Whole Food Testing

What is its place in GM crop risk assessment?

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Comparative safety assessment of GM crops

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2 Types of changes

- Intentional
  - The product of the transgene and its related metabolites
- Assess for
  - Toxicity
  - Allergenicity
  - Nutritional equivalence

Comparative safety assessment of GM crops

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2 Types of changes

- Unintentional
- Assess by
  - compositional analysis
  - agronomic equivalency
  - Whole Food (WF) toxicity studies

Question: What is the contribution of WF studies to safety assessment?

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- Food irradiation: Past precedent with whole food (WF) toxicity studies
  - Chemistry and toxicology
  - Risk assessment
  - Criticism of WF studies on irradiated food
- WF studies on GM crops
  - What has been done
  - Study design
  - Inherent limitations
  - Ethical considerations
  - Role of animal studies

IFBiC Task Force 10

Academia • Government • Industry

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- Sue Barlow (Consultant in Toxicology and Risk Assessment, UK)
- Andrew Bartholomaeus (University of Queensland and Canberra University)
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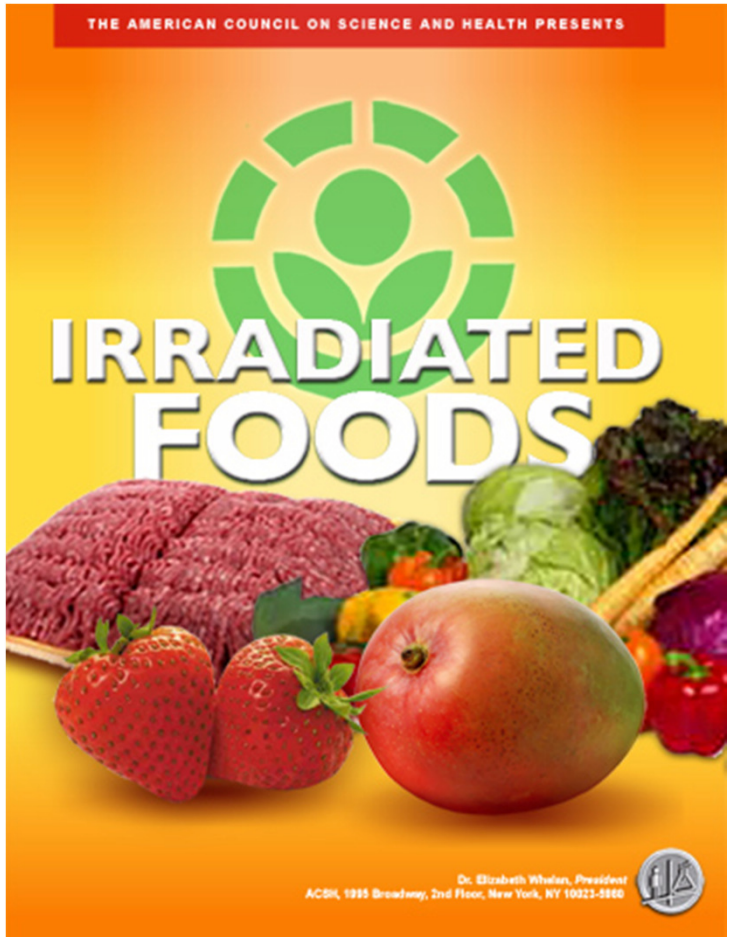
- Alaina Sauve (Syngenta)
- Kate Walker (ILSI-IFBiC)
- Flavio Zambrone (Planitox, ILSI Brazil)

The use of whole food animal studies in the safety assessment of genetically modified crops: limitations and recommendations

Accepted for publication in *Critical Reviews in Toxicology*

Safety of irradiated foods



- Concerns about the safety of irradiated foods are very similar to those for GM crops
  - altered nutrient content
  - production of unintended and unknown toxic products
  - induced radioactivity
- Adoption of food irradiation delayed

Chemistry of irradiated foods

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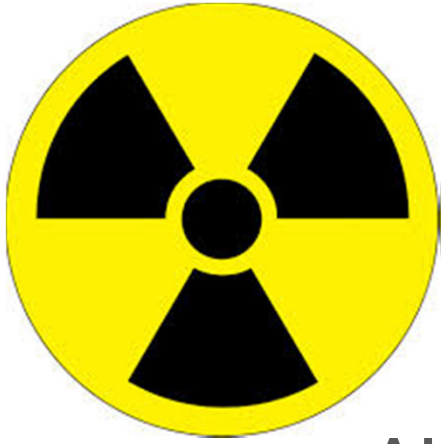
- Chemistry and toxicology studies of low-dose irradiated foods
  - predicted a purely chemical approach to wholesomeness evaluation of irradiated foods may prove possible
  - chemical changes smaller than those caused by heating



