Adopted: 1 July 2025

DOI: 10.2903/j.efsa.2025.9581

efsa JOURNAL

SCIENTIFIC OPINION

# Assessment of genetically modified soybean MON 94637 (application GMFF-2023-21116)

**EFSA Panel on Genetically Modified Organisms (GMO) | Josep Casacuberta | Francisco Barro | Albert Braeuning | Ruud de Maagd | Michelle M. Epstein | Thomas Frenzel | Jean-Luc Gallois | Frits Koning | Antoine Messéan | F. Javier Moreno | Fabien Nogué | Giovanni Savoini | Alan H. Schulman | Christoph Tebbe | Eve Veromann | Michele Ardizzone | Giacomo De Sanctis | Antonio Fernandez | Arianna Ferrari | Andrea Gennaro | José Ángel Gómez Ruiz | Tilemachos Goumperis | Sara Jacchia | Dafni Maria Kagkli | Paolo Lenzi | Aleksandra Lewandowska | Ana M. Camargo | Franco Maria Neri | Pietro Piffanelli | Tommaso Raffaello**

**Correspondence:** nif@efsa.europa.eu

The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

**Abstract**

Genetically modified soybean MON 94637 was developed to provide protection against targeted lepidopteran pests. These properties were achieved by introducing the *cry1A.2* and *cry1B.2* expression cassettes. The molecular characterisation data and bioinformatic analyses do not identify issues requiring food/feed safety assessment. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between soybean MON 94637 and its conventional counterpart need further assessment. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the Cry1A.2 and Cry1B.2 proteins as expressed in soybean MON 94637 and finds no evidence that the genetic modification would change the overall safety of soybean MON 94637, as food and feed. In the context of this application, the consumption of food and feed from soybean MON 94637 does not represent a nutritional concern in humans and animals, and no post-market monitoring of food/feed is considered necessary. In the case of release of processed soybean MON 94637 or accidental spillage of viable GM soybean seeds into the environment, this would not raise environmental safety concerns. The post-market environmental monitoring plan (PMEM) and reporting intervals are in line with the intended uses of soybean MON 94637. The GMO Panel concludes that soybean MON 94637 is as safe as its conventional counterpart and the tested non-GM soybean varieties with respect to potential effects on human and animal health, and the environment.

**KEYWORDS**

Cry1A.2, Cry1B.2, genetic engineering, GM, import and processing, MON 94637, soybean (*Glycine max*)

# CONTENTS

## SUMMARY

Following the submission of application GMFF-2023-21116 under Regulation (EC) No 1829/2003 from Bayer CropScience LP (referred to hereafter as 'the applicant'), the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') was asked to deliver a scientific opinion on the safety of genetically modified (GM) insect-resistant soybean (*Glycine max* L.) MON 94637 (Unique Identifier MON-94637-8) according to Regulation (EU) No 503/2013. The scope of application GMFF-2023-21116 is for import, processing and food and feed uses within the European Union (EU) of soybean MON 94637 and does not include cultivation in the EU.

In this scientific opinion, the GMO Panel reports on the outcome of its risk assessment of soybean MON 94637 according to the scope of the application GMFF-2023-21116. The GMO Panel conducted the assessment of soybean MON 94637 in line with the principles described in Regulation (EU) No 503/2013 and its applicable guidelines for the risk assessment of GM plants. The molecular characterisation data establish that soybean MON 94637 contains a single insert consisting of one copy of the *cry1A.2* and *cry1B.2* expression cassettes. The quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and complies with the requirements listed in the EFSA Technical Note. Bioinformatic analyses of the sequences encoding the newly expressed proteins (NEPs), the sequences corresponding to open reading frames (ORFs) within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise safety concerns. The stability of the inserted DNA and of the introduced trait is confirmed over several generations. The methodology used to quantify the levels of the Cry1A.2 and Cry1B.2 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and *Bacillus thuringiensis*-produced Cry1A.2 and Cry1B.2 proteins indicate that these proteins are equivalent, and the *B. thuringiensis*-derived proteins can be used in the safety studies.

Considering the selection of test materials, the field trial sites, the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between soybean MON 94637 and its conventional counterpart need further assessment. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the Cry1A.2 and Cry1B.2 proteins as expressed in soybean MON 94637. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of soybean MON 94637, as food and feed. In the context of this application, the consumption of food and feed from soybean MON 94637 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that soybean MON 94637 is as safe as the conventional counterpart and non-GM soybean reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

Considering the introduced traits, the outcome of the agronomic and phenotypic analysis and the routes and levels of exposure, soybean MON 94637 would not raise safety concerns in the case of accidental release of processed soybean MON 94637 or accidental spillage of viable GM soybean seeds into the environment. The PMEM plan and reporting intervals are in line with the intended uses of soybean MON 94637.

The literature searches did not identify any relevant publications on soybean MON 94637 that raised any safety concerns.

The GMO Panel concludes that soybean MON 94637, as described in this application, is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

# 1 | INTRODUCTION

The scope of the application GMFF-2023-21116 is for food and feed uses, import and processing of soybean MON 94637 but does not include cultivation in the European Union (EU). Soybean MON 94637 was developed to confer protection against targeted lepidopteran pests.

## 1.1 | Background

On 25 January 2024, the European Food Safety Authority (EFSA) received from the Competent Authority of the Netherlands application GMFF-2023-21116 for authorisation of soybean MON 94637 (Unique Identifier MON-94637-8), submitted by Bayer CropScience LP (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.[1] Following receipt of application GMFF-2023-21116, EFSA informed EU Member States (MS) and the European Commission and made the application available to them. Simultaneously, EFSA published the summary of the application.[2]

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013,[3] with the EFSA guidance documents, and, when needed, asked the applicant to supplement the initial application. On 29 April 2024, EFSA declared the application valid.

From validity date, EFSA and the Panel on Genetically Modified Organisms of the European Food Safety Authority (referred to hereafter as 'GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application GMFF-2023-21116. Such time limit was extended whenever EFSA and/or GMO Panel requested supplementary information from the applicant. According to Regulation (EC) No 1829/2003, any supplementary information provided by the applicant during the risk assessment was made available to the EU Member States and European Commission (for further details, see the Section 5, below). In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of EU Member States, including national Competent Authorities within the meaning of Directive 2001/18/EC.[4] The EU Member States had 3 months to make their opinion known on application GMFF-2023-21116 as of the date of validity.

## 1.2 | Terms of Reference as provided by the requestor

According to Articles 6 and 18 of Regulation (EC) No 1829/2003, EFSA and its GMO Panel were requested to carry out a scientific risk assessment of soybean MON 94637 in the context of its scope as defined in application GMFF-2023-21116.

According to Regulation (EC) No 1829/2003, this scientific opinion is to be seen as the report requested under Articles 6(6) and 18(6) of that Regulation. In addition to the present scientific opinion, EFSA was also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003, but not to give an opinion on them because they pertain to risk management.[5]

# 2 | DATA AND METHODOLOGIES

## 2.1 | Data

The applicant has submitted a confidential and a non-confidential version of a dossier following the 'EFSA guidelines for the submission of an application to comply with the specific provisions of Regulation (EU) No 503/2013',[3] and of the 'Administrative Guidance for the preparation of applications' (EFSA, 2021a, 2021b).

