The Pusztais' guide to GMOs and regulation

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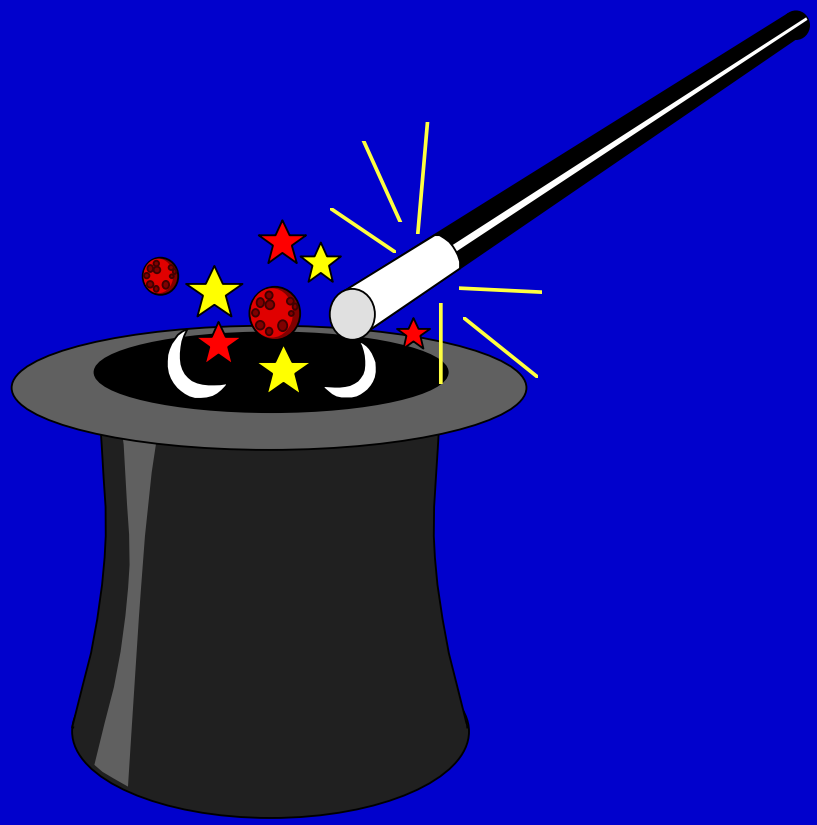
Substantial equivalence

How can a plant be novel and ‘the same’?

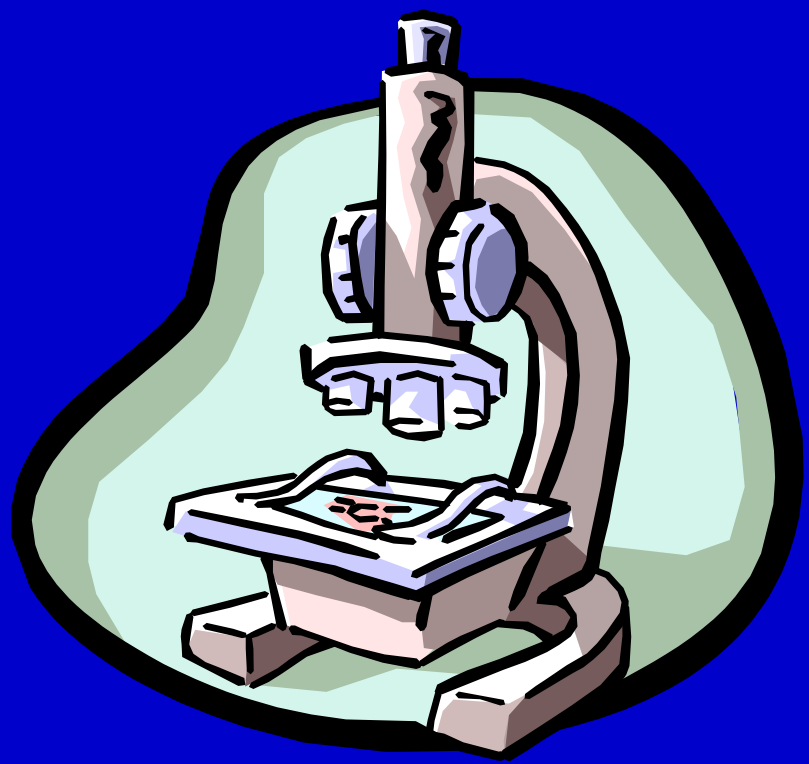


This is the reason for the use of substantial equivalence:

- **A plant should be novel to be patented (this is why you have to insert the new gene)**
- **The plant should be the same as its parents, so it does not need to be safety tested**

Substantial equivalence

- A BSE infected cow is substantially equivalent to a healthy cow
- Their chemical composition is the same, the only difference between them is that the conformation of a tiny protein (prion) component is different



Which one would you eat?

