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A Tripartite Perspective for Safety Assessment of Cultivated Food Products

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45th Anniversary

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Disclosure

- The views expressed in this presentation are mine and do not necessarily represent the views of ILSI or any other group.
- No conflict of interest to declare.

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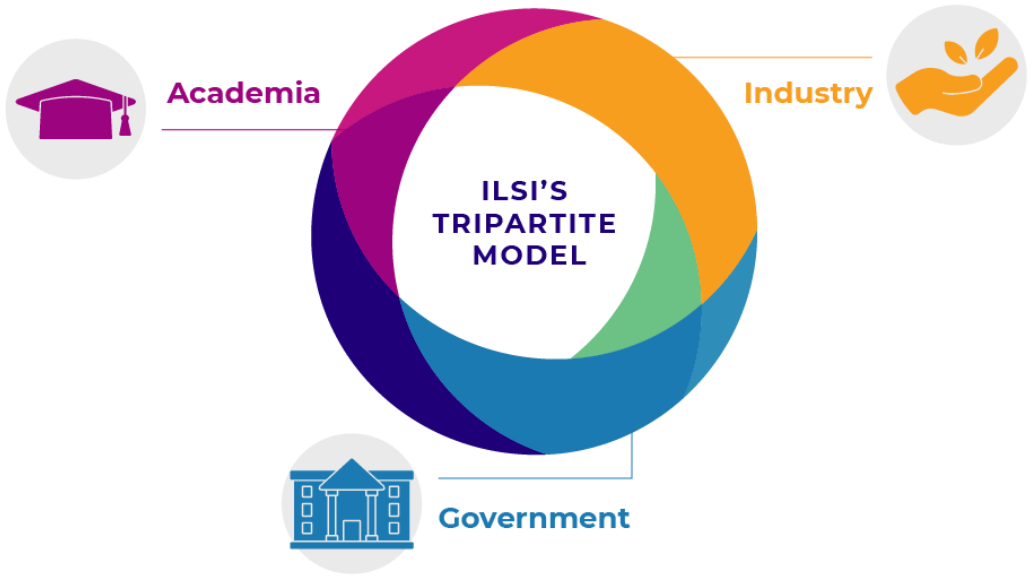
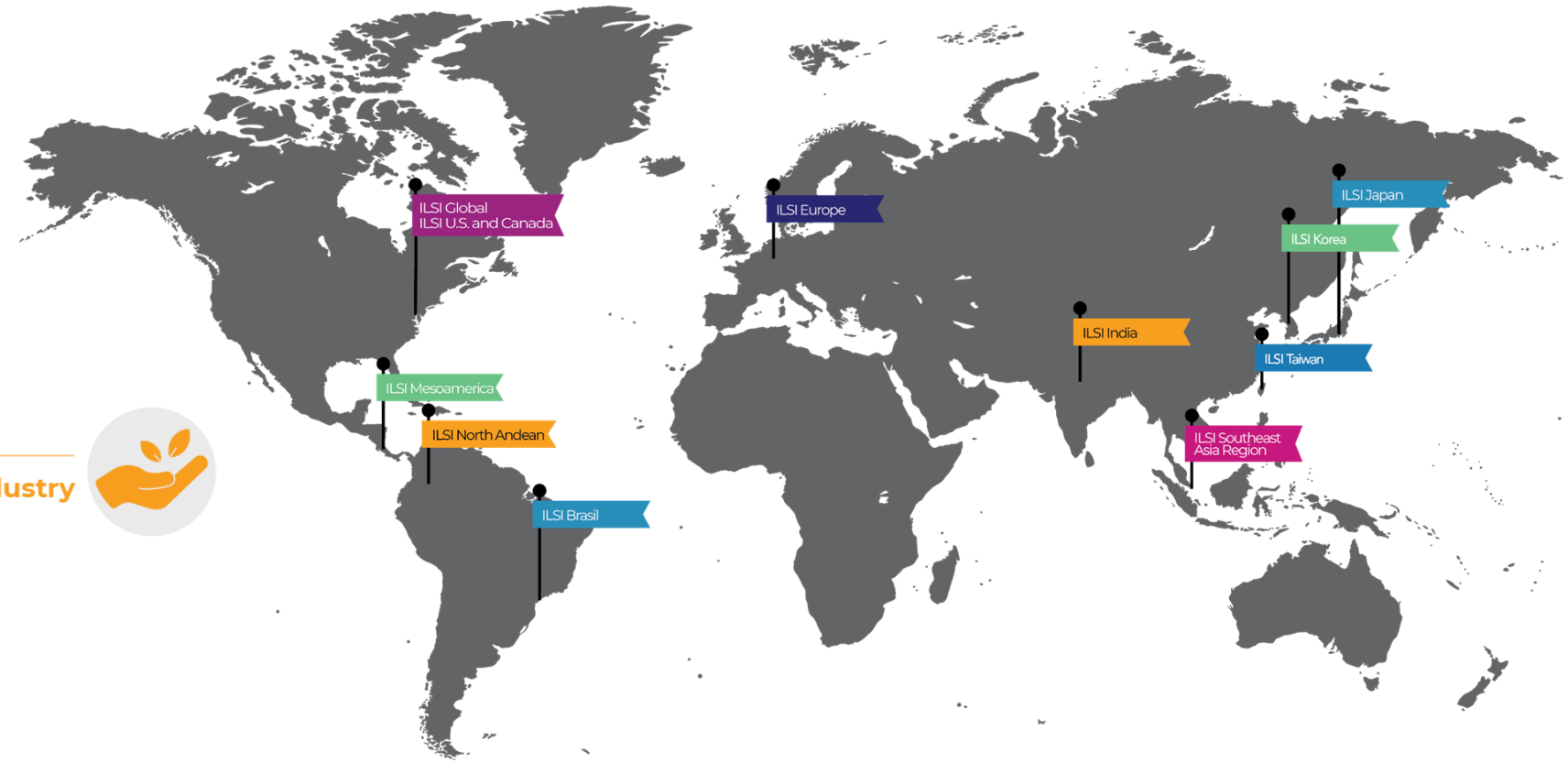
How does ILSI operate?