In accordance with Art. 38 of the Regulation (EC) No 178/2002[6] and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, the non-confidential version of the dossier has been published on OpenEFSA.[7]

[1]Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed. OJ L 268, 18.10.2003, pp. 1–23.

[2]Available online: https://open.efsa.europa.eu/dossier/GMFF-2023-21116.

[3]Commission Implementing Regulation (EU) No 503/2013 of 3 April 2013 on applications for authorisation of genetically modified food and feed in accordance with Regulation (EC) No 1829/2003 of the European Parliament and of the Council and amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. OJ L157, 8.6.2013, pp. 1–48.

[4]Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 12.3.2001, pp. 1–38.

[5]These particulars are available online at: https://open.efsa.europa.eu/dossier/GMFF-2023-21116.

[6]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–48.

[7]https://open.efsa.europa.eu/dossier/GMFF-2023-21116.

According to Art. 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations,[8] EFSA carried out a public consultation on the non-confidential version of the dossier from 10 January to 31 January 2025 for which no comments were received.

The GMO Panel based its scientific assessment of soybean MON 94637 on the valid dossier GMFF-2023-21116, additional information provided by the applicant during the risk assessment, scientific comments submitted by EU Member States and relevant scientific publications.

## 2.2 | Methodologies

The GMO Panel conducted its assessment in line with the principles described in Regulation (EU) No 503/2013, the applicable guidelines (i.e. EFSA GMO Panel, 2010a, 2011a, 2011b, 2015; EFSA Scientific Committee, 2011) and explanatory notes and statements (i.e. EFSA, 2010, 2014, 2017, 2018, 2019a, 2019b; EFSA GMO Panel, 2010b, 2018, 2021) for the risk assessment of GM plants.

For this application, the contractors performed preparatory work for the evaluation of the completeness and quality of DNA sequencing information (OC/EFSA/GMO/2020/01), the bioinformatic analyses (OC/EFSA/GMO/2021/06) and the analysis of the 28- and 90-day toxicity studies (EOI/EFSA/2022/01 – CT 17-2024 and EOI/EFSA/2022/01 – CT NIF 2023 02).

# 3 | ASSESSMENT

## 3.1 | Introduction

Soybean MON 94637 was genetically modified to confer protection against targeted lepidopteran pests through the expression of Cry1A.2 and Cry1B.2 chimeric proteins. The two NEPs have not been previously assessed by the GMO Panel.

## 3.2 | Systematic literature review

The GMO Panel assessed the applicant's literature searches on soybean MON 94637, which includes a scoping review, according to the guidelines given in EFSA (2010, 2019b).

A systematic review as referred to in Regulation (EU) No 503/2013 has not been provided in support to the risk assessment of application GMFF-2023-21116. Based on the outcome of the scoping review, the GMO Panel agrees that there is limited value in undertaking a systematic review for soybean MON 94637 at present.

The GMO Panel considers the overall quality of the performed literature searches acceptable. The literature searches did not identify any relevant publications on soybean MON 94637. Therefore, the GMO Panel does not identify any safety issues pertaining to the intended uses of soybean MON 94637.

## 3.3 | Molecular characterisation[9]

### 3.3.1 | Transformation process and vector constructs

Soybean MON 94637 was developed by *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*)-mediated transformation. Explants of soybean variety A3555 were co-cultured with a disarmed *Agrobacterium tumefaciens* strain AB30 containing the vector PV-GMIR527237. The plasmid PV-GMIR527237 used for the transformation contains two separate T-DNAs, each with a right and left border. T-DNA I carries two expression cassettes containing the following genetic elements:

- The *cry1A.2* expression cassette consisting of the promoter, leader and intron of the polyubiquitin gene *UBQ10* from *Arabidopsis thaliana*; the chimeric plant codon-optimised sequence of the *cry1A.2* gene, consisting of sequences encoding Cry1Ah, Cry1Ac and Cry1Ca domains from *Bacillus thuringiensis* (*Bt*); the 3′ UTR sequence of a gene encoding a putative zinc finger protein (*Zfp-Mt1*) from *Medicago truncatula*.
- The *cry1B.2* expression cassette consists of the promoter and leader of a chlorophyll a/b binding (CAB) protein from *Cucumis melo*; the chimeric plant codon-optimised sequence of the *cry1B.2* gene, consisting of sequences encoding Cry1Be, Cry1Ka2 and Cry1Ab domains from *Bacillus thuringiensis* (*Bt*), the 3′ UTR sequence of a lipoxygenase gene (*Lox-Mt1*) from *Medicago truncatula*.

T-DNA II carries two expression cassettes containing the following genetic elements:

[8]Decision available at: https://www.efsa.europa.eu/sites/default/files/corporate_publications/files/210111-PAs-pre-submission-phase-and-public-consultations.pdf.
[9]Dossier: Part II – Section 1.2; additional information: 5/6/2024, 29/7/2024, 19/12/2024, 23/1/2025, 24/4/2025 and 7/5/2025.

– The *aadA* expression cassette consisting of the enhancer from the 35S RNA of figwort mosaic virus (FMV), the promoter, leader and intron sequences of the EF–1α gene from *Arabidopsis thaliana*, the targeting sequence of the *ShkG* gene from *Arabidopsis thaliana*, the coding sequence of the *aadA* gene and the 3′ UTR sequence of the E9 gene from *Pisum sativum*.
– The *splA* expression cassette consisting of 5′ UTR leader, promoter and enhancer sequence of an unknown seed protein gene (*Usp*) from *Vicia faba*, the coding sequence of the *splA* gene from *Agrobacterium tumefaciens* encoding the sucrose phosphorylase protein and the 3′ untranslated sequence of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens*.

The vector backbone contained elements necessary for the maintenance and selection of the plasmid in bacteria.

T-DNA II was used for selecting the transformed plants and, after self-pollination of R0 plants, only those in which T-DNA II was segregated away were selected in the R1 generation.

### 3.3.2 | Transgene constructs in the GM plant

Molecular characterisation of soybean was performed by next generation sequencing (NGS) and junction sequence analysis (JSA), in order to determine insert copy number and to confirm the absence of plasmid backbone and T-DNA II sequences, and NGS sequencing on PCR-amplified fragments to determine size and organisation of the inserted sequences. The approach used is acceptable in terms of coverage and sensitivity. Overall, the quality of the sequencing methodology and data sets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA Technical Note (2018).

NGS/JSA of the whole genome indicated that soybean MON 94637 contains a single insert, consisting of a single copy of the T-DNA I in the same configuration as in the PV-GMIR527237 transformation vector. NGS/JSA also indicated the absence of plasmid backbone and T-DNA II sequences in the soybean genome.

The nucleotide sequence of the entire insert of soybean MON 94637 together with 1000 bp of the 5′ and 1000 bp of the 3′ flanking regions was determined. The insert of 12,240 bp is identical to the T-DNA of PV-GMIR527237, except for the deletion of 217 bp of the right border region and 172 bp of the left border region.

A comparison with the pre-insertion locus indicated that 14 bp was deleted from the soybean genomic DNA. The possible interruption of known endogenous soybean genes by the insertion in soybean MON 94637 was evaluated by bioinformatics analyses of the pre-insertion locus and of the genomic sequences flanking the insert. The T-DNA is inserted in an intergenic region between a predicted pseudogene and a predicted galactinol-sucrose galactosyltransferase gene. The results of these analyses do not indicate the interruption of any known endogenous gene in soybean MON 94637, and there are no indications from comparative agronomic performance and compositional analyses of any unintended effect caused by the insertion (Section 3.3).

