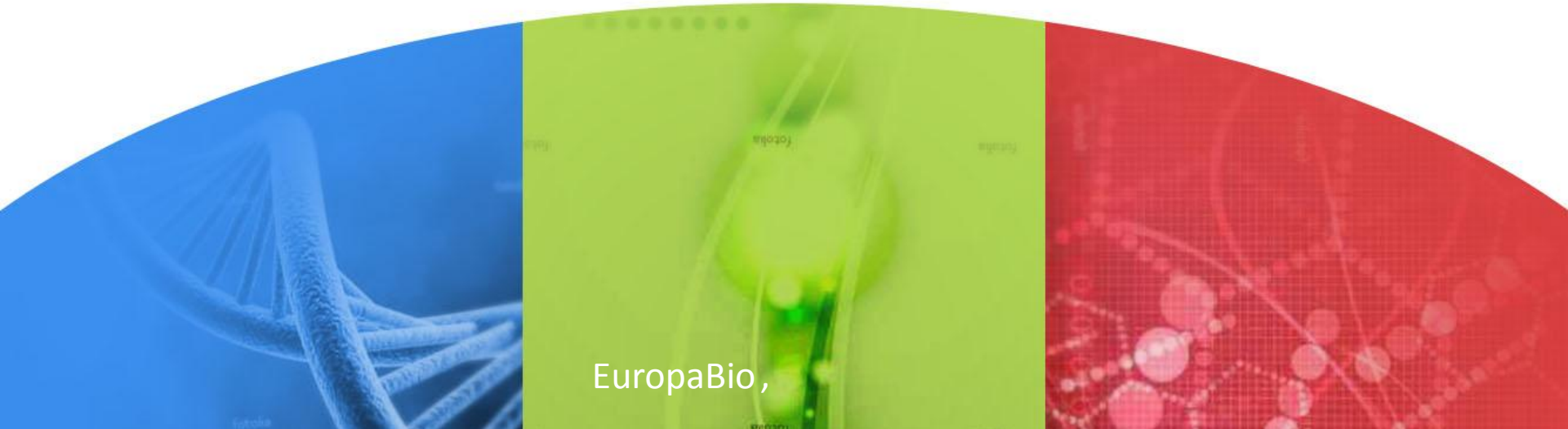
The European Association for Bioindustries

ALLERGENICITY ASSESSMENT

Applications for Authorisation of Genetically Modified Food and Feed Products submitted under Regulation (EC) No 1829/2003



EuropaBio,

Outline

1. Background information
2. Pepsin resistance
3. Non-IgE mediated immune adverse reactions to foods
4. Endogenous allergens
5. Concluding remarks
6. Implementation

1. Background information

Self-task mandate of the EFSA GMO Panel to establish a new working group to develop **supplementary guidelines** for the allergenicity assessment of GM plants to incorporate new developments
(published: July 2014; deadline: July 2016)

Background

Assessment of the two 2013 publications commissioned and published by EFSA

1. "Literature review: '*in vitro*' digestibility tests for allergenicity assessment" by E.N. Clare Mills *et al.*, 2013a
2. "Literature review: non-IgE-mediated immune adverse reactions to foods" by E.N. Clare Mills *et al.,* 2013b

Inclusion of certain allergens (as defined in OECD Consensus documents) in the compositional analysis and consequently, the quantitative measurement of individual allergens.

Proposal: Develop supplementary guidelines for the allergenicity assessment of GM plants to incorporate new developments

2. Pepsin resistance: *historical perspective*

- **Codex Guidelines** recommend testing for resistance to pepsin digestion since "*a correlation exists between resistance to digestion by pepsin and allergenic potential*"
- **Implementing Regulation (EU) No 503/2013** as well as **2011 EFSA** Guidance recommendations are focused on *physiological digestion* instead of *pepsin resistance*
- *In vitro* digestibility tests for allergenicity assessment (Mills *et al.,* 2013a) recommends standardisation of methodology and replication of the dynamic digestive process; the HESI sponsored efforts to standardise the pepsin resistance assay not discussed

EFSA guidance documents and Implementing Regulation (EU) No 503/2013 deviate from internationally agreed guidelines

