International Safety Assessment of Sweeteners

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Introduction

• There are many sweeteners used in foods and beverages around the world.

• Sweeteners are classified as either:
  – Food ingredient (Provides nutrition, calories)
    • Sucrose, fructose, honey, etc.
  – Food additive (Provides sweetness, few or no calories)
    • Aspartame, acesulfame K, cyclamate, saccharin, sucralose, stevia extracts, polyols (xylitol, etc.) and others.
    • Food additive petition required for approval of use
Risk Assessment of Food Additives

- Evaluated for safety by an independent scientific body (risk assessor) before being considered for authorization in a given market.
- At international level, the risk assessor is JECFA or the Joint FAO/WHO Expert Committee on Food Additives.
- At European level, the risk assessor is the European Food Safety Authority (EFSA) - previously - Scientific Committee on Food (SCF).
- Establishes Acceptable Daily Intake (ADI).
JECFA

• Established in 1950s, first meeting in 1955.
• Set out general principles for evaluation of food additives.
• Brought harmonization of the approach to safety assessment of food additives on a worldwide basis.
• Toxicological evaluations usually result in allocation of acceptable daily intake (ADI) for food additive.
JECFA General principles

- impossible to establish absolute proof of non-toxicity for all members of human population;

- critically designed animal studies provide reasonable basis for evaluating safety of food additives;

- safe level for food additive should be based on knowledge of minimum dietary level that produces no unfavorable response in test animals;

- fate of additive during food processing and preparation should be considered because of possible formation of toxic substances and interaction with components of food or other food additives;

- decisions on use of food additives must be based on judgment of qualified scientists that the intake of the additive will be below any level which could be harmful to consumers;

- consideration should be given to vulnerable sub-groups within the population;

- also consider performance of additive, and applicability of analytical methods, intake assessments, and specifications of purity.
International Programme on Chemical Safety

• A joint venture: United Nations Environment Programme, the International Labour Organisation, and WHO.

• Objective: to evaluate and disseminate evaluations of the effects of chemicals on human health and the environment.

• Updated 1987 monograph in 2009, with the publication of EHC240: Principles and methods for the risk assessment of chemicals in food

• Guidelines provide a comprehensive current review of the key issues considered by JECFA during their risk assessments of food chemicals
Risk assessment paradigm

Identify Hazard
Characterize Dose-Response

Assess Exposure

Estimate Acceptable Daily Intake (ADI)

Characterize Risk
Intakes of all users, including high users and special groups, must be below ADI

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Identify Potential Hazard of Proposed Sweetener

Comprehensive battery of studies are conducted in multiple species

- Genetic toxicity
- Pharmacokinetics
- Short term, sub-chronic, and chronic toxicity
- Carcinogenicity
- Reproductive toxicity and teratogenicity (birth defects)
- Human clinical studies
Use of Standardized Toxicology Protocols is Critical to Interpretation

• Protocols have been standardized (certified) by multiple organizations
  – Organization for Economic Cooperation and Development (OECD) –
    http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm
  – Redbook by the US Food and Drug Administration –
  – VICH- International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products
    • http://www.vichsec.org/index.htm
Is the sweetener mutagenic?

– To determine the potential of sweetener to induce DNA or chromosome defects (somatic cell) or heritable defects (germ cell)
– Usually is first test conducted on potential sweetener.
– If determined to be genotoxic, studies end as will not be approved as sweetener.
– Are many types of tests.
– Most common battery includes:
  • test for gene mutations in bacteria (e.g. Ames test);
  and
  • in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells;
  or
  • in vitro mouse lymphoma thymidine kinase +/- gene mutation assay;
  and
  • an in vivo test for chromosomal damage using mammalian hematopoietic cells. (e.g. Mammalian Erythrocyte Micronucleus Test).
What happens to the sweetener in the body? Pharmacokinetics (ADME)

Evaluates the rate and extent of:
- **Absorption** (how much? Where?)
- **Distribution** (does sweetener accumulate in any tissues?)
- **Metabolite** formation (any toxic metabolites?)
- **Excretion** of sweetener

**Key Parameters**
- AUC (Area Under Curve)
- Cmax (maximum plasma concentration)
- Tmax (Time Cmax is reached)
- Identification, quantification of metabolites
- Tissue concentrations
- Urine and fecal concentrations

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What happens to the sweetener in the body?