The results of the segregation analysis (see Section 3.3.5) are compatible with a single insertion in the nuclear genome, in agreement with the conclusions of the bioinformatic analyses.

Bioinformatics analyses of the amino acid sequence of the newly expressed Cry1A.2 and Cry1B.2 proteins reveal no significant similarities to toxins and allergens.

In addition, bioinformatic analyses of the newly created open reading frames (ORFs) within the insert and spanning the junctions between the insert and genomic DNA do not indicate significant similarities to toxins and allergens.

In order to assess the possibility for horizontal gene transfer (HGT) by homologous recombination (HR), the applicant performed a sequence identity analysis of the inserted DNA in soybean MON 94637, which consists of two expression cassettes containing plant codon-optimised NEP coding sequences, with microbial DNA. The likelihood and potential consequences of plant-to-bacteria gene transfer are described in Section 3.6.1.2.

### 3.3.3 | Protein characterisation and equivalence

Soybean MON 94637 expresses two new proteins: Cry1A.2 and Cry1B.2. Given the technical restraints in producing large enough quantities from plants, these proteins were recombinantly produced in *Bacillus thuringiensis*. A set of biochemical methods was employed to demonstrate the equivalence between the soybean and *B. thuringiensis*-derived Cry1A.2 and Cry1B.2. Purified proteins from these two sources were characterised and compared in terms of their biochemical, structural and functional properties.

**Cry1A.2 protein characterisation and equivalence**

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting analysis showed that both plant and *B. thuringiensis*-produced Cry1A.2 proteins had the expected molecular weight of ~124 kDa and were comparably immunoreactive to Cry1A.2 protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the Cry1A.2 proteins were glycosylated. Amino acid sequence analysis of the plant-derived and the previously analysed *B. thuringiensis*-produced Cry1A.2 proteins by mass spectrometry (MS) methods showed that both proteins matched the

deduced sequence as defined by the *cry1A.2* gene. Functional equivalence was demonstrated by an insect bioassay which showed that plant and *B. thuringiensis*-derived Cry1A.2 proteins had insecticidal activity against target organisms.

**Cry1B.2 protein characterisation and equivalence**

SDS-PAGE and western blotting analysis showed that both plant and *B. thuringiensis*-produced Cry1B.2 proteins had the expected molecular weight of ~135 kDa and were comparably immunoreactive to Cry1B.2 protein-specific antibodies. Glycosylation detection analysis demonstrated that none of the Cry1B.2 proteins were glycosylated. Amino acid sequence analysis of the plant-derived and the previously analysed *B. thuringiensis*-produced Cry1B.2 protein by MS methods showed that both proteins matched the deduced sequence as defined by the *cry1B.2* gene. In addition, the MS data showed that the N-terminal methionine of the plant-produced and *B. thuringiensis*-derived Cry1B.2 protein was truncated. Such modifications are common in eukaryotic proteins (e.g. Polevoda & Sherman, 2000). Functional equivalence was demonstrated by an insect bioassay which showed that plant and *B. thuringiensis*-derived Cry1B.2 proteins had insecticidal activity against target organisms.

The protein characterisation data comparing the biochemical, structural and functional properties of plant and *B. thuringiensis*-produced Cry1A.2 and Cry1B.2 proteins indicate that these proteins are equivalent, and the *B. thuringiensis*-derived proteins can be used in the safety studies.

### 3.3.4 | Information on the expression of the insert

Protein levels of Cry1A.2 and Cry1B.2 were analysed by an enzyme-linked immunosorbent assay (ELISA) in material harvested in a field trial across five locations in the United States during the 2021 growing season. Samples analysed included seeds (BBCH 89), flower (BBCH 60–65) and forage (BBCH 77). The mean values and standard error of protein expression levels in seeds ($n = 20$), flower ($n = 20$) and forage ($n = 20$) of the Cry1A.2 and Cry1B.2 proteins used to estimate human and animal dietary exposure (see Section 3.4.5) are reported in Table 1.

**TABLE 1** Mean values ($n = 20$) and standard error of NEP in seeds [μg/g dry weight (dw) and μg/g fresh weight (fw)], flowers and forage (μg/g dw) from soybean MON 94637.

| Tissue | μg/g dry weight (dw) | μg/g fresh weight (fw) |
|---|---|---|
| **Seed (BBCH 89)** | | |
| Cry1A.2 | 24[a] ± 2.2[b] (7.0–43)[c] | 21 ± 2.0 (6.4–39) |
| Cry1B.2 | 12 ± 0.75 (7.1–20) | 11 ± 0.69 (6.5–18) |
| **Forage (BBCH 77)** | | |
| Cry1A.2 | 84 ± 3.4 (59–120) | |
| Cry1B.2 | 55 ± 3.3 (36–96) | |
| **Flower (BBCH 60–65)** | | |
| Cry1A.2 | 260 ± 24 (110–610) | |
| Cry1B.2 | 180 ± 9.8 (130–300) | |

[a]Mean value.
[b]Standard error.
[c]Range.

### 3.3.5 | Inheritance and stability of inserted DNA

Genetic stability of soybean MON 94637 insert was assessed by NGS/JSA of genomic DNA from five consecutive generations (R3, R4, R5, R6, R7) and PCR-based segregation analysis from three generations (F2, F3, F4). The results indicate that all the tested plants retained the single copy of the insert and flanking regions, which were stably inherited in subsequent generations.

The results support the presence of a single insertion, segregating in a Mendelian fashion.

### 3.3.6 | Conclusion on molecular characterisation

The molecular characterisation data establish that soybean MON 94637 contains a single insert consisting of one copy of the *cry1A.2* and the *cry1B.2* expression cassettes. Bioinformatic analyses of the sequences encoding the NEPs, the sequences corresponding to ORFs within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise any safety concerns. The stability of the inserted DNA is confirmed over several generations. The methodology used to quantify the levels of the Cry1A.2 and Cry1B.2 proteins is considered adequate. The protein characterisation data comparing the biochemical, structural and functional properties of plant and *B. thuringiensis*-produced Cry1A.2 and Cry1B.2 proteins indicate that these proteins are equivalent, and the *B. thuringiensis*-derived proteins can be used in the safety studies.

## 3.4 | Comparative analysis[10]

### 3.4.1 | Overview of studies conducted for the comparative analysis

Application GMFF-2023-21116 presents data on agronomic and phenotypic characteristics, as well as on forage and seed composition of soybean MON 94637 (Table 2).

**TABLE 2** Overview of the comparative analysis studies to characterise soybean MON 94637 provided in the application GMFF-2023-21116.

| Study focus | Study details | Comparator | Non-GM reference varieties |
|---|---|---|---|
| Agronomic and phenotypic/compositional analysis | Field study, US, 2021, 8 Sites[a] | A3555 | 11[b] |

Abbreviation: GM, genetically modified.

[a]The field trial sites were located in Audubon County, Iowa; Clinton County, Illinois; Warren County, Illinois; Boone County, Indiana; Ingham County, Michigan; York County, Nebraska; York County, Nebraska and Miami County, Ohio.

