U.S. Endowment for Forestry and Communities

USDA Forest Service U.S. DEPARTMENT OF AGRICULTURE

Safer-by-Design Toolbox to Advance Functionalized Cellulose Nanomaterials: Toolbox Development and Life Cycle Risk Assessment

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Vireo Advisors, LLC
Raising the Bar on Sustainability in Innovation

UCDAVIS

Baylor University

TAPPI NANO DIVISION

Toolbox 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

3. 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*.

Structure of the Toolbox

The Toolbox is currently under development and organized into 10 worksheets, including an introduction to the Toolbox; an experimental overview; Compendium of physical, chemical and toxicological methods and protocols; as well as databases of physical, chemical and toxicological data generated to date for different cellulose surface chemistries.

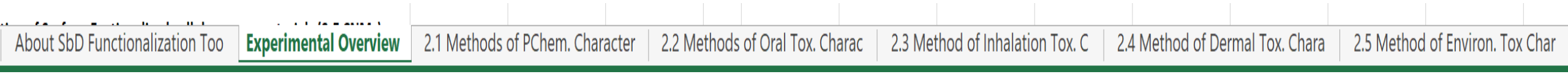
Tab 1.0 ➢ About the Functionalization toolbox (goals, structure and organization and how to use)

Tab 1.1 ➢ Experimental overview

Tab 2.2-2.5 ➢ Methods and protocols (Pchem Characterization; oral, inhalation, dermal and environmental toxicity characterization)

Tab 3.1-3.5 ➢ Database (Pchem data, Oral, Inhalation, Dermal, Environmental toxicity data)

Tab 4 ➢ Life Cycle Assessment



Toolbox Methods & Data Development

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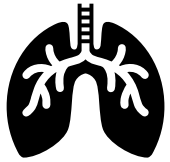
Methods and data to evaluate safety of CNs forms:



Inhalation

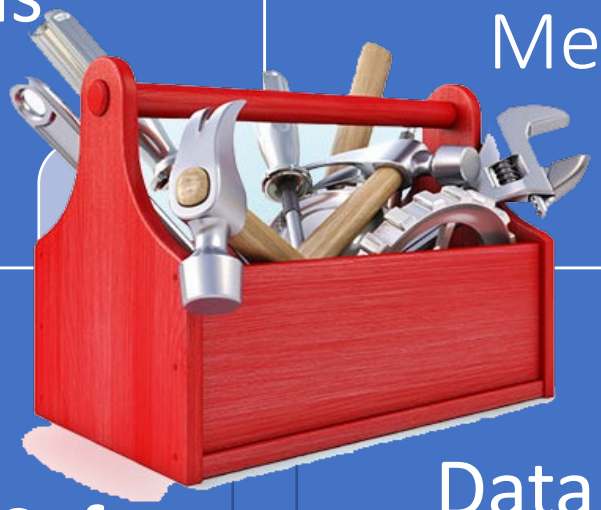
Oral

Dermal

Env.

ATS Safety Testing Methods

Physical Chemical Characterization Methods

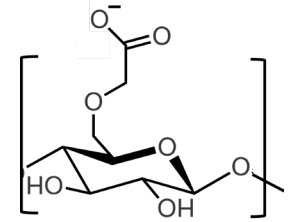


Database of Safety Data

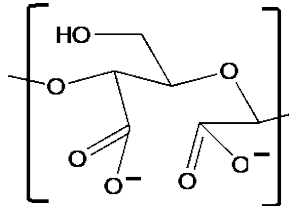
Database of Physical and Chemical Data

LCRA

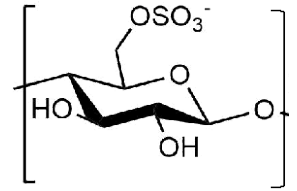
1st generation of modified CNs:



