulose in Sprague-Dawley rats, Toxicology Reports, 7, 174-182

Detection of cellulose

Use a label that is stable in the gastrointestinal tract/organism/test system, but also does not affect the structure/pchem of cellulose or the toxicity, and is detectable after the study

- Measure pH stability, changes in physical chemical properties, extent of labeling of cellulose

Fluorescent Labeling

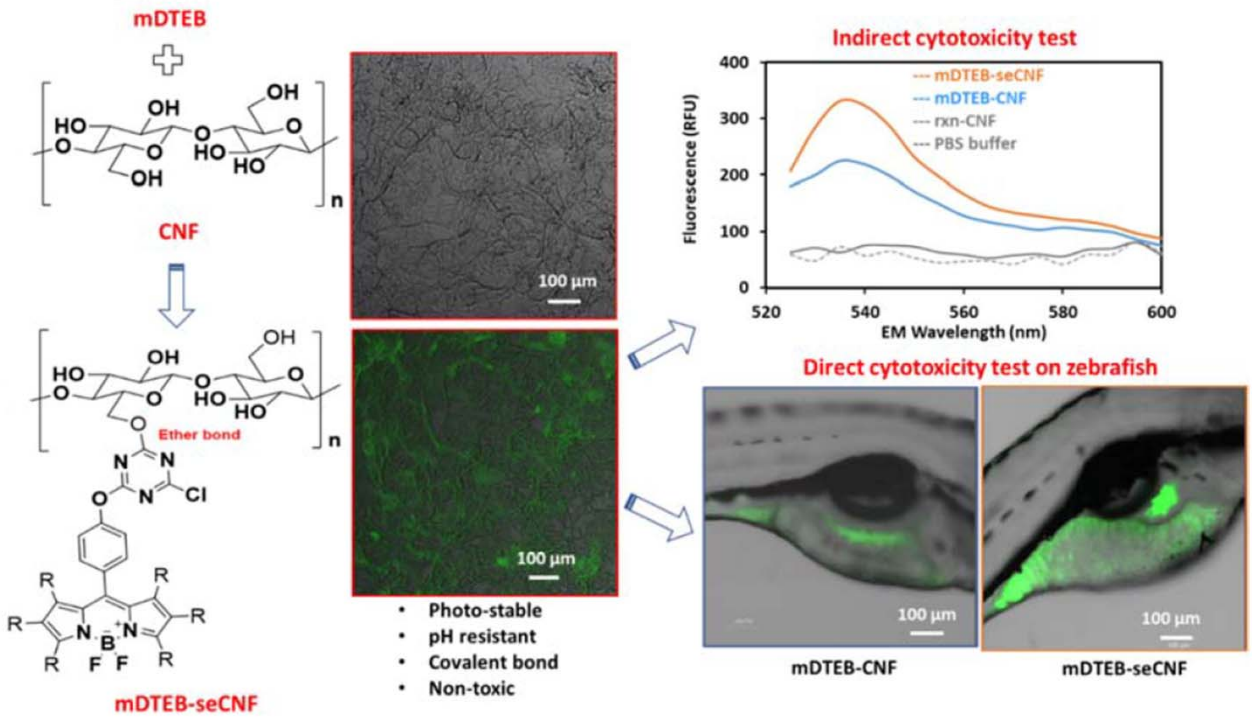
- Stable (low pH, enzyme, etc. of digestive tract)
- Low toxicity and cytotoxicity
- Difficulties in quantitatively detecting in biological fluids and tissue

Radiolabeling

- The standard for toxicokinetic testing
- Most commercial labs are equipped to do toxicokinetic testing with C14
- Challenge is to find a lab that can do relevant physical chemical characterization on radiolabeled materials

DETECTION - LABELED CNF

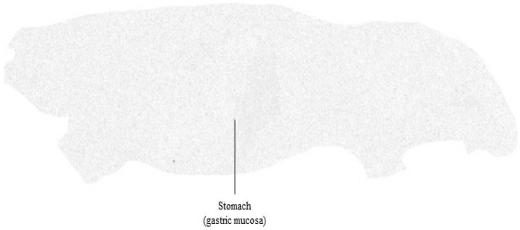
EHS studies - mDTEB-CNF



Patel et al. 2021. Fluorescently labeled cellulose nanofibers for environmental health and safety studies. Nanomaterials, 11, 1015.

Food Contact Materials Migration studies Texas Red - CNF

Toxicokinetic studies 14C - CNF



Vireo Advisors, LLC

ACKNOWLEDGEMENTS

P^{3}Nano, the U.S. Endowment for Forestry and Communities and the USDA US Forest Service Forest Product Laboratory (FPL).

AFSAC partners

Douglas Fox - American University

Jeff Gilman, Jeremiah Woodcock, Ilabahen Patel, Ryan Beams, Stephan Stranick, Ryan Nieuwendaal - NIST

Christie Sayes, Marina Mulenos – Baylor University

Philip Demokritou, Glen DeLoid, Maryam Salari – Harvard University

Stacey Harper, Bryan Harper – Oregon State University

Heli Kangas and Ilona Leppänen – VTT Technical Research Centre of Finland

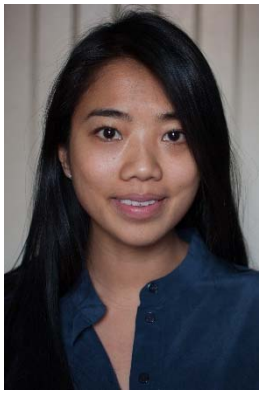
The Vireo Team

The Team at Vireo

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.

Patricia Hodgkinson is Vireo's research consultant with a background in Food Policy law.

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

Jerry Liu and Megan Roberts are interns and Ph.D. Students at the University of Toronto

Ana Diges is an intern and recent UW-Madison Graduate in Materials Science and Engineering

We're Always Looking for New Talent to Join Our Team!!

Vireo Advisors, LLC

Thank you!

Kimberly Ong, Ph.D.

kong@vireoadvisors.com

www.VireoAdvisors.com/blog

Twitter and LinkedIn
@Vireo_Advisors

Vireo Advisors works internationally to advise organizations about safety and build consortia for safe commercialization of new technologies such as bio-based materials, nanotechnologies and nanomaterial-enabled products.

Vireo Advisors, LLC