Toxicity Assessment of GM Crops

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Teosinte (ssp *parviglumis*)

Landraces

Artificial Selection

Inbred Lines

Conclusions

- Classical toxicology has very little role in GM crop risk assessment.
- Whole food studies are
  - Scientifically invalid
  - Uninterpretable
  - Unethical
  - Unnecessary
- Key considerations are
  - Known characteristics of the parent crop species
    - Chemical analysis for any natural toxins in the parent crop eg
      - toxic alkaloids in species of the Solanaceae (potatoes, tomatoes etc)
      - Cyanogenic glycosides in cassava
  - Characterisation of the transgene
  - Consideration of novel herbicide (or pesticide) residues from herbicide tolerance genes
    - Chemical analysis of residues
  - Evaluation of the crop development process
    - Backcrossing
    - Proven agronomic characteristics consistent with the parent crop and transgene
- Toxicology studies may be useful;
  - On novel herbicide or pesticide metabolite(s) not previously characterised
  - Where a truly novel active protein is to be introduced for a specific purpose (eg the first use of BT toxin)

Overview

- Sources of data
  - Previous experience with new technologies
  - Knowledge of the parent plant and characteristics of the transgene
  - Related species
  - Knowledge of natural variation
- Separating Real from False concerns
  - Sources of risk
  - Hazards from "Unintended" effects
  - Predictable risks
- Toxicity studies
  - When and what to test

Sources of data

Data Sources

- A toxicity assessment does not necessarily require in vivo animal toxicity studies
- A toxicity assessment starts with a consideration of the potential for toxicity, eg
  - we know a lot about corn (maize)
  - Corn naturally has multiple transposones and single nucleotide polymorphisms
  - Genetic variation amongst corn varieties is greater than between humans and chimpanzees
  - A toxic corn has never been observed either naturally or in the multiple GM strains developed using multiple transgenes from multiple sources
  - There is no plausible mechanism for the *de novo* generation of toxicity in corn through insertion of a transgene
  - **So, probability of producing a toxic corn, unrelated to the protein expressed by the transgene itself, solely through the method of insertion of a transgene, is zero**
  - **Therefore no requirement, or value, in toxicity studies on GM corn**

Data Sources – assessing the potential for toxicity

- Plant strain development
  - What do we know about the parent plant
    - Does any strain or line of that species produce toxic proteins or toxic secondary metabolites ?
      - If yes, then investigate presence and level using analytical chemistry
    - Does any closely related species produce a toxin
      - If yes then investigate using analytical chemistry
  - What do we know about the transgene
    - Does it come from an organism that produces a toxin or that has close relatives that produce a toxin
      - If yes then;
        - How well characterised is the transgene and its expressed product – can we exclude the carry over of sequences coding for the toxin
        - If in doubt investigate using analytical chemistry
  - How many backcrosses were involved:
    - every backcross to the parent line reduces the genetic material from the initial hybrid
  - Are the agronomic properties consistent with the parent line plus the transgene

