ESTABLISHING THE SAFETY OF CELLULOSE NANOMATERIALS FOR FOOD-RELATED USES

PRESENTED BY
JO ANNE SHATKIN, PHD
President
Vireo Advisors, LLC
OVERVIEW

• Current and potential food-related uses of cellulose nanomaterials (CNs)

• Current state of knowledge on the safety of CNs for food

• Vireo’s Food Safety Study
REGULATORY REQUIREMENTS
Authorization Procedures – Food use

If not on permitted food additive list, or if there is a new use:

U.S. – Food additive petition, GRAS, Significant New Use, Food Contact Notice

Canada – Food additive submission

E.U. – Food additive or food contact authorization, Novel Foods (starting 2018, food consisting of NMIs are considered novel foods)
REGULATORY POLICY AND FOOD CONTACT NANOMATERIALS

• An emerging situation

FDA’s Approach to Regulation of Products of Nanotechnology

EFSA Journal 2011;9(5):2140

SCIENTIFIC OPINION

Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain

EFSA Scientific Committee

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2
3
FOOD-RELATED USES OF CNs
## Technical Effects of CNs Used in the Food Industry

**1. Food Additives**

- **(i) Rheology Modifier**
  - e.g. Viscosity modifier; thixotropic; gelling agent

- **(ii) Stabilizer**
  - e.g. Carrier; emulsifier; stabilizing agent; thermal stability (high temperature processing; freeze thaw cycles); anti-caking agent

- **(iii) Low Calorie Substitute**
  - e.g. Non-caloric bulking agent; fat replacement

- **(iv) Fiber Supplement**
  - e.g. Source of dietary fiber

- **(v) Improved Food Qualities**
  - e.g. Control ice crystal growth; reduced fat absorption during frying; humectant; control ice crystal growth; improved mouthfeel to mimic full fat texture; adds body and creaminess; improved texture; improved flavor retention; opacifier

- **(vi) Processing aid**
  - e.g. Filtration aid in beverage processing; prevents boil-out; aids in extrusion; tableting aid
### Potential CN Food Contact Uses

#### Technical Effects of CNs for Packaging

<table>
<thead>
<tr>
<th>Category</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Food Coating</td>
<td>e.g. Protective coating to prevent spoilage</td>
</tr>
<tr>
<td>3. Paper and Paperboard Packaging</td>
<td>e.g. Food packaging composites; coating for paper and paperboard food-contact materials; additive to paper/paperboard products; food barrier; release/baking paper additive (greases proof paper); gas barrier (oxygen)</td>
</tr>
<tr>
<td>4. Plastic Food Packaging</td>
<td>e.g. Composite alternative to polystyrene foams; biodegradable plastic packaging</td>
</tr>
</tbody>
</table>
# Food-Related Use Matrix

## Potential CN Uses

<table>
<thead>
<tr>
<th>Intended Technical Effects</th>
<th>Rheology Modifier</th>
<th>Stabilizer</th>
<th>Low Calorie Substitute</th>
<th>Fiber Supplement</th>
<th>Improved Food Qualities</th>
<th>Processing Aid</th>
<th>Proposed Use Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Food Additives</td>
<td></td>
<td></td>
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<tr>
<td>(a) Baked goods and baking mixes</td>
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<td></td>
<td></td>
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<tr>
<td>Batters and bneadings</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>0.5-3%</td>
</tr>
<tr>
<td>Cake (fat-reduced)</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>5%</td>
</tr>
<tr>
<td>Fillings (Bakery products)</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>0.8-2.0%</td>
</tr>
<tr>
<td>Puffed snacks</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>2-5%</td>
</tr>
<tr>
<td>(b) Beverages, alcoholic</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Alcoholic formulations (e.g. piña colada mix)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>0.3-0.5%</td>
</tr>
<tr>
<td>(c) Beverages, non-alcoholic</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Soy milk beverages</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.28-0.4%</td>
</tr>
<tr>
<td>High fiber drinks</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.5-1.0%</td>
</tr>
<tr>
<td>Coffee beverage</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>0.4-0.6%</td>
</tr>
<tr>
<td>Nutritional beverage</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>0.4-0.8%</td>
</tr>
</tbody>
</table>
## CURRENT STATE OF KNOWLEDGE ON CN SAFETY

