

# Assessing the Safety of Pharmaceutical Proteins for Plant Expression

## Human Health Risk Assessment

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# Role of the UI in BIGMAP

The University of Iowa (UI) classifies plant made pharmaceutical proteins for which information is available in the public domain.

UI investigators are selected to represent the disciplines of

human and animal toxicology,  
plant physiology and genetics,  
biocatalysis and bioprocessing,  
agricultural safety and health,  
epidemiology and  
decision science.

# Members of the University of Iowa BIGMAP Study Group:

- Peter Thorne – Professor, Toxicologist
- Gabriele Ludewig – Assistant Professor, Toxicologist
- Andrea Adamcakova-Dodd – Research Assistant, Toxicologist
- Kelley Donham – Professor, Agriculture and Health Safety
- Erin Irish – Associate Professor, Plant Genetics
- John Rosazza – Professor, Biocatalysis
- James Torner – Professor, Epidemiologist
- Charles Whiteman – Professor, Decision Science

**BIGMAP, ISU** – name of the compound with basic information



The core UI BIGMAP Group  
literature review + draft preparation



Draft distribution to all other UI BIGMAP Group members



UI BIGMAP group meeting  
Discussion + comments



Members expertise added to the review



Final approval by all UI members



**Back to BIGMAP, ISU**

# Compounds Evaluated by UI BIGMAP Group

- Aprotinin
- E. coli Heat-labile Enterotoxin B subunit (LT-B)
- Lactoferrin
- Trypsin
- Lysozyme – in the process

**Risk ranking** will be performed when evaluation of all compounds is finalized.

# Literature Review

- Basic Information Summary from ISU
- Searches for Peer-Reviewed Literature – Medline, Pub Med, Ovid, SciFinder, ScienceDirect
- Iowa Drug Information Service (IDIS)
- Internet – using Google search engine
- Patent documents
- Pharmaceutical companies – Material Safety Data Sheet (MSDS) – supplementary information
- Gen/Protein databases; gene analysis software

## Problems:

- Significant deficiency in the literature
- Difficulties to find primary sources
- Sources with potential financial or other interest (omitted in the review)

# Is a Plant-Made Pharmaceutical (PMPs) a Health Hazard?

“A compound is a health hazard if it produces acute or chronic health effects in exposed individuals.”

The Potential to be a Hazard may depend on:

- the characteristics of the original protein
- crop and tissue in which PMP is produced – changed characteristics
- and the environment in which the crop is grown:
  - unexpected toxins or residues of pesticides;
  - negative effects on the natural environment (wildlife, soil microorganisms);
  - human exposure through unexpected routes, doses

# Potential Human Exposure

- **General population** (unintended exposure)
  - gene flow via pollen drift;
  - mix-up of PMP crops and food or feed crops; contaminated equipment
- **Occupational exposure** (farm workers and pharmaceutical workers)
  - Skin contact
  - inhaling pollen
  - breathing in dust at harvest or processing
- **Target population** (patients)
  - routes of administration and dosimetry issues

Risk: The likelihood or probability that a the potential harm may arise from some process. Usually the probability of that event and some assessment of its expected harm at the exposure level is combined in Risk Assessment

# Factors taken into account in the safety assessment I

## ➤ Comparison between PMP and conventional counterpart:

- similarity of chemical, physical and molecular properties (molecular weight, absorbance, solubility, potency etc.);
- substantial equivalence;
- effects on function, stability;
- transformation process;
- the recombinant DNA (e.g. stability of insertion, potential for gene transfer);
- novel protein expression (seeds, pollen, leaves, etc.);

# Substantial Equivalence

PMPs and existing counterpart can either be:

- substantially equivalent (Maize-derived Aprotinin and bovine Aprotinin);
- substantially equivalent except for certain defined differences (on which further safety assessments would then focus), or
- non-equivalent – more extensive safety testing would be necessary

# Factors taken into account in the safety assessment II

- Evaluating the original and GM compound
  - side effects found after application of currently used conventional counterparts (animal and clinical studies);
  - structural and evolutionary similarities (Structural Classification of Proteins - SCOP database <http://scop.mrc-lmb.cam.ac.uk/scop>) with known toxin;
  - potential toxicity or allergenicity (sequence homology, review of animal and clinical studies);
  - resistance to heat and gastric digestion;
- Effect of the environment
  - source of the protein;
  - routes of exposure/administration;

