Protein Safety:
Food Toxicology Paradigm

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Protecting and Promoting *Your* Health

All proteins are composed of the same genetically encoded amino acids…. but not all proteins are created equal

- Structural proteins, functional proteins
- Innocuous proteins, toxic proteins

Classic Toxicology Paradigm: chemicals

- Acute, subchronic, chronic toxicity
- Reproductive and developmental toxicity
- Mutagenicity
- Genotoxicity
- Exposure route
  - (dermal, oral, respiratory, systemic)
- Exposure substance (metabolite(s))

Hazard

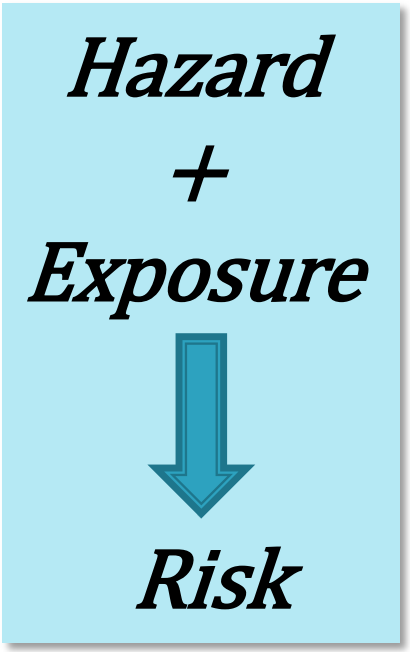
+

Exposure

Risk

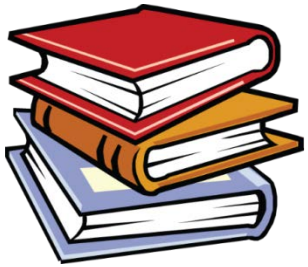
Food Toxicology Paradigm

- Focus on oral route exposure – food or gavage
- Integrates knowledge of the substance – or related substances that have been consumed safely in food



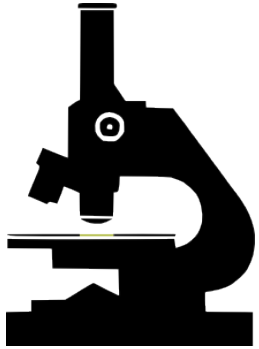
Approach to Food Toxicology

Source, function, and history



Estimates of Oral Exposure

Information from chemical toxicity studies (as needed)



Approach to Food Toxicology: proteins

Source, function, and history

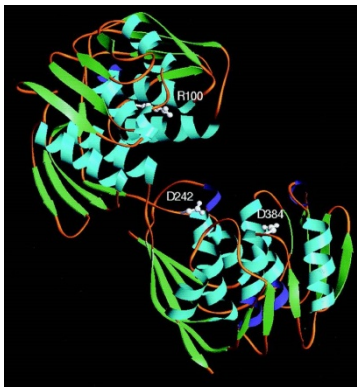
Estimates of Oral Exposure

Information from chemical toxicity studies (as needed)

- Is the source organism known to have toxic proteins?
- Is the new protein structural or functional?
- What is its mode of action?
- Is the new protein similar to known toxic proteins?

EPSPS: history of safe use

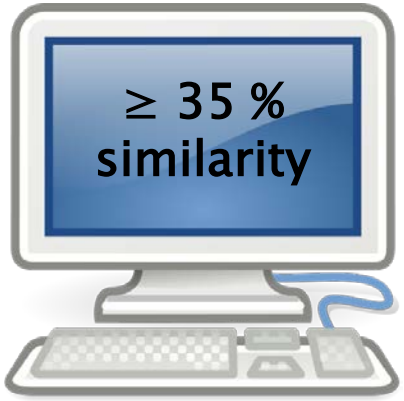
Source, function, and history



Identity	EPSPS – 5-enopyruvilskimate-3-phosphate synthase
Sources	Plant, bacteria, fungi
Function	Shikimate pathway; biosynthesis of aromatic amino acids
GE form	Amino acid substitutions that render it insensitive to inhibition by the herbicide glyphosate (*Agrobacterium* sp strain CP4 and *Zea mays*)

GE crops: Soy, corn cotton, wheat, sugar beet, alfalfa, canola

Bioinformatic Analysis:
comparative approach



The amino acid sequence similarity of the new protein is compared to known protein toxins

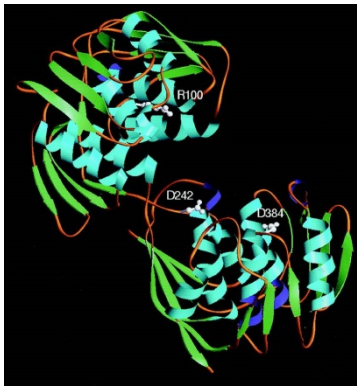
<u>Sequence Databases</u>

Three primary, inter-connected databases

- National Center for Biotechnology Information/GenBank (USA)
- European Bioinformatics Institute/EMBL (UK)
- DNA Databank of Japan (Japan)

Secondary, specialized databases are built by harnessing sequences of interest from these primary databases.

EPSPS: weight of the evidence



Sequence comparison	no relevant similarities to known toxins
Dietary consumption	low levels in edible plant tissues (ng to ug protein/g tissue)
Stability to heat	rapid denaturation in heat
Digestion Simulated Gastric Fluid	Rapid digestion in SGF (<30 seconds)
Data from oral toxicology study	NOAEL = 572 mg/kg (mouse)

Approach to Food Toxicology: proteins

Source, function, and history

Estimates of Oral Exposure

Information from chemical toxicity studies (as needed)

- What are the levels of the protein in edible plant tissues?
- How much of the protein will people or animals consume?
- Is it stabile to heat or food processing?
- Is it resistant to digestion in the GI tract?

Cry protein in pollen in honey: rough estimate of daily intake (EDI)

Estimates of Oral Exposure

~7500 grains of pollen/gram (g) of **RAW** honey

↓ *~2,000,000 grains/g pollen*

0.00375 g pollen/g of **RAW** honey

↓ *~10 µg Cry protein/gram of pollen*

0.0375 µg Cry protein/gram of **RAW** honey

↓ *Mean user: 3.3 g honey/person/day*
90% user: 7.6 g/p/d

EDI =

0.132 µg Cry protein/person/day (mean user)
0.304 µg Cry protein/person/day (90% user)

Cry protein in pollen in honey: rough estimate of daily intake

Estimates of Oral Exposure

If we convert EDI units : from µg to mg and p/d to kilogram bodyweight/d…..

Estimated Daily Intake (EDI)

0.000002 mg Cry protein/kg bw/d (mean user)
0.000005 mg Cry protein/kg bw/d (90% user)

Rodent acute oral toxicity study:

- No adverse effects observed at highest dose tested =1460 mg Cry protein/kg bw

Margin of Exposure

- the MOE for consumers (90%) of **RAW** honey is *at least*

$1460/0.000005 = 2.9 \times 10^8$!

Food toxicology for proteins includes consideration of

Source, function, and history

Estimates of Oral Exposure

Information from chemical toxicity studies (as needed)

- If the new protein is present in the food but is not similar to proteins that have previously been consumed safely
- If the estimated dietary exposure is higher than has previously been safely consumed
- If bioinformatic analysis suggests possible similarity to known toxin

For More Information

Internet:

FDA

http://www.fda.gov/GEplantfoods

Email:

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