



SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

No Observed Adverse Effect Level: Sucralose as a Case Study

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Conflict of Interest Statement

- This presentation is based on publicly available statements and publications, as well as my expert opinion. Relevant references are cited.
- I have not received funding for and have not been involved in any capacity in the research discussed in this presentation.
- Neither myself nor any member of my immediate family, has any financial interest with a commercial organization that has a direct or indirect interest in the subject matter.
- Neither I nor my employer currently hold any contractor agreements with any commercial organization with an interest in the subject matter.
- The Calorie Control Council provided financial support to my employer for my participation in writing an expert critical review of published literature on sucralose. This work is completed and has been submitted for publication.
- My employer received reimbursement for my travel expenses and speaker honoraria for presentations at 2 nutrition conferences in the past 2 years from Heartland Food Group. Sponsorship was disclosed at conferences.
- In summary, I have no conflict of interest for this presentation.



Overview

- Step-wise approach to assessment of adversity for a novel food additive
- Considerations in determining a NOAEL - Sucralose
- Importance of GLP studies and standardized animal testing methodologies
- Susceptible subpopulations
- New research
- Human relevance



Ultimate Goal: Ensure Proposed Uses are Safe for Humans



No Observed Adverse Effect Level (NOAEL)

- For a specific study:
 - the dose determined by empirical study at which no adverse effects induced by test article,
 - i.e., harmful anatomical, biochemical, or functional changes.
 - Some harmful findings may not be statistically significant, while some statistically significant changes may not cause harm.
- For an ingredient or test article:
 - NOAELs from multiple studies are considered together in defining most important adverse responses in most sensitive species.

Example of Definition of Adverse Response

- *In the context of a nonclinical toxicity study, an adverse effect is a **test item-related change** in the morphology, physiology, growth, development, reproduction or life span of the animal model that likely results in an **impairment of functional capacity** to maintain homeostasis and/or an impairment of the capacity to respond to an additional challenge.*

Step-wise Testing and Challenges

Initial Screening

Chemical characterization
In silico and *in vitro* methods
Genotoxicity studies
Digestibility
Stability in food matrices

Advantage of identifying significant adversity as early as possible, assists in prioritizing compounds for further product development and testing.



Adapted from
Blaauboer *et al.*, 2016

Step-wise Testing and Challenges

Initial
Screening



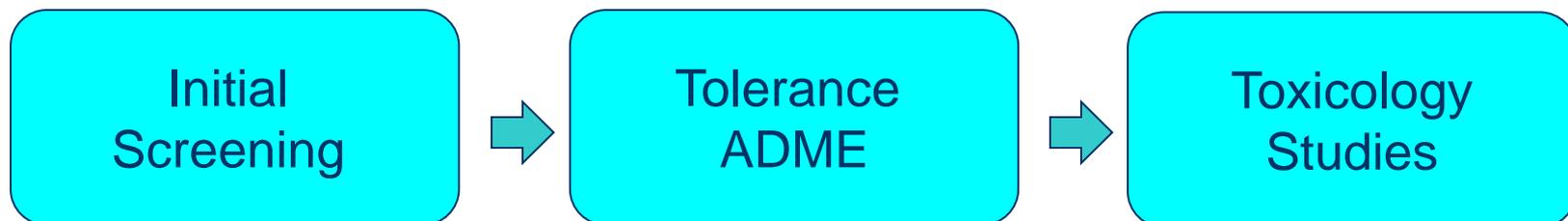
Tolerance
ADME

High doses needed to increase sensitivity of detection of adverse effects poses challenges for diet formulation and acceptability.