TEMPO



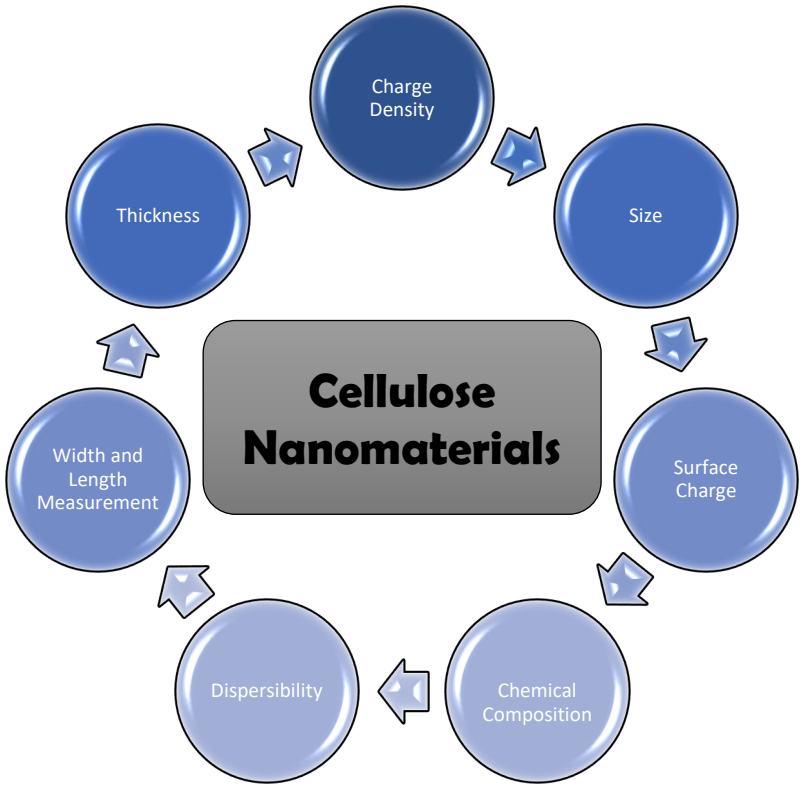
C2/C3 Carboxy



Sulfated

Toolbox Includes Physical Chemical Characterization Methods

Endpoints



SOPs for Physical Chemical characterization

Physical and chemical characterization of cellulose suspensions. Physical and chemical tests were conducted on the twelve cellulose suspensions.

Characterization of Pristine cellulose nanomaterials

Physio-chemical properties	Method	SOP	Link to SOP	Reference	Notes/Follow-up
Particle size, length and width	Atomic force microscopy (AFM)	The atomic force microscopy (AFM) technique was used for image analysis, specifically using an Asylum Research MFP-3D. OMCL-AC160TS standard silicon probes with a nominal tip radius of 7 nm and a spring constant of 26 N/m were used to conduct this analysis. To collect surface profiles, a few drops of diluted SCNF dispersion (approximately 0.0001 wt %) were placed on freshly cleaved mica discs, allowed to dry, and then analyzed under ambient conditions using tapping mode.			Review/Confirm with You-lo's manuscript on Pchem Characterization of "Pristine CNFs"
Thickness	Atomic force microscopy (AFM)	Sample suspension (10 mL, 0.002 wt%) was deposited onto a freshly cleaved mica surface, air-dried, scanned (Asylum-Research MFP-3D) in air under ambient condition using tapping mode with OMCL-AC160TS standard silicon probes. The scan rate was set to 1 Hz and image resolution is 512 * 512 pixels. The height images and profiles were processed with Igor Pro 6.21 loaded with MFP3D 090909 + 1409, and the average thickness was determined from ca. 200 individual nanofibrils.		Jiang, 2013 Patterson, 2020	
Width	Transmission electron microscopy (TEM)	Samples were prepared by placing a drop of diluted SMFC dispersion (ca. 0.0001 wt %) on a glow-discharged carbon grid and blotting away the excess after 10 min. Samples were negatively stained with 2 wt % uranyl acetate to enhance contrast. Micrographs were taken with a LaB6 electron source by using an accelerating voltage of 100 kV.		Pingrey, 2022	
	Transmission electron microscopy (TEM)	Sample suspension (8 ml, 0.01 wt%) was deposited onto glow-discharged carboncoated TEM grids (300-mesh copper, formvar-carbon, Ted Pella Inc., Redding, CA) with the excess liquid being removed by blotting with a filter paper after 10 min. The specimens were then negatively stained with 2% uranyl acetate solution for 5 min, blotted with a filter paper to remove excess staining solution and allowed to dry under the ambient condition. The samples were observed using a Philip CM12 transmission electron microscope operated at a 100 kV accelerating voltage. The width of CNFs was measured from ca. 200 individual nanofibrils using an image analyzer (ImageJ, NIH, USA). Again, a cut-off value of 5 nm was used for CNF1.5 and CNF3 to exclude the larger fibrils.		Jiang, 2013	

Characterization of simulated digested cellulose materials

Physio-chemical properties	Method	SOP	Link to SOP	Reference	Notes/Follow-up
Particle Size and Surface Charge Analysis	Dynamic light scattering with a Zetasizer Nanoseries Nano-ZS (Malvern Pananalytical, Almelo, Netherlands)	To determine the hydrodynamic diameter (HDD), dispersity index (DI), and zeta potential (ζ potential), DLS techniques were acquired with a Zetasizer Nanoseries Nano-ZS (Malvern Pananalytical, Almelo, Netherlands). A dilution of the stock solution was required for this analysis to ensure precision. Cellulose stock suspensions were diluted to 0.01% with ultrapure water and transferred to a disposable folded capillary cell DTS 1060 (Malvern Pananalytical, Almelo, Netherlands). For HDD and DI, each sample was scanned for 10 s, 11 times, in triplicate. A 173◦ backscatter angle was used in general purpose mode. For zeta potential measurements, the Helmholtz–Smoluchowski model was utilized at 25 runs, in triplicate, for each sample in auto report mode. Each sample had a mean count rate greater than 1000 counts per measurement to ensure accuracy.		Pradhan et al., 2020	This was the only pchem characterization description outlined in the methodology section of Sayes manuscript, just wanted to confirm its related to "Pristine CMs" and not Digested forms.

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Toolbox Includes Database of Physical Chemical Data

Physical and chemical properties of pristine CNs

For the sample identifiers (IDs), SCNF refers to sulfated CNF, TCNF refers to TEMPO CNF, and PCCNF refers to periodate-chlorite CNF. The a, b, c, and d notations represent four different functionalities within each CNF series. The stock concentrations were 1 w/v% for SCNF and 0.6 w/v% for TCNF and PCCNF.

