19 June 2019

Adjuvanticity/immunogenicity allergenicity assessment of proteins

Cry proteins as a case study

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Trusted science for safe food

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Immune adverse reactions to proteins

GUIDELINES FOR PREDICTION

Codex Alimentarius 2003-2009

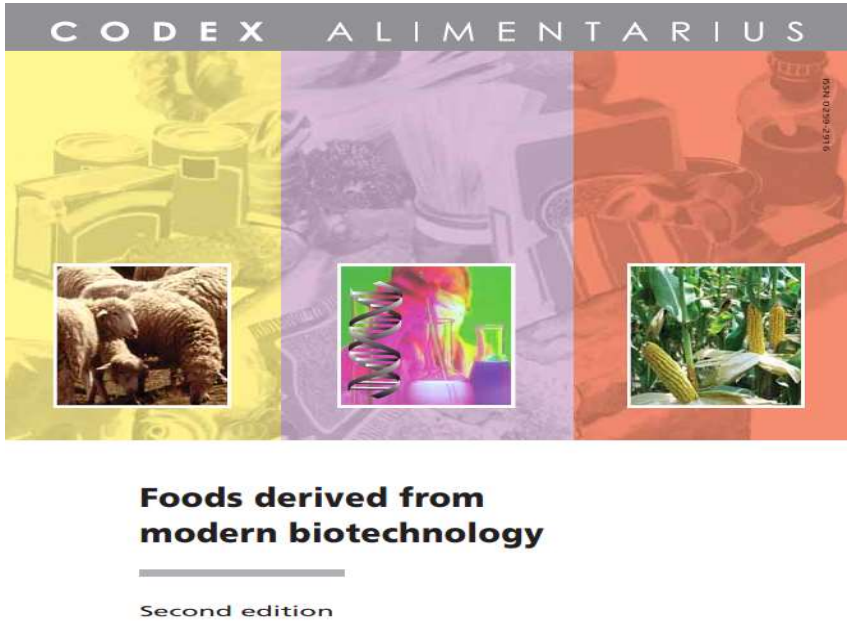
GMO Panel	- 2010
	- 2011
	- 2017
CEF Panel	- 2009
FEEDAP Panel	- 2008
	- 2017
NDA Panel	- 2016

Immune adverse reactions to proteins

What makes a protein an allergen?

- The assessment should consider likelihood for:
  - *de novo* sensitisation
  - elicitation of a reaction
- Different sources of information to be taken into account
- Weight of evidence (WoE)

Allergenicity assessment – Weight of evidence



- **The information in the WoE includes**:
  - Source of the protein
  - Amino acid sequence comparison
  - *In vitro* degradation studies
  - Specific serum screening
  - Cell based / *in vivo* assays

On a case-by-case basis

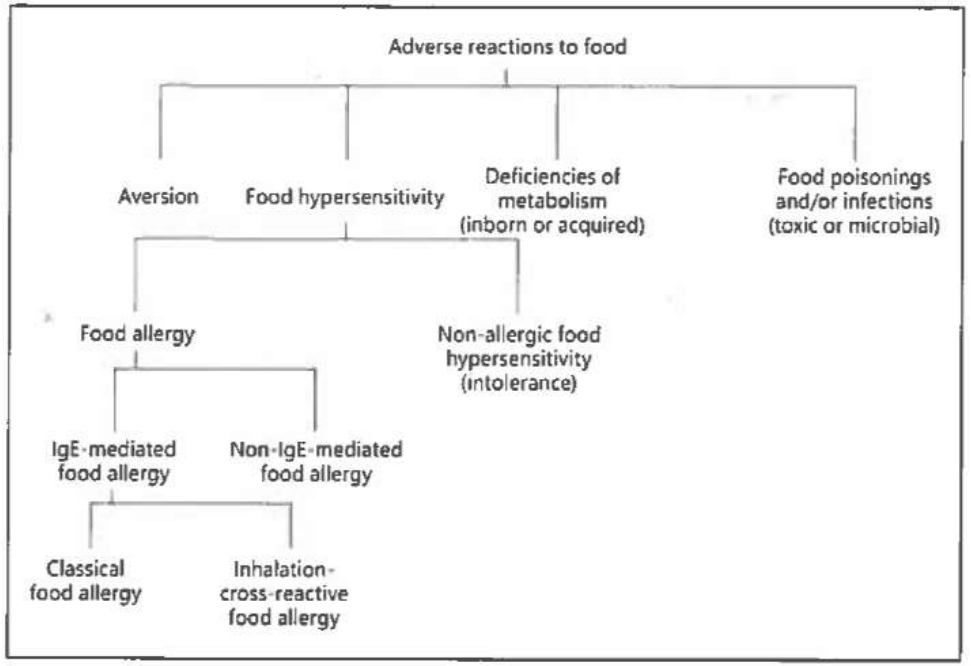
Innovative considerations – Weight of evidence

