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Celiac disease assessment

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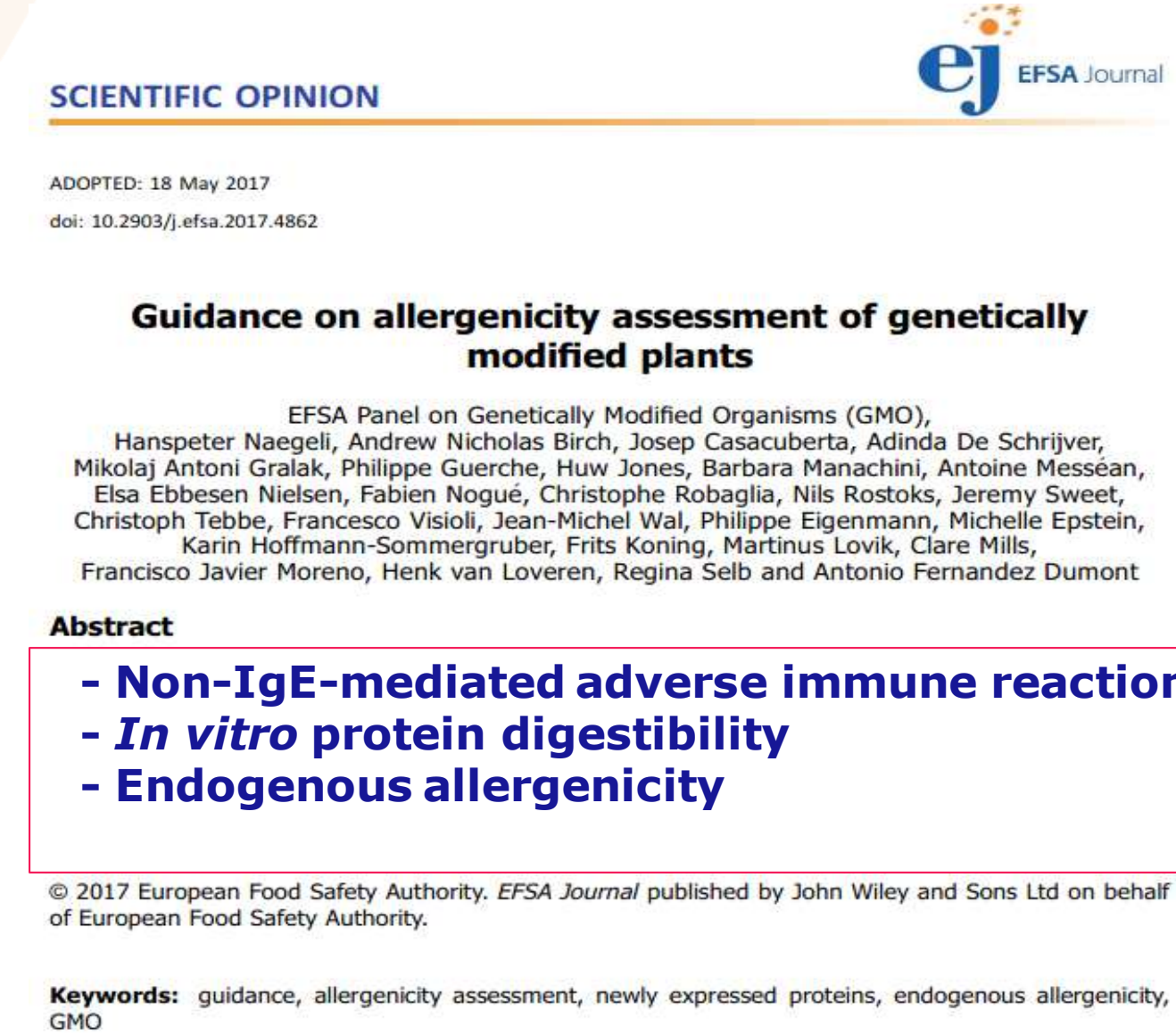
Allergenicity/immunogenicity assessment



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 18 May 2017

doi: 10.2903/j.efsa.2017.4862

Guidance on allergenicity assessment of genetically modified plants

EFSA Panel on Genetically Modified Organisms (GMO),
Hanspeter Naegeli, Andrew Nicholas Birch, Josep Casacuberta, Adinda De Schrijver, Mikolaj Antoni Gralak, Philippe Guerche, Huw Jones, Barbara Manachini, Antoine Messéan, Elsa Ebbesen Nielsen, Fabien Nogué, Christophe Robaglia, Nils Rostoks, Jeremy Sweet, Christoph Tebbe, Francesco Visioli, Jean-Michel Wal, Philippe Eigenmann, Michelle Epstein, Karin Hoffmann-Sommergruber, Frits Koning, Martinus Lovik, Clare Mills, Francisco Javier Moreno, Henk van Loveren, Regina Selb and Antonio Fernandez Dumont

Abstract

- Non-IgE-mediated adverse immune reactions
- *In vitro* protein digestibility
- Endogenous allergenicity

Keywords: guidance, allergenicity assessment, newly expressed proteins, endogenous allergenicity, GMO

- **Why?**
  - To consider new developments
  - To address MS/NGOs/EP/applicants comments
  - To assist implementation of regulatory requirements
- **Background information**
  - Literature reviews from external contractor and internally performed

