

Allergenicity risk assessment

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Declaration of interests

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UK Biological and Biotechnological Sciences Research Council

UK Innovate

European Food Safety Authority

In-kind sponsorship of students and collaborations

Waters Corporation, Romer Laboratories Ltd, Zess, Reacta Biotech Ltd.

Allergenic risks posed by transgenic plants – how it all began

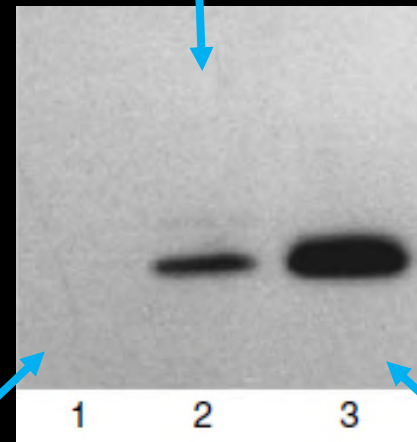


However, the work did not consider food allergy until IgE binding studies confirmed that the brazil nut 2S albumin retained its IgE binding properties.....

Transgenic soybean
expressing Brazil
nut protein

In order to improve the nutritional quality of soybean a transgenic lines was developed expressing the gene coding for a methionine-rich 2S albumin protein from Brazil nut.

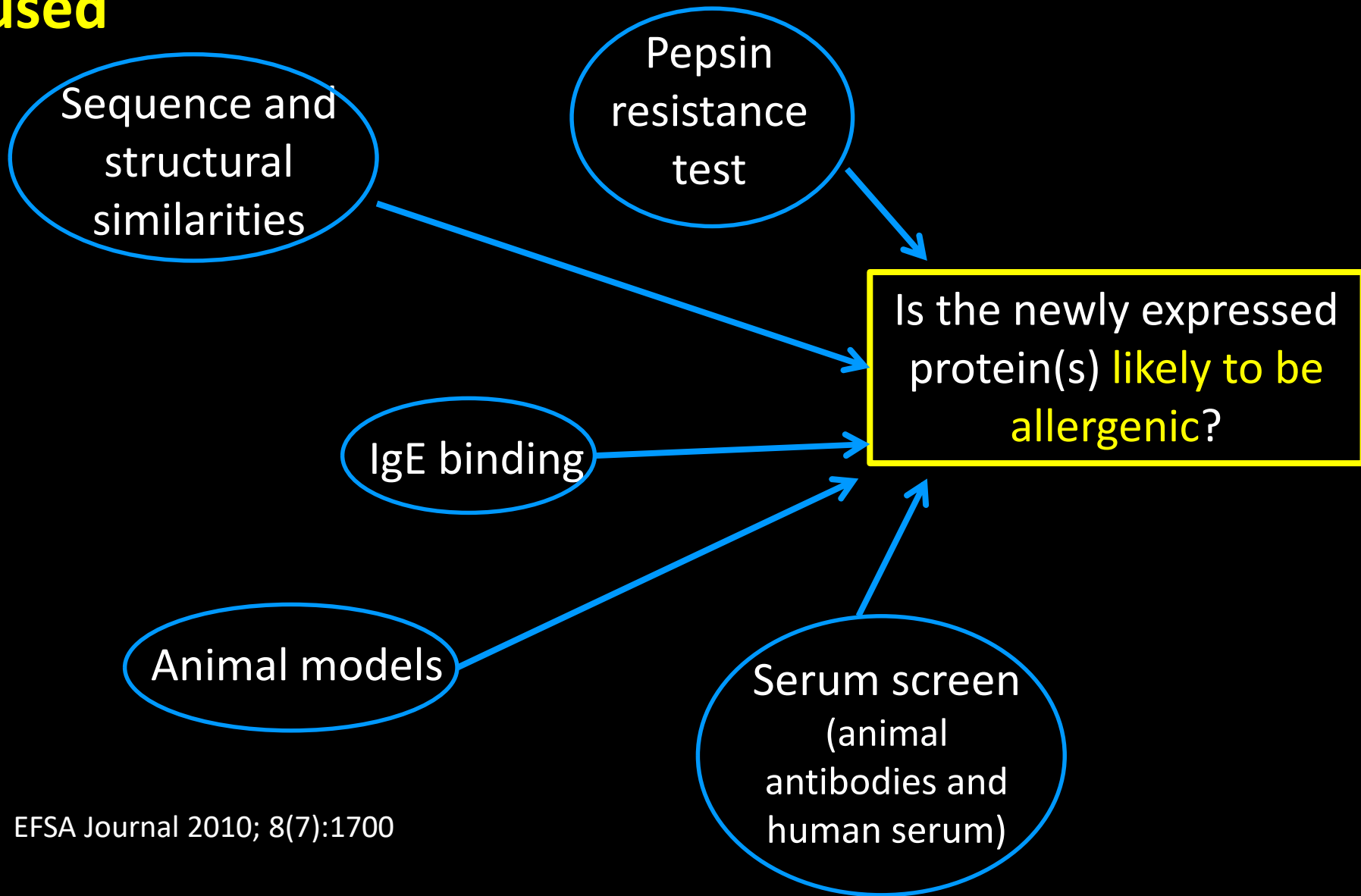
Non- transgenic
soybean



Brazil nut 2S
albumin

Making the ultimate “HIDDEN ALLERGEN”!

