Mechanistic and Epidemiological Studies and Their Role in the Safety Assessment of Low- and No-Calorie Sweeteners

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Conflict of Interest Statement

- No financial support provided for preparation of this presentation; work was conducted as normal course of employment
- ToxStrategies, LLC., provides consulting services to the public and private sector; the firm routinely supports food safety assessment initiatives (including sweeteners)
  - Some of the published research presented herein was supported by the American Beverage Association (i.e., reviews of mechanistic data for several LNCS)
Trends Over Time: Peer Review Publications

Global development and future trends of artificial sweetener research based on bibliometrics

Ziwei Chen, Zhiwei Shen, Zuolin Han, Xiaoqing Li

Fig. 2. Production, type proportion, cumulative trend and stage division of publications about ASs.
Increasing use of systematic review; increasing identification and consideration of epidemiological and mechanistic data as part of the evidence base.
Today’s Objective

Overview considerations of how to utilize mechanistic and epidemiological evidence in the evaluation of low-calorie sweetener safety
Background: Systematic Review

Common Structure of Systematic Review

- **Problem Formulation**
  - Scoping, scientific needs/objectives, feasibility
  - Develop PECO question/statement and context

- **Protocol Development**
  - Determine methods for selecting, appraising, and evaluating evidence
  - Document methods (*a priori*)

- **Identify Evidence Base**
  - Implement search strategy (syntax, databases, etc.)
  - Screen and select studies via inclusion/exclusion criteria

- **Individual Study Assessment**
  - Extract data
  - Critical appraisal for risk of bias (internal validity) and possibly other elements of study quality/relevance

- **Body of Evidence Assessment**
  - Qualitative synthesis and integration - includes assessment of confidence (consistency, magnitude, dose-response, etc.)
  - Quantitative synthesis and integration (e.g., meta-analyses, meta-regression)

- **Reporting**
  - Comprehensive documentation of approach, findings, and conclusions in a public forum
Epidemiological Data
Types of Epidemiological Data
Example of Considerations for Individual Studies:

- **Observational design (subject to confounding bias; recall bias; exposure misclassification bias)**
- **Used for assessing association (vs. causation)**

**Example: Debras et al. 2022**

- Reported associations between sweetener consumption and cancer incidence
  - Low magnitude of effect: e.g., adjusted Hazards Ratio, aHR 1.15 (95% CI 1.03–1.28]) despite large difference in estimated consumption
  - No dose response relationship
  - Potential for residual confounding and bias
  - Self-reported exposure
  - Sweetener group definitions (and analyses) were not mutually exclusive
  - Findings conflict with an earlier analysis of the same cohort
**Example of Risk of Bias: Toews et al. 2019**

*Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomized and non-randomized controlled trials and observational studies*

Supplementary file 2: Results of the assessment of risk of bias in included observational studies

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Example of Risk of Bias: Toews et al. 2019 (cont.)
Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomized and non-randomized controlled trials and observational studies

- “Of the few studies identified for each outcome, most had few participants, were of short duration, and their methodology and reporting quality was limited; therefore confidence in reported results was limited.”

- “Most studies did not contain enough information on the study design or lacked other reporting detail—that is, the sweetener used was not transparently reported,...”

- “Additionally, reported doses and outcomes measures were reported so differently that we could not assess the effect of dose on any outcome...”
Example: Review of Cross-Sectional Studies and Gut Microbiota

- (no formal critical appraisal)
- “All three clinical trials on polyols (i.e., xylitol) showed prebiotic effects on gut microbiota, but these studies had multiple limitations (publication date, dosage, duration) that jeopardize their validity.”

Further, an important limitation of cross-sectional studies in this review is that they only allow observation of an association, and not a causal effect, between LNCS consumption and gut microbiota composition. Cross-sectional studies cannot determine whether the microbial composition is directly due to LNCS consumption.
Tools to Help Assess Epidemiological Evidence for Sweeteners

- **Individual study level**
  - Establish clear criteria for inclusion based on assessment needs
  - Assess reliability (internal validity) of studies using risk of bias
  - Assess impact of systemic and residual bias (e.g., e-values)

- **Across epidemiological studies**
  - Assess confidence in the evidence, including risk of bias across studies
  - Assess causality; consider other evidence streams:
    - Experimental data in rodents
    - Mechanistic data (biological plausibility)
    - Toxicokinetics
Impact on Safety Assessment: Consideration of Causation