- **EFSA GD 2011 and Regulation No 503/2013**:

  - Adjuvanticity is first time introduced in RA documents

- **EFSA GD 2017**

  - Non-IgE-mediated adverse immune reactions



Poulsen K.L. 2015. Chem immunol Allergy. Basel Karger, 2015, vol101, pp 59-67

Cry proteins assessed by EFSA

Bacillus thuringiensis Cry1Ac Protoxin is a Potent Systemic and Mucosal Adjuvant

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(Received 18 August 1998; Accepted in revised form 15 December 1998)

Each experimental group contained five animals to which three antigen doses were applied on days 1, 7 and 14 either by the IP or IG route. Mice were sacrificed 7 days after the last immunization. The immunogens administered to determine the effect of Cry1Ac and CT on the immune response to HBsAg were: (1) 10 μg HbsAg; (2) 10 μg HBsAg plus 10 μg CT; (3) 10 μg HBsAg plus 10 μg Cry1Ac; and (4) 10 μg HBsAg plus 100 μg Cry1Ac. To determine the effect of Cry1Ac and CT on the

Cry proteins assessed by EFSA

IgG anti-BSA response of the small intestine significantly. We conclude that Cry1Ac is a mucosal and systemic adjuvant as potent as CT which enhances mostly serum and intestinal IgG antibody responses, especially at the large intestine, and its effects depend on the route and antigen used. These features make Cry1Ac of potential use as carrier and/or adjuvant in mucosal and parenteral vaccines.