Backcross theory

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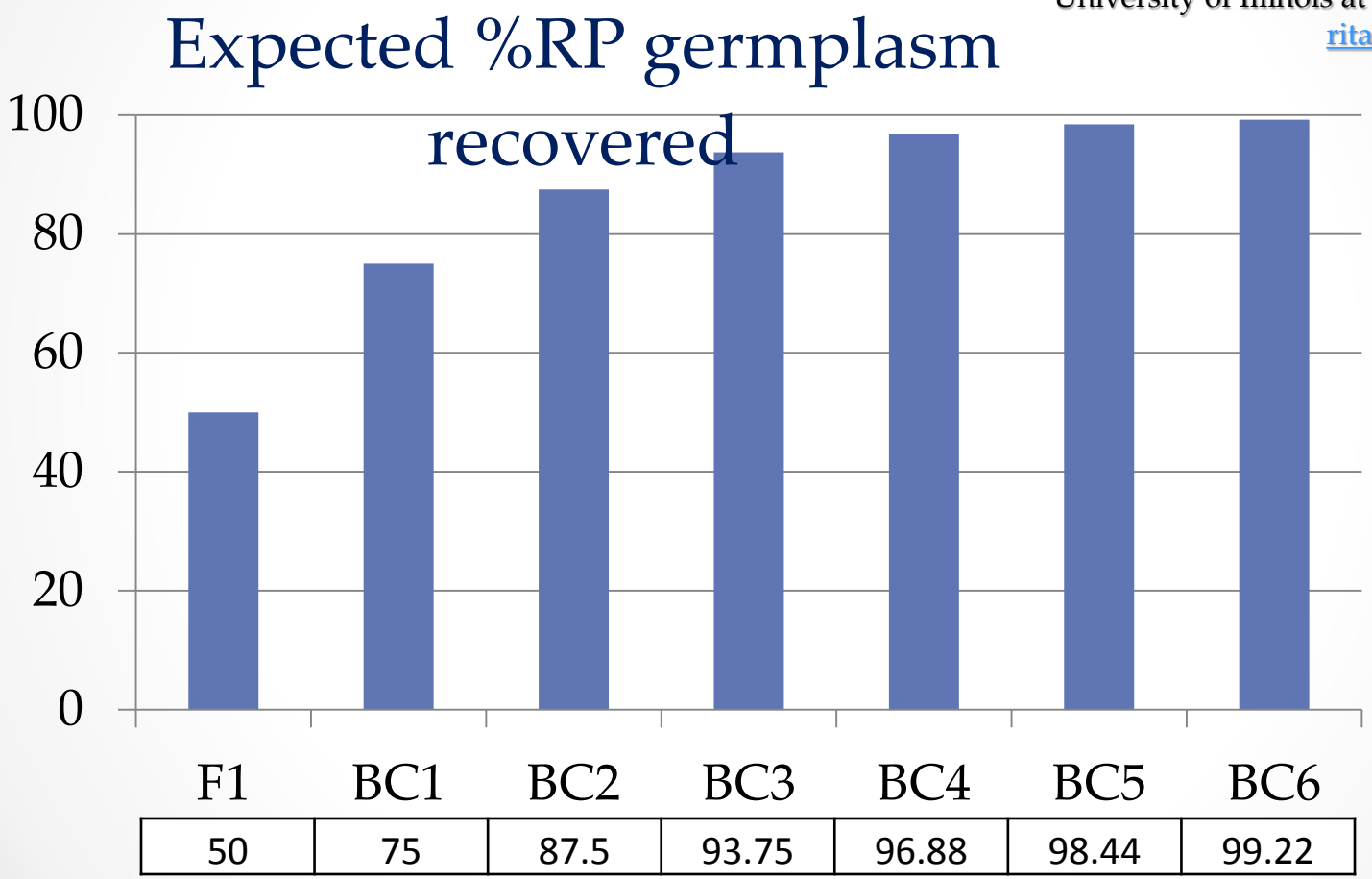

$$RP\%_{(n)} = 1 - \left(\frac{1}{2}\right)^{n+1}$$

RP%: percentage of recurrent parent germplasm recovered
n: backcross generation number

Classical Toxicity Studies

What place do they have in GM crop risk assessment

We have been here before

- Concerns around the safety of irradiated foods echo those expressed in regards to GM crops
  - Concerns
    - Reduced nutrition (radio-labile vitamins)
    - Production of unintended & unknown toxic/carcinogenic radio-lytic products
    - Induced radioactivity
  - Responses
    - Analytical chemistry
    - Toxicology studies of whole irradiated food to "test" unintended unknowns
- Adoption of the technology delayed for nearly half a century
  - Radioactivity discovered in 1895
  - First suggested as a food sterilant in 1895
  - First major applications in food in the 1960s
  - Still controversial for some

Chemistry

- Virtually all of the radiolysis products found in high-dose irradiated foods to date are either **naturally present in foods or produced in thermally processed foods.** This understanding of the radiation chemistry of foods is vital in assessing wholesomeness.
- **Compounds found in irradiated model systems that are either far different in composition from the foods of interest or have been irradiated under extreme conditions do not validly reflect the chemistry (or toxicology) of actual foods**, because competitive reactions will occur in the latter that make **the formation of such compounds very unlikely**.
- The commonality in the chemistry among the major protein, lipid and starch constituents, with minor chemical differences being accounted for by the slight differences in the composition of these constituents, **justifies use of the chemical analysis for granting broadly- based, generic approvals of high-dose irradiated foods.**

IAEA/FAO/WHO 1999 HIGH-DOSE IRRADIATION: WHOLESOMENESS OF FOOD IRRADIATED WITH DOSES ABOVE 10 kGy

Toxicology

- Tens of thousands experimental animals sacrificed over a 50 year period;
  - rats, mice, dogs, primates, chickens, quail
- No results not predictable from a knowledge of radiation chemistry