Focus group involvement

Immune-mediated adverse reactions

IgE-mediated	Immediate hypersensitivity reactions
Combined IgE- and non-IgE-mediated	Eosinophilic oesophagitis Eosinophilic gastroenteritis Eosinophilic gastroenterocolitis
Non-IgE-mediated	Food enteropathy Celiac disease Food protein induced enterocolitis Heiner syndrome

Adapted from: The University of Manchester, 2013. Literature review: EFSA supporting publication 2013:EN-527, 40 pp

Immune-mediated adverse reactions

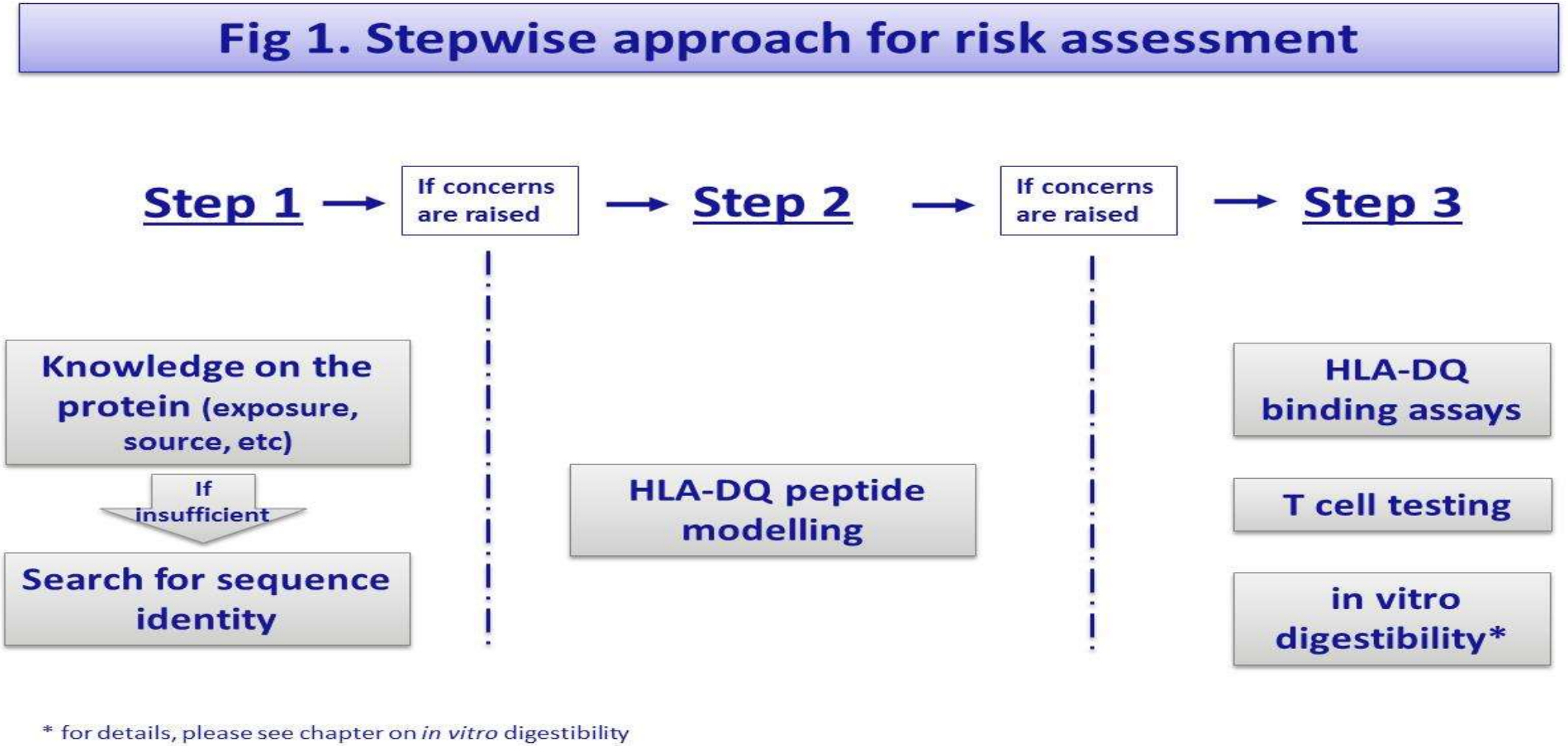
IgE-mediated — Immediate hypersensitivity reactions