14 years later...a weight-of-evidence approach is used

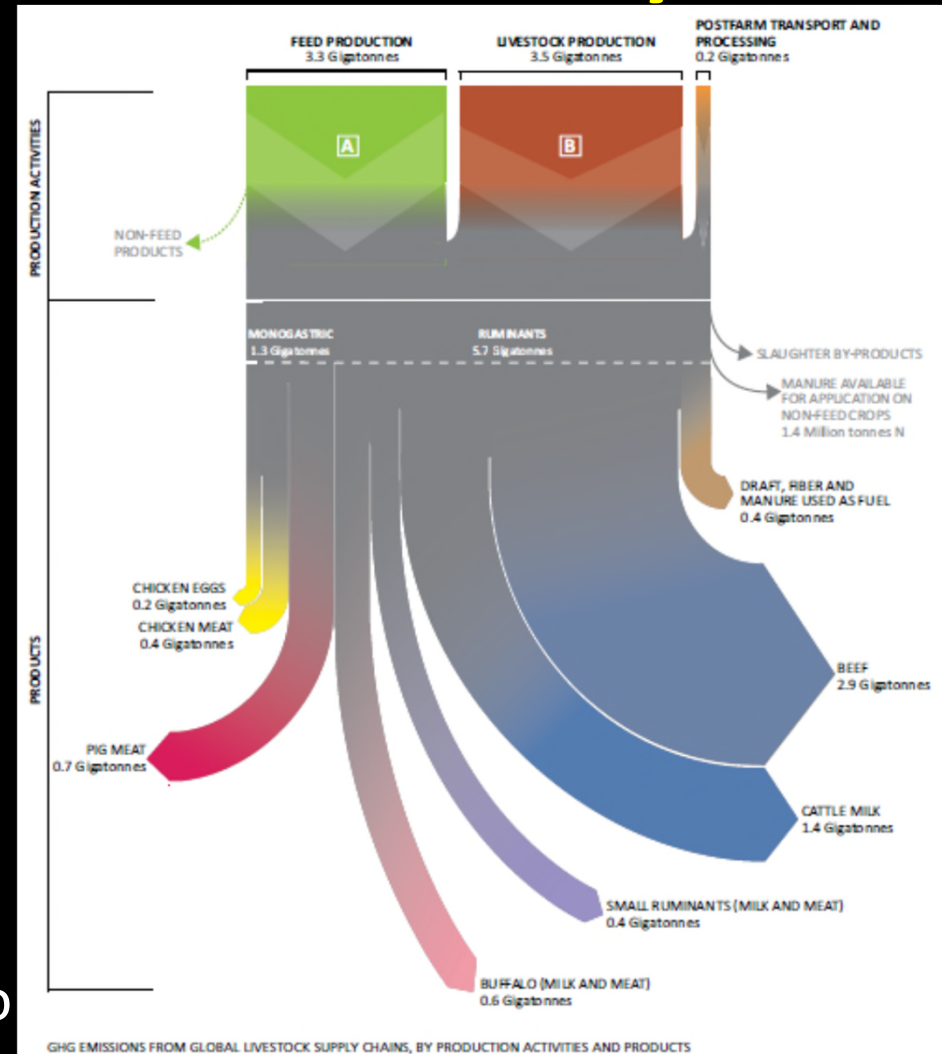




New technologies – needed for transformation of the food system

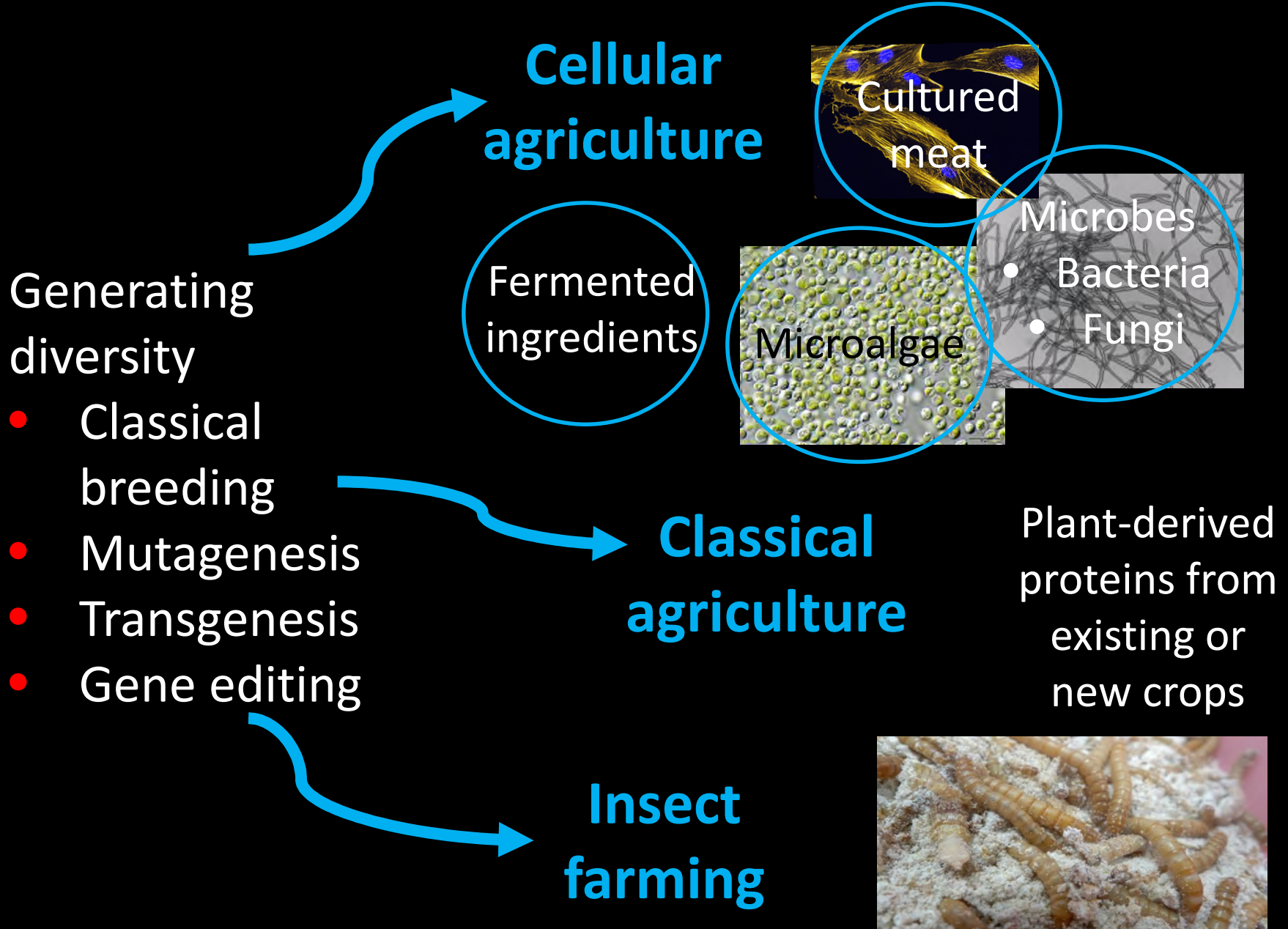
Challenges in the 21st Century!