[b]Non-GM soybean with their corresponding maturity group indicated in brackets were Asgrow A3253 (3.2); Pioneer P35A41 (3.5); DairyLand DSR-3200 (3.2); Pioneer P34A50 (3.4); Pioneer P38A10 (3.8); Asgrow A3956 (3.9); Stine 35J02 (3.5); LG Seeds C3848 (3.8); Specialty 3352C (3.3); LG Seeds C3400 (3.4) and CHANNEL 3341C (3.3).

### 3.4.2 | Experimental field trial design and statistical analysis

At each field trial site, the following materials were grown in a randomised complete block design with four replicates: soybean MON 94637, the comparator A3555 and four non-GM reference varieties.

The agronomic, phenotypic and compositional data were analysed as specified by EFSA GMO Panel (2010b, 2011a). This includes the application of a difference test (between the GM soybean and the non-GM comparator) and an equivalence test (between the GM soybean and the set of non-GM reference varieties). The results of the equivalence test are categorised into four possible outcomes (I–IV, ranging from equivalence to non-equivalence).[11]

### 3.4.3 | Suitability of selected test materials

#### 3.4.3.1 | *Selection of the test materials*

The comparator used in the field trials is the non-GM soybean A3555, which has the same genetic background as soybean MON 94637 (as documented by the pedigree) and is considered to be the conventional counterpart.

Soybean MON 94637 and the conventional counterpart belong to maturity group 3.5, which is considered appropriate for growing in environments across the US, where the comparative field trials were conducted.

Commercial non-GM reference varieties ranging from maturity group 3.2 to 3.9 were selected by the applicant and, at each selected site, four reference varieties were tested (see Table 2). On the basis of the provided information on maturity group classes, the GMO Panel considers the selected non-GM reference varieties appropriate for the comparative assessment.

#### 3.4.3.2 | *Seed production and quality*

Seeds of soybean MON 94637 and the conventional counterpart used in the 2021 field trials were produced from plants free of diseases, harvested and stored under similar conditions, before being sown in the field trial sites. The seed lots were verified for their identity via event-specific polymerase chain reaction analysis.

[10]Dossier: Part II – Section 1.3; additional information: 5/6/2024.

[11]In detail, the four outcomes are category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence).

The seeds were tested for their germination capacity under warm and cold temperature conditions.[12] Germination capacity of soybean MON 94637 was compared with its conventional counterpart and with four[13] commercial non-GM soybean reference varieties, and the results[14] of these studies indicate that the seed germination of soybean MON 94637 was not different from that of its comparators.

#### 3.4.3.3 | *Conclusion on suitability*

The GMO Panel concludes that the soybean MON 94637, the conventional counterpart and the non-GM reference varieties were properly selected and are of adequate quality. Therefore, the test materials are considered suitable for the comparative analysis.

### 3.4.4 | Representativeness of the receiving environments

#### 3.4.4.1 | *Selection of field trial sites*

The selected field trial sites were located in commercial soybean-growing regions of the United States. The soil and climatic characteristics of the selected fields[15] correspond to optimal, near optimal and sub-optimal conditions for soybean cultivation (Sys et al., 1993). The GMO Panel considers that the selected sites reflect commercial soybean-growing regions in which the test materials are likely to be grown.

#### 3.4.4.2 | *Meteorological conditions*

Maximum and minimum mean temperatures and sums of precipitation were provided for each site on a weekly basis; no exceptional weather conditions were reported at any of the selected sites. The GMO Panel considers that the meteorological data set falls within the historical range of climatic conditions normally occurring at these sites.

#### 3.4.4.3 | *Management practices*

The field trials included plots containing soybean MON 94637, plots with the conventional counterpart and plots with non-GM reference varieties, managed according to local agricultural practices.

The GMO Panel considers that the management practices, including sowing, harvesting and application of plant protection products, were appropriate for the selected receiving environments.

#### 3.4.4.4 | *Conclusion on representativeness*

The GMO Panel concludes that the geographical locations, soil and climatic characteristics, meteorological conditions and management practices of the field trial sites are typical for receiving environments where the tested materials could be grown.

### 3.4.5 | Agronomic and phenotypic analysis

Ten agronomic and phenotypic endpoints[16] plus information on abiotic stressors, disease incidence and arthropod damage were collected from the field trial sites (see Table 2).

The statistical analysis (Section 3.4.2) was applied to the 10 endpoints, with the following results: for soybean MON 94637, the test of difference identified a statistically significant difference with the conventional counterpart for final stand count, which fell under equivalence category I.

### 3.4.6 | Compositional analysis

Soybean MON 94637 seeds and forage harvested from eight sites (Table 2) were analysed for 76 constituents (seven in forage and 69 in seeds), including those recommended by OECD (2012). The statistical analysis was not applied to 11 seed constituents because their concentration in more than half of the samples was below the limit of quantification.[17]

[12]Warm temperature condition corresponds to around 25°C for 7 days and cold temperature to around 10°C for 7 days followed by 7 days at around 25°C.

[13]Non-GM reference varieties were: Stone 3915C; LG Seeds C3400; Specialty 3352C and Asgrow A3253.

[14]GM soybean showed a mean germination of 100% and 99.7% while the conventional counterpart showed a mean of 99.7% and 99.8% under warm and cold temperature conditions respectively.

[15]Soil types of the field trials were silt loam, silty clay loam and loam; soil organic matter ranged from 1.1% to 2.5%; pH ranged from 5.8 to 7.2; average temperatures and sums of precipitation during the usual crop growing season ranged, respectively, from 19.7 to 23.7°C and from 373 to 563 mm.

[16]Early stand count, days to flowering, days to maturity, plant height, lodging, fruit count, final stand count, moisture, seed weight and yield.

[17]Caprylic acid (C8: 0), capric acid (C10:0), lauric acid (C12:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), heptadecenoic acid (C17:1), γ-linolenic acid (C18:3), eicosadienoic acid (C20:2), eicosatrienoic acid (C20:3), arachidonic acid (C20:4).

The statistical analysis was applied to a total of 65 constituents (58 in seeds[18] and seven in forage)[19]; a summary of the outcome of the test of difference and the test of equivalence is presented in Table 3.

For soybean MON 94637, statistically significant differences with the conventional counterpart were found for eight endpoints (three in forage and five in seeds). All these endpoints fell under equivalence category I or II.

**TABLE 3** Outcome of the comparative compositional analysis in seeds and forage for soybean MON 94637. The table shows the number of endpoints in each category.

| | | Test of difference[a] | |
|---|---|---|---|
| | | **Not different** | **Significantly different** |
| **Test of equivalence**[b] | Category I/II | 52 | 8[c] |
| | Category III/IV | – | – |
| | Not categorised | 5[d] | – |
| | Total endpoints | 65 | |

[a]Comparison between soybean MON 94637 and its conventional counterpart.

[b]Four different outcomes: category I (indicating full equivalence to the non-GM reference varieties); category II (equivalence is more likely than non-equivalence); category III (non-equivalence is more likely than equivalence); and category IV (indicating non-equivalence). Not categorised means that the test of equivalence was not applied because of the lack of variation among the non-GM reference varieties.