- Survey and evaluate toxicology data
  Typically from guideline studies in experimental animals

- Evaluate causation
  Not necessary because of reliance on studies with experimental design and the ability to distinguish treatment-related effects

- Identify most sensitive effect/study
  Typically from a repeated-dose or chronic exposure rodents

- Develop toxicity reference value
  Typically by applying safety factors to a NOAEL

- When using experimental animal studies (typically conducted according to standard guidelines), there is confidence in exposure/dose-response relationships because of the controlled study design.
- Effects can clearly be established as “treatment-related” and thus a step to evaluate causation is typically not needed.
Additional considerations in the evaluation process are necessary when utilizing observational data. Specifically, risk assessors need to determine:

1. If there is a causal relationship that is unlikely to be explained by chance, bias, and confounding?
2. If so, is there confidence that the specific dose causes the specific response (beyond an association, is there confidence in the actual dose-response relationship)?
Beyond Risk of Bias—Use Existing Tools to Better Understand Associations (and Causation)

- **Qualitative techniques**—
  - Traditional qualitative assessment of causality - Bradford Hill (GRADE)
  - Triangulation of evidence
  - Develop a **directed acyclic graph (DAG)** to show the complex relationship between exposure, response, and other explanatory variables

- **Quantitatively** techniques E.g., show that the effect is probably causally/not causally related to exposure?

- Individual study data where covariates can be mapped to specific study participants
  - **Bayesian Network Analysis** could quantitatively measure the magnitude and direction of relationships in shown in the DAG
  - **Counterfactual analysis** – if we remove exposure from the model, do we get the same estimate of risk?

- Summary information from studies
  - E-Values (and others) can still quantitatively address the likelihood that exposure causes the observed outcome in observational epidemiological
Example: Jones et al. 2022—Liver Cancer

- Jones et al. 2022 “adjusts” for most co-factors (shown as white circles in the DAG)
- However, diet is measured as total caloric intake and does not quantify or account for intake of red/processed meats, vegetables, whole grains, or other dietary factors that are shown to impact liver cancer risks
  - If diet is defined by intake of food types (e.g., red meat), it is not adequately controlled and is a potential confounder (pink arrows)
- We can estimate the likelihood that this potential residual bias affects the observed exposure-response associations through E-values
  - Using red/processed meat as an example, the E-Value is lower magnitude than the potential confounder, increasing confidence that the observed HR is due to bias
  - Other examples could include saturated fat, and vegetable intakes, and other combinations
- This is supported through triangulation with McCullogh 2022, who found no significant increase in liver cancer risk after adjusting for vegetable and red/processed meat consumption at baseline

HR: 1.74
E-Value 1.15-1.16

HR: 1.13-1.17
Mechanistic Data
What Are Mechanistic Data?

Derived from multiple study types: in silico, in vitro, in vivo, epidemiological
Data may represent multiple levels of biological complexity

Chemical Properties
- Receptor/Ligand Interaction
- DNA Binding
  - Protein Oxidation
  - Cytotoxicity
  - Mitogenic

Organism Responses
- Lethality
  - Impaired Development
  - Impaired Reproduction
  - Cancer

Population Responses
- Structure
  - Recruitment
  - Extinction

Examples:
- Gene Activation
  - Protein Production
  - Altered Signaling
  - Protein Depletion

- Altered Physiology
  - Disrupted Homeostasis
  - Altered Tissue Development or Function

Chemical Initiator(s)

Initiating Event

Macro-Molecular Interactions

Cellular Responses

Organ Responses
Why Do Assessors Typically Look at Mechanistic Data?

- Biological plausibility of a hazard?
- Developing an AOP?
- MoA for risk assessment?
- New approach method development?
- Many others...
Constructs (Tools) in Practice for Mechanistic Data

**Key Characteristics (organizational)**
- Broad categorizations
- Not specific to a key event or event relationship

**Adverse Outcome Pathway**
- Chemical agnostic
- Outcome specific
- Domains of applicability, essentiality
- Key events and key event relationships

**Mode of Action**
- Chemical specific
- Outcome specific
- Population/tissue/dose specific

**Tool Selection Consideration:**
AOP and MoA are pathway-based constructs (what question do you need to answer?)
Consideration for Best Practice: Complimentary Nature of Constructs