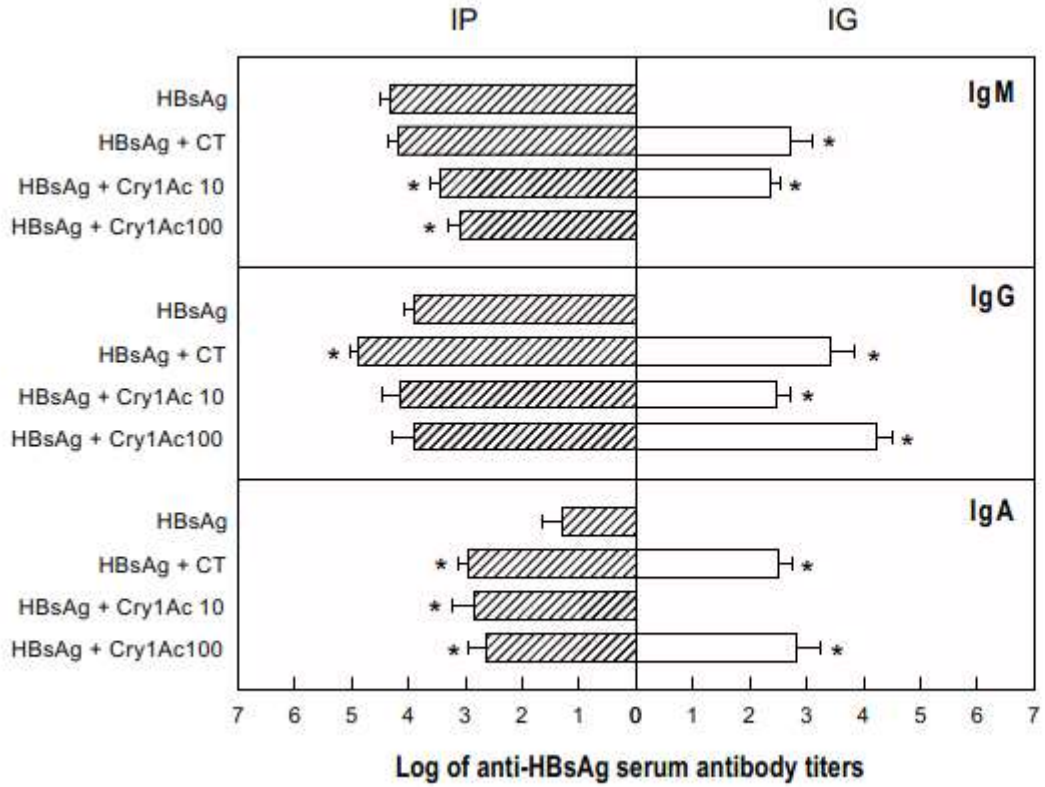

Fig. 1. Anti-HBsAg serum antibody titres. Mice were immunized via IP or IG with 10 μg HBsAg alone or co-administered with CT or Cry1Ac at doses of 10 μg (Cry1Ac 10) or 100 μg (Cry1Ac 100). The anti-HBsAg IgA, IgG and IgM serum antibody titres are expressed as the logarithm of their end-point dilution. Asterisks indicate significant differences ($P<0.05$) with the group of mice immunized with antigen alone.

Cry proteins assessed by EFSA

Intranasal Cry1Ac Protoxin is an Effective Mucosal and Systemic Carrier and Adjuvant of *Streptococcus pneumoniae* Polysaccharides in Mice

L. Moreno-Fierros,*† E. J. Ruiz-Medina,* R. Esquivel,* R. López-Revilla†‡ & S. Piña-Cruz†

Cry1Ac coadministered or conjugated to CPS6B: The immunogens intranasally administered to determine the effect of Cry1Ac coadministered or conjugated to CPS6B were: (i) 23.5 µg of capsular PS serotype 6B (CPS6B) alone; (ii) 23.5 µg CPS6B plus 10 µg of Cry1Ac; (iii) 23.5 µg of CPS6B plus 10 µg CT; and (iv) Cry1Ac–CPS6B conjugate (the amounts of each component in the conjugate were similar to those coadministered). Control mice received the vehicle (PBS) alone. We also immunized by intraperitoneal route, groups similar to (i), (ii) and (iv).

Cry proteins assessed by EFSA

Comparative study of the adjuvanticity of *Bacillus thuringiensis* Cry1Ab protein and cholera toxin on allergic sensitisation and elicitation to peanut

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(Received 21 August 2008; final version received 22 September 2008)

animal care and with permission 91–122 of the French Veterinary Services. Balb/c mice were intra-gastrically administered 200 µl of PE alone (0.5 mg/g of mouse b.w.) or mixed with either CT or Cry1Ab (0.6 µg/g of mouse b.w., i.e. ca. 10 µg of Cry 1Ab or CT /mouse) at days 1, 7, 13, 19 and 25 ($n = 16$ per group). Control mice ($n = 9$) received 200 µl of phosphate buffer saline (PBS).

In conclusion, Cry1Ab did not demonstrate adjuvant effects on oral sensitisation to peanut when compared to the effects of CT in similar conditions. However, this study

Cry proteins assessed by EFSA

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No Adjuvant Effect of *Bacillus thuringiensis*-Maize on Allergic Responses in Mice

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Abstract

Genetically modified (GM) foods are evaluated carefully for their ability to induce allergic disease. However, few studies have tested the capacity of a GM food to act as an adjuvant, i.e. influencing allergic responses to other unrelated allergens at acute onset and in individuals with pre-existing allergy. We sought to evaluate the effect of short-term feeding of GM *Bacillus thuringiensis* (*Bt*)-maize (MON810) on the initiation and relapse of allergic asthma in mice. BALB/c mice were provided a diet containing 33% GM or non-GM maize for up to 34 days either before ovalbumin (OVA)-induced experimental allergic asthma or disease relapse in mice with pre-existing allergy. We observed that GM-maize feeding did not affect OVA-induced eosinophilic airway and lung inflammation, mucus hypersecretion or OVA-specific antibody production at initiation or relapse of allergic asthma. There was no adjuvant effect upon GM-maize consumption on the onset or severity of allergic responses in a mouse model of allergic asthma.