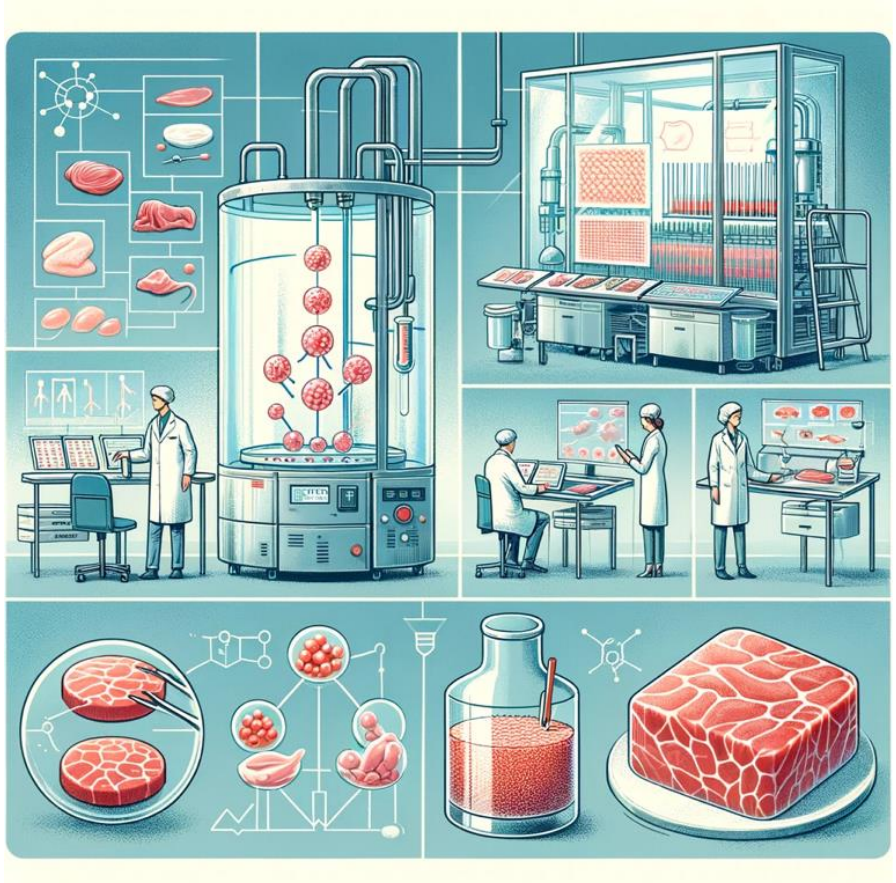
For 45 years, ILSI has specialized in convening scientists from academia, as well as the public and private sectors, to collaborate in a neutral forum on scientific topics of mutual interest. Projects are actualized through pooled funding and expertise.