# Determination of Allergenicity Potential

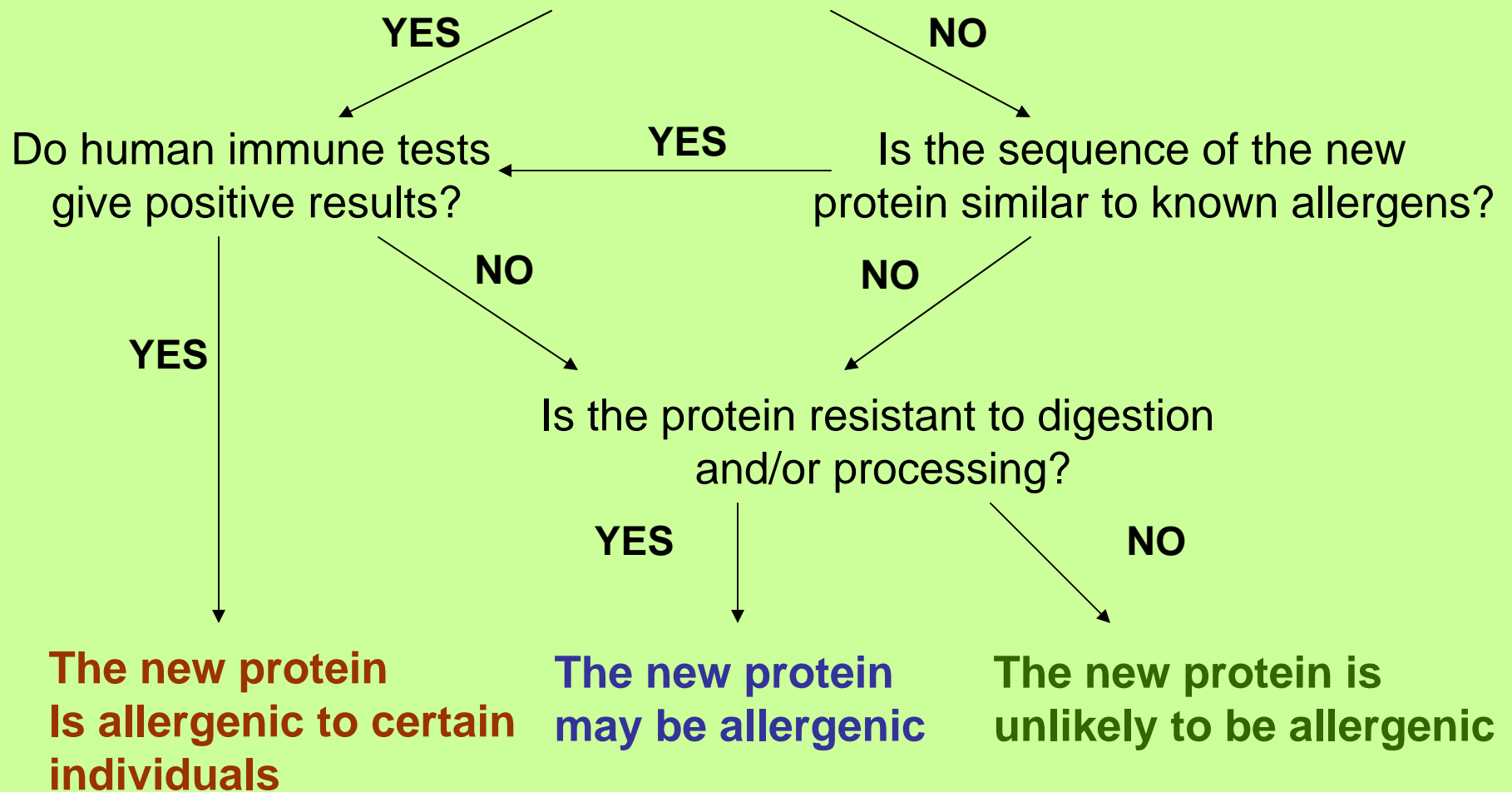
The potential allergenicity of PMPs can be assessed by examining:

- source of the protein;
- amino acid sequence with known allergens (using BLASTP);
- stability to digestion;
- heat or processing stability;
- glycosylation status using NetOGlyc 3.0 Server and based on the literature review;
- stability of the protein based on the literature review and protein properties; and
- literature review of clinical studies reporting allergic reaction after administration of conventional counterpart

# Decision-tree approach for the assessment of the potential allergenicity

In 1996, the International Food Biotechnology Council, in conjunction with the Allergy and Immunology Institute of the International Life Sciences Institute, developed a decision-tree approach for the assessment of the potential allergenicity of GM foods (Metcalf et al 1996).

## Is the new protein from an allergenic source?



Simplified version was reproduced from:

Metcalfe, D. D., Astwood, J. D. et al. (1996). "Assessment of the allergenic potential of foods derived from genetically engineered crop plants." Crit Rev Food Sci Nutr 36 Suppl: S165-86.

# Plant-derived Aprotinin

- Protease inhibitor “*bovine pancreatic trypsin inhibitor*” – inhibits serine proteases;
- Use: reduction of risk of blood loss in surgery and of inflammatory reactions;
- Routes of administration: intravenous + dermal
- **Plant-derived Aprotinin – biochemically and functionally equivalent to Aprotinin from bovine organs;**
  - ❖ AproliZean™ – (ProdiGene – maize);
  - ❖ Apronexin™ – (produced by Large Scale Biology Corp. for Sigma Aldrich);

## Potential for adverse reactions:

- Allergic reactions especially with re-exposure;
- May cause renal insufficiency – caution in patient with this disorder;
- More studies are warranted for potential exposure through respiratory system (pollen).