Combined IgE- and non-IgE-mediated

Non-IgE-mediated

Celiac disease
Clear cause-effect relationship

RA of proteins: celiac disease



* for details, please see chapter on *in vitro* digestibility

Celiac disease – Applicant's safety assessment

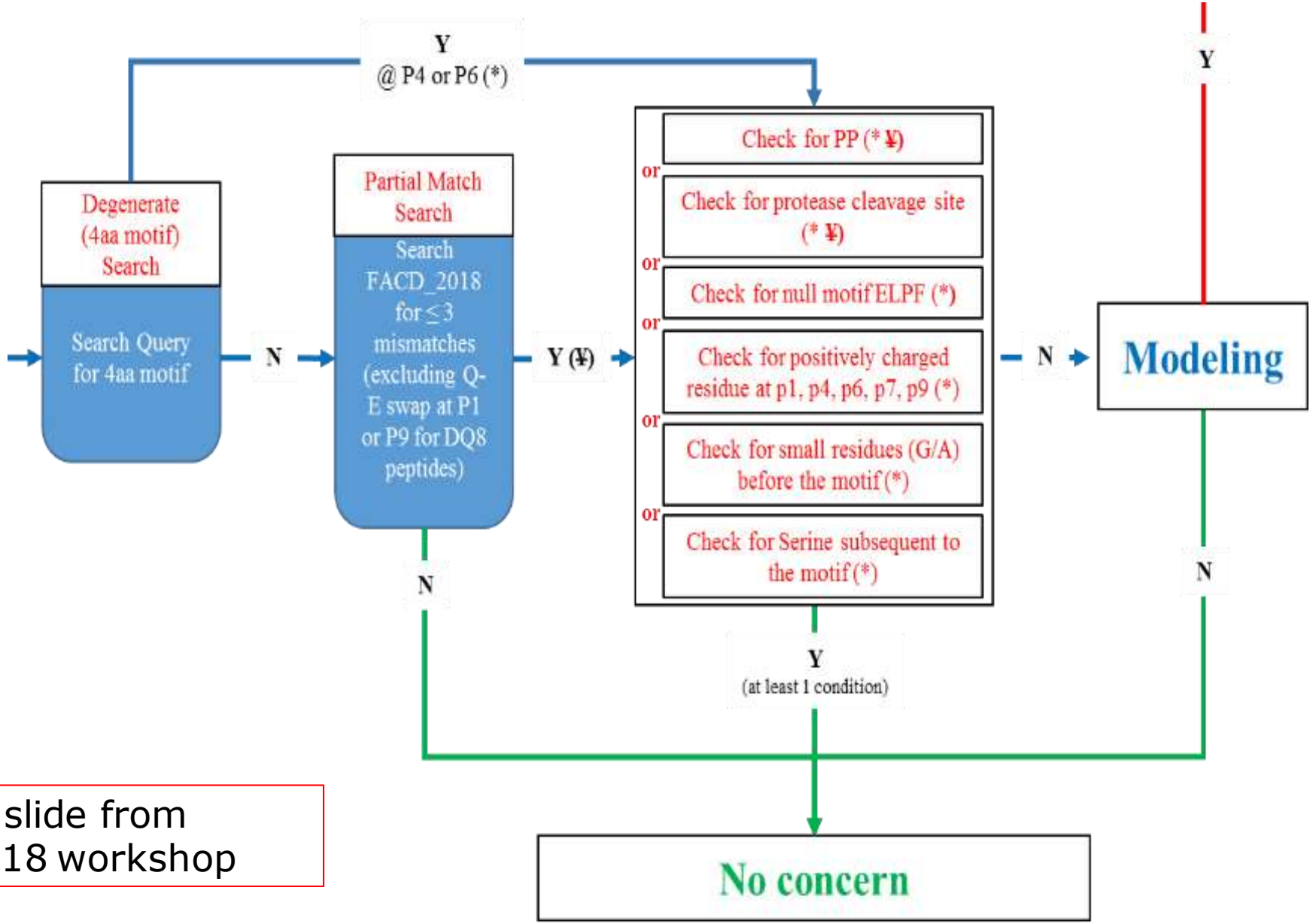
Main aspects to highlight

- Real harmonisation between applicants
- Overall approach proposed by applicants in line with EFSA 2017 guidance document
- Stepwise approach:
  - Knowledge on the source and the protein
  - Searches { with perfect match / with partial match } { with motif / without motif }
  - HLA-DQ modelling
  - *In vitro* testing

Aspects requiring additional discussion

- **Possible assessment criteria**
  - EFSA described few assessment criteria
  - Additional assessment criteria needed?
  - Tool should be practical keeping the case-by-case philosophy
- **Development of a publicly available database**
  - Few databases used/inclusion criteria
- **HLA-DQ-peptide modelling**
  - EFSA procurement
- ***In vitro* testing**

Applicant's proposal – Assessment criteria



Applicant's slide from October 2018 workshop

Applicant's proposal – Database

CD Peptide Sequences

DQ2 restricted epitopes

Epitope	Motif	Reference
DQ2.5-glia-α1a	PFPQPQLPY	Arentz-Hansen et al. (2000)
DQ2.5-glia-α1b	PYPQPQLPY	Arentz-Hansen et al. (2002)
DQ2.5-glia-α2	PQPQLPYPQ	Arentz-Hansen et al. (2000)
DQ2.5-glia-α3	FRPQQPYPQ	Vader et al. (2002b)
DQ2.5-glia-γ1	PQQSFPQQQ	Sjöström et al. (1998)
DQ2.5-glia-γ2	IQPQQPAQL	Qiao et al. (2005), Vader et al. (2002b)
DQ2.5-glia-γ3	QQPQQPYPQ	Arentz-Hansen et al. (2002)
DQ2.5-glia-γ4a	SQPQQQFPQ	Arentz-Hansen et al. (2002)
DQ2.5-glia-γ4b	PQPQQQFPQ	Qiao et al. (2005)
DQ2.5-glia-γ4c	QQPQQPFPQ	Arentz-Hansen et al. (2002)
DQ2.5-glia-γ4d	PQPQQPFCQ	Qiao (unpublished)
DQ2.5-glia-γ5	QQPFPQQPQ	Arentz-Hansen et al. (2002)
DQ2.5-glia-ω1	PFPQPQQPF	Tye-Din et al. (2010)
DQ2.5-glia-ω2	PQPQQPFPW	Tye-Din et al. (2010)
DQ2.2-glut-L1	PFSQQQQPV	Vader et al. (2002b)
DQ2.5-glut-L2	FSQQQQSPF	Stepniak et al. (2005), Vader et al. (2002b)
DQ2.5-hor-1	PFPQPQQPF	Tye-Din et al. (2010), Vader et al. (2003)
DQ2.5-hor-2	PQPQQPFPQ	Vader et al. (2003)
DQ2.5-sec-1	PFPQPQQPF	Tye-Din et al. (2010), Vader et al. (2003)
DQ2.5-sec-2	PQPQQPFPQ	Vader et al. (2003)
DQ2.5-ave-1	PYPEQQEPF	Arentz-Hansen et al. (2004), Vader et al. (2003)
DQ2.5-ave-1b	PYPEQQQPF	Arentz-Hansen et al. (2004), Vader et al. (2003)