- Feeding a global population expected to reach nearly 10 billion by 2050
- Reducing reliance on livestock which contribute 14.5% of anthropogenic green house gas emissions [cattle contributing 9.4% of the total]
- Make more effective use of land – livestock currently estimated to take 70% of agricultural land use
- Conserve water resources



FAO Tackling climate change through livestock;
<http://www.fao.org/3/i3437e/i3437e00.htm>

New technologies for food protein production

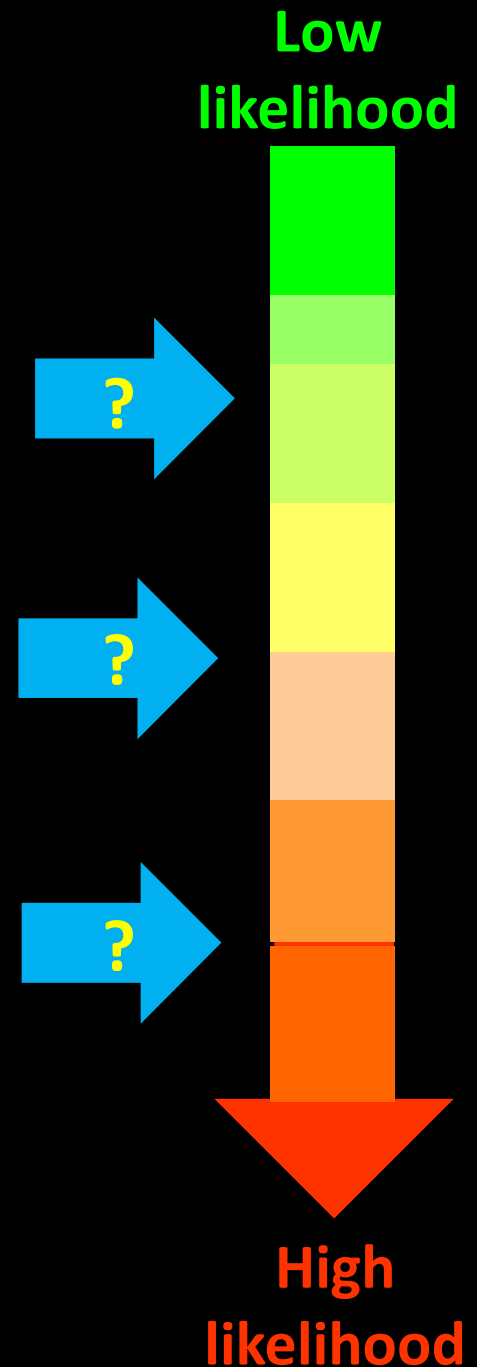


Assessing allergenic risks

FACET 1: Changes in the allergenicity of an existing priority allergenic food

FACET 2: Risks posed to existing allergic population through cross-reactivity

FACET 3: Risks posed of de novo sensitisation to develop a new allergenic protein y



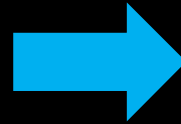
**FACET 1: Impact on potency of existing
priority allergenic foods**

Effect of GM on endogenous allergenicity

Purpose of the assessment: to ensure that no overexpression of endogenous allergens has taken place which may result in greater risk to consumers

Allergen profile determined of

- non-GM reference varieties
- non-GM conventional counterpart
- New GM line



Statistical evaluation to identify whether allergen profile is changed in the new GM line

Assessment is part of the GM compositional analysis

Soybean as an example

Which allergens?

Methods to assess changes in allergen levels

IgE-binding proteins	Allergen nomenclature	Molecular weight (kDa)	Family
Hydrophobic proteins	Gly m 1 ¹	7.0-7.5	Lipid transfer protein
Defensin	Gly m 2 ¹	8.0	Storage protein
Profilin	Gly m 3 ¹	14	Profilin
SAM22	Gly m 4 ¹	16.6	Pathogenesis related protein PR-10
P34	Gly m Bd 30 K	34	Protease
Unknown Asn-linked glycoprotein	Gly m Bd 28 K	26	Unknown
β-Conglycinin (vicilin, 7S globulin)	Gly m 5 ¹	140-170	Storage protein (with subunits)
Glycinin (legumin, 11S globulin)	Gly m 6 ¹	320-360	Storage protein (with subunits)
2S albumin	Not assigned	12	Prolamin
Lectin	Not assigned	120	Lectin
Lipoxygenase	Not assigned	102	Enzyme
Kunitz trypsin inhibitor	Not assigned	21	Protease inhibitor
Unknown	Not assigned	39	Unknown
Unknown	Not assigned	50	Homology to chlorophyll A-B binding protein
P22-25	Not assigned	22-25	Unknown

Source: adapted from L'Hocine and Boye, (2007); updated with information from WHO/IUIS (2011)