Cultivated meat, cultured meat or lab-grown meat: a form of cellular agriculture where meat is produced by cultivating animal cells in controlled environments

Some background on cultivated meat…



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Some positive, or less positive aspects of cultivated food products

Positive aspects:

- Ethical Benefits
- Environmental Sustainability
- Health and Safety
- Efficiency and Scalability
- Innovation in Food Technology

Aspects that need attention:

- Cost
- Consumer Acceptance
- Environmental Impact
- Scaling and Infrastructure
- Trained Personnel

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ILSI US and Canada 'Risk Assessment of Cultivated Food Products' Vanguard Committee

- **Melanie Abley**, PhD – Office of Chief Scientist, Senior Advisor for Food Safety, Nutrition and Human Health, **USDA**, USA
- **Jeff Farber**, PhD – Adjunct Professor, Dept. of Food Science, **University of Guelph**, Canada
- **Andrew Janis**, PhD – Chief Scientific Officer, **Vow**, Australia
- **Scott Lowe** – Senior Director of Regulatory Affairs, **Ajinomoto Health & Nutrition North America**, USA
- **Haley Oliver**, PhD – Professor of Food Science, **Purdue University**, USA
- **Andrew Stout**, PhD – Research Scientist Metabolic and Tissue Engineering, **Tufts University**, USA
- **David Prescott**, PhD – Research Scientist Chemical Safety, **Health Canada**, Canada
- **Jayadev Raju**, PhD – Research Scientist Chemical Safety, **Health Canada**, Canada
- **René Viñas**, PhD, DABT – Global Regulatory & Public Policy, Senior Manager, Toxicologist, **UPSIDE Foods**, USA