DQ8 restricted epitopes

Epitope	Motif	Reference
DQ8-glia-α1	QGSFQPSQQ	van de Wal et al. (1998b)
DQ8-glia-γ1a	QQPQQPFPQ	Tollefsen et al. (2006)
DQ8-glia-γ1b	QQPQQPYPQ	Tollefsen et al. (2006)
DQ8-glut-H1	QGYYPTSPQ	van de Wal et al. (1999)

The single letter code for amino acids is used. A characteristic Q-X1-P-X2 motif is present in the large majority of HLA-DQ2 epitopes (in bold). This sequence is a target sequence for TG2, which yields E-X1-P-X2. Due to the introduction of the negatively charged amino acid glutamate, the peptides become high affinity binders for HLA-DQ2. glia-α = α-gliadin; glia-γ = γ-gliadin; hor = hordein; sec = secalin; ave = avenin; glut-L = LMW-glutenin, glut-H = HMW-glutenin.

Table adapted from: EFSA 2017 and Sollid et al., 2012

1378 total peptides

// Guidance document

// Propepper

// Allergenonline

Exact 9 amino acid match, a likelihood of hazard

CD Peptides found in proteins from wheat, barley, rye and oats

Applicant's slide from October 2018 workshop

Database development

EFSA guidance statements

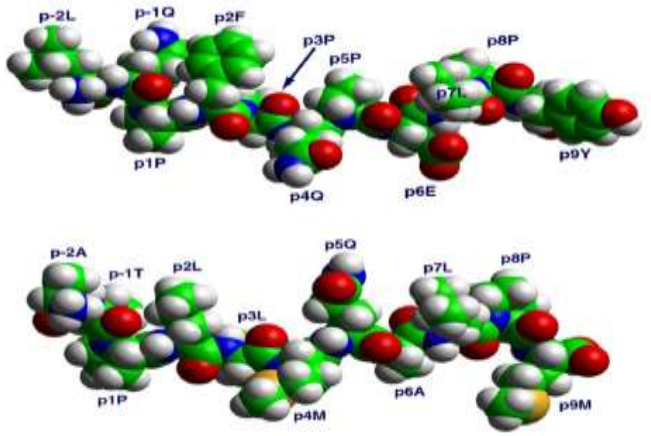
- Recognition of gluten peptides by CD4+ T cells from one or more CD patients has been a prerequisite for defining toxic CD peptides (Sollid et al., 2012)
- Several listings/databases of the known T-cell stimulatory sequences identified in gluten, hordeins, secalins and avenins are available including the https://propepper.net database and the http://www.allergenonline.org/database.
- An overview of the best characterised epitopes along with a unified nomenclature is presented in Sollid et al. (2012)
- Could these prerequisites be used as starting points to define what inclusion criteria should be applied for the purpose of building up a database?

Database development

EFSA GD 2017

Development of a comprehensive database that is publicly available, curated regularly and appropriately built and designed for risk assessment purposes is an important aspect to be further investigated

HLA-DQ peptide modelling



- EFSA exploring possibilities to develop a software tool for peptide modelling
- Applicability to safety assessments involving proteins – not only useful for GM plants
- Tool publicly available
- International cooperation and recognition needed