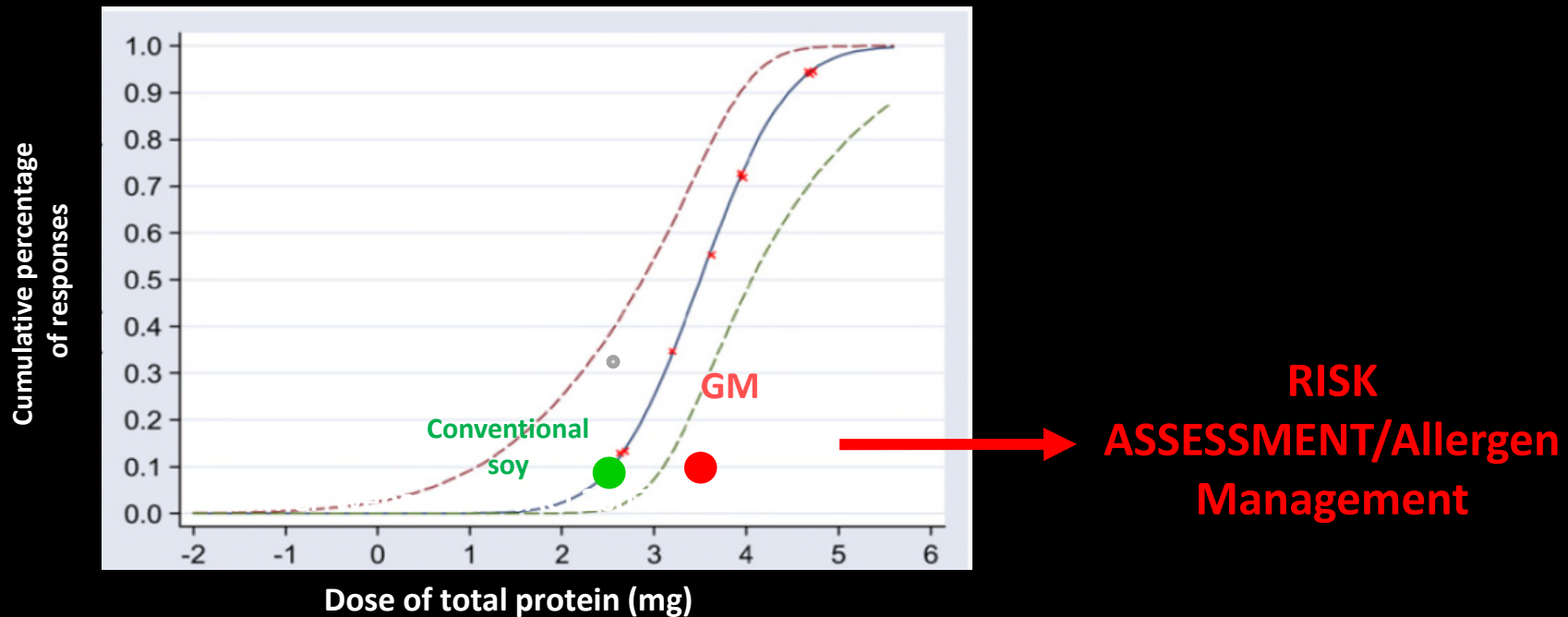
¹ WHO/IUIS (2011) Allergen nomenclature recognized by WHO and IUIS

OECD consensus soy 2012

Requires a database of natural levels of endogenous allergens determined using standardised methodology to support a harmonised risk assessment process.

- Mass spectrometry using targeted analysis
- 2D electrophoresis and immunoblotting
- 2D electrophoresis and spot identification by mass spectrometry
- Immunoassays with human serum IgE

What to do if allergen content is increased?



Use threshold dose – distribution curves?

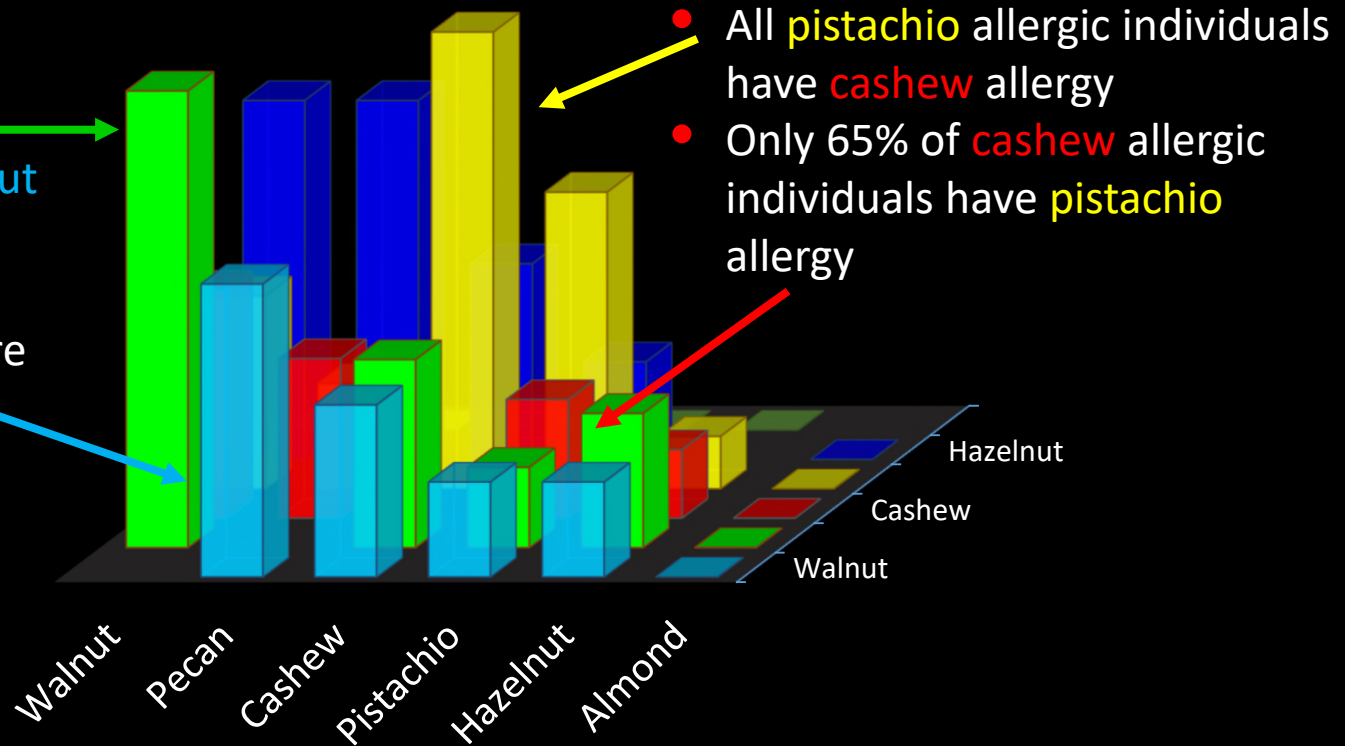
- Available for total soy protein and data very limited at present
- Takes a population approach – so some allergic individuals tested are not uniformly allergic to all relevant soy allergens

This is a relevant consideration for allergenic foods modified by gene editing

FACET 2: Assessing risks for cross-reactive allergies in the allergic population

Tree nut co-allergies are complex...