- Palatability
- Diet formulation for nutrient balance
- ADME to identify species most similar to human



Step-wise testing and challenges

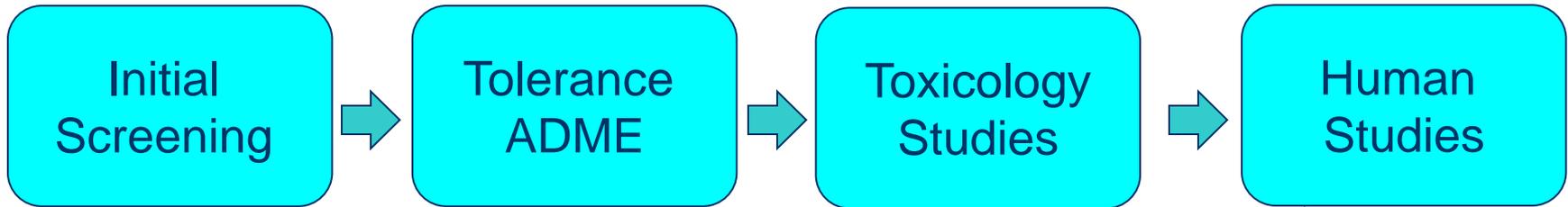


Does exposure to compound affect any health parameter throughout life stages?
At what dose?
Extensive resources and time required.

Subchronic 90-day,
Chronic – 1-2 yr for rodents
Reproductive -2 generation
Carcinogenicity
Immunotoxicity
Neurotoxicity,
Other specialized studies

Adapted from
Blauboer *et al.*, 2016

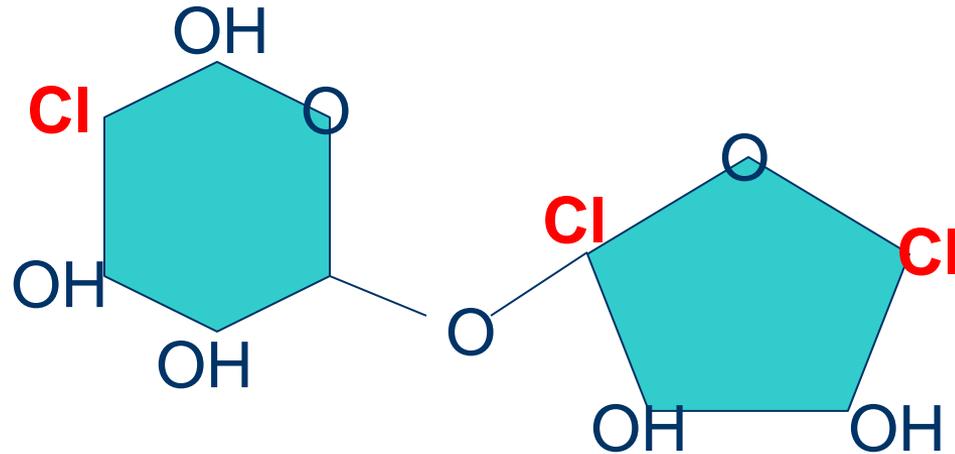
Step-wise Testing and Challenges



- May be conducted, after establish safety in animal studies.

- Confirm tolerance of expected human exposure levels
- Any susceptible subgroups with special considerations?

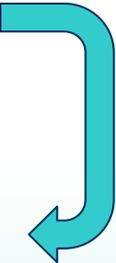
Sucralose - Case study



Disaccharide (sucrose)
3 Cl replace 3 OH

- Non-caloric – sweetness potency 600x sucrose; not digested or metabolized for energy.
- Highly stable in food and beverage matrices.
- Does not breakdown during typical heat processing and baking of foods.

Sucralose: Toxicokinetic Studies

- *In vitro* studies: microbial and plant glycosidases, and mammalian intestinal extracts, no evidence of hydrolysis of sucralose by any enzymes
 - *In vivo*: 5 species tested
 - Mouse
 - Rat
 - Rabbit
 - Dog
 - Human
- 
- Sucralose toxicokinetics in rat most similar to those observed in humans**