EFSA procurement

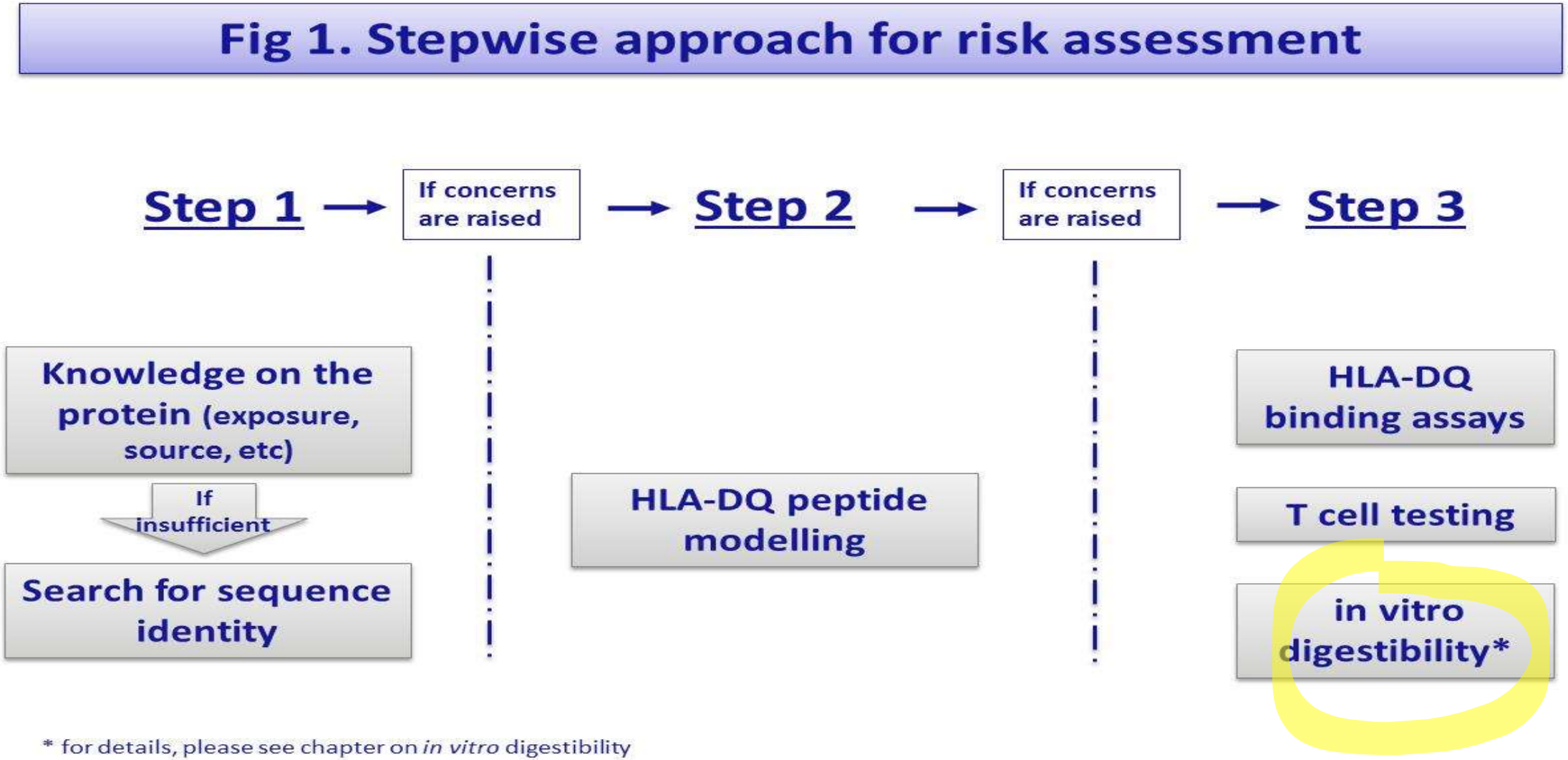
HLA-DQ peptide modelling

OC/EFSA/GMO/2019/01

HLA-DQ peptide modelling software – Developing a software tool for peptide modelling regarding its capacity to bind to HLA-DQ molecules and to activate T-cells

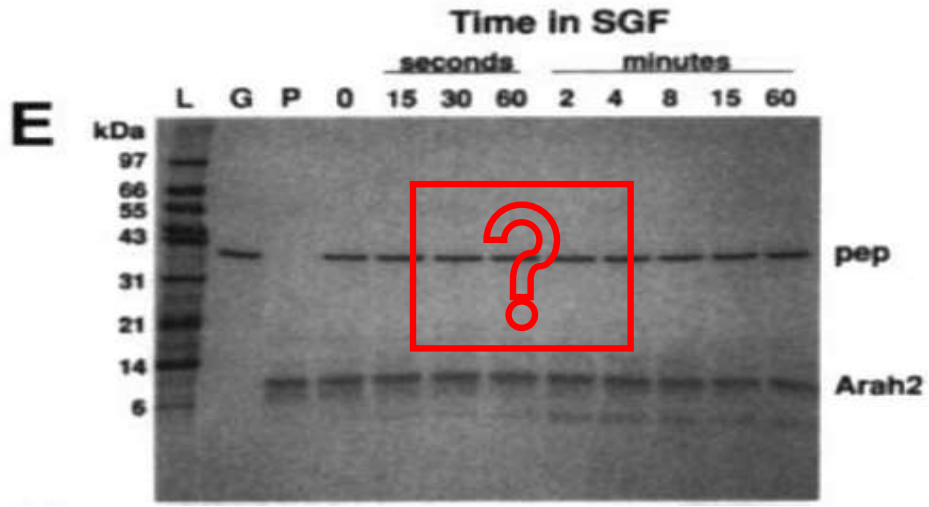
1. Collecting info on tools available for protein modelling related to celiac disease
2. Developing a software tool for predicting the capacity of a protein to cause celiac disease based on peptide binding to specific molecules and activation of immune cells
3. Testing and adjusting the software tool

RA of proteins: celiac disease



In vitro protein degradation

- Pepsin test questioned by:
  - More recent scientific developments
  - Member States
  - EP / NGOs



Astwood et al 1996. Nat Biotechnol 14, 1269-1273

MANCHESTER 1824
The University of Manchester

EFSA supporting publication 2013:EN-529

EXTERNAL SCIENTIFIC REPORT

Literature review: '*in vitro* digestibility tests for allergenicity assessment'[1]

E.N. Clare Mills[1], Justin T. Marsh[1], Phil E. Johnson[1], Robert Boyle[2], Karin Hoffmann-Sommergruber[3], Didier DuPont[4], Joan Bartra[5], Serafim Bakalis[6], John McLaughlin[7], Peter R. Shewry[8]

In vitro protein degradation: EFSA GD (2017)