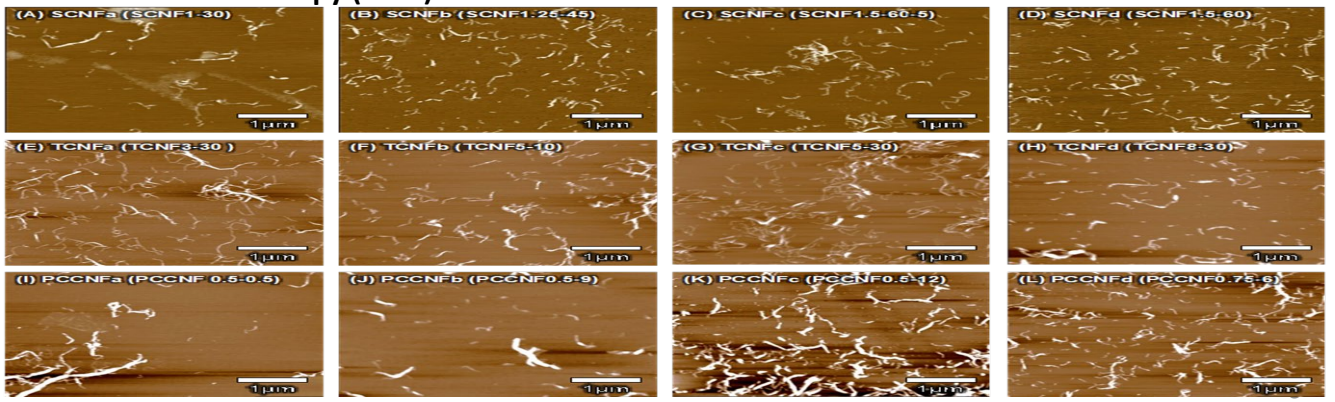
Sample ID	Reagent, concentration (mmol/g), time (min)	Blending time (min)	Charge (mmol/g)	Width (nm)	Length (nm)
CNF					
SCNFa	HSO3Cl, 1, 30	30	1.49	3.2+0.9 n=56	693±330 n=166
SCNFb	HSO3Cl, 1.25, 45	30	1.84	4.0+1.0 n=73	577±294 n=175
SCNFc	HSO3Cl, 1.5, 60	5	2.23	3.2+0.7 n=103	501±295 n=100
SCNFd	HSO3Cl, 1.5, 60	30	2.23	NA	365±194 n=100
TCNFa	NaClO, 3, 30	30	1.1	6.5±2.2 n=50	551±200 n=50
TCNFb	NaClO, 5, 50	10	1.42	6.1±1.6 n=47	486±174 n=50
TCNFc	NaClO, 5, 60	30	1.42	4.6±1.6 n=30	530±145 n=50
TCNFd	NaClO, 8, 80	30	1.48	4.9±2.0 n=50	486±206 n=50
PCCNFa	NaIO4, 3.08, 4; NaClO2, 6.16, 30	30	0.72	5.9±1.6 n=50	452±162 n=30
PCCNFb	NaIO4, 3.08,4 NaClO2, 6.16, 9h	30	0.82	5.7±1.6 n=47	533±275 n=30
PCCNFc	NaIO4, 3.08, 4; NaClO2, 6.16, 12h	30	0.91	5.5±1.6 n=30	381±150 n=30
PCCNFd	NaIO4, 4.62, 4; NaClO2, 6.16, 6h	30	1.04	5.6±1.7 n=50	344±170 n=30

Physical and chemical characterization before and after simulated digestion

The table reports the average hydrodynamic diameter (HDD) ± the standard deviation, dispersity index (DI) for each digestion period. No other statistical significance was observed.) DIs (DI) ± the standard deviation, and zeta potential (ZP) ± the standard deviation. ∗P < 0.05 for unmodified CN against all functionalized forms in ultrapure water and after simulated digestion.

	In ultrapure water			After simulated digestion		
Sample ID	HDD (nm)	DI (unitless)	ZP (mV)	HDD (nm)	DI (unitless)	ZP (mV)
CNF	14,400 ± 104	0.827 ± 0.16	-33.1 ± 0.945	977 ± 154	0.750 ± 0.068	-25.2 ± 0.321
SCNFa	846 ± 250	0.965 ± 0.061	-61.2 ± 6.59	436 ± 32.4	0.453 ± 0.052	-39.1 ± 2.12
SCNFb	1,170 ± 322	0.978 ± 0.038	-36.6 ± 3.66	403 ± 17.5	0.498 ± 0.032	-46.2 ± 0.265
SCNFc	1,440 ± 217.3	0.980 ± 0.035	-60.9 ± 3.74	522 ± 23.52	0.526 ± 0.123	-38.8 ± 1.42
SCNFd	602 ± 40.6	0.886 ± 0.054	-57.3 ± 1.89	477 ± 51.43	0.602 ± 0.02	-39.6 ± 1.42
TCNFa	1,160 ± 167.4	0.998 ± 0.002	-53.9 ± 0.643	489 ± 63.23	0.551 ± 0.05	-37.9 ± 1.25
TCNFb	1,440 ± 217.3	0.980 ± 0.035	-60.9 ± 3.74	522 ± 23.52	0.526 ± 0.123	-38.8 ± 1.42
TCNFc	602 ± 40.6	0.886 ± 0.054	-57.3 ± 1.89	477 ± 51.43	0.602 ± 0.02	-39.6 ± 1.42
TCNFd	625 ± 53.75	0.867 ± 0.084	-53.2 ± 3.57	455 ± 35.29	0.540 ± 0.029	-41.6 ± 0.643
PCCNFa	1,160 ± 167.4	0.998 ± 0.002	-53.9 ± 0.643	489 ± 63.23	0.551 ± 0.05	-37.9 ± 1.25
PCCNFb	1,440 ± 217.3	0.980 ± 0.035	-60.9 ± 3.74	522 ± 23.52	0.526 ± 0.123	-38.8 ± 1.42
PCCNFc	602 ± 40.6	0.886 ± 0.054	-57.3 ± 1.89	477 ± 51.43	0.602 ± 0.02	-39.6 ± 1.42
PCCNFd	625 ± 53.75	0.867 ± 0.084	-53.2 ± 3.57	455 ± 35.29	0.540 ± 0.029	-41.6 ± 0.643