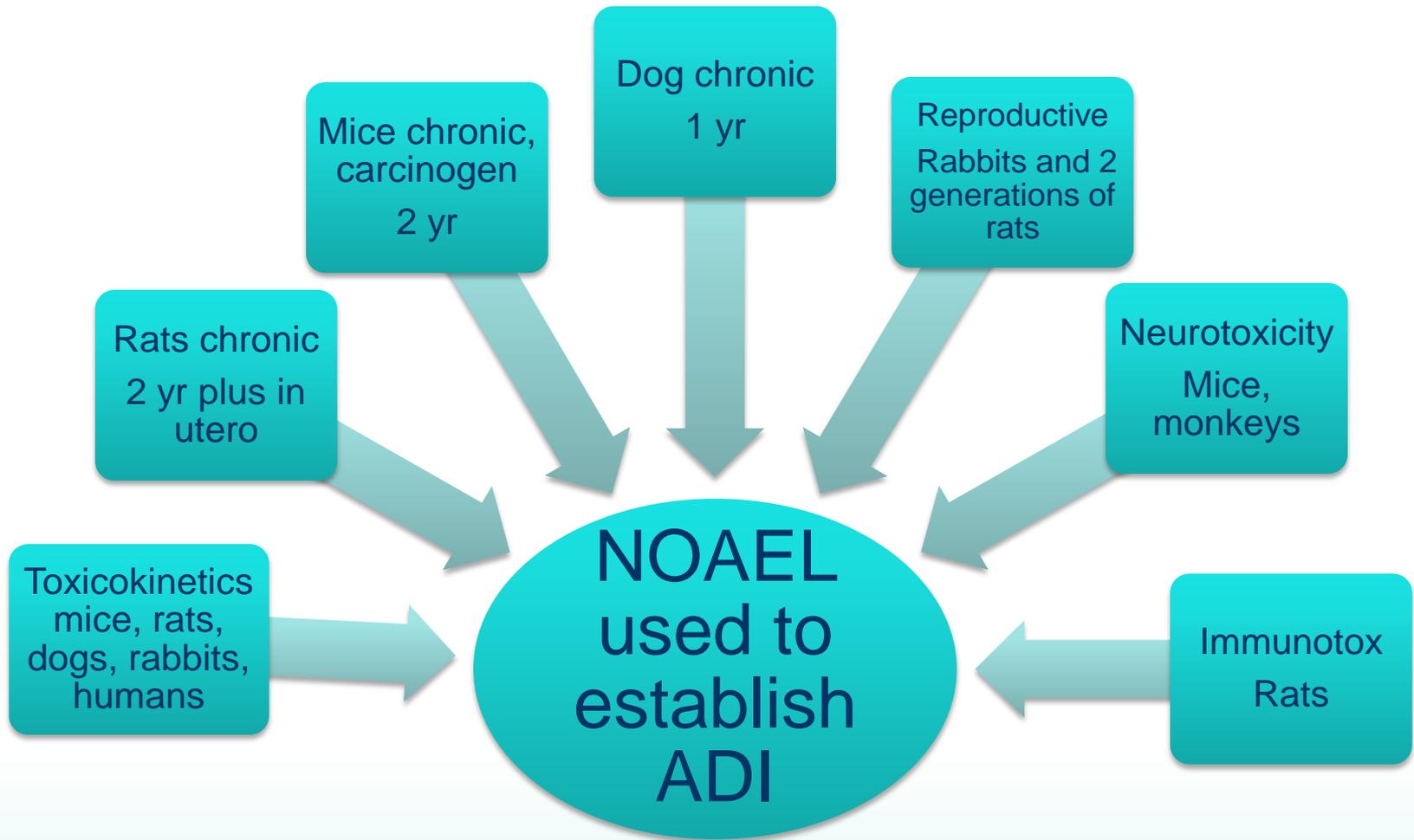
Sucralose: Toxicokinetic studies

Absorption into blood	Poorly absorbed in all species. ~15% in humans. Remainder unchanged in feces
Digestive enzymes, gut microflora	No digestion into monosaccharides No metabolism by gut microflora
Metabolism	Majority: not metabolized. ~2% glucuronidated by liver.
Excretion	Majority: Unchanged sucralose in feces. Absorbed sucralose: excreted in urine unchanged and glucuronide metabolites.