Cry proteins assessed by EFSA&VKM

EFSA/GMO/472
Parma, 13 November 2009

BILATERAL TECHNICAL MEETING BETWEEN MEMBERS OF THE EFSA PANEL ON GENETICALLY MODIFIED ORGANISMS AND THE VKM NORWEGIAN DELEGATION

According to Article 30.2 of the Regulation (EC) No 178/2002

ADJUVANTICITY OF CRY PROTEINS

epithelial intestinal cells was shown to be not specific and did not induce damage. It was agreed that an adjuvant effect of Cry proteins has indeed been demonstrated in animals; however, the studies were performed using relatively high doses and routes of administration that are different from those occurring during intake of Bt maize by human consumers. Moreover, the adjuvant effect of Cry1Ac enhanced the immune response to co-administered proteins but was not shown to induce an allergic reaction or an IgE response. A recent publication by Guimaraes *et al.* (2008)[1] was presented in which it is shown that the mechanisms involved in the adjuvanticity of Cry proteins and cholera toxin are different.

VKM report on Cry proteins

Summary of the health risk assessment of the adjuvant effects of Cry proteins from genetically modified plants used in food and fodder

Opinion of the Panel on Genetically Modified Organism of the Norwegian Scientific Committee for Food Safety

Only two of the 10 Cry proteins that are currently used in genetically modified plants, Cry1Ab and Cry1Ac, have been studied experimentally regarding adjuvant effects. Therefore, this risk assessment is based upon immunological observations from these studies. To the knowledge of the Panel, adjuvant effects have not been investigated for the other eight Cry proteins used in GM plants, or for other groups of Cry proteins.

VKM report on Cry proteins

It is important to emphasise that only a limited number of publications from a few research groups are dealing with the adjuvant effects of these two Cry proteins.

There are many knowledge gaps related to assessment of adjuvants. Most of the immunologic adjuvant experiments have been performed using Cry1Ac. Whether the other Cry proteins have similar adjuvant properties is unknown.

One element of uncertainty in exposure assessment is the lack of knowledge concerning exposure via the respiratory tract and the skin, and also the lack of quantitative understanding of the relationship between the extent of exposure to an adjuvant and its effects in terms of development of allergies.

Despite several uncertainties, the Panel concludes on the basis of current knowledge that it is very unlikely that the Cry proteins in food pose an increased health risk in the amounts that would be ingested by eating processed GM maize or soya, compared with eating food based on isogenic non-modified plants.

Cry proteins assessed by EFSA

Immunological and Metabolomic Impacts of Administration of Cry1Ab Protein and MON 810 Maize in Mouse

Karine Adel-Patient[1]*, Valeria D. Guimaraes[1], Alain Paris[2], Marie-Françoise Drumare[1], Sandrine Ah-Leung[1], Patricia Lamourette[3], Marie-Claire Nevers[3], Cécile Canlet[4], Jérôme Molina[4], Hervé Bernard[1], Christophe Créminon[3], Jean-Michel Wal[1]

metabolic information. Our results confirm the immunogenicity of purified Cry1Ab without evidence of allergenic potential. Immunological and metabolomic studies revealed slight differences in mouse metabolic profiles after i.g. administration of MON810 *vs* its non-GM counterpart, but no significant unintended effect of the genetic modification on immune responses was seen.

Cry proteins assessed by EFSA

European Food Safety Authority

Investigations of immunogenic, allergenic and adjuvant properties of Cry1Ab protein after intragastric exposure in a food allergy model in mice

Monica Andreassen[1,2,6*], Thomas Bøhn[1,3], Odd-Gunnar Wikmark[1,4], Johanna Bodin[2], Terje Traavik[1,3], Martinus Løvik[5] and Unni Cecilie Nygaard[2]

Results: In contrast to results from previous airway investigations, we observed no indication of immunogenic properties of trypCry1Ab protein after repeated intragastric exposures to one dose, with or without CT as adjuvant. Moreover, the results indicated that trypCry1Ab given by the intragastric route was not able to promote allergic responses or anaphylactic reactions against the co-administered allergen lupin at the given dose.