Atomic force microscopy (AFM)



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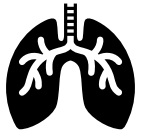
Toolbox Includes Alternative Testing Strategies (ATS)

ATS developed for safety assessment



- Simulated inhalation exposure: alveolar lung model

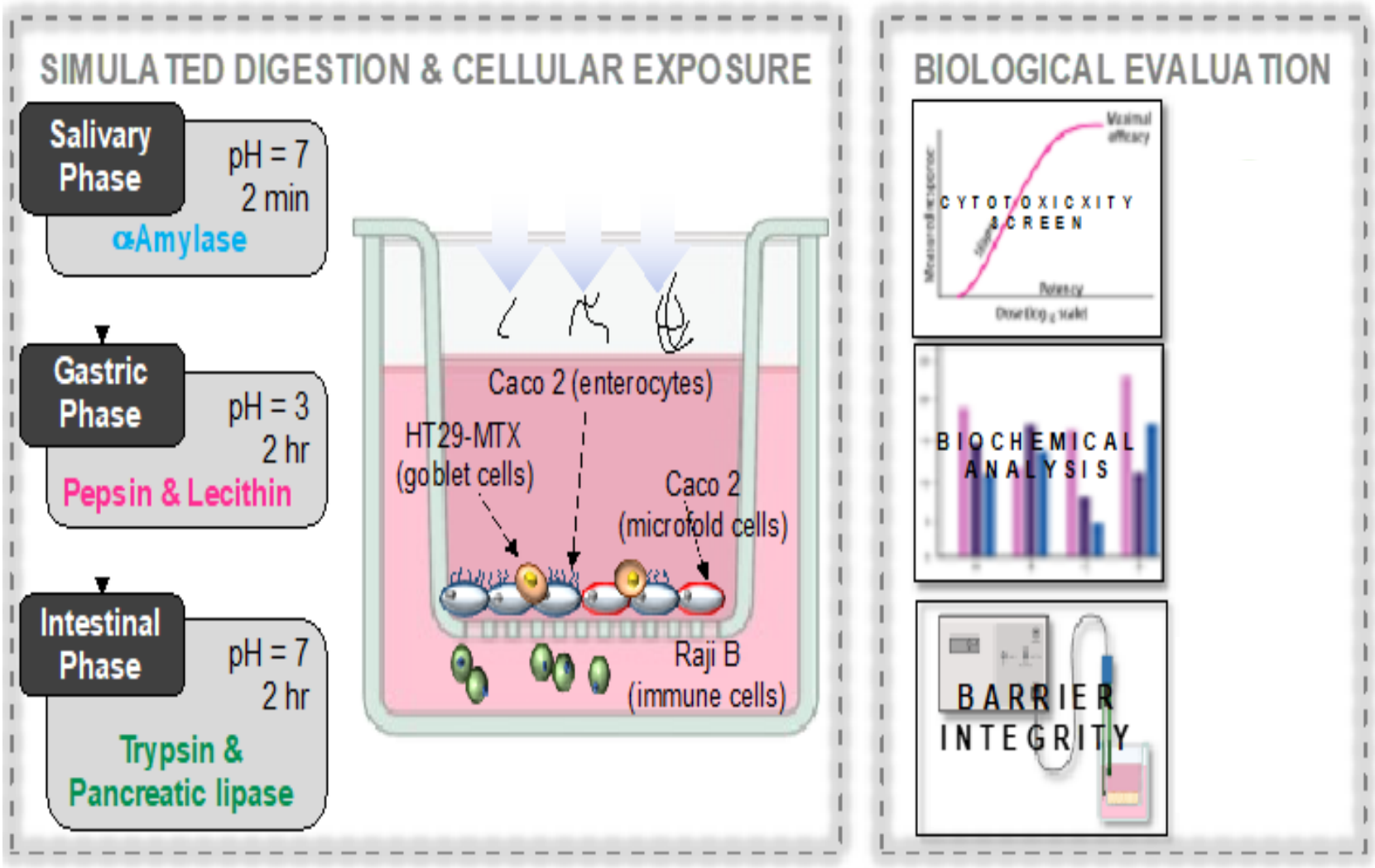
- Simulated oral exposure: gastrointestinal model