- All **pecan** allergic individuals have **walnut** allergy
- Only 68% of subjects with **walnut** allergy are allergic to **pecan**

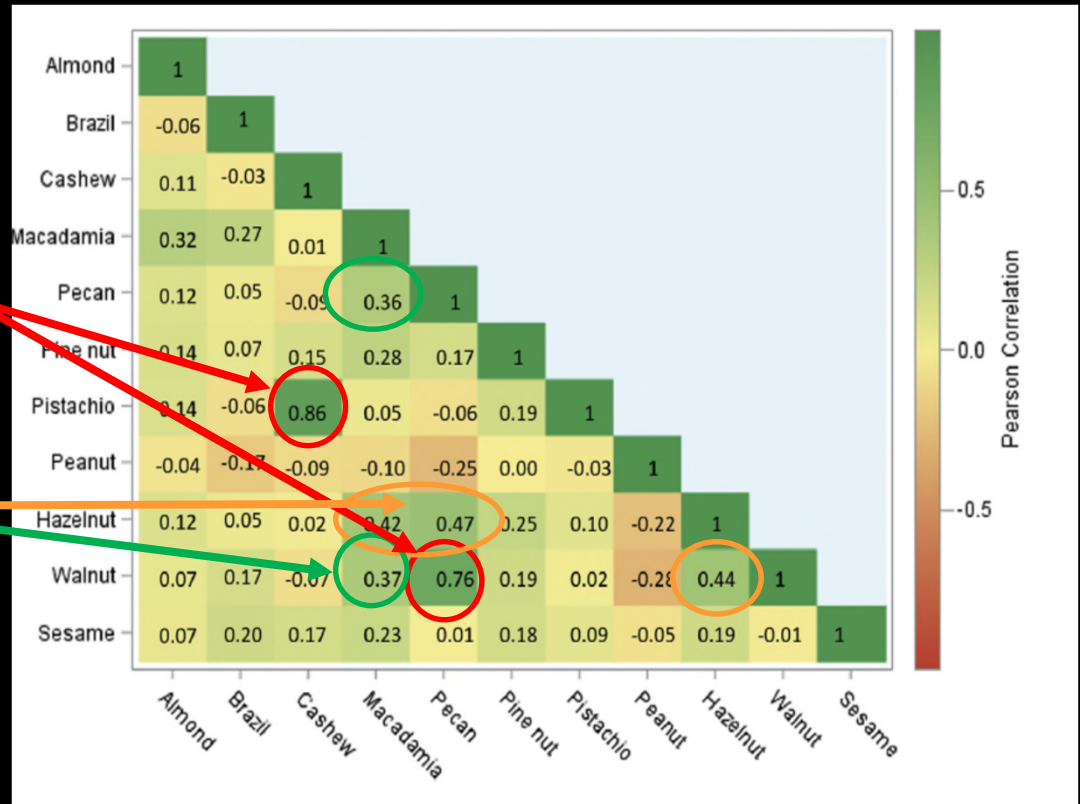


- All **pistachio** allergic individuals have **cashew** allergy
- Only 65% of **cashew** allergic individuals have **pistachio** allergy

Elizur et al Allergy 73, 593-601.

All tree nuts are seeds which underlies tree nut co-allergies

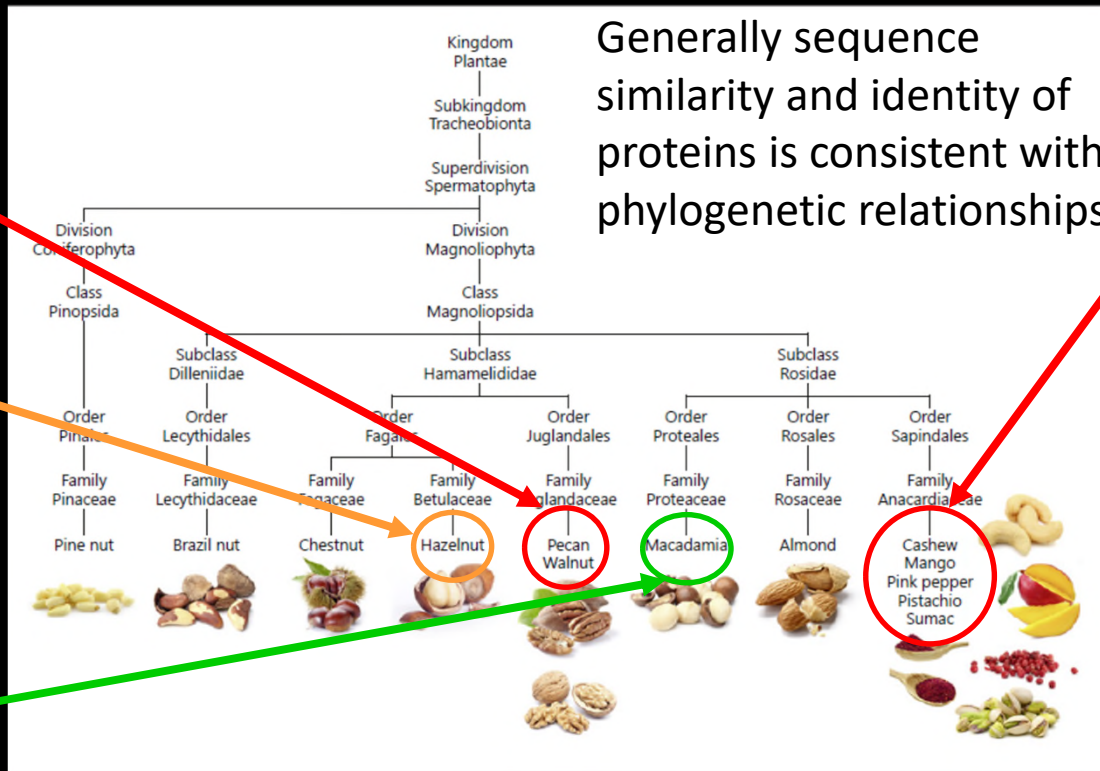
- The multi-centre Pronuts study showed 60.7% of children had more than one nut or seed allergy
- It shows similar patterns to other studies relating cashew-pistachio and walnut-pecan allergies
- Hazelnut allergy was also correlated with walnut and pecan allergy as was macadamia although to a lesser extent



The frequency of an index allergy was a function prevalence and consumption

Phylogenetic relationships between tree nut species help explain clinical reactivity – and maybe identify new allergens...

Generally sequence similarity and identity of proteins is consistent with phylogenetic relationships.