There is a need for biological testing!

SUBSTANTIAL EQUIVALENCE

- All major and nutritionally important minor components, known antinutrients, toxins and allergens in a large number of samples of GM- and parent-line plants grown side-by-side and harvested at the same time must be measured in parallel by reliable analytical methods
- Data must be provided for transgene stability, and equivalence must be shown by proteomics, mRNA finger-printing, metabolomics, etc

COMPOSITIONAL ANALYSIS

- **To establish that no unintended changes occurred in the plant on genetic modification the composition of the GM plant must be compared with that of the parent line grown under identical conditions (same location: soil, water, rainfall, temperature, sunlight, etc.) and harvested at the same time**
- **Comparison with the same conventional plant on the market place or other commercial varieties is invalid**

Antibiotic-resistance markers

ANTIBIOTIC-resistance MARKERS

- **<u>Assertion:</u> One should not worry about antibiotic resistance.**
- **Only one bacteria in a billion takes up the marker-gene**

Number of bacteria

The truth: In humans:

10^{12} bacteria/g colon	10^{12}
10^{3} g gut/person	10^{15}
1 in 10^{9} bacteria is transformed	10^{6}
efficiency of 1%	10^{4}

= 10,000 transformed bacteria/person

In animals (cow):

10^{13} bacteria/g tissue	10^{13}
10^{4}g gut/animal	10^{17}
1 in 10^{9} bacteria is transformed	10^{8}
efficiency of 1%	10^{6}

=1,000,000 transformed bacteria/cow

After acquiring RESISTANCE to ONE antibiotic

bacteria may become resistant to other antibiotics in a much shorter time-period

In the presence of antibiotics resistance is a competitive advantage

Bt-crops

- **Question: Why people object to the use of *Bt* in GM crops when it has been used in organic farming for decades and nobody objected?**
- **Answers: In *Bt* crops not the bacteria, but the effective part of the bacterial toxin is encoded**
- **In organic farming the bacteria is sprayed only at high insect infestation**
- **The bacteria is only present on the surface of the plant and destroyed by heat and rain or can be washed off**
- **In the *Bt*-GM crops *every* cell expresses the toxin *all the time*.**

Bt-crops

- **Assertion:** ***Bt*** **Cry proteins bind to specific receptors in the midgut of sensitive insects but exert no toxicity in species that lack these receptors**
- **Question: What species have been checked? Humans? Animals, such as pigs, sheep, cows, birds, etc.? Why is it then that in the published literature there are reports that some** ***Bt*** **Cry toxins bind to receptors in the mammalian (mice, rat) gut?**

Bt-crops

- **The bacterial protoxin (which converts to the active toxin only in the gut of the insect) is safe. But this does not necessarily prove that the active toxin in the Bt-crops is safe too?**

- **Regulatory evaluation by FDA or EPA means only opinions as these agencies do not have laboratories. The FDA only consider the data presented to them by the biotech companies during a non-compulsory consultation process.**

Bt-crops

- **If all Bt-toxins are different from each other for patent purposes, then their mode of action, safety, toxicity, specificity and other characteristics might also be different. Therefore each should be tested *separately* and data gained with one cannot be used to justify the release of another without testing!**
- **It is also necessary to test GM plants expressing several stacked Cry genes, even if the individual Cry genes and their products had been separately tested!**

ROUND-UP READY-CROPS

- Assessment to glyphosate resistance is based on criteria by Benbrook (1991) but ignoring later data and analysis by Benbrook (2003)
- A supposed advantage of RR use that it leaves minimal residue in the soil..., but this is not so
- Spread of RR-resistance is helped by repeated use of RR on the same field and no tillage
- Only 3 locations have been confirmed as having RR-resistant weed population - true but these locations are countries, such as Australia, Canada, California and South-Africa!!!