FAO/IAEA/WHO conclusions

- " the determination of wholesomeness for a representative food could be extrapolated to other foods of similar composition on the basis of available chemical data" (ie without animal testing) and
- "the committee also recognised the value of chemical studies as a basis for evaluating the wholesomeness of irradiated foods."
- although "several different chemical bonds in the constituents are broken or formed, leading to either desired or undesired effects..... it is through a consideration of the radiation chemistry of food that these chemical differences and their implications for wholesomeness and product quality can be understood."
- Thus, although vast numbers of animal studies were conducted on a wide range of irradiated foods using various levels of irradiation;
  - "none of the toxicological studies....had produced evidence of adverse effects..."
- These studies had continued to be conducted despite the understanding that:
  - "Knowledge of the nature and concentration of these radiolytic products indicated that there was no evidence of a toxicological hazard"
- Indeed in earlier deliberations, the committee concluded that the animal studies were supporting evidence for the chemical analyses rather than the other way around.

Criticisms of WF studies on irradiated foods

Elias (1980) The wholesomeness of irradiated food. Ecotox. Env. Safety 4, 172–183

- " …the impossibility of physically or chemically identifying what was being tested;
- the inability to incorporate sufficient irradiated food into the animal diet without seriously disturbing the nutrition of the test animals giving rise to secondary toxicological findings totally unrelated to irradiation effects, and
- the obvious impossibility of using sufficiently large numbers of animals in each experimental group to permit ascribing with an acceptable degree of statistical confidence any observed variations to the effect of radiolytic products present in minute amounts".
- "…It is more convincing to be able to state that certain likely effects have been searched for and found absent than to admit that one did not know quite what to look for – but found it absent nevertheless".

Whole Food Studies on GM Crops

Peer-reviewed studies of GM feeds in livestock

Test species	Test crop	Study duration	Control	Reference Group[e]	% in feed[f]	References
Cattle (dairy cows)	HT soy (Gly)[b]	28 d	Parental	1	10.2	Hammond et al. (1996)
	Bt maize	21-28d	Iso	0	75-80	Donkin et al. (2003)
	HT maize (Gly)	28d	Iso	2	63	Grant et al. (2003)
	Bt maize	28d	Iso	2	66.7	Grant et al. (2003)
	HT maize (Gly)	28d	Iso	2	57.3	Ipharraguerre et al. (2003)
	Bt maize	35d	Conventional	0	35	Yonemochi et al. (2003)
	HT maize (Gluf)	84d	Iso	2	33.1	Phipps et al. (2005)
	Bt + HT maize (Gly)	28d	Iso	0	45.1	Calsamiglia et al. (2007)
	HT alfalfa (Gly)	28d	Conventional	2	39.7	Combs and Hartnell (2008)
	Bt cottonseed	28d	Iso	0	40	Mohanta et al. (2010)
	Bt maize	25 months	Iso	0	71	Steinke et al. (2010)
	Bt + HT maize (Gluf)	28d	Iso	0	44	Brouk et al. (2011)
	Bt cottonseed	28d	Iso	0	40	Singhal et al. (2011)
Cattle (steers)	HT maize (Gly)	92d	Iso	2	75	Erickson et al. (2003)
	HT maize (Gly)	94d	Iso	2	73	Erickson et al. (2003)
	HT maize (Gly)	144d	Iso	2	79.5	Erickson et al. (2003)
Cattle (calves)	Bt maize	84d	Iso	0	43.3	Shimada et al. (2006)
Swine	HT soy (Gly)	4 months[c]	Iso	0	14-24.3[f]	Cromwell et al. (2002)
	HT maize (Gly)	103d	Iso	2	68.1-81.8	Hyun et al. (2004)
	HT maize (Gly)	NI[c]	Iso	2	65-77	Hyun et al. (2004)
	HT rice (Gluf)	98d	Iso	1	72-85.8	Cromwell et al. (2005)
	Bt maize	104d	Iso	2	68.7-82.5	Hyun et al. (2005)
	Bt maize	NI[c]	Iso	2	65-76	Hyun et al. (2005)
	Bt maize	NI[c]	Combined[d]	0	78-83	Custodio et al. (2006)
	Bt maize	NI[c]	Combined[d]	0	70-76.5	Custodio et al. (2006)
	HT wheat (Gly)	NI[c]	Iso	4	70-85	Peterson et al. (2008)
	Bt + HT maize (Gluf)	4 months[c]	Iso	1	69.1-81.9	Stein et al. (2009)
	Bt maize	NI[c]	Iso	0	70	Yonimochi et al. (2010)
	Bt maize	30d	Iso	0	38.9	Walsh et al. (2012)