[c]Endpoints with significant differences between soybean MON 94637 and its conventional counterpart falling in equivalence category I–II. For seeds: total fat, lysine, palmitoleic acid (C16:1), behenic acid (22:0), trypsin inhibitor; for forage: moisture, carbohydrates and protein.

[d]Endpoints not categorised for equivalence and without significant differences between the soybean MON 94637 and its conventional counterpart. For seeds: methionine, phenylalanine, soybean lectin and stachyose; for forage: NDF.

The GMO Panel assessed all the significant differences between the soybean MON 94637 and the conventional counterpart, taking into account the potential impact on plant metabolism and the natural variability observed for the set of non-GM reference varieties. No endpoints with outcomes under category III/IV were identified.

### 3.4.7 | Conclusion on comparative analysis

Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic–phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis.

Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that:

- None of the differences identified in agronomic and phenotypic characteristics between soybean MON 94637 and the conventional counterpart needs further assessment.
- None of the differences identified in forage and seed composition between the soybean MON 94637 and the conventional counterpart needs further assessment regarding food and feed safety.

## 3.5 | Food/feed safety assessment[20]

### 3.5.1 | Overview of overarching information for food and feed assessment

#### 3.5.1.1 | *Compositional analysis*

The compositional analysis of soybean MON 94637 and the conventional counterpart provided by the applicant and assessed by the GMO Panel is described in Section 3.4.6.

[18]Proximates and fibre content (ash, carbohydrates, moisture, protein, total fat, acid detergent fibre (ADF) and neutral detergent fibre (NDF)), minerals (calcium and phosphorus), amino acids (alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine), fatty acids (myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), and behenic acid (22:0)), vitamins (α-tocopherol, and phylloquinone), isoflavones (daidzein, genistein and glycitein), endogenous allergens (gly m 1, gly m 3, gly m 4, gly m 5 (β-conglycinin), gly m 6 (glycinin), gly m 7, gly m 8, gly m Bd 28k, gly m Bd 30k, Kunitz trypsin inhibitor), other compounds (phytic acid, raffinose, soybean lectin, stachyose, trypsin inhibitor).

[19]Moisture, protein, total fat, ash, carbohydrates, acid detergent fibre (ADF) and neutral detergent fibre (NDF).

[20]Dossier: Part II – Sections 1.4, 1.5, 1.6, 2, 3 and 4; additional information: 5/6/2024, 29/7/2024, 1/10/2024, 26/11/2024, 23/1/2025, 10/6/2025.

#### 3.5.1.2 | *Newly expressed proteins*

Two proteins, Cry1A.2 and Cry1B.2, are newly expressed in soybean MON 94637. The Cry1A.2 and Cry1B.2 proteins have not been previously assessed by the GMO Panel.

##### 3.5.1.2.1 | *Molecular characterisation*

The protein characterisation of the newly expressed Cry1A.2 and Cry1B.2 proteins provided by the applicant and assessed by the GMO Panel is described in Section 3.3.3. Furthermore, the equivalence between the soybean MON 94637 and the *B. thuringiensis*-derived proteins was demonstrated.

##### 3.5.1.2.2 | *History of safe use for consumption as food and feed of the NEPs*

a. Information on the source organism

The Cry1A.2 and Cry1B.2 proteins gene source organism is an environmentally ubiquitous bacterium (*B. thuringiensis*) and has been reported to protect plants by producing Bt toxins that inhibit insect and nematode growth. Furthermore, Bt microbials are used as sprayed pesticide for pest control in agriculture.

b. Information on structure, function and mode of action

The insecticidal proteins Cry1A.2 and Cry1B.2 confer protection against certain susceptible lepidopteran pests when expressed in plants by causing disruption of the midgut epithelium. It is reported that the gastrointestinal tract of mammals, including humans, lacks receptors with high specific affinity for Cry proteins (Hammond et al., 2013; Jurat-Fuentes & Crickmore, 2017; Koch et al., 2015).

c. Information on identity/homology of NEPs to other proteins/constituents in conventional food and feed sources

The GMO Panel is not aware of any information on identity/homology of Cry1A.2 and Cry1B.2 to other proteins in conventional food and feed sources.

d. Overall conclusion of the history of safe use

The GMO Panel considers the above information not sufficient to duly document the history of safe use for consumption of the Cry1A.2 and Cry1B.2 proteins.

##### 3.5.1.2.3 | *Stability of the NEPs*

Protein stability is one of several relevant parameters to consider in the weight-of-evidence approach in protein safety assessment (EFSA GMO Panel, 2010c, 2011a, 2017, 2021). The term protein stability encompasses several properties such as thermal stability, pH-dependent stability, proteolytic stability and physical stability (e.g. tendency to aggregate), among others (Li et al., 2019). It has been shown that when characteristics of known food allergens are examined, a relevant trait attributed to food allergens is protein stability (Breiteneder & Mills, 2005; Costa et al., 2021; Foo & Müller, 2021; Helm, 2001).

a. Effect of temperature and pH on NEPs

The applicant provided experimental studies on the effects of temperature on the Cry1A.2 and Cry1B.2 proteins as expressed in soybean MON 94637, using a microbial recombinant system. Independent samples of the Cry1A.2 and Cry1B.2 proteins were incubated for 15 or 30 min at 25, 37, 55, 75 and 95°C followed by SDS-PAGE or by a bioassay measuring their functional activity. No functional activity of the Cry1A.2 and Cry1B.2 proteins was observed at temperatures of 75°C or above. In relation to the effect of pH on the Cry1A.2 and Cry1B.2 proteins, the molecular mass and immunoreactivity of the proteins were unchanged at pH 1.2 and 7.

b. In vitro protein degradation by proteolytic enzymes

The applicant provided independent studies on in vitro protein degradation (i.e. resistance to pepsin in solutions at pH ~1.2) of the Cry1A.2 and Cry1B.2 proteins from microbial recombinant systems. The integrity of the test proteins in samples of the incubation mixture taken at various time points was analysed by SDS-PAGE followed by protein staining or by western blotting. The Cry1A.2 and Cry1B.2 proteins were degraded by pepsin within 0.5 min of incubation. Transient peptide fragments of low molecular weight, i.e. ~3.5 and 6 kDa in the case of Cry1A.2 and of 4 kDa for Cry1B.2, were observed at different time points by SDS-PAGE. Furthermore, the applicant provided additional studies where the Cry1A.2 and Cry1B.2 proteins were subjected to a sequential digestion, pepsin followed by pancreatin. The transient peptide fragments seen

after 2 min in the pepsin analysis were degraded within 0.5 min of exposure to pancreatin when analysed by SDS-PAGE. The sequential addition of digestive enzymes – gastric digestion conditions followed by an intestinal in vitro digestion – has been proposed as part of several alternative protocols to the classical pepsin resistance test to simulate more closely, within the inherent limitations of in vitro models, the physiological conditions of gastrointestinal digestion (EFSA GMO Panel, 2021, 2022). This is in line with Codex Alimentarius which indicates that alternative in vitro digestion protocols may be used, where adequate justification is provided (Codex Alimentarius, 2009).

#### 3.5.1.2.4 | *Synergistic and antagonistic interactions among the NEPs*

The potential for a functional interaction among the Cry1A.2 and Cry1B.2 proteins has been assessed with regard to human and animal health. Based on current scientific knowledge on the biological function of the two proteins (Section 3.5.1.2.2), no synergistic or antagonistic interactions between these two proteins are expected that could raise safety concerns for food and feed from soybean MON 94637.