Classical pepsin resistance test → *In vitro* protein digestibility test reflecting more physiological conditions

Proposed gastric conditions:

Elderly/adults in fasted state
Elderly/adults in fed state
People with impaired gastric function
People taking antacids
Infants

→ High pH / Low [Pepsin] (biosurfactants)

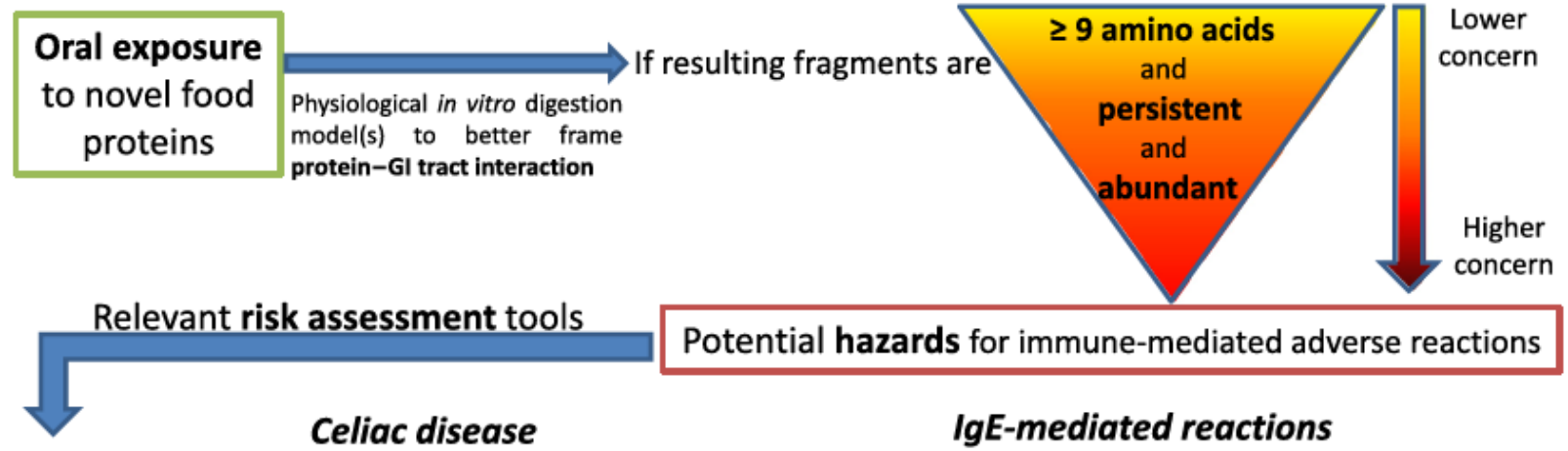
→ Low pH / High [Pepsin] (biosurfactants)

→ **Intestinal digestion**

Interim phase ongoing to evaluate the efficacy of the proposed revisions

Finalisation: end 2019
Further discussion on the way forward needed

Future developments



	Celiac disease	***IgE-mediated reactions***
In silico	Optimized (see Box 1)	In use: sequence identity (for predicting cross-reactivity). *To be developed*: validated bioinformatic approaches (consideration of amino acid physico-chemical properties, etc.).
In vitro	Optimized (see Box 1)	In use: pepsin-resistance test and specific human sera screen (for predicting cross-reactivity). *To be developed*: validated (i) digestion models (considering additional steps with small intestinal brush border enzymes); (ii) gut epithelial cell-based approaches (antigen-presenting cells, MHC-associated peptide proteomics [MAPPs], etc.).
In vivo	Not foreseen	Oral challenges in allergic humans. *To be developed:* (i) HLA-phenotyping in humans (key for more effective MAPPs assays); (ii) validated animal models (relevant for sensitization and for the establishment of dose–response relationship).

Trends in Biotechnology

Fernandez et al 2019. Trends in biotech. In press. Doi:10.1016/j.tibtech.2019.03.010.

Scouting for Naturally Low-Toxicity Wheat Genotypes by a Multidisciplinary Approach