- And in poultry

Test species	Test crop	Study duration	Control	Reference Group[e]	% in feed[f]	References
Poultry (broiler chickens)	HT soy (Gly)	42d	Parental	0	26.6-32.9	Hammond et al. (1996)
	Bt maize	38d	Iso	0	61.4-67.4	Brake and Vlachos (1998)
	HT maize (Gly)	38-40d	Parental	5	50-60	Sidhu et al. (2000)
	Bt maize	49d	Conventional	0	70	Yonemochi et al. (2002)
	Bt maize	42d	Iso	1	48.2-63.6	Brake et al. (2003)
	Bt maize	42d	Iso	5	57.1-62.7	Taylor et al. (2003)
	Bt + HT maize (Gly)	42d	Iso	5	55.2-60.5	Taylor et al. (2003)
	HT canola (Gly)	42d	Iso	6	25	Taylor et al. (2004)
	Bt maize	39d	Iso	0	60	Aeschbacher et al. (2005)
	IP (VIP3A) maize	49d	Iso	2	55.0-66.0	Brake et al. (2005)
	Bt maize	42d	Iso	0	48.7-62.7	Rossi et al. (2005)
	Bt + HT maize (Gly)	43-44d	Iso	5	54.7-59.4	Taylor et al. (2005)
	HT soy (ALSi, Gly)	42d	Iso	3	22.5-31	McNaughton et al. (2007)
	HT soy (Gly)	42d	Iso	6	61.4-64.8	Taylor et al. (2007a)
	Bt maize	42d	Iso	4	55.1-59.6	Taylor et al. (2007b)
	Bt + HT maize (Gly)	42d	Iso	4	54.8-58.5	Taylor et al. (2007b)
	Bt + HT maize (Gly)	42d	Iso	6	57.3-59.4	Taylor et al. (2007c)
	HT maize (ALSi, Gly)	42d	Iso	3	58.5-71.5	McNaughton et al. (2008)
	HT maize	42d	Iso	3	50-60	Herman et al. (2011)
	HT soy	42d	Iso	3	32-40	Herman et al. (2011b)
	HT maize + HT soy	42d	Iso	3	91.5-94.2	McNaughton et al. (2011a)
Poultry (laying hens)	Bt maize	6 months	Iso	0	60	Aeschbacher et al. (2005)
	Bt + HT maize (Gluf)	3 months	Iso	1	64.8	Jacobs et al. (2008)
	High oleic soy	3 months	Iso	2	23.5	Mejia et al. (2010)
	HT maize + HT soy	3 months	Iso	3	84.6-86.3	McNaughton et al. (2011b)