GLYPHOSATE

- **The statement by WHO that glyphosate is not carcinogenic, mutagenic or teratogenic was given in 1994 - What has happened since?**
- **It was stated that glyphosate (Round-up) has minimal environmental impact because of its lack of persistence. It was claimed to present low risk of ground water contamination and no significant runoff to surface water and negligible soil erosion. - Most of this is not true. See Danish Ministry of Health website, www ..?**

GLYPHOSATE

- **Assertion: Weed control by glyphosate will reduce the total herbicide use in agriculture**
- **Answer - This was true only for the first 2 years. Since then the usage has increased. See Benbrook (2003) *AgBiotech InfoNet Technical Paper No. 6.*** http://www.biotech-info.net/technicalpaper6.html
- **Assertion: It poses minimal risk to human health**
- **Answer: Where are the data?**

HOW TO READ THE DOSSIER?

What should you look for?

FUTURE TENSE

- Any statement relating to the future implies something that has not yet happened. There is no guarantee, that it ever will.

Examples:

The next step in the regulatory process ***will be the drafting*** by the EU Commission a decision…

…proposals will be made for consultation concerning the ***possible authorization***

…it is ***unlikely that it will have*** an adverse effect

LOOK FOR EXPRESSIONS, SUCH AS:

- ***A comprehensive environmental assessment was not conducted*** **- this means that in the file any references to environmental effects have no scientific basis**
- ***The processing and food and feed uses of the GM plant is unlikely to have any adverse effect on human and animal health*** **- without data and description of the methods used this is just an unsupported assertion**

LOOK FOR EXPRESSIONS, SUCH AS:

- ***Risk assessment was done to assess the safety of foods and food ingredients derived from a GM plant*** **- But without actual valid data this has no value; it does not vouchsafe the environmental safety of the GM plant either**
- ***In the safety evaluation the potential toxicity of the gene products and their metabolites were considered*** **- But without risk assessment on the GM-plant its safety cannot be claimed**

LOOK FOR EXPRESSIONS, SUCH AS:

- ***The GM food/plant is safe because the expressed GM protein in it showed no homology to known toxins or allergens*** **- But one cannot assess the safety of any new toxin generated by the gene transfer when, by definition, one does not know what to look for?**
- ***The significant differences found are within normal biological variability*** **- how to define this and by whom? What is normal, in this sense?**

LOOK FOR EXPRESSIONS, SUCH AS:

- ***…extensive testing demonstrated*** **- But without specifying the tests and giving their results this is meaningless**
- ***... long history of safe use in human foods and animal feeds*** **- But the first GM crop was only released in the mid-nineties, about 10 years ago**
- ***…poses no meaningful risk to the environment*** **- What does this mean? How was it done and by whom? Who decides?**

LOOK FOR EXPRESSIONS, SUCH AS:

- **…*may suggest*… - but does not prove**
- **…*is homologous*… - but not identical**
- **…*is unlikely to be biologically significant* - without actual work this is only an opinion, and not a scientific statement**
- **…*the values were within the range observed for commercial lines or historical values* - The only relevant scientific comparison is with the isogenic parent line!**

LOOK FOR EXPRESSIONS, SUCH AS:

- *… the structure of the GM protein is virtually identical with the original* - **but not the same!**
- *…encodes a selectable marker* - **NPTII is an antibiotic-resistance marker gene, phased out in the EU.**
- *particle acceleration method* - **gene gun**
- *… corn/maize does not produce significant quantities of toxins, allergens or antinutritional factors…* - **what about the GM-maize/corn?**

LOOK FOR EXPRESSIONS, SUCH AS:

- *…was determined by calculation…* **-why not measured?**
- ***In x cases of the total y comparisons there were no significant differences…*** **- this means that in the remaining (y-x) cases the changes were significant! Significance must be determined in all comparisons!**

LOOK FOR EXPRESSIONS, SUCH AS:

- **…*visual inspection of the alignment* - actually this means no proper evaluation of the data**
- **…*a truncated fragment of the protein* - it means that there are differences in several amino acids between the two proteins…**
- **… *the isolated GM protein was full length* - this means that the protein can be purified from the GM-crop, therefore this protein should be used for all safety studies**