2. Pepsin resistance: *current status*

- Most dietary **proteins are digested** and provide nutritional value
- The ability to resist pepsin digestion is an **intrinsic property** of the protein
- Current pepsin resistance assay provides **information to predict** the potential dietary exposure to a protein
- Pepsin's enzymatic activity is optimal between pH 1.5-2.0. Not clear how assays outside of this **optimal range** will further inform on protein safety
- **Codex guidelines** do **not require** pepsin resistance assays to strictly mimic **physiological conditions** of human digestion
  - No validated *in vitro* method exists that can mimic human digestion conditions
  - Human digestion is highly variable and depends on many factors including age, diet, genetic make-up

3. Non-IgE mediated immune adverse reactions to foods: *historical perspective*

- Non-IgE-mediated immune adverse reactions to foods (Mills *et al*., 2013b) reviews the science of identifying gluten and related food proteins that cause T-cell mediated Celiac Disease
- Testing scheme proposal presented for *in vitro* or *in vivo* immunoreactivity when/if a trigger is identified. However,
  - **No testing platforms** are identified and/or validated
  - **No predictive tests** for novel proteins and the likelihood of T-cell stimulation are available.
  - **No serum test** for celiac disease is available
- Mills *et al.*, 2013b paper is consistent with Martel *et al.*, 2010 paper which looked at examining enzymes. Also consistent with 2012 International Symposium in Prague (PATC/HESI) that examined the state of the science in sensitisation

Not clear how the risk assessment process and hypothesis-driven analyses would be used effectively for GMO protein risk assessment

3. Non-IgE mediated immune adverse reactions to foods: *current status*

- University of Nebraska food allergen **database FARRP** (www.allergenonline.org) includes gluten and related proteins (since inception)

- Risk management of significant "*in silico*" bioinformatic alignments to gluten proteins is consistent with alignments to any allergen proteins; would require a closer look into risk or avoidance of novel protein
  - Current industry practices would **not** take any novel proteins with alignments to allergens through development
  - Current weight-of-evidence approach encompass celiac disease risks for novel proteins

4. Endogenous allergens

- Endogenous allergens are assessed only in commonly **allergenic crops** such as soybean
- Several soybean allergen lists exists, but only some of the proteins from these lists had **sufficient clinical data** to suggest they cause soybean allergic disease
- MS, ELISA-, and gel separation-based **methods** have been developed and can provide comparative measurements of allergen levels
- Methods using **human serum** are not appropriate for the measurement of allergens in a comparative assessment because of the limited supply of critical reagents
- How to **interpret** the data?
  - Allergic individuals already avoid the offending foods
  - No allergen threshold information exists for sensitisation
  - No clear link between the amount of allergen exposure and sensitisation
  - Information on the natural variability of allergen expression levels is necessary to establish a context of safety