• Data from studies allow understanding of the following questions:
  – Does the animal used in toxicology testing (e.g. rat) have similar ADME as human?
  – How much variability between individuals?
  – Any potential susceptible subpopulations identified based on absorption, metabolism etc.?
    • Will be used in establishing ADI
  – May provide data on possible mechanism or mode of toxicity at high concentrations.
Does the sweetener cause adverse effects? At what doses?

• All compounds (natural and man-made) will be toxic at some dose!
• Goal is to establish dose that is safe when consumed every day or the No Observed Effect Level (NOEL)
  – Extensive testing conducted in animals, using a minimum of 3 doses, which are above expected human exposure levels.
  – Ideally, want:
  • low dose with no observed effect (NOEL or NOAEL)
  • high dose that produces mild toxicity (i.e. 10% reduction of body weight)
  • mid-range dose(s) so establish dose response
  • For non-toxic, high dose studied may be set at an accepted limit dose, such as 5% addition to the diet.
- Short term studies conducted to ensure no adverse effects, and to establish doses, before investing in long term and cancer studies.
Does the sweetener cause cancer?

All sweeteners must be tested before approval.

**Usually require:**
- 2-year study in rats plus 18-month study in mice
- 20-25 animals/sex per group; at least 3 dose levels plus control group.

**Any evidence of dose response in:**
- incidence of tumors vs. controls
- time of tumor development
- tumors/animal
- benign vs. malignant
- site of tumor development

**Must consider:** relevance to humans of an increase in certain types of neoplasms, background incidence, and possible threshold effects at high doses.

**Studies that do not use approved methods may not be valid for risk assessment.** Chronic infection, improper pathology, improper housing, unknown background diet all impair ability to interpret results and make studies invalid. (see EFSA report on aspartame studies)
Does sweetener have any effect during reproduction, pregnancy and development?

**Objectives:** To determine potential adverse effects on conception, pregnancy, delivery, lactation, neonatal survival and vitality.

Sweetener fed to parents before mating, to mother during pregnancy and lactation, then to subsequent generation.

**Measurements:**
- % mothers in F0 and F1 that get pregnant
- # of pregnancies that go full-term
- litter size, # dead/alive
- pup weight
- viability (# alive after 4 days)
- lactation - # survive 21 day lactation period
- Parents - necropsy and histopathology with special attention to reproductive organs
Toxicity study to assess safety during pregnancy and development

Exposure before mating, during mating period, pregnancy, lactation & lifetime

Exposure in utero, via breast milk, & via diet for lifetime

Exposure in utero, lactation & growth

No exposure, second generation examined for adverse effects

Parent Generation F0

First Generation F1

Second Generation F2

Unselected weaned pups

Weaned pups selected for teratology study

Unselected weaned pups
Do results from studies indicate need for any additional tests for susceptible populations?

Examples:
- Because low-calorie sweetener often used frequently by diabetics, additional studies on effects of sweetener on insulin and glucose control conducted.
- For steviol glycosides, additional studies on individuals with low blood pressure required.
- For aspartame, extensive testing on neurotoxicity and behavior has been conducted (Magnuson et al., 2007)

Other possible tests:
Immunotoxicity, Mechanistic studies, Endocrine effects.
Acceptable Daily Intake (ADI)

Amount “that can be ingested daily over a lifetime without appreciable health risk” (WHO)

- Based on the most sensitive critical health outcome, in most sensitive species.
- Established to protect the most sensitive subpopulation, including pregnant women and children, but not infants (<12 weeks).
- As is based on body weight, is adjusted for age and size.
- Takes into account the differences in sensitivities in human populations, particularly from genotypic and phenotypic variations.
Establish ADI

ADI (mg/kg body weight/day) = \textbf{NOAEL} safety factors

NOAEL is found by study or observation, and is the highest dose level producing no detectable adverse alterations of morphology, functional capacity, growth, development or life-span.