LOOK FOR EXPRESSIONS, SUCH AS:

- **…*comparable molecular weight* - but not identical; comparable is not a scientific term**
- **… *considerable overlap within 95% confidence intervals* - but not full overlap, which is needed for identity; not the same mean value, not the same error, not the same range**

REFERENCE TO MISSING DATA

- In some submissions there are pages marked as page 1 of 22. This means that this page is from a longer report, but the other 21 pages are not given
- There are references in the text as (Figure X) or (Table X), but these are not given in the files. Where are these data? Why are they not given?

ANIMAL STUDIES

- In nutritional studies no E. coli recombinant proteins may be used
- It is not allowed to replace animals which die during the experiment
- Differences in starting parameters (weight, etc) of the animals must be less than 5% to allow the detection of significant differences by the end

FEEDING STUDIES

- **The composition of the diets must be specified and confirmed by actual analysis. *All diets* must have the same protein and energy content, and should be supplemented with all required vitamins and minerals**
- **All animals should be singly housed and fed the *same amount of diet*. If not, their growth cannot be compared**

CONTROLS IN FEEDING STUDIES

- ***All*** **control diets must contain the same amount of protein and energy, as the test diet**
- **Two control diets must be used (EFSA!)**
  - **1. The *parent line* grown and harvested the same way as the GM**
  - **2. As above but *supplemented with the gene product isolated* from the GM plant**

FEEDING STUDIES

- **To establish the effect of the diet on animal growth the experiment should be carried out with *young, rapidly-growing animals,* as the organ- and body weights of older animals are less sensitive to dietary changes**
- **Animal starting weights should be close; their *differences must not exceed 3%,* or it will be difficult to detect statistically significant differences in their growth, particularly in the short-term and with small group sizes**

FEEDING STUDIES

- **Look out for in the submission whether…**
  - **...the growth of groups of pair-fed rats was monitored, and samples of urine and faeces for nitrogen and dry weight balance and blood for immune- and endocrine tests were taken.**
  - **... at the end the gut and other organs were removed from the dissected rat bodies, weighed (wet and dry), sections for histology taken, and DNA and enzyme tests, etc were performed?**
- **If not, you can ignore the data!**

NUTRITIONAL EVALUATION

- **When human safety of GM-foods is evaluated the calculations are based on food consumption data characteristic of the American population. The diet eaten by Americans is meat-based, energy and protein rich, and more varied than the diets eaten in the Third World. For safety evaluation only the food consumption patterns in your country are relevant!**

SAFETY ASSESSMENT OF THE TRANSGENIC PROTEIN

- **The safety assessment of a gene product is invalid if it is performed using E. coli recombinant - and not the transgenic proteins isolated from the GM plant. Since the post-tranlational processing of proteins emerging from the ribosomes is different in organisms at different levels of the evolutionary process, it is likely that the recombinant proteins produced by the plant and the bacteria are structurally and functionally different**

STATISTICAL EVALUATION

- **The *GM food is unsafe* if its effects on rats are significantly different from that of the non-GM parental line control diet**
- **If the effects of feeding rats with parent line control diet are changed on spiking with the transgene product, the *transgene is unsafe***
- **If effects of the GM-plant, and the parent line control spiked with the gene product differ, the problem is likely due to *transgene insertion or position***

DIGESTIBILITY

Scientifically unacceptable to use E.coli-recombinant form of the gene product instead of the protein isolated from the plant for establishing its stability

- **use a simulated gastric digestibility test in vitro (in a test tube with enzymes) to show whether the gene product survives digestion in the gut**

STABILITY TO DIGESTION OF TRANSGENIC PROTEINS

- **Because recombinant proteins expressed in E. coli or in the GM plants can be different the use of E. coli surrogates in digestibility studies is scientifically invalid**
- **Protein digestion in the alimentary tract cannot be simulated by *in vitro* digestion assays because the gut surface and its digestive enzymes are absent in the test tube, and the pH, the concentration and distribution of the enzymes are different in the two systems**