- **Stephane Vidry**, PhD – Global Executive Director, **ILSI**, USA

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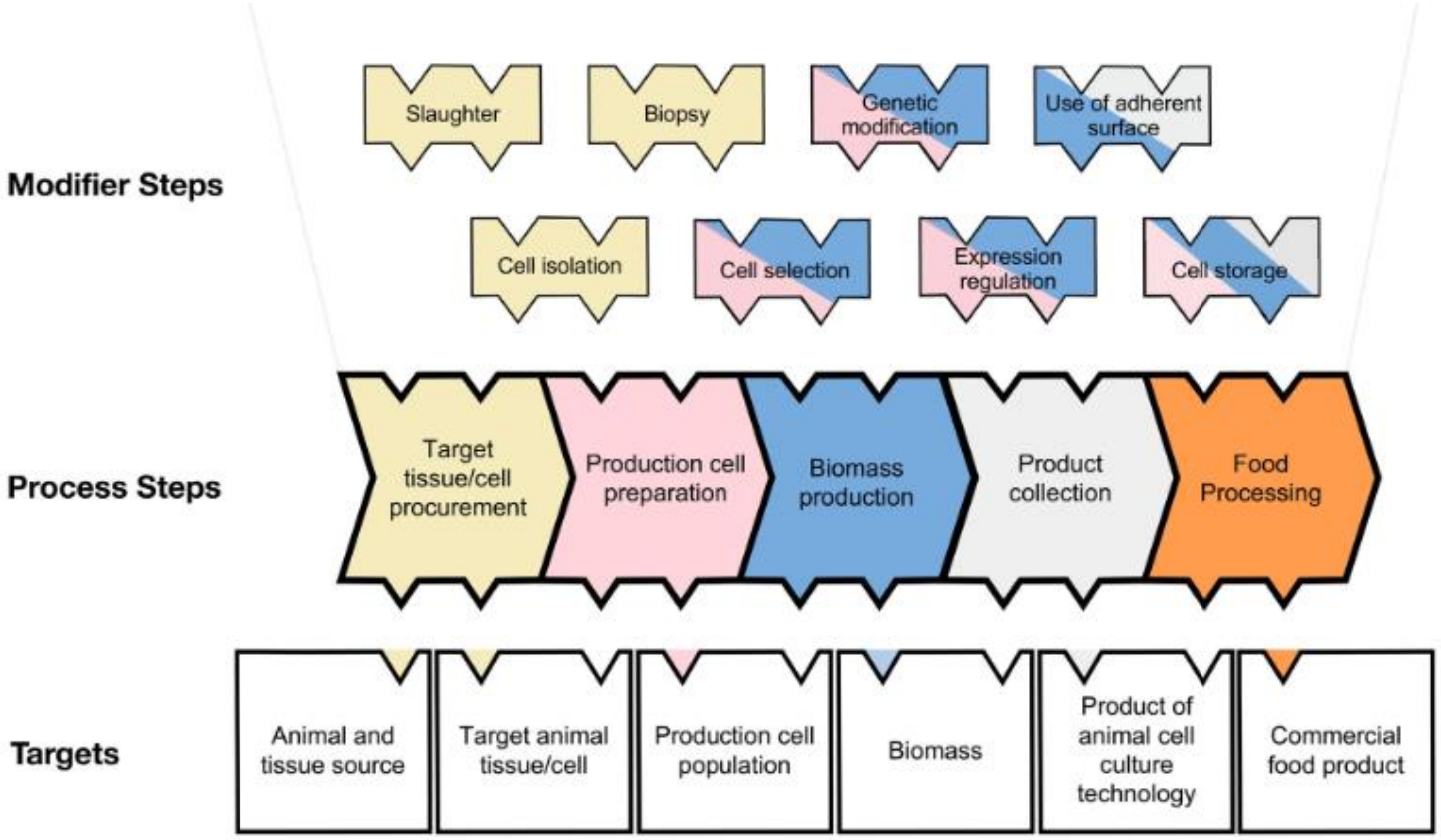
Hazard Identification

Understanding the process of producing cultivated meat allows us to identify specific points at which hazards can be introduced.

It also allows us to break products down into ingredient categories that can be assessed for hazards individually.

- Cells
- Culture Medium
- Scaffolding Material
- Processing Materials
- Equipment



Key steps in the cultured meat and seafood development and manufacturing process

Ong et al., ***Comp Rev Food Sci Food Safe***, **Volume: 20, Issue: 6, Pages: 5421-5448, First published: 10 October 2021, DOI: (10.1111/1541-4337.12853)**

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White Paper – **'Categorization of Manufacturing Components in Cellular Agriculture: A Tripartite Perspective for Safety Assessment'**

Beefy-9: A model CellAg culture medium.

communications biology

ARTICLE

https://doi.org/10.1038/s42003-022-03423-8 OPEN

Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat

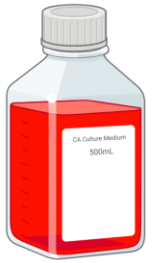
Andrew J. Stout[1], Addison B. Mirliani[1], Miriam L. Rittenberg[1,2], Michelle Shub[1], Eugene C. White[3], John S. K. Yuen Jr.[1] & David L. Kaplan[1]✉