#### 3.5.1.3 | *Effects of processing*

Soybean MON 94637 will undergo existing production processes used for conventional soybean. No novel production process is envisaged. Based on the outcome of the comparative assessment, processing of the GM crop into food and feed products is not expected to result in products being different from those of conventional non-GM reference varieties currently in the EU market.[21]

### 3.5.2 | Toxicology assessment

The strategies to assess the toxicological impact of any changes on the GM soybean MON 94637 food and feed resulting from the genetic modification focus on the assessment of (i) NEPs; (ii) new constituents other than NEPs; (iii) altered levels of food and feed constituents; and (iv) the whole genetically modified food and feed.

#### 3.5.2.1 | *Assessment of NEPs*

Two proteins (Cry1A.2 and Cry1B.2) are newly expressed in soybean MON 94637.

#### 3.5.2.1.1 | *NEP never assessed before*

A weight-of-evidence approach was followed by the GMO Panel to assess the toxicological profile of the newly expressed Cry1A.2 and Cry1B.2 proteins, taking into account all of the information relevant for their hazard assessment, including molecular characterisation, substrate specificity, history of safe use for consumption as food and feed of the NEPs, stability of the NEPs and synergistic or antagonistic interactions (Section 3.5.1.2), updated bioinformatic analyses for similarity to toxins and in vivo toxicity studies.

#### 3.5.2.1.2 | *Bioinformatic analyses*

Updated bioinformatics analyses of the amino acid sequence of the Cry1A.2 and Cry1B.2 proteins revealed no significant similarities to known toxins (Section 3.3.2).

#### 3.5.2.1.3 | *In vivo toxicity studies*

For the assessment of the Cry1A.2 and Cry1B.2 proteins, the applicant provided a 28-day toxicity study with each protein. The outcome of the in vivo toxicity studies with the Cry1A.2 and Cry1B.2 proteins is described below.

*28-day repeated dose toxicity study with Cry1A.2 protein*

The 28-day repeated dose toxicity study in mice with the Cry1A.2 protein was conducted in accordance with OECD TG 407 (2008) and to the principles of good laboratory practice (GLP).

Groups of Crl:CD-1 mice (20/sex per group), 9- to 10-week-old at the start of dosing, were allocated to five groups. Groups were administered by gavage: the test substance (Cry1A.2 protein) at targeted nominal doses of 1000, 100 or 10 mg/kg body weight (bw) per day (high, medium and low Cry1A.2 protein groups); 1000 mg/kg bw per day of bovine serum albumin (BSA control group) and the vehicle.

[21]On-going assessment of novel food applications (https://food.ec.europa.eu/food-safety/novel-food/authorisations/summary-applications-and-notifications_en).

Mice were randomised to treatment groups (males and females separately) using a stratified randomisation block designed to achieve similar group mean body weights (± 20% of the mean for each sex). The GMO Panel notes that animals were housed individually.

The test substance used in this study was produced by a recombinant system and reported as 91% pure. The amino acid sequence analysis of the *Bacillus thuringiensis*-produced Cry1A.2 used in this 28-day toxicity study by mass spectrum fingerprint analysis matched the deduced sequence as defined by the *cry1A.2* gene. This protein had the expected molecular weight and immunoreactivity to Cry1A.2-specific antibodies, was not glycosylated and showed functional activity.

In-life procedures and observations and terminal procedures were conducted in accordance with OECD TG 407 (2008).

Deviations to the protocol reported in the study were considered minor deviations with no impact on the study results.

An appropriate range of statistical tests was performed on the results of the study and a detailed description of the methodology and of statistically significant findings identified in mice is reported in Appendix A.

There were no Cry1A.2-related incidents of mortality or clinical signs. Four deaths occurred during the study – 2 high-dose males (on day 1 and day 4; replacement animals added to the group) with findings consistent with gavage error; one low-dose male and a control female. No Cry1A.2-related adverse findings were identified in any of the investigated parameters. No Cry1A.2-related clinical observations or ophthalmology findings were seen. A small number of statistically significant findings were noted, but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[22] for the parameter in mice of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;
- exhibited no consistent pattern with related parameters or endpoints;
- exhibited no consistency with increasing dose levels.

No gross pathology findings related to the administration of the test item were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence or severity of the histopathology findings related to the administration of the test item compared to the vehicle group.

The GMO Panel concludes that no adverse effects were observed in mice in this 28-day toxicity study on *Bacillus thuringiensis* produced Cry1A.2 protein, at 10 mg/kg bw per day up to 1000 mg/kg bw per day.

*28-day repeated dose toxicity study with Cry1B.2 protein*

The 28-day repeated dose toxicity study in mice with the Cry1B.2 protein was conducted in accordance with OECD TG 407 (2008) and to the principles of good laboratory practice (GLP).

Groups of Crl:CD-1 mice (20/sex per group), 9- to 10-week-old at the start of dosing were allocated to five groups. Groups were administered by gavage: the test substance (Cry1B.2 protein) at targeted nominal doses of 1000, 100 or 10 mg/kg body weight (bw) per day (high, medium and low Cry1B.2 protein groups); 1000 mg/kg bw per day of bovine serum albumin (BSA control group) and the vehicle.

Mice were randomised to treatment groups (males and females separately) using a stratified randomisation block designed to achieve similar group mean body weights (± 20% of the mean for each sex). The GMO Panel notes that animals were housed individually.

The test substance used in this study was produced by a recombinant system and reported as 100% pure. The amino acid sequence analysis of the *Bacillus thuringiensis* produced Cry1B.2 used in this 28-day toxicity study by mass spectrum fingerprint analysis matched the deduced sequence as defined by the *cry1B.2* gene. This protein had the expected molecular weight and immunoreactivity to Cry1B.2-specific antibodies, was not glycosylated and showed functional activity.

In-life procedures and observations and terminal procedures were conducted in accordance with OECD TG 407 (2008).

Deviations to the protocol reported in the study were considered minor deviations with no impact on the study results.

An appropriate range of statistical tests was performed on the results of the study, and a detailed description of the methodology and of statistically significant findings identified in mice is reported in Appendix A.

There were no Cry1B.2-related incidents of mortality or clinical signs. No Cry1B.2-related adverse findings were identified in any of the investigated parameters. No Cry1B.2-related clinical observations or ophthalmology findings were seen. A small number of statistically significant findings were noted, but these were not considered adverse effects of treatment for one or more of the following reasons:

- were within the normal variation[22] for the parameter in mice of this age;
- were of small magnitude;
- were identified at only a small number of time intervals with no impact on the overall value;

[22]Although animals used in a toxicology study are of the same strain, from the same supplier and are closely matched for age and body weight at the start of the study, they exhibit a degree of variability in the parameters investigated during the study. This variability is evident even within control groups. To help reach a conclusion on whether a statistically significant finding in a test group is treatment-related, account is taken of whether the result in the test group is outside the normal range for untreated animals of the same strain and age. To do this, a number of sources of information are considered, including the standardised effect size, the standard deviations and range of values within test and control groups in the study and, if applicable, data from other studies performed in the same test facility within a small timeframe and under almost identical conditions (Historic Control Data).

– exhibited no consistent pattern with related parameters or end points;
– exhibited no consistency with increasing dose levels.