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Fibrillated cellulosics</th>
<th>Crystalline cellulosics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolism and Excretion</strong></td>
<td>No available data</td>
<td>Cellulose Nanocrystals (CNC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Intravenous injection of 35 mg/L CNC rapidly cleared in mice (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Oral gavage of 5000 mg/kg/day for 90 days in rats showed no tissue accumulation or intestinal uptake (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microcrystalline Cellulose (MCC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Oral intake of 150 g MCC in human was fully recovered from the feces within 2 days (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Oral intake of 20% MCC in rats was fully recovered in feces, and no evidence of degradation (2)</td>
</tr>
</tbody>
</table>
## Current State of Knowledge on CN Safety

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<th>Endpoint</th>
<th>Fibrillated cellulosics</th>
<th>Crystalline cellulosics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute oral/intraperitoneal studies</td>
<td>No adverse effects observed after:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Oral gavage of 300 mg/kg TEMPO oxidized and mechanically derived fibrillated cellulose in mice (5)</td>
<td></td>
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<tr>
<td></td>
<td>- Oral gavage and intraperitoneal injection of 2000 mg/kg bacterial cellulose in rats (6)</td>
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<tr>
<td></td>
<td></td>
<td>Crystalline celluloses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No adverse effects observed after:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Oral gavage of 2000 mg/kg CNC in rats (7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Feeding of 5000 mg/kg 85% MCC in rats (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No adverse effects observed after:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Oral gavage of 3160 mg/kg alpha-cellulose in rats (2)</td>
</tr>
</tbody>
</table>
## Current State of Knowledge on CN Safety

<table>
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<tr>
<th>Endpoint</th>
<th>Fibrillated cellulosics</th>
<th>Crystalline cellulosics</th>
</tr>
</thead>
</table>
| Subacute, subchronic, and chronic oral/dietary toxicity studies | No adverse effects observed after:  
- Oral feeding of a 5331 mg/kg bacterial cellulose mixture (60% with 20% Na-CMC, 20% sucrose) for 28 days (8) | Crystalline celluloses  
No adverse effects observed after Oral gavage  
- of 5000 mg/kg MCC for 90 days in rats (4)  
- 2000 mg/kg CNC for 28 days in rats (7)  

- Feeding:  
  - 30g/day MCC for 5 weeks to humans (2)  
  - 7.5% MCC for 17 weeks to rats (9)  
  - 20% MCC for 21 days in rats (10)  
  - 20% MCC for 4 weeks in rats (11)  

- Other - No adverse effects observed after feeding:  
  - 50% cellulose for 45 days in sheep (12)  
  - 30% dry, gel, or fibrous cellulose for 72 weeks in rats (13) |
## Current State of Knowledge on CN Safety

### Endpoint | Fibrillated cellulosics | Crystalline cellulosics
---|---|---
Genotoxicity studies | in vivo erythrocyte micronucleus assay  
- Oral gavage or intraperitoneal injection of 2000 mg/kg bacterial cellulose in mice cause no adverse effects (6)  
- Ames test  
- Exposure to 2000 mg/L MFC causes no mutagenicity (14)  
- Exposure to 240 mg/L CNF causes no mutagenicity (15)  
- in vitro Comet & micronucleus (MN) assays  
- Exposure to 950 mg/L (Comet) and 1250 mg/L (MN assay) in vitro causes no adverse effects (16) | in vivo erythrocyte micronucleus assay  
- Exposure to 2000 mg/kg CNC in mice cause no adverse effects (7)  
- Chromosomal damage  
- Exposure to 5000 mg/L CNC in hamster ovary cells cause no chromosomal damage (7)  
- in vitro micronucleus (MN) assay  
- Exposure to 100 mg/L CNC or MCC for 48h cause no MN induction (17)
# Current State of Knowledge on CN Safety