Vitamins

Biotin	Pyridoxine hydrochloride
Choline chloride	Riboflavin
D-Calcium pantothenate	Thiamine hydrochloride
Folic Acid	Vitamin B12
Niacinamide	i-Inositol

Inorganic Salts

Calcium chloride	Potassium chloride
Cupric sulfate	Sodium bicarbonate
Ferric nitrate	Sodium chloride
Ferric sulfate	Sodium phosphate
Magnesium chloride	Zinc sulfate
Magnesium sulfate	



Serum Substitute

Neuregulin	Insulin
FGF-2	Transferrin
Sodium Selenite	TGF-β3
rAlbumin	2-Phospho-L-ascorbic acid

Energy Sources and Molecular Building Blocks

D-Glucose	Putrescine 2HCl
Hypoxanthine Na	Sodium Pyruvate
Linoleic Acid	Thymidine
Lipoic Acid	Free Amino Acids

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Medium Component Triage Exercise

- Each component of Beefy-9 medium is assessed for its safety
  - Based on known hazards, history of use, and the ability to mitigate risk
- Components are then assigned a risk tier
  - **Tier 1** – ingredients that are approved for use in food
  - **Tier 2** – ingredients without a history of use in food but are likely to be safe to consume
  - **Tier 3** – ingredients that pose unknown risk due to a lack of safety data
  - **Tier 4** - ingredients that are not considered safe to ingest
- Intended as an example exercise that companies can follow when submitting their safety evaluation packages.

D-Calcium pantothenate (Vitamin B5) 2.24 mg/L	Purpose	An integral component of Coenzyme A, a primary carrier of acyl groups for various cellular processes (10.1016/s0083-6729(08)60684-6). Also plays a role in fatty acid synthesis.	Tier 1
	Hazard	Little to no acute oral toxicity in rats (up to 10g/kg), dogs, and primates (both 1g/kg). Mice exhibited an LD50 of 10g/kg (https://doi.org/10.3181/00379727-45-11664P). Very high doses (1.2g daily for 12 weeks in children) have led to increased serum transaminase levels in some patients, indicative of minor liver injury (https://doi.org/10.1542/peds.74.1.103), while doses between 10-20g/day in adults induced nausea and diarrhea in some patients (Flodin, Pharmacology of Micronutrients, 1988). No UL, NOAEL or LOAEL have been established due to the lack of toxicity evidence.	
	History of Use	Found abundantly in the diet (average western diet contains an estimated 5.8mg/day of pantothenate). 10.1093/ajcn/34.7.1328 Higher doses (up to 2g/day) have been used in clinical trials without displaying any adverse effects (U.S. Practitioner Research Group, 1980. Practitioner 224, 208–2015)	
	Mitigation	N/A, Vitamin B5 is safe and regularly consumed	

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Tier 1 Example: Niacinamide (Vitamin B3)

Tier 1: Ingredients approved for use in food

- **Concentration in DMEM-F-12/Beefy-9**: 2.02 mg/L
- **Purpose**: Precursor to Nicotinamide Adenine Dinucleotide (NAD), an electron-donating cofactor required by hundreds of enzymes
- **Hazard**: Very high dose Vitamin B3 can induce gastrointestinal symptoms, thrombocytopenia, and potentially hepatotoxicity
- **History of Use**: Vitamin B3 occurs naturally in foods, is regularly found in vitamin supplements, and is included as fortification in flour and other foods. Its upper tolerable limit of daily intake (UL) is recommended to be 35mg/day for adults in the United States
- **Mitigation**: The UL of niacinamide is contained in approximately 17.5L of DMEM/F-12 culture medium. This medium is washed away during processing, and it is unlikely that levels approaching the UL will be absorbed into the final cellular product.