Pilolli et al 2019. Nature/Scientific Reports,9:1646

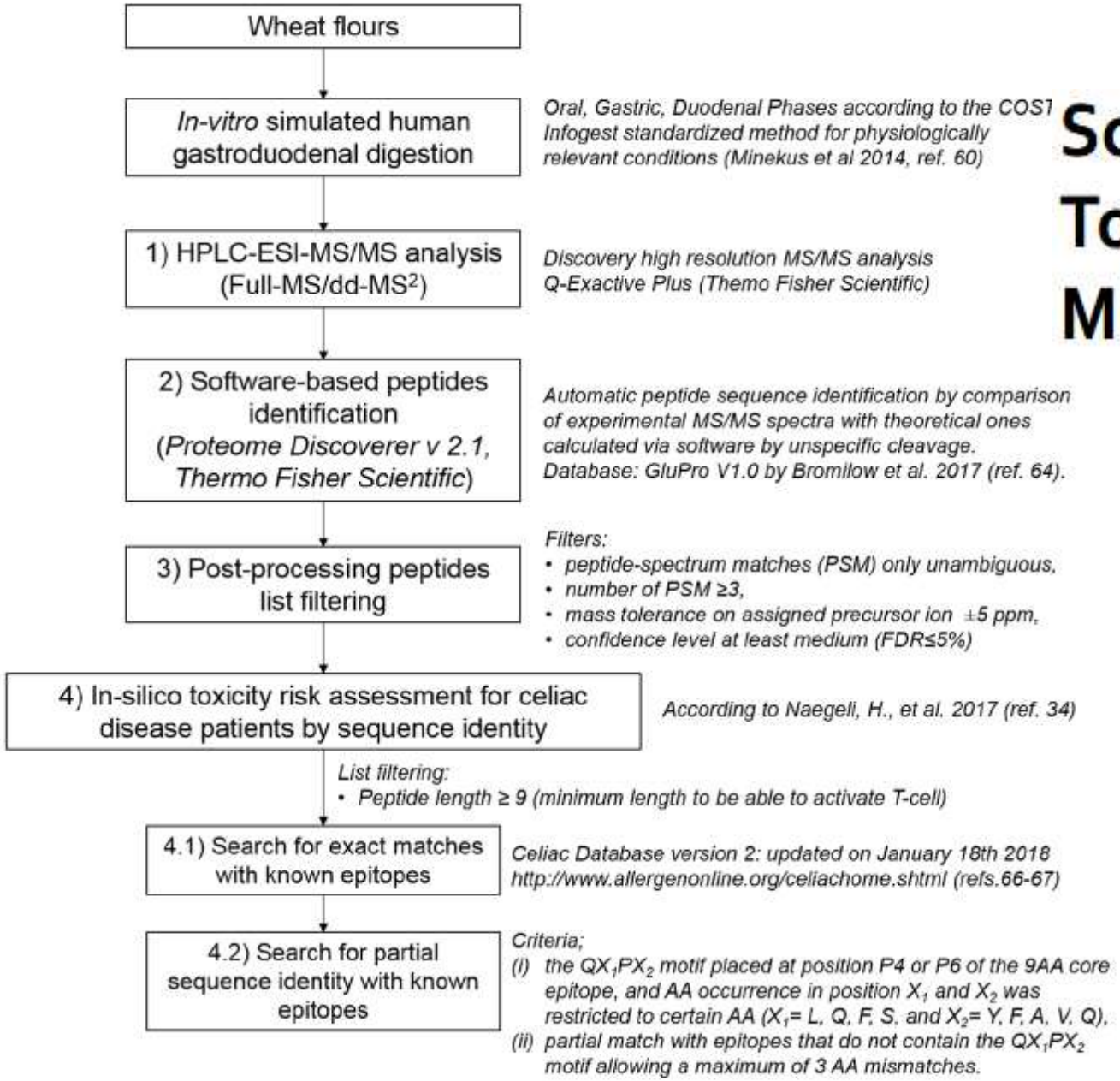
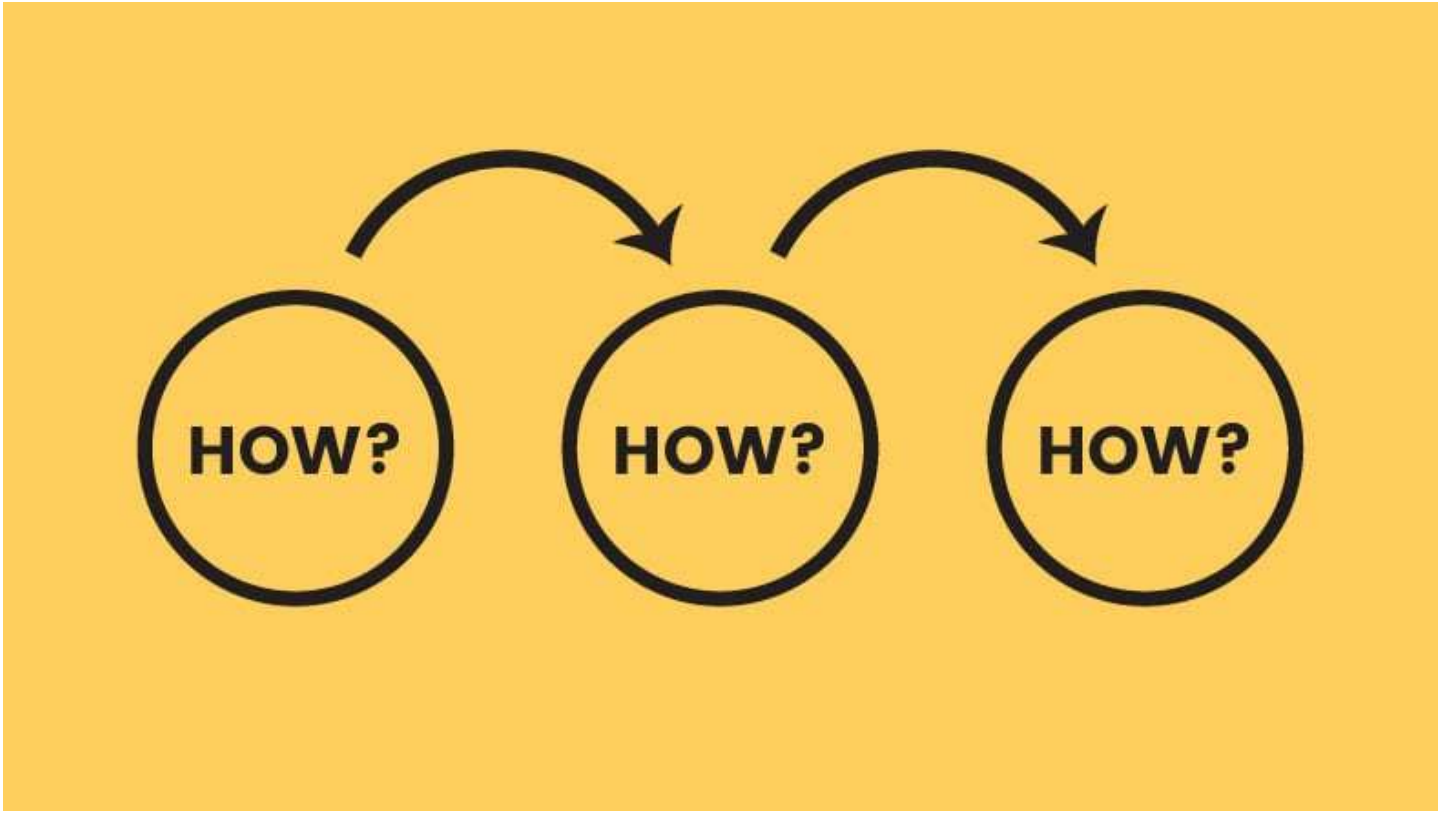