No gross pathology findings related to the administration of the test item were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence or severity of the histopathology findings related to the administration of the test item compared to the vehicle group.

The GMO Panel concludes that no adverse effects were observed in mice in this 28-day toxicity study on *Bacillus thuringiensis* produced Cry1B.2 protein, at 10 mg/kg bw per day up to 1000 mg/kg bw per day.

*Overall conclusion of the toxicological assessment of the NEPs*

Based on the above information, the GMO Panel did not find any indication that the Cry1A.2 and Cry1B.2 proteins raise food and feed safety concerns in humans, farmed and companion animals.

#### 3.5.2.2 | *Assessment of new constituents other than NEPs*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, no new constituents other than the NEPs have been identified in seeds and forage from soybean MON 94637. Therefore, no further food and feed safety assessment of components other than the NEPs is required.

#### 3.5.2.3 | *Information on altered levels of food and feed constituents*

Based on the outcome of the studies considered in the comparative analysis and molecular characterisation, none of the differences identified between soybean MON 94637 and its conventional counterpart in seeds and forage composition requires further assessment.

#### 3.5.2.4 | *Assessment of the whole genetically modified food and feed*

Based on the outcome of the molecular characterisation, toxicological and comparative analysis assessment, no compositional modifications or indications of possible unintended effects relevant to food and feed safety have been identified for soybean MON 94637. Therefore, animal feeding studies with food/feed derived from soybean MON 94637 are not considered necessary by the GMO Panel (EFSA GMO Panel, 2011a). In accordance with Regulation (EU) No 503/2013, the applicant provided a 90-day feeding study in rats fed with diets containing meal (toasted and defatted) derived from soybean MON 94637.

In this study, pair-housed Crl:CD(SD) rats (16 per sex per group; 2 rats per cage) were allocated to three groups using a randomised complete block design with eight replications per sex.

Groups were fed diets containing soybean MON 94637 meal at 30% and 15% of inclusion level (the latter supplemented with 15% of the conventional counterpart) and the conventional counterpart (inclusion level 30%).

The study was adapted from OECD test guideline 408 (OECD, 2018), aligned with EFSA Scientific Committee guidance (EFSA Scientific Committee, 2011) and EFSA Explanatory statement (EFSA, 2014) and complied with the principles of GLP with some minor deviations described in the study report, not impacting the study results and interpretation.

The stability of the test and control materials was not verified; however, in accordance with product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the treatment. The GMO Panel considers this justification acceptable. Diet preparation procedures and regular evaluations of the mixing methods guaranteed the homogeneity and the proper concentration of the test or control substances in them.

Event-specific PCR analysis confirmed the presence of the event in both the GM seeds and diets and excluded the presence of the event in the respective controls. Both the GM and control seeds and diets were analysed for nutrients, antinutrients and potential contaminants. Balanced diets were formulated based on the specifications for LabDiet® Certified Rodent Diet 5002. Feed and water were provided ad libitum. In-life procedures and observations and terminal procedures were conducted in accordance with OECD TG 408 (2018).

An appropriate range of statistical tests was performed on the results of the study. A detailed description of the methodology and of statistically significant findings identified in rats given diets containing meals derived from soybean MON 94637 is reported in Appendix A.

There were no test diet-related incidents of mortality or clinical signs. No test diet-related adverse findings were identified in any of the investigated parameters. A small number of statistically significant findings were noted, but these were not considered adverse effects of treatment for one or more of the following reasons:

– were within the normal variation[22] for the parameter in rats of this age;
– were of small magnitude;
– were identified at only a small number of time intervals with no impact on the overall value;
– exhibited no consistent pattern with related parameters or endpoints;
– exhibited no consistency with increasing incorporation levels.

No gross pathology findings related to the administration of the test diet were observed at necropsy, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence or severity of the histopathological findings related to the administration of the test diet compared to the control group.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no treatment-related adverse effects were observed in rats after feeding diets containing soybean MON 94637 meals at 30% or 15% for 90 days.

### 3.5.3 | Allergenicity

The strategies to assess the potential risk of allergenicity focus: (i) on the source of the recombinant protein; (ii) on the potential of the NEP to induce sensitisation or to elicit allergic reactions in already sensitised persons; and (iii) on whether the transformation may have altered the allergenic properties of the modified plant. Furthermore, the assessment also takes into account potential adjuvant properties of the NEPs, which is defined as the ability to enhance an allergic reaction.

#### 3.5.3.1 | *Assessment of allergenicity of the NEPs*

A weight-of-evidence approach was followed, taking into account all of the information obtained on the NEP, as no single piece of information or experimental method yielded sufficient evidence to predict allergenicity (Codex Alimentarius, 2009; EFSA GMO Panel, 2011a, 2017; Regulation (EU) No 503/2013).

The *cry1A.2* and *cry1B.2* genes are derived from *B. thuringensis* (see Section 3.3.1), which is not considered a common allergenic source.

Updated bioinformatic analyses of the amino acid sequences of the Cry1A.2 and Cry1B.2 proteins, using the criterion of 35% identity in a sliding window of 80 amino acids, revealed no significant similarities to known allergens.

The studies on protein stability of the Cry1A.2 and Cry1B.2 proteins have been described in Section 3.5.1.2.3. In addition, the GMO Panel did not find an indication that the newly expressed Cry1A.2 and Cry1B.2 proteins at the levels expressed in soybean MON 94637 might be adjuvants.

Furthermore, the applicant provided information on the safety of the Cry1A.2 and Cry1B.2 proteins regarding their potential hazard to cause a coeliac disease response.[23] For such assessment, the applicant followed the principles described in the EFSA GMO Panel guidance document (EFSA GMO Panel, 2017). The assessment of the Cry1A.2 and Cry1B.2 proteins identified no perfect or relevant partial matches with known coeliac disease peptide sequences.

In the context of this application, the GMO Panel considers that there are no indications that the newly expressed Cry1A.2 and/or Cry1B.2 proteins in soybean MON 94637 may be allergenic.

#### 3.5.3.2 | *Assessment of allergenicity of the whole GM plant or crop*

Soybean is considered a common allergenic food (OECD, 2012).[24] Therefore, any potential change in the endogenous allergenicity of the GM plant should be assessed (Regulation (EU) No 503/2013). For such assessment, the applicant included in the comparative analysis specific allergens relevant for soybean (Section 3.4.6) quantified using liquid chromatography with tandem MS, which has been previously considered acceptable (EFSA GMO Panel, 2010c, 2017; Fernandez et al., 2013; Selb et al., 2017). These allergens were selected based on the list of potential soybean allergens described in the pertinent OECD document (OECD, 2012), and a scientific rational supporting their selection was provided by the applicant and considered acceptable by the GMO Panel. No changes in the levels of endogenous allergens raising concern are identified by the GMO Panel.

In the context of this application, the GMO Panel considers that there is no evidence that the genetic modification might substantially change the overall allergenicity of soybean MON 94637 when compared with that of the conventional counterpart and the non-GM reference varieties tested.

### 3.5.4 | Dietary exposure assessment to new constituents

In line with Regulation (EU) No 503/2013, the applicant provided dietary exposure estimates to Cry1A.2 and Cry1B.2 proteins newly expressed in soybean MON 94637. Dietary exposure was estimated based on protein expression levels reported in this application for soybean MON 94637, the currently available consumption data and feed practices, the foods and feeds currently available on the market, and the described processing conditions.