- Cashew, and pistachio are closely related again explaining cross-reactivity

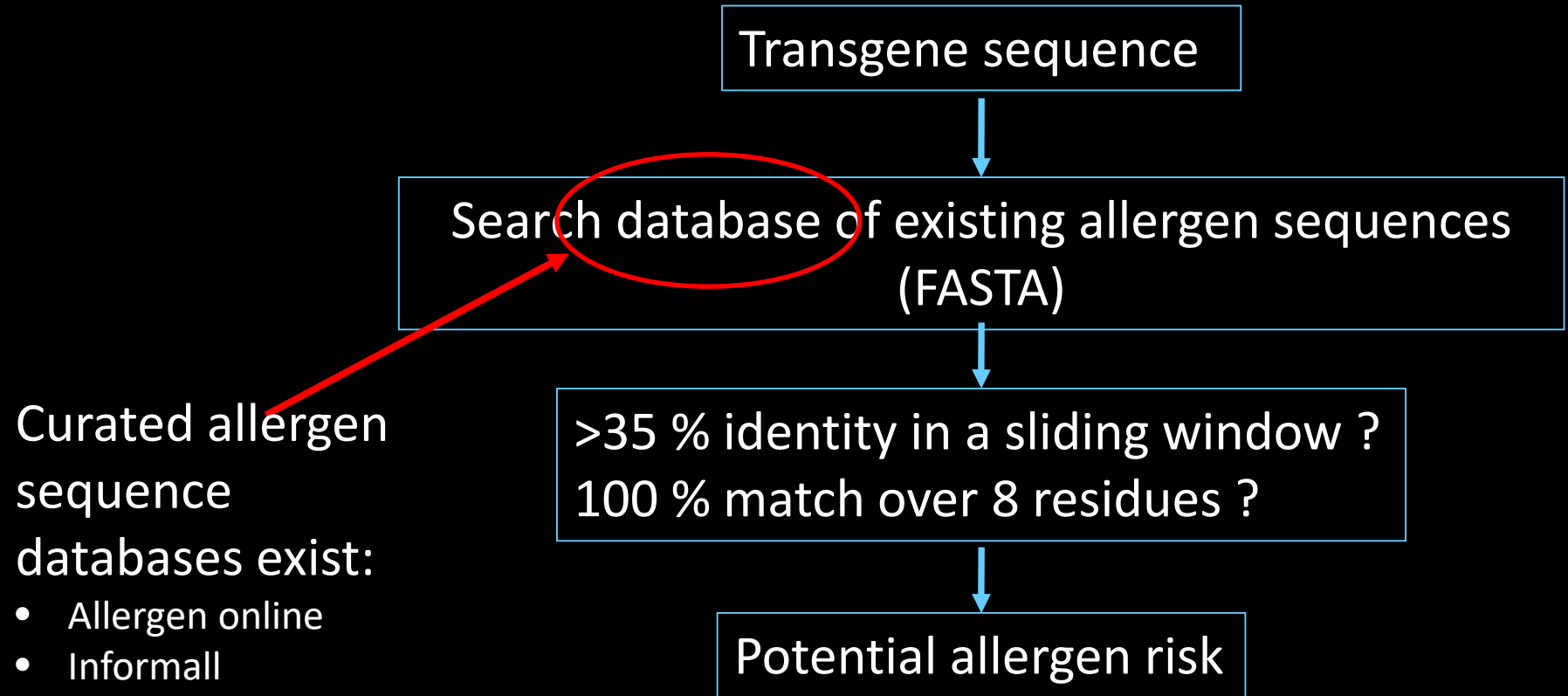
- Are pink peppercorns and the spice sumac issues for the future?

- Pecan and walnut are closely related explaining their clinical co-reactivity

- Hazelnut is more distantly related explaining its lower reactivity

- Macadamia is more closely related botanically to cashew and pistachio

Assessing risks for cross-reactive allergies – informatics assessment



Curated allergen sequence databases exist:

- Allergen online
- Informall
- SDAP
- Others?

Most databases are in academic organisations and their future is uncertain

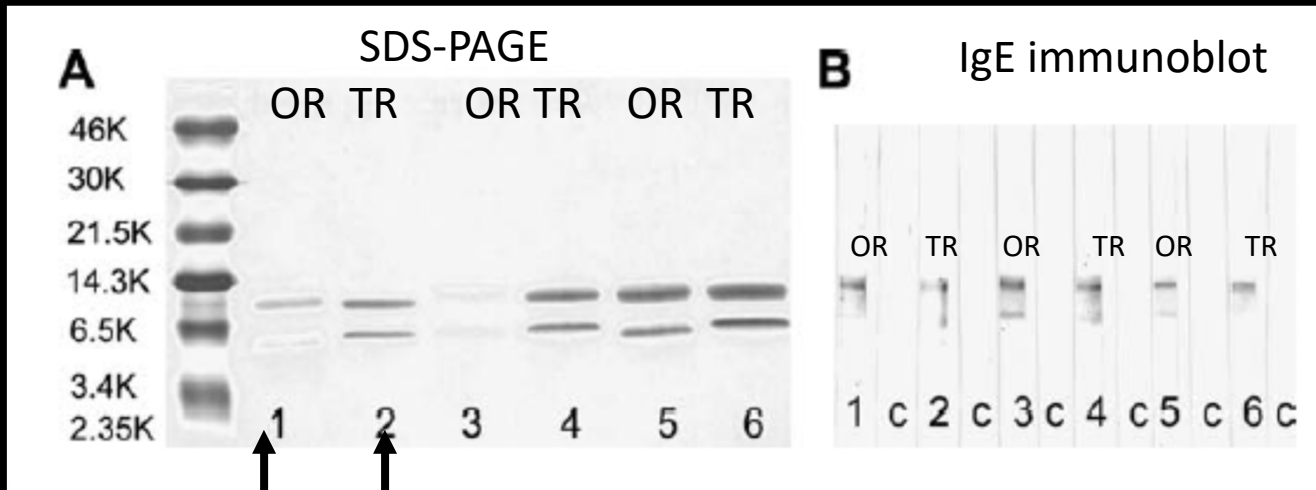
Proteins from plant sources

Protein powder from oil seed rape (*Brassica rapa* L. and *Brassica napus* L.)



- Also originally sold as animal feed
- Hazards such as erucic acid and glucosinolates were addressed by
 - Using cultivars naturally low in these compounds
 - Developing a production process that further reduced these compounds and anti-nutritional factors such as phytates.