Conclusion: The study suggests no immunogenic, allergenic or adjuvant capacity of the given dose of trypCry1Ab protein after intragastric exposure of prime aged mice.

Cry proteins assessed by EFSA

Study of the allergenic potential of *Bacillus thuringiensis* Cry1Ac toxin following intra-gastric administration in a murine model of food-allergy

Karla I. Santos-Vigil, Damaris Ilhuicatzi-Alvarado, Ana L. García-Hernández, Juan S. Herrera-García, Leticia Moreno-Fierros*

this has been associated with food allergy and intestinal inflammation. Although the adjuvant and allergenic potential of CT were higher than the effects of Cry1Ac, the results show that applied intra-gastrically at 50 µg doses, Cry1Ac is immunogenic, moderately allergenic and able to provoke intestinal lymphoid hyperplasia. Moreover, Cry1Ac is also able to induce anaphylaxis, since when mice were intragastrically sensitized with increasing doses of Cry1Ac, with every dose tested, a significant drop in rectal temperature was recorded after intravenous challenge.

EFSA's technical report on Cry1Ac

- GM plants_ Cry1Ac assessed by EFSA (6 cotton and 3 soybean)

- Safety assessment in line with Codex Alimentarius and relevant EFSA GD and European Regulation

- Weight-of-evidence approach followed

- Cry proteins effects on the immune system (mainly Cry1Ac and Cry1Ab)

- EU-funded projects MARLON and GRACE, raising the need to develop validated and standardized models for allergenicity assessment (humans and animals)

EFSA's technical report on Cry1Ac

No new elements that would lead the EFSA GMO Panel to reconsider the outcome of its previous opinions

- Comparison of two proteins (OVA and Cry1Ac) at different doses without appropriate control(s) → limited relevance in RA

- Unclear if findings are linked to Cry1Ac only or if other proteins would behave similarly under conditions tested

Contrasting evidence on Cry1Ac and Cry1Ab from literature

- Hypothesis: differences in amino acid sequences, doses, routes of administration, animal models, experimental protocols, matrices

- To be understood: if there is a dose-response relationship, item to test, *in vivo/in vitro* model

Additional background information

EXTERNAL SCIENTIFIC REPORT

Literature review in support of adjuvanticity/immunogenicity assessment of proteins

Marco Daniele Parenti, Aurelia Santoro, Alberto Del Rio, Claudio Franceschi

Innovamol Srl, Modena, Italy; University of Bologna, Bologna, Italy

emerged that: i) a clear classification of adjuvant and immunogens of proteins cannot be done; ii) structural features able to modulate adjuvanticity and immunogenicity are mainly ascribed to therapeutic proteins and in the context of allergenicity and cross-reactivity; iii) factors affecting the propensity of a protein to stimulate immune response are aggregation, thermal processing, digestion, food matrix, among others; iv) different proteins are described to have immunomodulatory effects; v) risk assessment of adjuvant and immunogenic behaviour of proteins requires specific methodologies that can be adapted from other fields; vi) adjuvanticity and immunogenicity of Cry proteins in certain experimental conditions seems plausible but due to low dosage, oral route of administration, food and feed processing and digestion, it is unlikely to emerge as a safety issue in food and feed; vii) eliciting an immune response is a very complex matter as the body responds to immune offence by inducing many processes. Based on these considerations, it is expected that the availability of new humanized animal models and the possibility to deploy artificial intelligent systems on the vastity of human data

