Explanatory note on the determination of Newly Expressed Protein levels

GMO Ad hoc meeting with Applicants
Parma, Italy – 23 & 24 October 2019

efsa
European Food Safety Authority

Use of Newly Expressed Protein (NEP) levels data in GM plant RA

- According to relevant EFSA GMO Panel guidelines and legislation for GM plant RA, information on NEP levels is needed for:
  - molecular characterisation of the event(s)
  - potential interactions in stacked events affecting NEP levels
  - assessment of the exposure to these NEPs in the context of food/feed and environmental safety
- No details on how to produce these data; submission of information is not harmonised
- EFSA was self tasked to produce an explanatory note

Terms of reference and data/methodologies

- An explanatory note on NEP levels determination to provide details on key methodological aspects to be considered by applicants in order to harmonise the information in submitted GM plant applications
- Based on:
  - EFSA GMO Panel guidelines on Food & Feed and ERA for GM plants
  - relevant legislation (e.g. Regulation (EU) 503/2013)
  - other bioanalytical method validation documents
  - scientific literature
  - gained experience from already assessed EFSA GM plant applications
- Not intended to recommend specific protocols

Content

- Two steps in protein quantification methodology:
  - protein extraction
  - analytical method employed
- Main methods discussed (ELISA, WB and MS) with particular attention on ELISA (most widely method used)
- Recommendations on what/how information should be reported (‘checklist’)

Protein Extraction

- Efficiency
  - total NEP levels are needed for the RA; chosen method might have limited 'extractability'
  - 'complete' extraction step under strong denaturing ('harsh') conditions to determine the remaining NEP amount in the 'insoluble' fraction
  - NEP extraction efficiency estimated relative to total NEP amount
- Tissue disruption/cell lysis (NEP molecular stability)
  - appropriate buffer-to-tissue ratio
  - fresh or lyophilised material is acceptable
- Extraction buffer (NEP molecular stability)
  - buffer compatible with quantification method
  - protease inhibitors should be used; if not justification should be provided

Analytical method (NEP quantification)

- Validation parameters (and data quality considerations)
  - Sensitivity; LOD, LOQ, standard curve
  - Matrix effects; method should be sufficiently accurate in the presence of tissue matrix components (for each tissue analysed)
  - Specificity; antibody cross reactivity for ELISA/WB, peptide interference for MS-based methods
  - Repeatability; variation in inter-assay and intra-assay measurements, CV

Analytical method (NEP quantification)

- Additional elements such as:
  - information on the reference standards (e.g. full-length proteins/equivalence, IS for MS)
  - antibody information (e.g. monoclonal/polyclonal, antigen used)
  - MS-specific (e.g. digestion efficiency)
  - processed food/feed (e.g. choice of antibodies)

Presentation of results

- Information on the samples, e.g.
  - number of analysed plants and how plant material was collected
  - sufficient description of plant/tissue treatment (herbicides)
  - any sample contamination and impact on results
- Information on the methods, e.g.
  - Method description (protocols)
  - all critical methodological parameters
- Data analysis and reporting, e.g.
  - % extraction efficiency should be applied (and described)
  - presented values in both FW and DW (conversion should be explained)
  - all acceptance criteria should be described and justified and the impact of data falling out of these criteria should be discussed

Additional considerations/Clarifications

- LOD: critical for NEPs expressed at low levels, less important for NEPs expressed at higher levels
- NEP quantification for stacks: acceptable that a previously validated method is applied as long as information on what material was used is provided; NOTE: Antibody cross-reactivity should be re-evaluated
- Use of extraction efficiency/correction factor: total NEP levels are needed for the RA
- Reference protein concentration determination methods: choice of method(s) is up to applicants however use of two methods is recommended for higher accuracy
- NEP levels in fresh weight(fw): used in dietary exposure estimations taking into account consumption data expressed also on a fw basis

Transition period (publication date: 20/08/2018)

- In line with the indicative timelines for submitting molecular datasets that require the generation of plant material, the recommendations will be applicable for GM plant applications submitted **24 months** after its publication (effective date:20/08/2020)
- A transition period of **two months** has been applied to elements for which only the provision of information was recommended (effective date: 20/10/2018)

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