- From long-term studies;
- Most susceptible species and endpoint;

Apply “safety factor” of usually 100 to account for

- differences between individuals (10 X);
- differences between humans and animals (10 X);

Example: Lifetime study NOAEL is 4000/body weight/day
Then ADI= 40 mg/body weight/day
What are the consequences of Sweetener Intakes above ADI?

Question was investigated by an international expert panel.

- Depends on by how much ADI exceeded.
  - High intensity sweeteners are self-limiting as products with high levels have unacceptable taste.
- Depends for how often and how long – the longer ADI exceeded, greater risk.
- The true threshold for biological effect is between the NOAEL and dose that shows adverse effects. This difference may be 10-fold or more, depends on interval between doses used.
- This difference corresponds to an extra safety factor in addition to that normally applied to a NOAEL.
- **Conclusion:** High level of conservatism built into ADI so low risk of occasional intakes above ADI by small amount.  
  
  (ILSI, 1998)
Different ADI values for different subgroups?

No.

ADI is intended to cover exposure of older infants and children, the foetus during pregnancy, and the neonatal and young infant during the nursing period.

- If there is scientific evidence that infants and children are more sensitive to the sweetener, that evidence **must be used in derivation of ADI**.

- ILSI International workshop strongly recommended that special ADIs should not be established, ADI must be safe for all.

ILSI, 1998
Conclusions

• There is international harmonization on the approaches and methods used for safety assessment of low calorie sweeteners.

• Toxicity testing of low calorie sweeteners is extensive. Sweeteners are not approved until all safety questions are addressed, and estimates of exposure are below ADI for even highest users.

• Review of post-market emerging data is ongoing.

• ADIs are established to protect the entire population, and susceptible subpopulations are carefully considered in determinations.
References and Resources

• EFSA. 2012. Guidance for submission for food additive evaluations. EFSA Journal;10(7):2760
• ISLI Europe. 1998. Significance of Excursions of intake above the ADI.
• JECFA. http://whqlibdoc.who.int/trs/WHO_TRS_144.pdf
• Magnuson et al., 1997. Crit Reviews in Toxicology
• Magnuson et al., 2013. Food Additives and Contaminants.
Thank you!

Questions?

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Species-specific tumor development not relevant to humans

- Alpha 2u-globulin and renal cancer (male rats)
- Leydig cell tumors (male rats)
- Forestomach tumors (rodents)
- Thyroid tumors due to differences in T3/T4 metabolism (rodents)
- Liver tumors due to peroxisome proliferation (rodents)
Subacute and Subchronic toxicity tests

**Purpose**: Identify delayed effects due to repeated exposure

1. Dose selection for subchronic and chronic studies,
2. Determine NOELs for certain toxicity endpoints, and
3. Permit design of future tests with emphasis on identified target organs.
4. Characterize dose-response relationships following repeated doses

**Short-term or subacute**: ~28-days repeated exposure.
- In mice and rats; both sexes
- Often conducted to establish doses for subchronic and chronic tests, but can give an indication of cumulative toxicity

**Parameters monitored and recorded are:**
- Behavioral observations
- Physiological (body weight, food consumption, organ weights)
- Biochemical (blood chemistry, hematology, urine analysis)
- Macroscopic (necropsy) and microscopic observations (tissue histology)
Subchronic toxicity tests

Duration:
- Rodents – 90 days
- Dogs - 90 days to 26 weeks

Number of animals:
- 20 rodents per sex per group
- At least 4 dogs per sex per group

Doses: consider likely human exposure; minimum 3 doses plus control group
- high dose that produces toxicity (i.e. 10% reduction of body weight)
- low dose with no observed effect (NOEL or NOAEL)
- mid-range dose(s)
- For non-toxic, high dose studied may be set at an accepted limit dose, such as 5% addition to the diet.

Parameters monitored and recorded:
- Same as for subacute.
- May not see NOEL, but only obtain LOAEL (Lowest observed effect level)