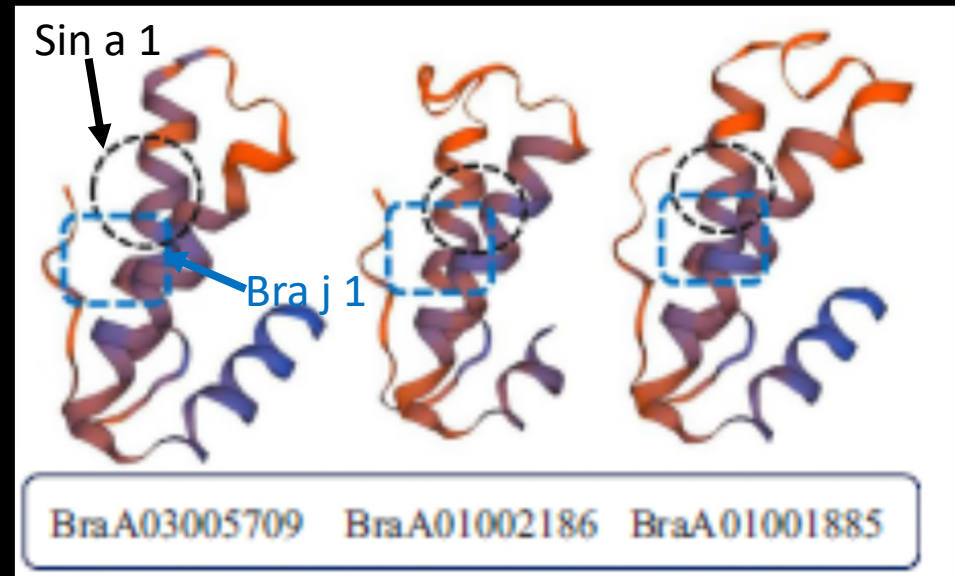
Allergenicity of oilseed rape/canola isolate



Napin, the 2S albumin of oilseed rape has been shown to be an allergen

Crude extract
Oilseed rape
Turnip rape
Purified fractions

Molecular modelling shows the presence of common epitopes with mustard allergens Sin a 1 and Bra j 2



Allergenicity of oilseed rape/canola isolate

- EFSA Panel assessment “*..the risk of sensitisation to rapeseed cannot be excluded and that it is likely that rapeseed trigger can allergic reactions in mustard allergic subjects.*”
- FSANZ Assessment “*An allergy assessment concluded that rapeseed protein isolate has the potential to induce allergic responses in individuals who are allergic to mustard.*”

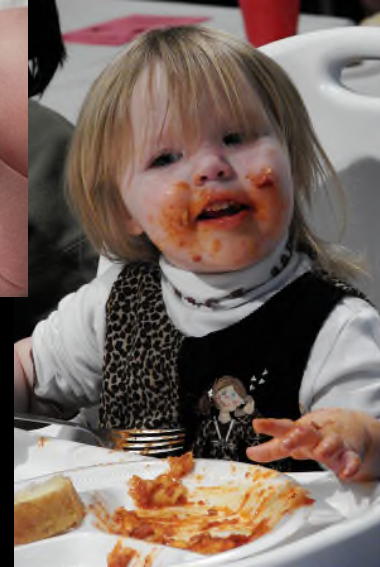
However, generally considered safe to consume

FACET 3: De novo sensitisation

Acquiring and maintaining tolerance

Environmental agents

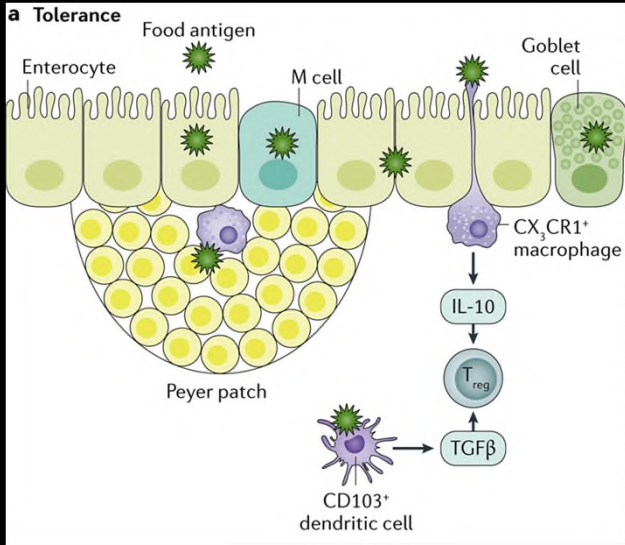
Microbes



Dietary proteins and constituents

- Starts in utero with the first 1000 days of life important for programming and health outcomes later in life
- Maintaining tolerance is a life-long process

Allergic disease – a function of the host and not simply the allergen



Tolerance

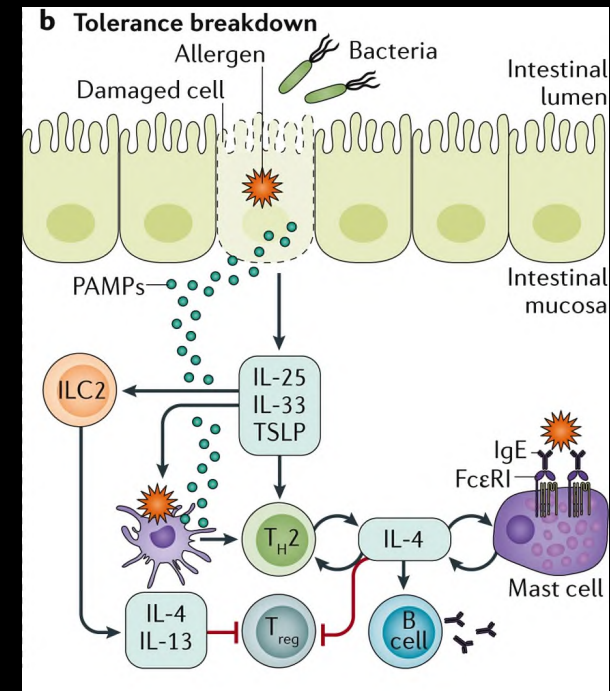
- Treg induction

Sensitisation

- Pro-allergic T_H2 effector cells

Drivers to Sensitisation

- Microbial products (PAMPs)
- Injury to the gut epithelial barrier
- Allergen exposure to e.g. the skin



Understanding presentation to the gut immune system

In vitro protein digestibility assays provide useful information on the form in which proteins maybe presented to the gut immune system

- **Simulated Gastroduodenal Digestion** provides information relevant to understanding the context of how a protein is presented to the immune system in a physiologically relevant context

- **The Pepsin Resistance Test** is a distinctly different biochemical test which provides complimentary information on the biochemical stability of a protein which may be predictive of allergenic potential

...and linking that to assessing sensitisation capacity

- Animal models are currently the only way to understand capacity new proteins to sensitise
- They can reproduce many aspects of sensitisation such as Th2 immune responses, IgE production and mast cell activation...
- BUT they are imperfect and cannot completely recreate clinical food allergy found in humans and are unable to predict allergenic potential

There is a need to develop a reference set of proteins of different allergenic potential to support development of improved predictive models for risk assessment.