ASSESSMENT OF THE ORAL TOXICITY AND NUTRITIONAL VALUE OF GM PROTEINS

- **Should be carried out with the transgene protein purified from the transgenic plant. The use of E. coli recombinant surrogate is not scientifically valid**

PROTEIN STRUCTURE

- **Assertion:** ***The protein expressed in a GM plant is indistinguishable from the original by western blot analysis with polyclonal antibodies***
- **Answer: This method is qualitative and only indicates similarity but not identity. Reaction with monoclonal antibodies indicates the identity of only one epitope.**
- **Assertion:** ***The same transgene produces the same protein whether in a GM plant or E. coli***
- **Answer: DNA is only coding for the amino acid sequence but not necessarily for the conformation, function, and biological activity of the protein**

PROTEIN STRUCTURE

- **Assertion:** ***Identity of the amino acid sequences in the active site of an enzyme with that in the GM enzyme proves their identity***
- **Answer: The identity of a small part of the amino acid sequence of two proteins does not necessarily show the identity of the rest or that their conformation and stability are the same**
- **Assertion:** ***Substitution of one amino acid by another does not alter the protein structure***
- **Answer: Without stability and conformational studies this is just an unsupported opinion**

PROTEIN STRUCTURE

- **Assertion:** ***Bands in similar positions on an SDS- (or other) gels prove the identity of two proteins***
- **Answer: SDS-gel electrophoresis is a crude method for the determination of the molecular weight; it is unsuitable to determine the structural-, and even less the functional similarity of two proteins**

ALLERGENICITY

- **No adequate animal model exist to test the allergenicity of a protein**
- **Allergic reaction is a defensive, usually harmful response of the immune system of an *individual* (human or animal) to exposure to an external irritant (protein, muco/lipo-polysaccharide, etc.)**
- **different persons might be allergic to different proteins from the same plant, or to different parts of the same protein**

ALLERGENICITY

- **Using databases to establish the lack of allergenicity from the lack of sequence identity of eight consecutive amino acids in the GM protein and a known allergen is not sufficient.**
- **Allergic reaction is to an epitop (a steric structure) on the allergen which, in most cases, is made up of non-consecutive amino acids.**
- **Occasionally six or even less amino acid identity is enough to evoke allergic reactions.**

ALLERGENICITY

- Prediction of allergenicity based on structural features of the protein, such as glycosylation, size or stability to proteolysis in a simulated digestion assay, is at best tentative. Present databases are not sufficiently large or inclusive to contain all toxins and allergens either
- Thus, for allergenicity testing, in addition to the decision-tree approach, in vivo immune-tests are needed, such as anti-gene product antibody tests (humans and animals) and immunization model studies (Brown Norway rats, etc.)

ENVIRONMENTAL SAFETY ASSESSMENT

- **Only data obtained under conditions identical to your own country can be considered.**
- **Out-crossing should be studied using your country's own flora**
- **For the existence of wild-relatives the flora of your country should be considered**
- **Work to establish the disease susceptibility of plants should be carried out under conditions found in your country**

EFFECTS ON NON-TARGET ORGANISMS

- **Conditions and fauna of your own country must be considered**
- **With Round-up Ready plants it is said that they are natural because there are many different EPSPS enzymes found in nature. This is true, but the mEPSPS in these GM plants is different; their biological activity is different although they may only differ by 1 amino acid**

ASSESSMENT OF AGRONOMICAL PERFORMANCE

- Conditions and agricultural practices in your country should only be considered
- Your environmental conditions and local production methods are likely to be different from that of the USA
- Results of field trials often relate to other countries, different conditions and may have objectives different to your own

DATA ANALYSIS

- **The only proper control for a GM-plant is its parent line**
- **A wide range of data referring to commercial varieties in the submission are just simply irrelevant!**
- **Look for significant differences/trends; p value < (less than) 0.05 means significant differences; p<0.001 is biologically highly significant**
- **Of all the significant alterations only 5% can be explained by chance alone**

REFERENCES

- Technical Reports of Monsanto, or other companies do not count as references. They are not openly available, and may be biased
- Unpublished studies conducted by Monsanto or others cannot be used as references
- Committee Reports are not references
- Opinions in published papers without data to support them can only be regarded as opinions
- Only peer-reviewed and published papers with experimental data count as proper references