- Dermal exposure: dermal and epidermal cells

- Environmental toxicity: zebrafish

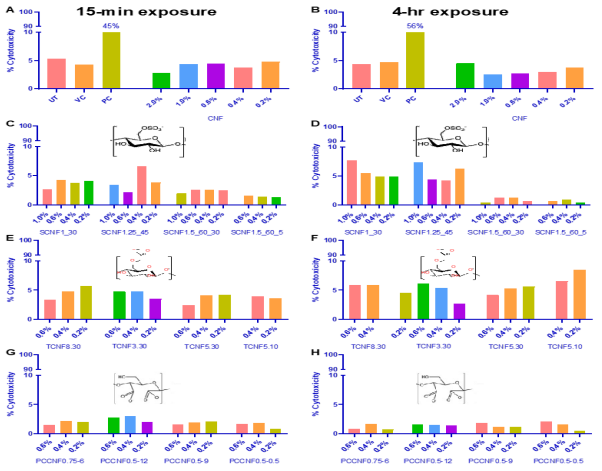
Toolbox Includes ATS Methods & Protocols

E.g. Oral Exposure Safety Assessments

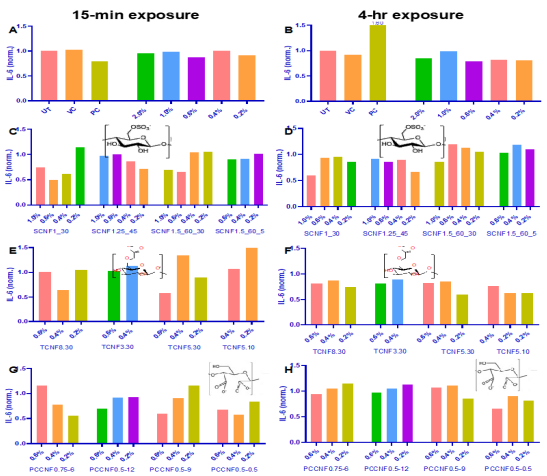


Toolbox Includes Database of Safety Data

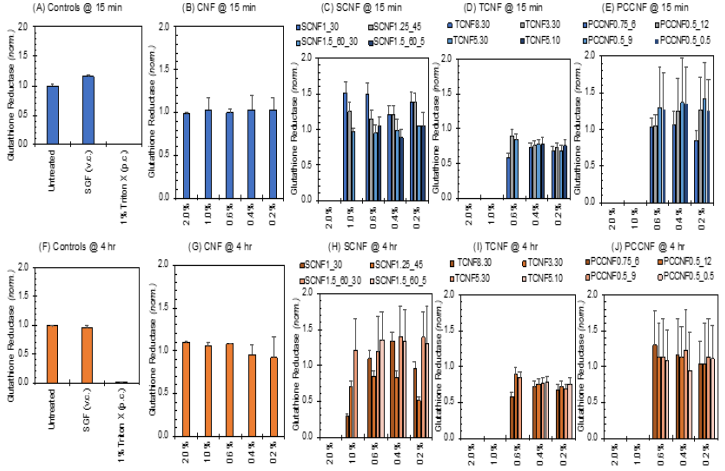
Cytotoxicity



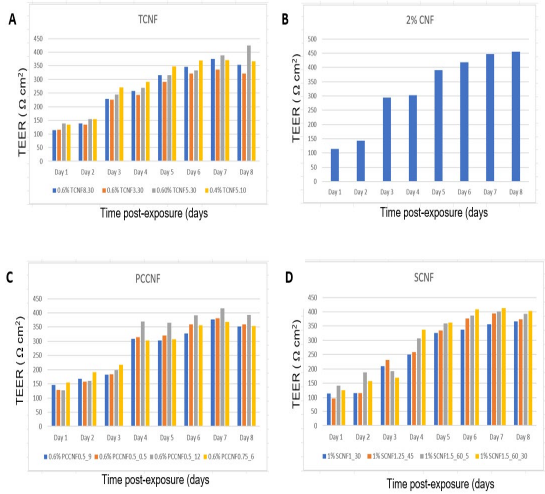
Cellular pro-inflammatory response



Cellular oxidative stress response



Cytoplasmic membrane integrity



RAW DATA

Endpoint	Concentration	1% CNF	2% CNF	TCNF3.30	TCNF5.10	TCNF5.30	TCNF8.30	SCNF1_30	SCNF1.25 45	SCNF1.5_60_5	SCNF1.5_60_30	PCCNF 0.5-0.5H	PCCNF 0.5-9H	PCCNF 0.5-12H	PCCNF 0.75-6H	1% CNF	2% CNF	TCNF3.30	TCNF5.10	TCNF5.30	TCNF8.30	SCNF1_30	SCNF1.25 45	SCNF1.5_60_5	SCNF1.5_60_30	PCCNF 0.5-0.5H	PCCNF 0.5-9H	PCCNF 0.5-12H	PCCNF 0.75-6H
		15 min														4hrs													
Viability (LDH)	1%	2.36	2.54	x	x	x	x	2.68	3.45	X	0.67	X	X	X	X	1.8	2.54	x	x	x	x	5.93	8.78	x	0.67	x	x	x	x
	0.6%	1.89	1.08	2.83	x	2.44	3.4	2.58	2.2	0.92	1.95	3.06	2.72	2.01	1.06	2.25	1.08	7.27	x	4.26	6.76	6.25	4.77	0.92	1.95	3.06	2.72	2.01	1.06
	0.4%	2.03	1.25	2.63	2.57	2.5	3.09	1.85	2.69	1.11	1.8	2.46	1.89	2.01	1.98	2.75	1.25	6.13	8.08	6.06	7.14	5.74	4.45	1.11	1.8	2.46	1.89	2.01	1.98
	0.2%	2.22	2.84	1.87	2.25	2.64	2.54	2.28	2.46	0.6	0.91	0.56	1.73	0.4	1.27	3.45	2.84	2.31	5.77	6.76	5	5.74	7.67	0.6	0.91	0.56	1.73	0.4	1.27
Oxidative Stress (GR)	1%	0.9	1.04	x	x	x	x	1.51	1.25	x	0.97	x	x	x	x	0.91	1.06	x	x	x	x	0.29	0.7	x	1.21	x	x	x	x
	0.6%	1.31	1.01	1.44	x	0.92	1.15	1.49	1.15	1.04	0.95	1.27	1.3	1.04	1.03	1.2	1.08	0.9	x	0.84	0.58	1.08	0.84	1.35	1.19	1.08	1.12	1.12	1.3
	0.4%	1.17	1.04	1.44	1.01	1.3	1.46	1.21	1.22	0.88	0.99	1.34	1.37	1.24	1.06	0.58	0.95	0.75	0.79	0.77	0.72	1.33	0.84	1.34	1.39	0.96	1.24	1.14	1.17
	0.2%	1.33	1.04	0.98	x	x	1.2	1.39	1.37	1.05	1.06	1.25	1.42	1.26	0.85	1	0.92	0.72	0.76	0.69	0.67	0.95	0.51	1.31	1.39	1.12	1.14	1.05	1.04
NOEC																													