Roberts *et al.*, 2000.

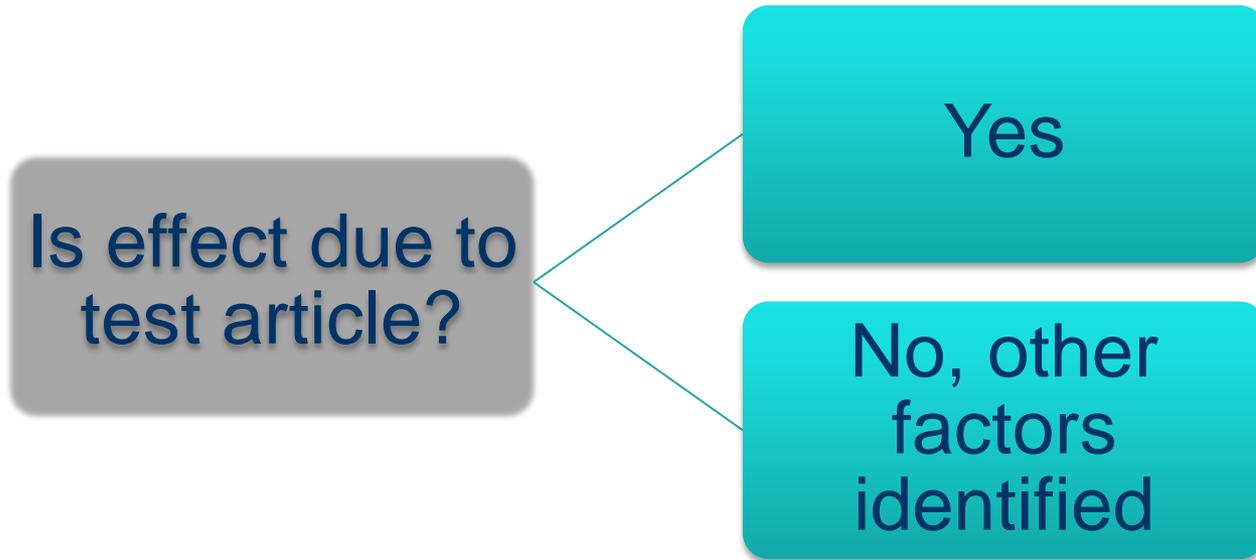
Grice and Goldsmith, 2000.

Sucralose Toxicology Studies: Multiple Species, Multiple Endpoints



ADI = Acceptable Daily Intake

Considerations When Effect Observed



Adapted from Kerlin *et al.*, 2015



Regulatory Guidelines for Studies Increase Ability to Assess if Effect Due to Test Article

- Multiple doses needed
 - Does response increase with dose?
 - Relationship between effect and dose?
 - Range of dose that has no adverse effect
- Is response different from control group?
 - Range of natural variation? Historical control data?
 - Biological plausibility?
- Consistently observed?
 - In multiple studies? More than one species? Both sexes?
- Conduct using Good Laboratory Practices (GLP)



Sucralose Example: Due to Test Article?

- Consistent effect: rats and mice fed sucralose in diet had **significantly reduced body weight** and **food consumption**, greatest effect at highest level (3%).
- Due to reduced palatability, reduced food intake?
Or due to sucralose itself?
- Subsequent studies conducted:
 - Same doses of sucralose administered by **gavage**,
 - **Pair-feeding** study to assess effect of reduced food consumption on growth.