- Almost all radiolysis products found in high-dose irradiated foods
  - are naturally present or
  - are also found in foods processed by other means
    - Joint FAO/IAEA/WHO Study Group, 1981 and 1999

Toxicology of irradiated foods

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- Many subchronic and chronic animal feeding studies conducted over a 50-year period
  - include teratogenic, mutagenic, carcinogenic endpoints
- Thousands of experimental animals, multiple species
  - rats, mice, dogs, non-human primates, chickens, quail
- Found no unpredictable results
  - no toxic effects due to food irradiation
  - few adverse effects in some studies related to nutrient degradation or nutrient deficiencies not related to irradiation

Conclusions of the Joint FAO/IAEA/WHO Study Group

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- "The determination of wholesomeness for a particular food could be extrapolated to other foods of similar composition on the basis of available chemical data"
- Although "several different chemical bonds…are broken or formed…it is through a consideration of the radiation chemistry of food that these chemical differences and their implications for wholesomeness…can be understood"
- The strengths and limitations of WF toxicity studies were not directly addressed but
  - "none of the toxicological studies…had produced evidence of adverse effects"
- On the surface, the lack of adverse effects observed in animal studies confirmed the results of analytical studies….

Report of a Joint FAO/IAEA/WHO Study Group

Criticism of WF toxicity studies on irradiated foods

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- Professor Peter Elias (1980): *The wholesomeness of irradiated food*. Ecotox. Env. Safety 4, 172-183
- Limitations
  - the impossibility of physically or chemically identifying what was being tested
  - the inability to add enough irradiated food into the diet without disturbing the nutrition of the test animals
    - gives rise to secondary toxicological effects unrelated to food irradiation
  - the impossibility of using sufficiently large numbers of animals to attribute any observed variations to the effect of radiolytic products with statistical confidence

Whole food toxicity studies on GM crops

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- Purpose of WF toxicity studies is extrapolation to humans for hazard characterization

- IFBiC TF10 reviewed WF studies published in the peer-reviewed literature
  - > 50 studies using domestic animals
  - > 40 rodent studies
    - including several controversial studies that report adverse effects

Whole food toxicity studies with rodents on GM crops

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- TF10 examined subchronic (90 d) and shorter (21-30 d) studies
  - majority of recent studies are 90 d
  - based on OECD Guideline 408
  - majority of studies used rats

Whole food toxicity studies on GM crops

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Crop	Sponsor	Dose group	Group size	Reference group	Control	% in diet
Bt tomato[b]	RIKILT	1	12/sex	0	Iso	10
HT soy (Gly)	Japan	1	5/sex	0	Iso	30
Bt maize	Japan	1	8/sex	0	Iso (AIN93M)	5/50
HT soy (Gly)	China	3	10/sex	0	Iso	30/60/90
Ht maize (Gly)	Monsanto	2	20/sex	6	Iso (PMI)	11/33
Bt/HT maize (ECB/Gly)	Monsanto	2	20/sex	6	Iso (PMI)	11/33
Bt/HT maize (CRW/Gly)	Monsanto	2	20/sex	0	Iso (PMI)	11/33
Bt/HT maize (ECB/CRW/Gly)	Monsanto	2	20/sex	0	Iso (PMI)	11/33
HS potato (amylopectin)	BASF	3	5/sex	0	Iso	5
Bt maize (ECB)	Monsanto	2	20/sex	6	Iso (PMI)	11/33
Bt maize (CRW)	Monsanto	2	20/sex	6	Iso (PMI)	11/33
Bt/HT maize (ECB/Gluf)	Pioneer	2	12/sex	3	Iso (PMI)	33
Bt cotton	Dow	1	12/sex	3	Iso (PMI)	10
Bt rice	EU and Canada	1	16/sex	0	Iso	60
Lectin rice (snowdrop)	EU, China, India	1	16/sex	0	Iso	60
Lectin rice (PHA-E)	EU and China	1	8/sex	0	Iso (AIN93)	60
Bt/HT maize (CRW/Gluf)	Pioneer	1	12/sex	2	Iso (PMI)	35
HT maize (Gly)	Syngenta	2	12/sex	0	Iso	10/41
Bt/HT maize (CRW/Gly)	Monsanto	2	20/sex	6	Iso (PMI)	11/33
HT soy (Gly)	Monsanto	2	20/sex	6	Iso (PMI)	15
Bt maize (CRW)	Pioneer	2	10/sex	0	Iso (AIN93)	20
High oleic soy	Pioneer	1	12/sex	3	Iso (PMI)	20
HT soy (Gly/ALSi)	Pioneer	1	12/sex	3	Iso (PMI)	20
HT maize (Gly/ALSi)	Pioneer	1	12/sex	3	Iso (PMI)	35–38
Bt/HT maize (ECB/CRW/Gluf)	Pioneer	1	12/sex	3	Iso (PMI)	34
Lysine maize	Pioneer	2	10/sex	0	Iso (AIN93)	30/76
rhIGF-1 rice	China	2	16/sex	0	Iso	20
High amylose rice	China	1	10/sex	0	Iso	70
HT soy (ALSi)	BASF	2	10/sex	4	Iso	11/33
Bt/HT maize (Gluf)	China	3	10/sex	1	Iso	12.5/25/50
High oleic/HT soy (Gly)	Pioneer	3	10/sex	0	Iso	7.5/15/30
HT maize (Gly)	China	3	10/sex	0	Iso	12.5/25/50