## Endpoint Fibrillated cellulosics Crystalline cellulosics

### Carcinogenicity studies
- Dietary exposure to 9% MCC in rats reduces the incidence of colonic neoplasia and tumors (18, 19)

### Other
- Dietary exposure to 5% cellulose does not cause spontaneous disease or neoplasia in mice or rats (2)
- Dietary exposure to 30% cellulose for 72 weeks does not cause increased tumors (2)

### Teratogenicity and reproductive study
- Exposure to 0.2 mg/L CNF in cow embryos has no effects on hatching, degeneration, nor apoptosis (20)
- Exposure to 500 mg/L CNF in C. elegans did not affect reproduction (15)

### Crystalline cellulosics
- No teratogenic/reproductive effects after:
  - Dietary exposure to 50 g/kg MCC mix (85% MCC and 15% guar gum) in pregnant rats (2)
  - Dietary exposure to 50 g/kg MCC mix (85% MCC and 15% sodium carboxymethyl cellulose) (2)
  - Dietary exposure to 30% MCC in rats over 4 generations (2)
VIREO IS ORGANIZING A STUDY ON THE SAFETY OF CN IN FOOD/CONTACT APPLICATIONS

**STRUCTURE**: Pre-commercial/collaborative public/private project funding, planned to leverage government grant funds

**HYPOTHESIS**: Diverse forms of Cellulose Nanomaterials (CN) behave similarly in the gastrointestinal tract/simulated GI tract to conventional celluloses that are affirmed as Generally Regarded As Safe (GRAS) by the US Food and Drug Administration (FDA).

**GOALS**: Demonstrate safety by conventional and novel testing focused on oral exposure pathways.

- Develop data set with studies of absorption, distribution, metabolism and excretion (ADME) for existing GRAS and novel materials.
- Side by side conventional animal testing (*in vivo*) and 21st Century toxicity testing (*in vitro; ex vivo*).
- Leverage significant data for conventional cellulose safety in read-across to demonstrate similar safety levels
**PROJECT OBJECTIVES**

• Focused Oral Toxicity Studies to address gaps
  – Build a set of methods and a data set demonstrating safety
  – Conduct studies in commercial testing labs to compare oral uptake of New GRAS celluloses to Existing GRAS;
  – Develop a suite of lab assays to address nanoscale exposure and toxicity;

• Tiered testing approach – minimize initial effort
  – Yet achieve demonstration of safety

• Industrial collaboration to co-fund studies
**PLANNED TESTING STRATEGY**

**Tier 1 (2016-17)**
Methods development for *in vitro* assays

- Physical/chemical properties
  - E.g. size and distribution; aggregation state; aspect ratio; surface area; zeta potential; surface chemistry

- Detect/quantify
  - In test/biol media

**Tier 2 (2017-2018)**
ADME testing

- 14-day toxicokinetic study (mass balance after ingestion)

- *In vitro* testing
  - Cell viability, barrier co-culture models (*e.g.* Caco-2, B-cell & Raji coculture)
  - Simulated gut cellular toxicity assays
  - FDA recommended tests, *e.g.* mouse lymphoma thymidine kinase gene mutation assay; mutagenicity (*i.e.* OECD 476); micronucleus assay (OECD 487); digestion assays

**Tier 3 (2017-18?)**
- Oral gavage assays;
- **Other testing as necessary**
- (TBD in Tier 1)

Alternative Test Methods/Strategy
COLLABORATORS

• Collaborators:
  – Jo Anne Shatkin, Vireo Advisors – Project Coordinator
  – Harvard School of Public Health
  – Oregon State University
  – American University/NIST
  – Baylor University School of Medicine
  – Commercial testing laboratory - GLP Studies
  – Independent Peer reviewers
  – Former FDA Scientist as Advisor
  – Agenda 2020 Nanocellulose committee

• Anticipated funding partners:
  – P3Nano
  – Industry – producers/end users
  – US Govt. Granting Agencies (e.g. USDA NIFA; NIH/NIEHS)
  – International collaborators
Thank you

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