Figure 4. Workflow of the analytical strategy carried out for the identification by untargeted HR-MS/MS analysis of GD resistant peptides and in-silico toxicity risk assessment for celiac disease patients.

"EFSA guidance for allergenicity risk assessment in genetically modified plants provides very useful recommendations that should be followed in light of a harmonization of the methods"

Celiac disease assessment



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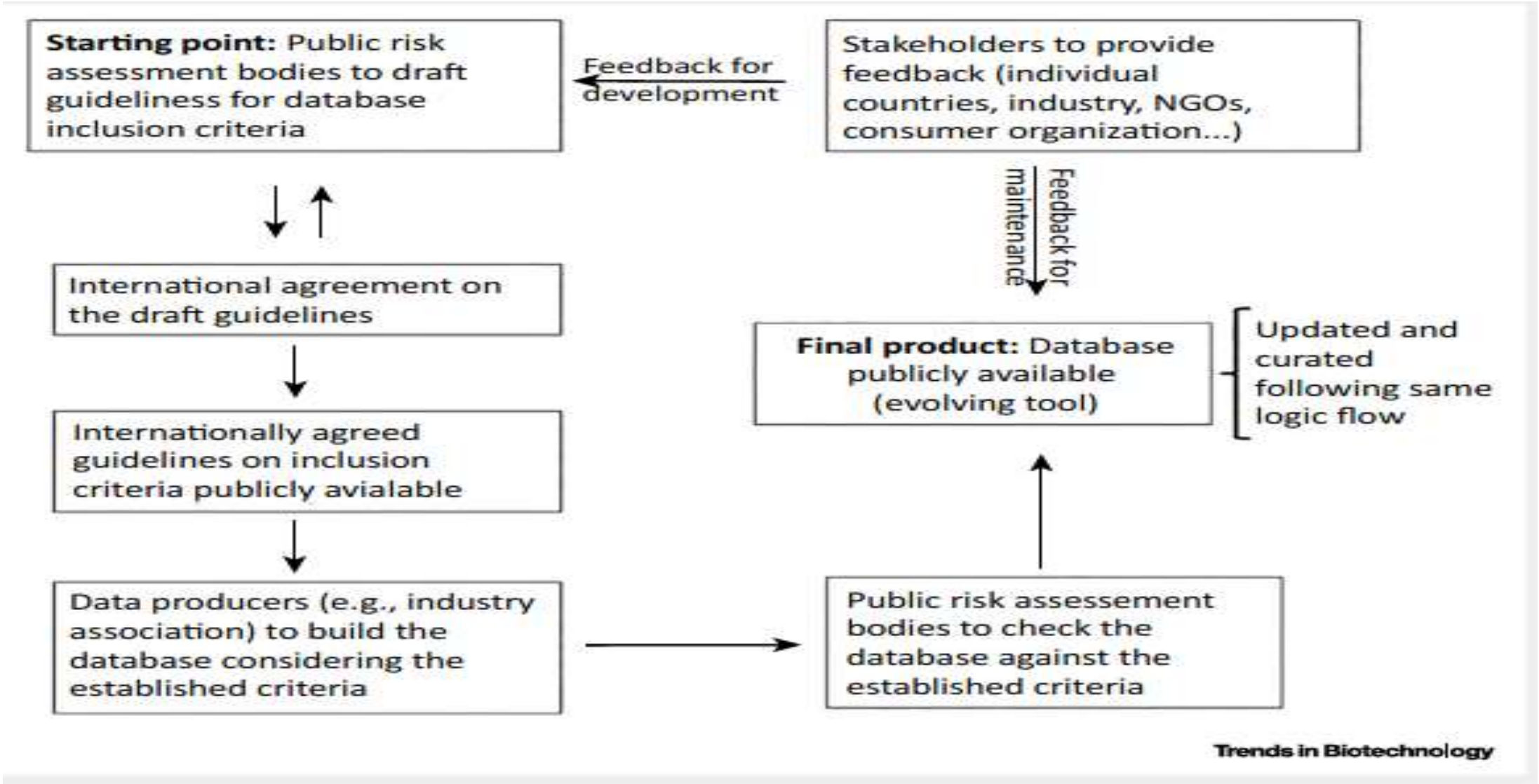
Database development



Fernandez and Paoletti 2018 https://doi.org/10.1016/j.tibtech.2018.03.005