Predicting sensitisation? Chia seeds as an example

- Does it contain protein? **Yes**
- How large is it? **Full complement of seed proteins**
- Is it from a food on Annex 2? **No**
- What is the taxonomy? **Not related to a known allergen or allergen family**

Reports of Chia seeds as allergens – since being allowed on the market

- Patient ate chia every day in yoghurt and developed eczema
- Multiple sensitisation to inhalants and foods including a range of tree nuts and legumes
- Likely sensitised to storage proteins especially 2S albumins

TOMAS-PEREZ, et al 2018. *J Investig Allergol Clin Immunol*, 28, 46-47

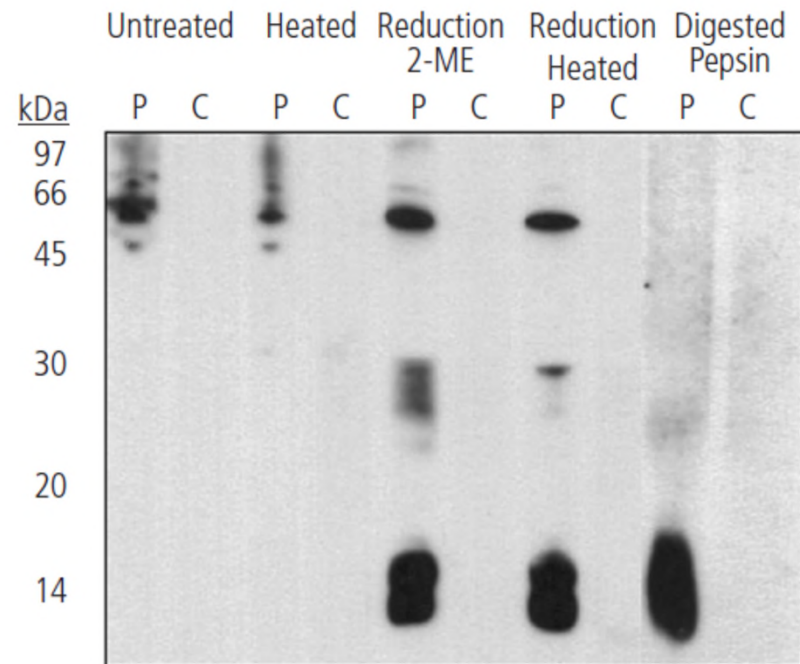
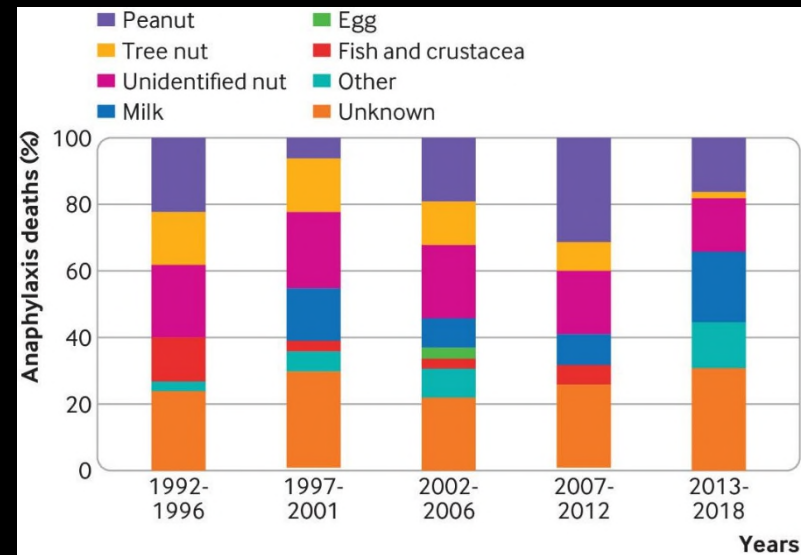


Figure. SDS-PAGE and IgE-immunoblotting performed with an extract of crushed and defatted chia seeds that underwent heating at 100°C for 5 minutes, reduction with 5% 2-mercaptoethanol at 100°C for 5 minutes, and digestion with pepsin at 37°C for 30 minutes. Lane P, patient's serum; lane C, pool of sera from nonatopic controls.

Predicting sensitisation and Post-market monitoring

- We urgently need better models, including cell models, that can predict sensitisation potential
- There is a need for a better way of monitoring food allergies after introducing new foods onto the market to identify and manage any emerging allergens
- Labelling for consumers needs to address this to enable that monitoring and a joined up approach with patient groups and the clinical community.



Alessia Baseggio Conrado et al. BMJ 2021;372:bmj.n251

Changes to priority allergens – will it change allergenicity assessment?



Food and Agriculture
Organization of the
United Nations



World Health
Organization

Ad hoc Joint FAO/WHO Expert Consultation on Risk Assessment of Food Allergens
Part 1: Review and validation of Codex priority allergen list through risk assessment

Virtual meeting: 30 November – 11 December 2020. 28 January 2021. 8 February 2021

nuts (almond, cashew, hazelnut, pecan, pistachio and walnut). Due to the lack of data on prevalence, severity and/or potency, or due to regional consumption of some foods, the Committee recommended that some of the allergens, such as buckwheat, celery, lupin, mustard, oats, soybean and tree nuts (Brazil nut, macadamia, pine nuts), should not be listed as global priority allergens but may be considered for inclusion on priority allergen lists in individual countries. Since current dietary trends include an increased

CODEX global priority list of allergens no longer includes soybean, mustard and Brazil nut.....how will this affect allergenicity risk assessment of GMOs and Novel foods?

**Thank you for listening – any
questions?**