Future perspectives in the area

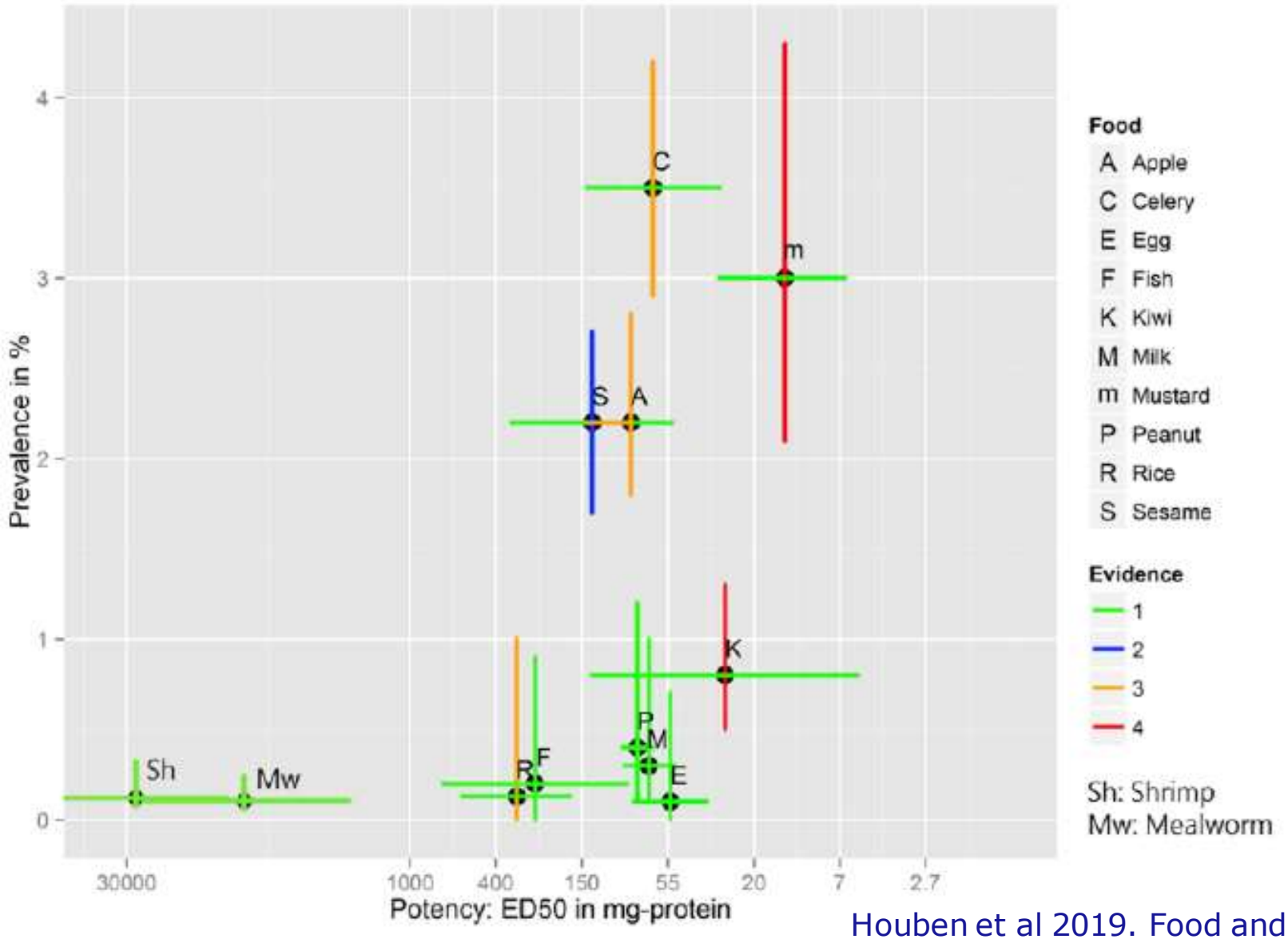
- Sounder studies for testing adjuvant and allergenic potential of proteins are desirable → EU funded projects largely contributing

- Future studies should consider limitations of current models, using relevant routes and methods of administration, doses, appropriate control proteins, realistic exposure regimes (effects of processing and the matrices)

- Strategy for ranking the allergic potential of known proteins as a way forward (FAO/WHO, 2001; EFSA GMO Panel, 2017; Remington et al 2018)