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Tier 2 Example: Hypoxanthine Sodium Salt

Tier 2: Ingredients without a well-document history of use in food that are expected to be safe

- **Concentration in DMEM-F-12/Beefy-9**: 2.39mg/L
- **Purpose**: Purine nucleobase that provides source material for nucleic acid synthesis by the salvage pathway.
- **Hazard**: The metabolism of hypoxanthine generates reactive oxygen species (ROS) that can induce pathology when produced in significant quantities. The purine that is most closely associated with development of gout following excess consumption. Mice receiving 20mg/g/day over the course of 4 weeks developed muscle fatigue and weakness.
- **History of Use**: Appears naturally in a number of foods at levels of up to 400-500mg per 100g of product. Occurs naturally in DNA/RNA supplements that are commercially available. Has not been examined as an intentional additive/ingredient in a food safety capacity.
- **Mitigation**: One liter of culture medium contains significantly less hypoxanthine than what would be found in food. Moreover, this medium will be washed away during production. Levels of hypoxanthine in the final product should still be monitored.

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Tier 3 Example: Insulin

Tier 3: Ingredients with an unknown risk

- **Concentration in DMEM-F-12/Beefy-9**: 5µg/ml (approximately 125 mU/ml)
- **Purpose**: Regulates metabolism and activates cellular signaling involved in the differentiation and proliferation of muscle cells
- **Hazard:** Bovine insulin is potentially immunogenic, which could result in cross-reactivity to human insulin and promote the progression of type 1 diabetes. Oral administration of insulin-like growth factor to mice has been shown to raise serum levels of this hormone, so alterations in hormonal balance of the consumer must also be considered.
- **History of Use:** Bovine insulin is found at concentrations between 50-300ng/ml in cow's milk, depending on the pregnancy status of the cow.
- **Mitigation:** The use of recombinant human insulin, rather than bovine insulin, may alleviate the potential for immunogenicity. Insulin is also a surface receptor-bound hormone, and thus may be more easily removed by washing of the final product than a factor that is taken up by cells. Finally, insulin is heat labile, and any potential residual activity would be removed from the product by cooking.

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Future Directions

- **Understand how media components, their sourcing and processing, and cell culture bioprocesses affect the residual presence of potential allergens**. (Questions: Are any components potential allergens? Does preparation of media pose any possible contamination with hazardous components? How do various preparation steps either remove or accumulate potential allergens? How do potential allergens (if any) need to be communicated to consumers and regulators?)

- **Understand the presence, accumulation and bioactivity of media components in final cultured food products**. (Questions: Under different bioreactor regimes, are any components leached or deposited in cell culture media, such as microplastics or metal ions? Do any deposited components accumulate in cells? If any components do accumulate, what (if any) health hazard do they pose for consumers? What potentially orally bioactive components are present in cell culture media? What residual concentration of these components is left in the final products? Do individual media components require additional independent risk assessment?)

- **Establish a shared database of common media components**. (This would allow regulators and scientists to better engage with the industry to develop standards and technologies to advance cultivated food understanding and safe development.)

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1. **Cultivated Meats: Pioneering Safety in Next-Gen Proteins**
2. **Deep Space Food Challenge: Insights into the Future of Food Production Systems**
3. **Artificial Intelligence: Enhancing Food Supply, Nutrition and Health into the Future**
4. **Advances in Healthy Aging: Innovative Approaches and Technological Breakthroughs for Promoting Health Span**
5. **Food Safety and Nutrition: Current and Future Trends in the Context of Global Climate Change and Sustainability Initiatives**
6. **Climate-Resilient Soil Management Strategies**

Two roundtable discussions

1. **Food Safety and Quality**
2. **Technological Advancements & Future Prospects & Consumer Perception**

Co-Chairs:

- **Melanie Abley**, PhD, Office of the Chief Scientist, **USDA**, USA
- **René Viñas**, PhD, **UPSIDE Foods**, USA

Panelists:

- **David Kaplan**, PhD, **Tufts University School of Engineering**, USA
- **Mathew Lau**, PhD, **A*STAR**, Singapore *(remote from Singapore)*
- **David Prescott**, PhD, Health Products and Food Branch, **Health Canada**, Canada
- **David B. Schmidt**, **AOAC International**, USA

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