Toxicokinetics
- e.g., gene activation, altered signaling
- KC: Reactive Electrophile
- KC: “Alters DNA repair/causes genomic instability”

Molecular initiating event
- e.g., protein production cell death; proliferation
- KC: “Alters cell proliferation”

Cellular response
- e.g., altered tissue development or function; hyperplasia
- KC: “Alters DNA repair/causes genomic instability”

Tissue response
- e.g., organ weight changes, organ malfunction, tumours
- KC: “Genotoxic”

Organ response
- e.g., increased tumour incidence

Population Response

Stressor
- Toxicokinetics

MOA

KC: Reactive Electrophile
KC: “Alters DNA repair/causes genomic instability”
KC: “Genotoxic”

AOP
Example: Search Strategies for Mechanistic Data


[23x347]Example: Search Strategies for Mechanistic Data


Example Approach to Identify and Assemble Mechanistic Data
(Subsequently Applied to Aspartame, Sucralose, and Stevia)

Step 1. Individual Study Assessment
Component 1: Reliability (Internal validity)
How well was the study designed/reported to evaluate the endpoint?
(1/2/3)
Component 2: Strength (External validity)
How good is the model at characterization outcome relative to cancer/KCC?
(1/2/4/8)
Component 3: Activity
Result of assay by model (Active/Inactive)
(1/0)

Step 2. Body of Evidence Integration by Individual KCC

Step 3. Body of Evidence Integration
Integration of all KCC data with adverse outcome data from other streams

Individual KCC Evaluation (KCC Score)
Algorithm used to accommodate components 1-3
Numerical score developed for each KCC (range -1 to 1)
Translated into overall designation of strong/mod/weak/NA for each KCC

Body of Evidence Integration (Integrate KCC data with adverse outcome data from other evidence streams)
Bridge KCC with commonly shared characteristics
Evaluate relevance to AO
Build AOP/AOP networks

Figure 1. Framework for evaluation of KCC mechanistic data and integration relative to potential carcinogenicity.

Result: Step 1—KC Activity Profile: Steviol Glycosides
(Used to Inform Pathway Assessment)

https://doi.org/10.1016/j.fct.2021.112045

G.A. Chappell, M.M. Heintz, S.J. Borghoff, C.L. Doepker, D.S. Wikoff. Lack of potential carcinogenicity for steviol glycosides - Systematic evaluation and integration of mechanistic data into the totality of evidence. Food and Chemical Toxicology, Volume 150, 2021,
Results: Steps 2 and 3—KC Activity Profile: Steviol Glycosides

Systematically identified literature, extracted by key characteristic, appraised reliability, and applied a quantitative framework to integrate by characteristic and identify activity for further revaluation.

Integration: Consider “linkages” of activity or events and possible pathway connections.

Integration: Consider activity in context of adverse outcomes from animal and human data – plausible biological pathways?

https://doi.org/10.1016/j.fct.2021.112045
Example: Assessment of Biological Plausibility (Aspartame)

**Review of >1360 mechanistic datasets**
- Represent broad array of study types (*in vivo, in vitro* including HTS), and *in silico* and species (human, mammalian, non-mammalian).
- Represent broad array of endpoints that range in biological complexity (molecular, cellular, organismal, etc.)

**Oxidative stress activity observed**
- Activity was equivocal in human models; more consistently observed in experimental animals in vivo (at high dose)
- Mixed results across a broad array of assay/endpoints; no clear key event identified
- Not linked to activity in other KCC; cannot be used alone to predict cancer outcome

**Immunosuppression activity** in assays mapped to this KCC was limited to experimental animals (at high dose and typically not predictive of responses in humans)

> No biologically plausible pathway was identified for aspartame and cancer

Integrated Mechanistic Profile

Note: Important to consider ADME of Aspartame when interpreting these data especially when tested in *in vitro* assays without metabolic capability.

Summary and Conclusions
Conclusions

- Going beyond traditional toxicology approaches
  - Increasing number and variety of study types, study models endpoints/outcomes, etc., being evaluated for sweeteners

- Requires pragmatic adaptation to risk assessment
  - Utilize and integrate existing tools in epidemiology, modern causal frameworks, computational toxicology etc.
References


- Toews I, Lohner S, Küllenberg de Gaudry D, Sommer H, Meerpohl JJ. Association between intake of non-sugar sweeteners and health outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. BMJ. 2019 Jan 2;364:k4718. doi: 10.1136/bmj.k4718.


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