	Post-exposure time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Cellular barrier Integrity (TEER)	Untreated	111	153	201	302	318	394	418	412
	Vehicle control (SGF)	110	115	158	293	294	279	344	366
	50 µM rotenone (pos control)	186	128	105	96	137	181	153	115
	2% CNF	114	143	294	303	391	418	447	455
	1% SCNF1_30	114	116	210	250	326	337	357	366
	1% SCNF1.25_45	96	115	231	259	335	376	394	374
	0.6% TCNF8.30	114	139	228	258	316	346	375	353
	0.6% TCNF3.30	116	134	226	243	291	321	336	322
	0.60% TCNF5.30	138	154	245	270	316	333	389	424
	0.4% TCNF5.10	134	154	271	291	347	370	371	366
	1% SCNF1.5_60_5	142	187	192	307	360	387	402	392
	1% SCNF1.5_60_30	126	158	170	337	363	409	413	403
	0.6% PCCNF0.5_9	146	168	182	309	303	327	377	352
	0.6% PCCNF0.5_0.5	128	158	183	314	320	359	381	359
	0.6% PCCNF0.5_12	127	161	199	370	365	391	416	393
	0.6% PCCNF0.75_6	154	191	217	303	307	356	368	354

Life-Cycle Risk Assessment (LCRA)

1. LCRA is an iterative and adaptive screening-level risk assessment framework
   - Develop qualitative exposure scenarios that describe potential exposures impacting workers, the public or the environment based on intended use across the product life-cycle.
   - These scenarios are ranked by applying exposure criteria to identify priority pathways for evaluation.
   - The risk assessment uses simplified worst-case assumptions.
   - Each iteration identifies what information is needed to make a better decision.

1. Identify and Characterize Potential Hazards

2. Assess Exposure Potential and Risk

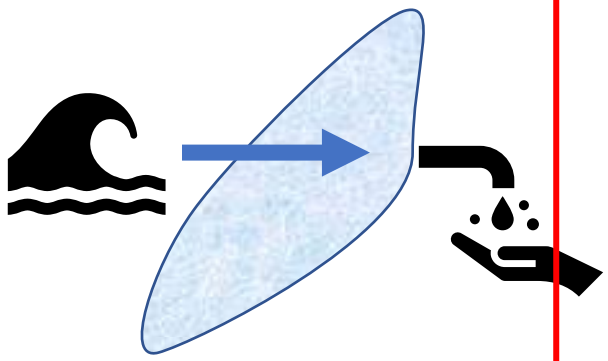
3. Prioritize Data Gaps

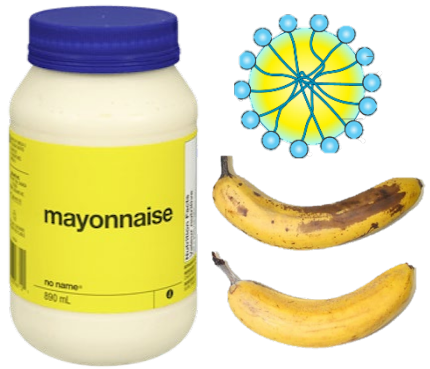
4. Address Data Gaps (E.g., Toxicity Studies)

Output: **Safety Demonstration**

LCRA

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

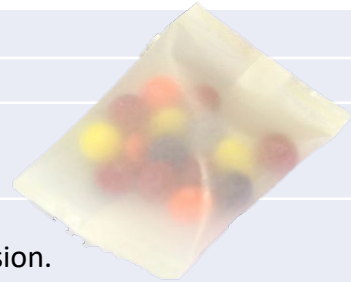




CS1: Carboxylated CNF Water Filtration Membranes	**CS2.1**: Carboxylated CNF Food Packaging	**CS2.2**: Sulfated CNF Food Packaging	**CS3**: Carboxylated CNF Food Additive