Different Interpretation & Conclusions

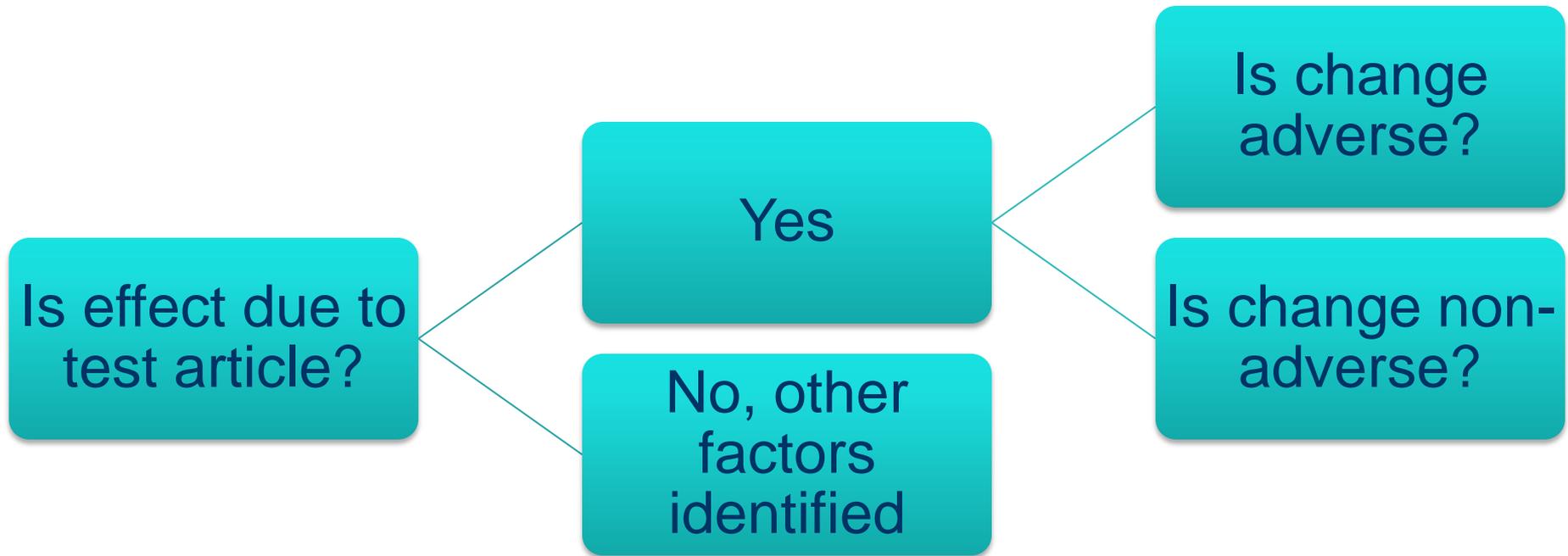
- WHO Joint Expert Committee on Food Additives –
 - due to poor palatability and low food consumption.
 - Conclude chronic rat study NOAEL= 1500 mg/kg/d.
- US FDA
 - reduction in food consumption accounts for reduced body weight at 1% of diet, but not at 3%.
 - Conclude for same study NOAEL= 500 mg/kg/d.

WHO Technical Report Series 806.

Federal Register. Vol. 63, No. 64. April 3, 1998



Considerations When Effect Observed



Adapted from Kerlin *et al.*, 2015



Changes Considered “Nonadverse”

1. No alteration in the **function** of the test organism or affected organ/tissue is noted;
2. Change represents an **adaptive** response;
3. Finding is **transient**;
4. Severity **limited**, below thresholds of concern;
5. Effect is **isolated** or independent (No changes in other parameters usually associated with effect);
6. Effect is not a precursor lesion;
7. Result is a **secondary** consequence; and
8. Effect arises from some inherent **biological property** of the animal model. (Lewis *et al.*, 2002)

Sucralose Example: “Nonadverse”

No alteration in the function of organ;

- 26 week study in rats, observed increased kidney weight relative to body weight in high dose group,
- BUT no change in function – no change in kidney tissues morphology and no effect on plasma electrolytes.

Sucralose Example: “Nonadverse”

Change represents an adaptive response;

- Dose-related enlargement of cecum due to sucralose in several rodent studies.
- This is widely recognized as a normal **physiological adaptation** to consumption of large amounts of poorly absorbed dietary components.