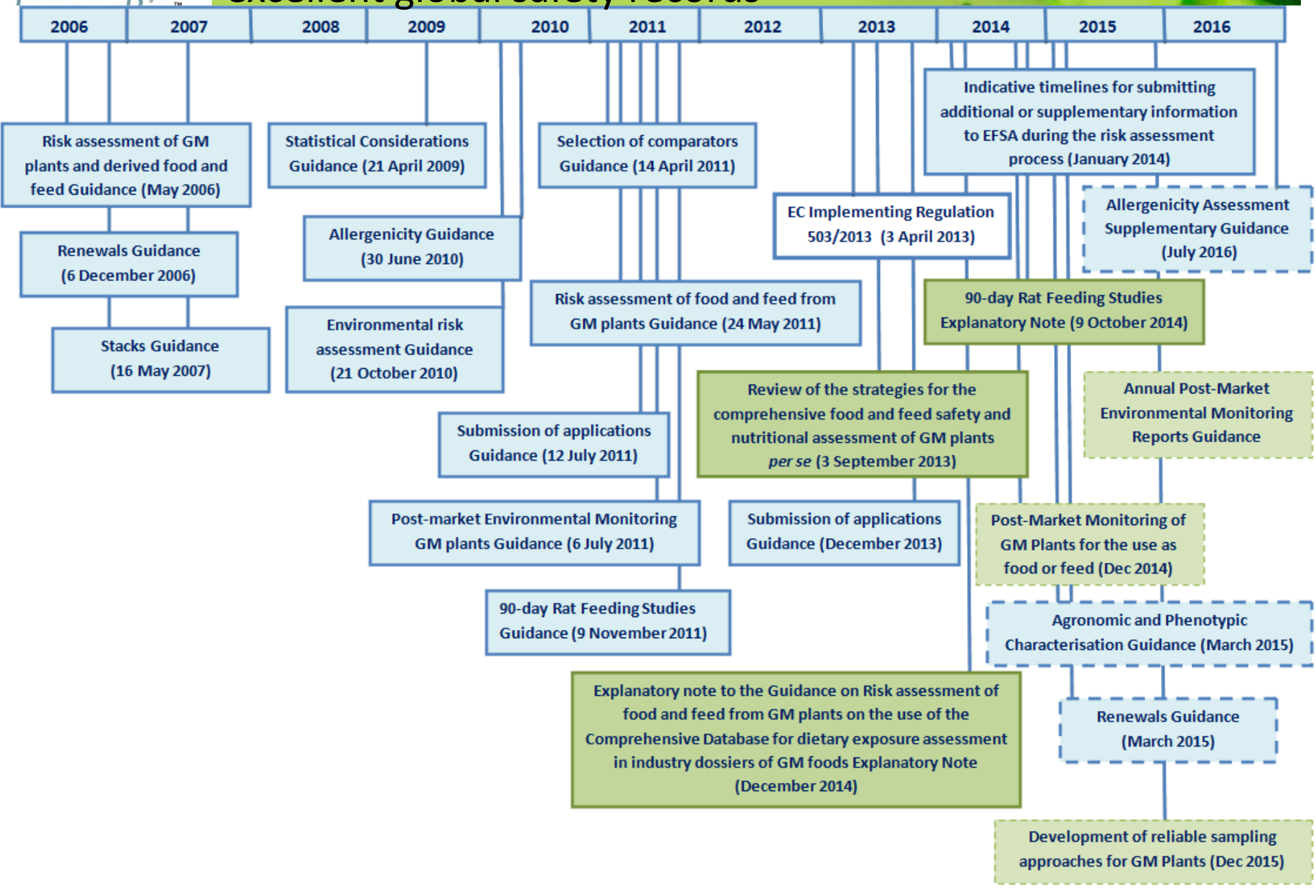
5. Concluding remarks

- **Codex guidelines** recommend testing for resistance to pepsin digestion through a validated method
- **Weight-of-evidence approach** can cover the potential celiac disease risks for novel proteins
- **No serum testing for celiac disease** is available
- **OECD consensus** document for soybean does not recommend testing of endogenous allergens as part of the comparative assessment
- **No predictive testing** for *de novo* sensitisation
- Current allergenicity data package sufficient to conclude on **negligible risk**

Implementation

- Need for **a standard procedure** for **clear and timely communication** of new requirements to applicants
- Need for **sufficient and realistic transition periods** before attaining mandatory compliance with the requirements set out in new guidance documents
- EFSA guidance should **not** be applied retroactively
- Need to justify the **relevance for risk assessment** (hazard x exposure)

Exponential increase in requirements in the EU despite excellent global safety records



Thank you!

Additional Information

1. Background information (continued)

Risk Assessment

- **Risk** is a function of **hazard** and **exposure**; the allergenicity assessment addresses both portions of this equation to identify risk.
- The risk assessment paradigm has to be acknowledged in terms that are consistent with the context of the hazard and exposure characterisations performed in the allergy weight-of-evidence approach.

Hazard	defined adverse effects from the newly expressed proteins in GM crops or from the transformation of the parental crop
Exposure	a measure of the likelihood that the hazard comes into significant contact with humans/animals such that the hazards can have a physiological impact
Characterisations	typical of the allergy assessment. These assess whether there are biophysical similarities between a novel protein and known allergens. There is no single, fully predictive test; therefore, the sum or "weight-of-evidence" from all of the characterisation studies is taken into account.
Study designs	should have a hypothesis that can be tested and have endpoints with interpretable outcomes.

1. Background information (*continued*)

How do allergy studies fit in with risk assessment?

Key points:

1. There is no single, fully predictive test for *de novo* allergy.
2. Because no characterisation studies are perfectly consistent among known allergens, all of the information for newly expressed proteins is assessed for allergenic potential , there is no single test to predict risk.
3. Many of the characterisation studies (assessments) feature an evaluation of exposure

Characterize protein hazard

- Not an allergen
- Similarity with known allergens
- Source organism
- Molecular characterisation identifies intended protein