Life-cycle Mapping & Exposure Scenario Development

Manufacture of sulfated/carboxylated cellulose 'nanopaper' food packaging case study		
Life-cycle stage	**Activity**	**Assumptions (By Life Cycle Stage)**
Raw Material	The starting material is chipped or shredded wood, obtained from lumber mills.	Assumes no cellulose nanomaterial is produced.
Product Manufacturing	The raw material is treated and processed according to the specific Case Study chemistry.	Assume sulfated and carboxylated cellulose production. Assume original batch formulation made is 2% C-CN and S-CN suspension. This stage takes place indoors with limited environmental release.
Product Application	CS2.1 follow a simple casting method, where the CNM dispersion is simply left to dry on a die to produce a film. CS2.2 instead sprays this dispersion onto a food packaging substrate to form a coating.	Assumes at least one transportation step to another facility or designated manufacturing space. (Possible) redispersion step from powder form. Loose powder exposures are possible. Assume casting 2% or greater concentration wet suspension, then left to cure/dry (up to 100%). This stage takes place indoors with limited environmental release.
Product Use	Intended to be single-use products.	Assumes intended use and practices will result in lower hazard (nanoparticles better bound in matrix) and frequency (assumes user will practice correct packaging use).
Re-use/Recycling	Remain in use beyond their recommended lifespan or modified through a variety of repurposing activities for use in new consumer products.	Assumes one transportation activity to remanufacturing facility. Dilution of the C-CN and S-CN product with other substances during new product formulation activity accounts for a lower magnitude of potential exposure.
Disposal	The original or reprocessed CNM products are disposed of as waste.	Consumer products are likely to end up in a landfill or incinerated for heat recovery. The products may also be discarded intentionally or unintentionally in an uncontrolled environment.



Scenario Development

- **Sulfated/carboxylated cellulose 'nanopaper' food packaging**

Life Cycle Stage	LC Stage Code	LC Stage #	Scenario #	Scenario	Receptor	Exposure Route
Raw Material	RM	1	1	Harvesting, chipping/shredding softwood	occupational	
Product Manufacturing	PM	2	1	Cleaning out synthesis equipment	occupational	inhalation
Product Manufacturing	PM	2	2	Cleaning out synthesis equipment	occupational	dermal/eye
Product Manufacturing	PM	2	3	Cleaning out synthesis equipment	environmental	direct
Product Manufacturing	PM	2	4	Incidental release of cellulose nanomaterial from synthesis equipment	occupational	inhalation
Product Manufacturing	PM	2	5	Incidental release of cellulose nanomaterial from synthesis equipment	occupational	dermal/eye
Product Manufacturing	PM	2	6	Incidental release of cellulose nanomaterial from synthesis equipment	environmental	direct
Product Manufacturing	PM	2	7	Accidental spill of cellulose nanomaterial from synthesis equipment	occupational	inhalation
Product Manufacturing	PM	2	8	Accidental spill of cellulose nanomaterial from synthesis equipment	occupational	dermal/eye
Product Manufacturing	PM	2	9	Accidental spill of cellulose nanomaterial from synthesis equipment	environmental	direct
Product Manufacturing	PM	2	10	Dried formulation extraction and handling (for powder NC ingredients)	occupational	inhalation
Product Manufacturing	PM	2	11	Dried formulation extraction and handling (for powder NC ingredients)	occupational	dermal/eye
Product Manufacturing	PM	2	12	Dried formulation extraction and handling (for powder NC ingredients)	environmental	direct

CS2.2: Sulfated CNF Food Packaging

CS2.1: Carboxylated CNF Food Packaging

Exposure Scenario Ranking

1. Scenarios are ranked based on hazard and exposure potential to determine overall relative risk.

2. Exposure estimates are then developed where possible, with focus on the highest priority pathways.

3. Four dimensions of exposure were used as a screen to rank the developed exposure scenarios.

   1) Directness of exposure, which relates to potential for direct contact of MFC (i.e., how easily are particles released);
   2) Magnitude, which relates to relative size of exposures based on percentage of MFC;
   3) Likelihood, which prioritizes intentional exposures over unintentional or accidental ones; and
   4) Frequency, an estimate of how often an exposure is expected.

	Directness of exposure	Magnitude	Likelihood	Frequency
Low (1)	Covalently bound MFC in substrate.	Exposure is to article where one component is ≤ 1% MFC.	Direct contact mitigated.	Infrequent—Exposure possible < 10 times per year.
Medium (2)	MFC potentially releasable from substrate.	Exposure to material > 1% to ≤ 10% MFC.	Unintentional—exposure possible based on activity.	Incidental—Use 10-50 times per year.
High (3)	Dried MFC in powder form.	Exposure to material > 10% MFC.	Intentional—repeat exposure during normal use.	Regular—Greater than 50 times per year.