Clinical Studies: Diabetic Population

Potential high users, different susceptibility?

Single dose, acute glycemic response

- Type I and Type II diabetic patients

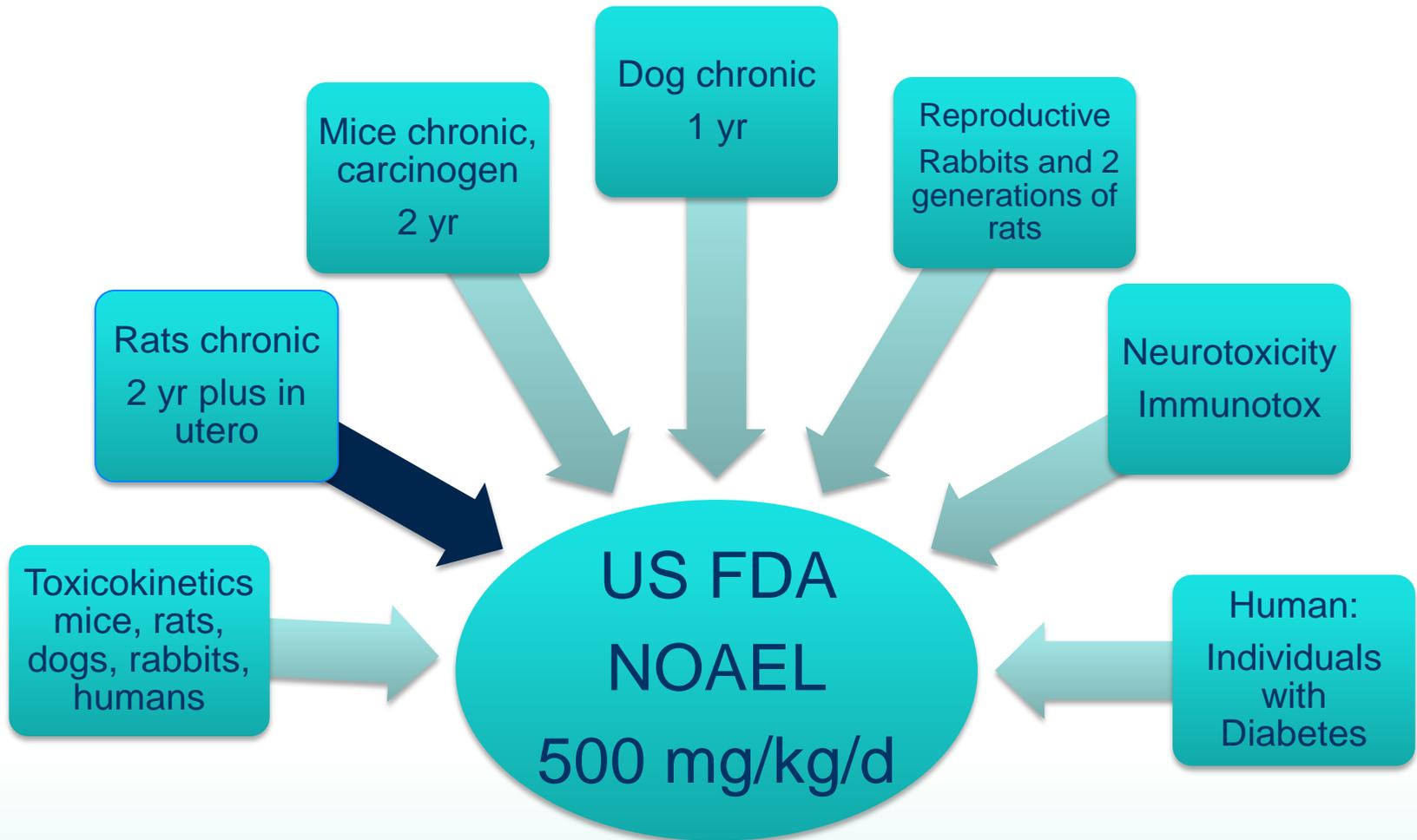
6 month repeated dose, glycemic control

- Type II diabetic patients (n=41)