## *E. Coli* Heat-labile **Enterotoxin B Subunit (LT-B)**

- Enterotoxigenic *E. coli* (ETEC) – causes secretory diarrhea by producing a heat labile toxin (A and B subunits);
- Use: B subunit (LT-B) is not toxic but can induce an immune response;
- Routes of administration: intranasal, intragastric, oral;
- **Maize-derived LT-B has features of native bacterial LT-B;**
  - Animal studies - maize-derived LT-B vaccine is efficacious and safe;
  - Clinical trials – well tolerated, more studies warranted;

### **Potential for adverse reactions:**

- Tolerance induction – Is it a function of LT-A?
- Neurologic toxicity – Bell's palsy (facial paralysis) after intranasal application of influenza vaccine containing;

*Example:*

## Determination of Allergenicity Potential of Aprotinin and Enterotoxin B subunit (LT-B)

<b>Characteristic #</b>	<b>Allergens</b>	<b>Aprotinin</b>	<b>LT-B</b>
Allergenic source of gene	Yes	Yes	No
Molecular weight 10-70 kDa	Yes	Yes	Yes
Glycosylation *	Yes	No	No
Similar sequence to allergens	Yes	Yes	No
Ability to induce IgE-response	Yes	Yes	No
Stable to digestion	Yes	Yes	Yes
Stable to processing	Yes	Yes	Yes
Prevalent protein in food	Yes	Yes	No

# Allergen characteristics according to Taylor (2002)

\* Typically, but not absolutely

# Plant-derived Lactoferrin (LF)

- Found in body excretions (milk, saliva, mucus, GI fluids etc.)
- Use: related to its antimicrobial, iron absorption, antioxidant, anti-inflammatory, immune modulating, anti-cancer activity;
- Routes of administration: oral application
- **No side effects observed in animals or humans after human or bovine LF administration;**
  - ❖ Ventria Bioscience – ExpressTec™ production system for human LF (using crops of rice and barley);
  - ❖ Meristem® Therapeutics – LF production from maize;

## Potential for adverse reactions:

- Induction of antibody response (patients with autoimmune diseases);
- Growth stimulation of specific pathogens – (*Trichomonas vaginalis*, *H. pylori*);
- Neurodegenerative disorders – LF accumulation in the brain tissue (Alzheimer's and Parkinson's disease, Down syndrome, or multiple sclerosis) – more studies warranted.

# Plant-derived Trypsin

- GI enzyme that digests proteins;
- Use: production of insulin, human and veterinary vaccines, wound care, cell culture
- Routes of administration: oral and intravenous;
- Trypsin from transgenic maize has shown equivalent activity to bovine trypsin (which is classified as occupational hazard);
  - ❖ TrypZean™ – bovine trypsin from transgenic maize (ProdiGene)

## Potential for adverse reactions:

- Inhalant allergen (occupational asthma);
- Acute pancreatitis and ulcers;
- Trypsin appears to be necessary for cancer cells to invade normal tissue;
- Epidermal proliferation and inflammation.

*Example:*

## Determination of Allergenicity Potential of Lactoferrin and Trypsin

<b>Characteristic #</b>	<b>Allergens</b>	<b>Lactoferrin</b>	<b>Trypsin</b>
Allergenic source of gene	Yes	Yes	Yes
Molecular weight 10-70 kDa	Yes	<b>No</b>	Yes
Glycosylation *	Yes	Yes	<b>No/?</b>
Similar sequence to allergens	Yes	<b>No</b>	Yes
Ability to induce IgE-response	Yes	?	Yes/?
Stable to digestion	Yes	Yes	Yes
Stable to processing	Yes	Yes	Yes
Prevalent protein in food	Yes	Milk products	<b>No</b>

# Allergen characteristics according to Taylor (2002)

\* Typically, but not absolutely

# Evaluation of Potential Adverse Effects

## Development of Ranking System

- Cardiovascular system;
- Respiratory system;
- Digestive system;
- Nervous system;
- Renal system;
- Hepatic system;
- Immunotoxicity/allergenicity;
- Mutagenicity and carcinogenicity;
- Organ toxicity (pancreas, lungs...);
- Reproductive-endocrine disruptors;
- Skin

# Summary Statement concerning Toxicity Evaluation

- The potential toxicity of the PMP product must be considered on a case-by-case basis;
- Particular attention must be paid if the product to be produced is a known toxin (allergen) or similar to a known toxin
- Potential co-exposure to plant toxins have to be considered;

Each new transgenic product must be evaluated on its own merits based on its potency in causing any toxic effects and routes + levels of exposure, as is typical of current risk assessment paradigms for chemical agents.



Thank You! Have a wonderful day!



**The University of Iowa College of Public Health**  
**Department of Occupational & Environmental Health**  
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