Exposure Scenario Ranking

- **Sulfated/carboxylated cellulose 'nanopaper' food packaging**

Life Cycle Stage	LC Stage Code	LC Stage #	Scenario #	Scenario	Receptor	Exposure Route	Hazard Potential	Magnitude	Likelihood	Frequency	Score
Product Manufacturing	PM	2	10	Dried formulation extraction and handling (for powder CN ingredients)	occupational	inhalation	3	3	3	3	12
Product Manufacturing	PM	2	11	Dried formulation extraction and handling (for powder CN ingredients)	occupational	ingestion/dermal/eye	3	3	3	3	12
Product Application	PA	3	7	S/C-CN rehydration (if powder CN ingredient)	occupational	inhalation	3	3	3	3	12
Product Application	PA	3	8	S/C-CN rehydration (if powder CN ingredient)	occupational	ingestion/dermal/eye	3	3	3	3	12
Product Application	PA	3	20	S/C-CN drying (film formation)	occupational	ingestion/dermal/eye	2	3	3	3	11
Product Application	PA	3	22	Surface treatment of S/C-CN film (hot press, wax coating, other)	occupational	ingestion/dermal/eye	2	3	3	3	11
Product Application	PA	3	24	Physical treatment of S/C-CN film (e.g. forming, bending, other)	occupational	ingestion/dermal/eye	2	3	3	3	11
Product Use	PU	4	1	Food packaging use (release, migration)	consumer	ingestion	2	3	3	3	11
Product Use	PU	4	3	Food packaging handling/interaction (release)	consumer	dermal/eye	2	3	3	3	11
Re-use/Recycling	RR	5	2	Collection and transport to re-use facility of used food packaging	occupational	ingestion/dermal/eye	2	3	3	3	11
Re-use/Recycling	RR	5	5	Physical breakdown of used food packaging (e.g. tearing)	occupational	ingestion/dermal/eye	2	3	3	3	11
Re-use/Recycling	RR	5	8	Chemical breakdown of used food packaging (e.g. pulping)	occupational	ingestion/dermal/eye	2	3	3	3	11
Re-use/Recycling	RR	5	12	Composting used food packaging	environmental	direct	2	3	3	3	11
Re-use/Recycling	RR	5	14	Bio-conversion (anaerobic digestion)	occupational	ingestion/dermal/eye	2	3	3	3	11
Disposal	D	6	2	Collection and transport to final end-of-life location	occupational	ingestion/dermal/eye	2	3	3	3	11
Disposal	D	6	6	Long-term MSW landfill storage	environmental	direct	2	3	3	3	11

Application of the Toolbox for Hazard Assessment

1. Toolbox aims to characterize hazards of functionalized forms by compiling database of physical, chemical, and toxicological data. Database for several surface chemistries already populated.
2. Goal is to use the toolbox to 'group' materials and 'read-across' safety data using the high-throughput/ATS methods in the toolbox.
    - Similar physicochemical characteristics
    - Act and behave similarly biologically or in the environment
        - E.g. Following simulated oral exposure in the gastrointestinal tract: cell viability, inflammation, oxidative stress, gut barrier integrity
    - Read-across via grouping to substantial database of safety studies available for conventional cellulose
    - Evaluation of safety of new surface functionalizations using grouping & read-across without expensive and time consuming animal testing.
3. Pre-commercial assessment, which can be used to inform safe- and sustainable by design considerations for new forms of cellulose

Life-Cycle Risk Analysis (LCRA)

1. Toolbox methods and data will be used to evaluate hazards, which is used in LCRA to evaluate the safety of next generation of CNs.

2. Current status: Toolbox oral exposure toxicity data received; being incorporated into LCRA analysis

1. Identify and Characterize Potential Hazards

2. Assess Exposure Potential and Risk

3. Prioritize Data Gaps

4. Address Data Gaps (E.g., Toxicity Studies)

Output: **Safety Demonstration**

Toolbox Next Steps:

- Finish toolbox development
  - Additional toxicological assays (inhalation, dermal, environmental exposures)
  - Build database of developed physical, chemical, and toxicological data
  - LCRA methodology demonstrating application of the toolbox to demonstrate safety
  - Make toolbox publicly available
- Finish LCRA case studies demonstrating application of the toolbox to evaluate safety of carboxylated and sulfated CNs for applications as food contact materials, food additives, and chemical applications (e.g. water filtration)
- Standardize and publish safety methods; data
- Build methods and data sets for NEXT GENERATION of modified materials
- Promote commercial and regulatory acceptance for these applications
  - Fill data gaps
  - Build ‘safer-by-design’ considerations and recommendations into Toolbox

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

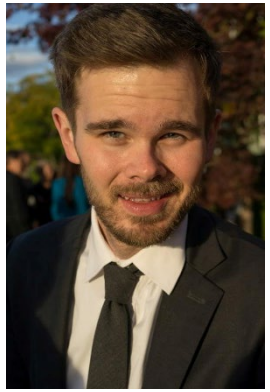
TAPPI Nano Division

The Vireo Team

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.

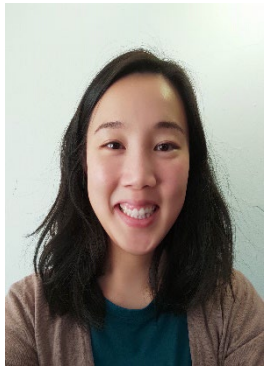
Dr. Shaun Clancy is a chemist with over 30 years experience in the chemicals industry, directing programs in health, safety, and regulatory affairs in major corporations. He is ANSI Co-Chair and participates in ISO TC229 and other international safety committees.

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.

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.

Yueyang (Brian) Zhang is a toxicologist and a post doctoral fellow at University of Alberta and a MITACS Fellow with Vireo.



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Thank you

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