3 month repeated dose, larger study

- Type II diabetic patients (n=136) managed with either insulin (n=64) or oral hypoglycemic drugs (n=72);

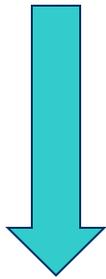
Most Appropriate Long-Term Study



New Research on Sweet Taste Receptors Raises New Questions



Taste
receptors
on tongue

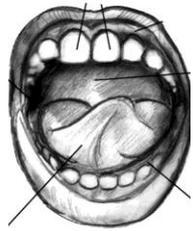


Activation
by sweet
compounds

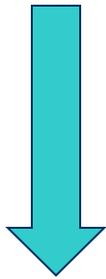
Signals to brain
Perceive sweetness



New Research on Sweet Taste Receptors Raises New Questions



Taste receptors on tongue

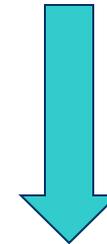


Activation by sweet compounds

Signals to brain
Perceive sweetness



Taste receptors in gut cells



Activation by glucose and other sugars

Release of gut hormones



-Do non-nutritive sweeteners stimulate these gut receptors?
- Functional significance?

Sucralose and Gut Hormones

- *In vitro* studies:
 - Sucralose activates of receptors, release of gut hormones
- Subsequent acute animal and human feeding studies on gut hormones and function:
 - Different designs, healthy and diabetic subjects,
 - Most report no effect on gut hormones,
 - No **adverse effect** on functions related to gut hormones including blood glucose and insulin levels, appetite, and gastric emptying.
- Confirms long-term daily consumption studies:
 - No adverse effects of use in healthy individuals and individuals with diabetes.

Reviewed in Bryant & McLaughlin, 2016;
Meyer-Gerspach *et al.*, 2016.

Carcinogenicity of Sucralose – Recent Conflicting Report

Studies conducted and reviewed by regulators

- Genotoxicity
 - *In vitro*, *In vivo*
 - Non-mutagenic in most, weakly mutagenic in some
- Mouse study
 - Guideline-compliant, GLP
 - No treatment-related change in neoplasias
- Rat, including *in utero*
 - Guideline-compliant, GLP, data reviewed by FDA
 - No treatment-related change in neoplasias

Carcinogenicity of Sucralose – Recent Conflicting Report

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- Mouse and Rat studies
 - Guideline-compliant protocol, GLP
 - No treatment-related change in neoplasias

Soffritti *et al.*, 2016 –

- Reported increase in hematopoietic neoplasias in male mice, not in females.

Results carefully reviewed by experts: conclude not reliable due to many factors: study conditions, history of chronic infection in animals, questionable pathology diagnoses and statistical analysis, not GLP, and more.

Relevance to Humans?



Human Relevance Framework

- Is the **weight of evidence** sufficient to establish a Mode of Action (MOA) in animals?
 - Consistent with all data available? Dose-response? Biological plausibility?
- **If yes:** Is animal MOA biologically plausible in humans?
 - Anatomical, physiological, biochemical differences?
- **If yes:** Is the MOA in humans, plausible when consider differences in kinetics/dynamic factors,?
 - Is high dose needed? Relevance to human exposure? Differences in formation of metabolites? And others.

Summary: Critical Considerations for Determination of Adversity

From *in silico*, *in vitro* and animal testing:

- Ensure effect due to test compound.
 - Understanding of effect and context,
 - Historical controls, severity, incidence, and correlations with other factors.
 - Statistical versus biological significance?
 - Overall weight of evidence, consistency across studies and biological plausibility.
- And – ultimately relevance to humans!



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Questions

At this time, please limit questions to those specific to this presentation, and save general questions for the Roundtable Discussion with all the presenters.