Characterize protein exposure

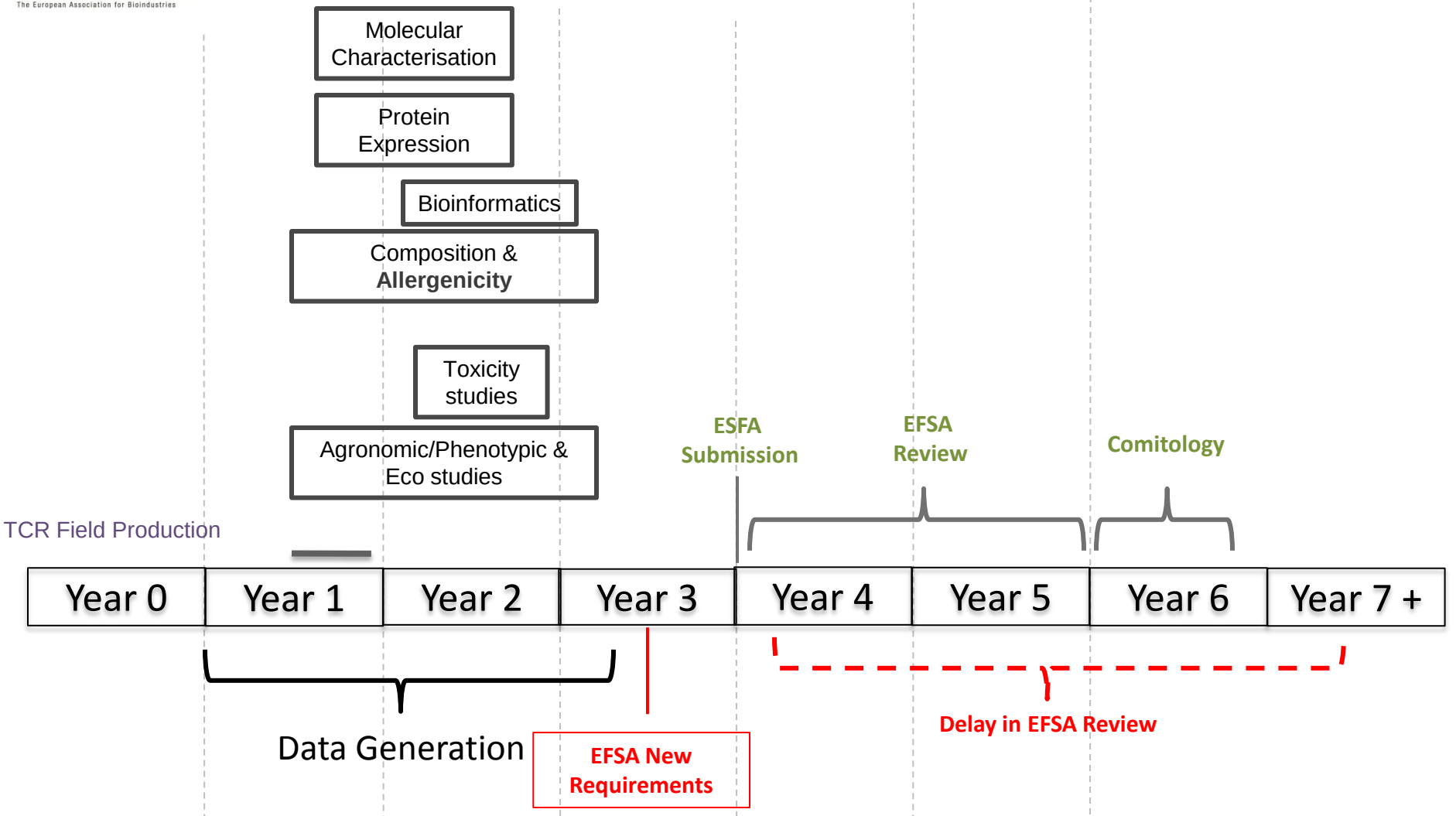
- History of safe exposure - oral
- Pepsin (SGF) - oral
- Heat Denaturation - oral
- Abundance in Food/Feed - oral

1. Background information (*continued*)

Key points in achieving consistent risk assessment

- Should make use of the full background knowledge regarding the novel protein's characterisation and intended use in food/feed.
- Should maintain a *case-by-case* approach
- In general, no specific characterisation should be taken as proof of risk in exclusion of the rest of risk assessment. Specific knowledge that supports safety should be utilized:
  - *Examples specific to allergy*: bacterial proteins not generally known as allergens; enzymes shown clinically not to act as allergens by oral route
- Achieve consistency in risk assessment
  - Industry provides dietary exposure assessment, but EFSA promotes "worst case" exposure assessment by assuming maximal exposure; this is undefined. It reduces the importance of recognizing the typical, very low concentrations of novel proteins in grain, enzyme clinical studies, etc.

6. Impact of any new development



Sufficient and realistic transition period warranted

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