Whole food toxicity studies on GM crops: commonalities

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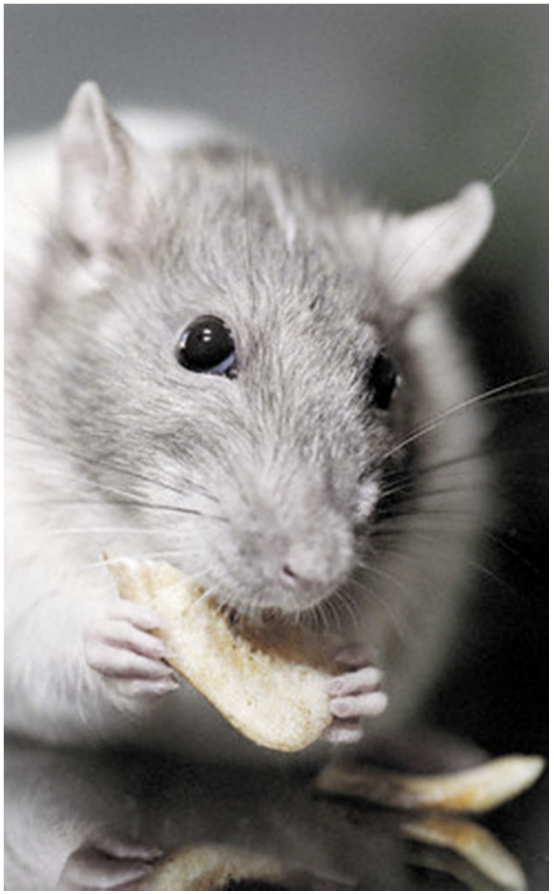

- GM crop incorporated into rodent diet
  - 15-70%, depending on the crop
  - usually two dose groups
- Near-isogenic crop used as comparator
  - multiple “reference” comparators may be used
- Historical control data sometimes considered for health endpoints
  - as in a toxicology study
- Interpretation of study based on
  - statistical comparisons
  - weight of evidence, as in a chemical toxicity study

Feed safety studies on GM crops

- WF studies conducted using domestic animals
  - cattle (dairy cows, steers, calves),
  - swine, poultry (broilers, laying hens)
- What they really are is "Nutritional non-inferiority" or "performance" studies
  - not serve as human health risk assessment
  - test species is the species that will be exposed in feed
  - >50 studies

Test species	Test crop	Study duration	Control	Reference group	% in feed	References
	HT soy (Gly)	28 d	Parental	1	10.2	Hammond et al. (1996)
	Bt maize	21–28 d	Iso	0	75–80	Donkin et al. (2003)
	HT maize (Gly)	28 d	Iso	2	63	Grant et al. (2003)
	Bt maize	28 d	Iso	2	66.7	Grant et al. (2003)
	HT maize (Gly)	28 d	Iso	2	57.3	Ipharraguerre et al. (2003)
	Bt maize	35 d	Conventional		35	Yonemochi et al. (2003)
	HT maize (Gluf)	84 d	Iso	2	33.1	Phipps et al. (2005)