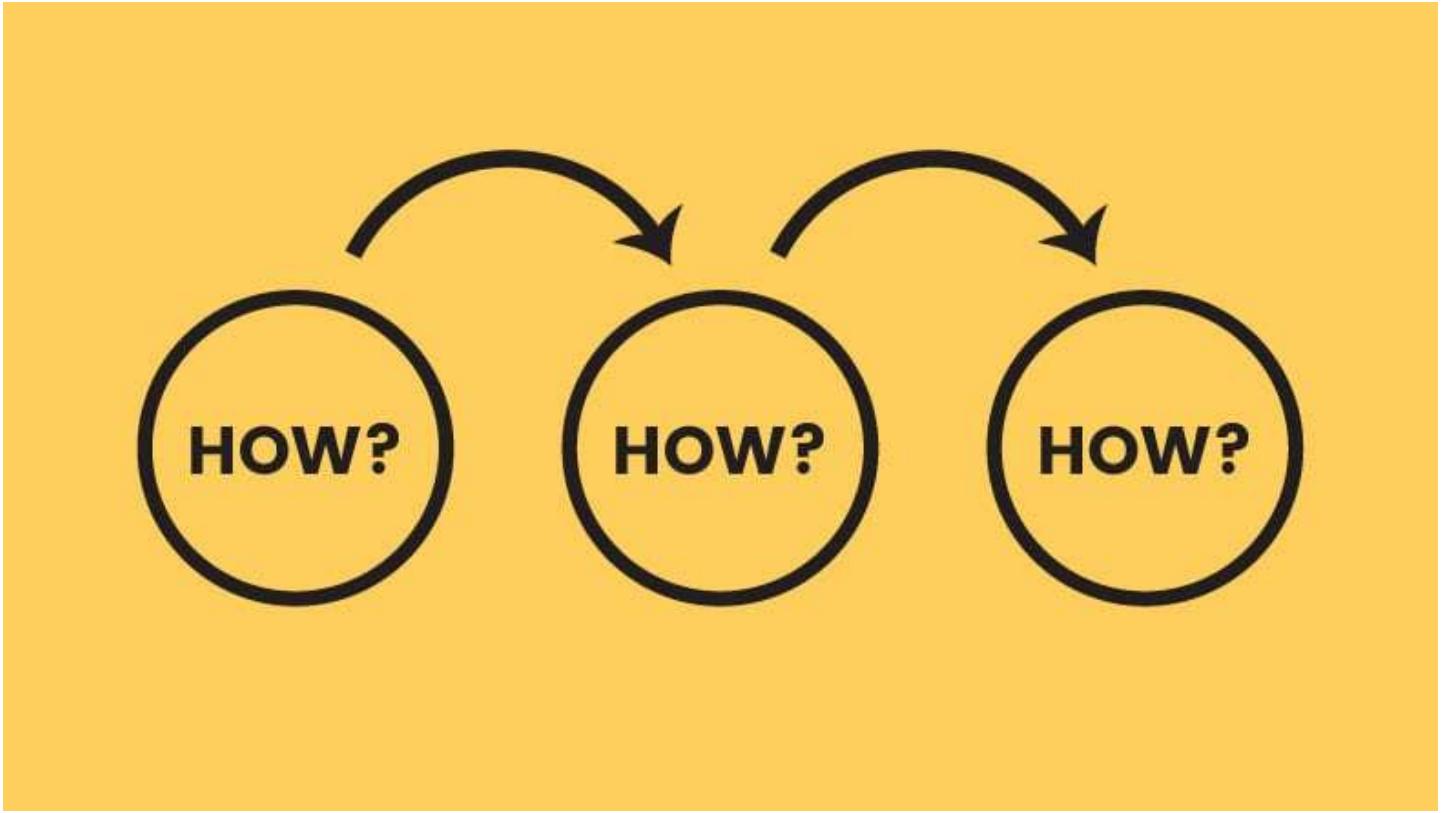
Fostering interaction/cooperation with Member States needed

Scaling allergy risks of foods relatively



Houben et al 2019. Food and chemical toxicology, 127, pp 61-69

Future perspectives in the area



Any views? Ideas?

Thank you very much

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