PUBLISHED, CREDIBLE, SUBCHRONIC RODENT WF TOXICOLOGY STUDIES CONDUCTED ON GM CROPS

Crop	Sponsor	Dose group	Group size	Ref. group[b]	Control	% in diet[c]	References
Bt tomato	RIKILT	1	12/sex	0	Iso	10	Noteborn et al. (1995)
HT soy (RR soy)	Japan	1	5/sex	0	Iso	30	Teshima et al. (2000)
Bt corn	Japan	1	8/sex	0	Iso (AIN 93M)	5/50	Teshima et al. (2002)
HT soy (RR)	China	3	10/sex	0	control	30/60/90	Zhu et al. (2004)
Ht corn (RR)	Monsanto	2	20/sex	6	Iso (PMI)	11/33	Hammond et al. (2004)
Bt/HT corn (ECB/RR)	Monsanto	2	20/sex	6	Iso (PMI)	11/33	EFSA (2005a)
Bt/HT corn (CRW/RR)	Monsanto	2	20/sex	0	Iso (PMI)	11/33	EFSA (2005b)
Bt/HT corn (ECB/CRW/RR)	Monsanto	2	20/sex	0	Iso (PMI)	11/33	EFSA (2005c)
HS potato (amylopectin)	BASF	3	5/sex	0	Iso	5	EFSA (2006)
Bt corn (ECB)	Monsanto	2	20/sex	6	Iso (PMI)	11/33	Hammond et al. (2006a)
Bt corn (CRW)	Monsanto	2	20/sex	6	Iso (PMI)	11/33	Hammond et al. (2006b)
Bt/HT corn (ECB/Gluf)	Pioneer	2	12/sex	3	Iso (PMI)	33	MacKenzie et al. (2007)
Bt cotton	Dow	1	12/sex	3	Iso (PMI)	10	Dryzga et al. (2007)
Bt rice	EU and Canada	1	16/sex	0	Iso	60	Schroder et al. (2007)
Lectin rice (snowdrop)	EU China India	1	16/sex	0	Iso	60	Poulsen et al. (2007a)
Lectin rice (PHA-E)	EU and China	1	8/sex	0	Iso (AIN93)	60	Poulsen et al. (2007b)
Bt/HT corn (CRW/Gluf)	Pioneer	1	12/sex	2	Iso (PMI)	35	Malley et al. (2007)
HT corn (RR)	Syngenta	2	12/sex	0	Iso	10/42	EFSA (2007)
Bt/HT corn (CRW/RR)	Monsanto	2	20/sex	6	Iso (PMI)	11/33	Healy et al. (2008)
HT soy (RR2Y)	Monsanto	2	20/sex	6	Iso (PMI)	5/15	EFSA (2008b)
Bt corn (CRW)	Pioneer	2	10/sex	0	Iso (AIN93)	50/70	He at al. (2008)
High oleic soy	Pioneer	1	12/sex	3	Iso (PMI)	20	Delaney et al. (2008a)
HT soy (GAT)	Pioneer	1	12/sex	3	Iso (PMI)	20	Appenzeller et al. (2008)
HT corn (GAT)	Pioneer	1	12/sex	3	Iso (PMI)	35–38	Appenzeller et al. (2009b)
Bt/HT corn (ECB/CRW)	Pioneer	1	12/sex	3	Iso (PMI)	34	Appenzeller et al. (2009a)
Lysine corn	Pioneer	2	10/sex	0	Iso (AIN93)	30/76	He et al. (2009)
rhIGF rice	China	2	16/sex	0	Iso	20	Tang et al. (2011)
HT soy (CV127)	BASF	2	10/sex	4	Iso	11/33	Chukwudebe et al. (2012)

Predictable Outcomes

- Uniquely in the field of toxicology, risk analysis based on in vitro, in silico and process evaluation (principally agronomic & compositional analysis) is 100% concordant with WF studies in experimental animals
- Reflects both
  - Negligible potential for accidental generation of unknown, unexpected toxic substances through gene insertion
  - High LOD of bioassays for unknowns
- Rats are a poor substitute for a HPLC (GCMS etc)

What about studies purporting to show harm

- Even critical studies from anti GM activists groups tend to support GM safety when analysed honestly
  - Austrian study
    - The study itself was actually quite well conducted and extensively reported
    - Biased, selective and inept interpretation misrepresented the findings
    - When reviewed by experts the study revealed no evidence of reproductive toxicity
  - Seralini studies
    - The most positive statement that can be made is that these studies can always be used as bad examples !

Compositional analysis

- Scientific basis for even this requirement is now highly questionable
- Hugely expensive with no evidence that it adds anything to public health and safety
- Clear evidence that considerable variation due to environment often exceeds genetic influence
- During GM commercialization backcrossing of elite hybrid with parent eliminates > 99.9 % of hybrid genetics (repetitive selection for introduced trait)
- Requirements for GM crops but not "conventionally" bred crops, which have greater genetic alteration, is irrational, logically inconsistent, discriminatory

What if a 90 day study in rats was actually meaningful ?

- How could it be enough ?
  - Species specificity
    - Pharmacokinetics
    - Toxicodynamics
    - Differential physiology/biochemistry
    - Life stages, reproduction etc
- Why not test pesticides the same way ?
  - Spray a crop
  - Wait till harvest
  - Test WF in a 90 day rat
  - No observed effects
  - End of testing !!!
  - Great savings $$$$$

Possible Roles for animal Toxicity Testing

- Toxicological characterisation of introduced novel secondary metabolite(s) or protein
  - Test pure or purified substance
- Characterisation of herbicide or pesticide metabolites unique to the transgenic variety
  - Test pure metabolite(s)
- ?

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