Whole food toxicity studies on GM crops

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- None of the properly conducted WF toxicity studies conducted on commercialized GM crops identified an adverse effect
  - Nothing to question the adequacy, sufficiency and accuracy of a risk assessment based on agronomic and compositional analyses
- High concordance (100% ?) between animal studies and agronomic/compositional analyses

Whole food toxicity studies on GM crops

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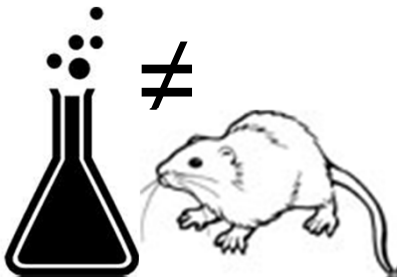
- If concordance between animal studies and compositional/agronomic analyses is high
  - does that mean that WF toxicity studies are predictive of safety?
- Not really. It actually reflects
  - the very low potential for accidental generation of unknown, toxic substances in GM crops
  - the insensitivity of a WF toxicity study for
    - unknowns of high toxicity at low levels
    - unknowns of low toxicity at any level



Why are WF toxicity studies insensitive?

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- Normal sources of toxicological power are unavailable
  - dose escalation (including a maximum tolerated dose) is not possible
  - pharmacokinetics studies not possible
- These strategies are normally used to overcome
    - statistical limitations due to small group sizes
    - species-related insensitivity
    - variations in individual responses

A "no-effect" level is not useful without an "effect" level

What about WF toxicity studies that claim harm?

- Some are well-conducted and extensively reported but interpretation is questionable
  - differences fall within normal ranges
- Some are not conducted according to standards required by regulatory agencies
  - atypical study design and/or analyses
  - non-standard endpoints
  - significant flaw in one or more aspects of the study design
  - incomplete, insufficient or vague reporting of study design and results
- Snell, C., A. Bernheim, J-B. Berge, M. Kuntz, G. Pascal, A. Paris, and A.E. Ricroch. 2012. Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials: A literature review. Food Chem Toxicol 50:1134-1148

Many whole-food studies have not used the proper diets/comparators

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Conducted by people without the necessary background or expertise

- Differing isoflavone levels in soy can lead to false results, such as
  - Mice fed glyphosate-tolerant soy had liver cell problems
  - Mice fed glyphosate-tolerant soy had unexplained changes in testicular cells

Risk assessment of genetically modified crops for nutrition and health

Javier A Magaña-Gómez and Ana M Calderón de la Barca

Lead article, *Nutrition Reviews* 2009. 67:1–16

Are WF toxicity studies ethical ?…

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- If there is no plausible biological explanation for the production of a toxic substance that is unrelated to the parent crop or the source of the transgene?
- If comparative assessment raises no concerns?
- If WF toxicity studies are not sensitive enough to detect potentially toxic unknown substances in food?
- If WF toxicity studies cannot provide the expected protection of consumers despite all effort to standardize, optimize or extend them? (Kolar and Rusche, 2010)

The 3 R's:
- Replacement
- Reduction
- Refinement

Possible roles for animal toxicity testing in GM crop safety assessment

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- Hazard characterization of a novel secondary metabolite or protein
  - not present in another food crop
  - not previously subjected to toxicity testing
- No HOSU for the novel substance, or no HOSU at the levels present in a GM crop
  - evaluate digestibility/degradation and other attributes of a novel substance
  - characterize potential adverse effects from purified novel substance or a food fraction enriched for the novel substance

Conclusions

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- Whole food toxicity studies are not sufficiently sensitive to detect unknowns in GM crops
- Increasing the duration of a WF toxicity study from 28 or 90 d to a chronic study does not correct for the inherent limitations
- Analytical approaches provide more meaningful data for GM crop risk assessment than WF toxicity studies
- Toxicology studies (not WF studies) may be useful
  - on a novel substance or metabolite introduced intentionally (or unintentionally) to a GM crop
- The lessons learned from irradiated foods should be heeded!

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Plant Biotechnology Journal

Plant Biotechnology Journal (2013) **11**, pp. 781–784 doi: 10.1111/pbi.12091

Commentary

New EU legislation for risk assessment of GM food: no scientific justification for mandatory animal feeding trials

Harry A. Kuiper[1,*], Esther J. Kok[2] and Howard V. Davies[3]

"…it is much more convincing to be able to state that certain likely effects have been searched for and found absent than to have to admit that one did not know quite what to look for – but found it absent nevertheless."

Peter Elias, 1980
The wholesomeness of irradiated food
Ecotox. Env. Safety 4, 172-183

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Thanks!

Questions?