For estimating dietary exposure, the levels of the NEPs in soybean MON 94637 seeds, forage and flowers[25] were derived from material harvested in a field trial across five locations in the United States during the 2021 growing season (Table 1, Section 3.3.4).

[23]Dossier: Part II – section 2, additional information 10/06/2025.

[24]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004.

[25]Flowers (growth stage BBCH 60–65) were used as surrogates of pollen for the analysis of the NEPs in soybean MON 94637.

#### 3.5.4.1 | *Human dietary exposure*

Chronic and acute estimations of dietary exposure to Cry1A.2 and Cry1B.2 proteins newly expressed in soybean MON 94637 were provided. The applicant followed the methodology described in the EFSA Statement 'Human dietary exposure assessment to newly expressed protein in GM foods' to anticipate human dietary exposure making use of summary statistics of consumption (EFSA, 2019a).

Human dietary exposure was estimated across European countries on different population groups: young population (infants, toddlers, 'other children'), adolescents, adult population (adults, elderly and very elderly) and special populations (pregnant and lactating women). Since no specific consumption data were available on commodities containing, consisting of or obtained from soybean MON 94637 seeds, a conservative scenario with 100% replacement of conventional soybean by the GM soybean was considered. Consumption figures for all relevant commodities (e.g. soya bread, textured soy protein, soya drink, tofu, etc.) were retrieved from the EFSA Comprehensive European Food Consumption Database.[26]

Mean protein expression values on fresh weight basis are considered as the most adequate to estimate human dietary exposure (both acute and chronic) when working with raw primary commodities that are commonly consumed as processed blended commodities (EFSA, 2019a). Different recipes and factors were considered to estimate the amount of soybean seeds in the consumed commodities before assigning NEP levels to the relevant commodities.[27] No losses in the NEPs during processing were considered.

The highest anticipated acute dietary exposure (highly exposed population) was in the age class 'infants' with estimates between 151.5 μg/kg bw per day for Cry1B.2 and 289.3 μg/kg bw per day for Cry1A.2. The main contributor to the exposure in the dietary survey with the highest estimates would be 'Follow-on formula, soya-based, powder'. In the dietary exposure scenario anticipating acute consumption of soybean-derived protein supplements, the highest estimates would range between 190.5 μg/kg bw per day for Cry1B.2 and 363.6 μg/kg bw per day for Cry1A.2 proteins (in the adult population).

The highest anticipated chronic dietary exposure (highly exposed population) was in the age class 'Infants' with estimates between 50.9 μg/kg bw per day for Cry1B.2 and 97.2 μg/kg bw per day for Cry1A.2. The main contributor to the exposure in the dietary survey with the highest estimates would be 'Follow-on formula, soya-based, powder'. In the dietary exposure scenario anticipating chronic consumption of soybean-derived protein supplements, the highest estimates would range between 123.8 μg/kg bw per day for Cry1B.2 and 236.4 μg/kg bw per day for Cry1A.2 proteins (in the adult population).

The GMO Panel considers pollen supplements as a possible contributor to the dietary exposure to Cry1A.2 and Cry1B.2, under the assumption that these supplements might be made of pollen from soybean MON 94637. Due to the technical challenges related to the collection of pollen from soybean flowers (e.g. close flowers, low amounts produced), the applicant analysed the presence of the NEPs in flowers as pollen surrogates (see Table 1). Following EFSA's request, detailed information was provided on how the flowers were collected and the parts included (sepals, petals, pistils and stamens).[28] The GMO Panel acknowledges the substantial uncertainty of the use of flowers as surrogates of pollen adds to the expression levels and, therefore, to the dietary exposure assessment. However, in the absence of identified hazards posed by the NEPs, these expression levels are accepted as surrogates.

Consumption data on pollen supplements are available for few consumers across seven different European countries.[26] The low number of consumers available adds uncertainty to the exposure estimations which should be interpreted with care and only allows the estimation of dietary exposure for average consumers. The highest mean acute dietary exposure would be between 125.4 μg/kg bw per day for Cry1B.2 and 181.2 μg/kg bw per day for Cry1A.2 proteins, in the elderly population. Similarly, the highest mean chronic dietary exposure in consumers of pollen supplements would be between 83.6 μg/kg bw per day for Cry1B.2 and 120.8 μg/kg bw per day for Cry1A.2 proteins, also in the elderly population.

#### 3.5.4.2 | *Animal dietary exposure*

Anticipated dietary exposure to Cry1A.2 and Cry1B.2 proteins in soybean MON 94637 was estimated across farmed and companion animals, assuming the consumption of soybean products commonly entering the feed supply chain, as below described.

Estimations were based on the recommendations provided in EFSA GMO Panel (2023).

A conservative scenario with 100% replacement of conventional soybean products by the soybean MON 94637 products was considered.

Mean levels (dry weight) of the NEPs in seeds and forage/silage from the soybean MON 94637 used for animal dietary exposure are listed in Table 1.

Mean levels (dry weight) of the NEPs in meal (49%), hull and protein concentrate (70%–90%) were calculated to be, respectively, 1.32, 0.34 and 2.07-fold higher than in seeds, based on conversion factors that take into account the protein content in these feed materials relative to soybean seeds and assuming that no protein is lost during their production or processing. Values are reported in tables of Appendix B.

[26] https://www.efsa.europa.eu/en/applications/gmo/tools. From version updated in March 2022.

[27] Example: 100 grams of tofu contains approximately 26 g of soybean seeds. This results in ~5.5 μg of Cry 1A.2 per gram of tofu as compared to the 21 μg/g (fw) reported as Cry 1A.2 mean concentration in soybean seeds (see Table 1; Section 3.3.4).

[28] Additional information 21/05/2025.

The applicant estimated dietary exposure in ruminants (dairy cow, beef cattle, dairy sheep and dairy goat) and other herbivorous animals (rabbit and horse), in pigs (fattening pig, lactating sow, piglet), in poultry (broiler, laying hens, turkey), in fish (salmon, carp) and in companion animals (cat and dog).

The dietary exposure to Cry1A.2 and Cry1B.2 proteins was estimated via the consumption of soybean forage/silage, full-fat seeds, meal, hulls and/or protein concentrate in standard diets or rations.

Estimated dietary exposure in the concerned animals is reported in Appendix B.

### 3.5.5 | Nutritional assessment of endogenous constituents

The intended trait of soybean MON 94637 is resistance against certain lepidopteran insect pests, with no intention to alter nutritional parameters. Comparison of the composition of the soybean MON 94637 with its conventional counterpart and the non-GM reference varieties did not identify differences that would require further safety assessment. From these data, the GMO Panel concludes that soybean MON 94637 is nutritionally equivalent to its conventional counterpart and the non-GM reference varieties used.

### 3.5.6 | Post-market monitoring of GM food/feed

Soybean MON 94637, as described in this application, does not raise any nutritional concern and is as safe as its conventional counterpart and the non-GM reference varieties tested. The GMO Panel concludes that based on the information considered in its safety assessment, a post-market monitoring plan for food and feed is not necessary.

### 3.5.7 | Conclusions on the food/feed safety assessment

The newly expressed Cry1A.2 and Cry1B.2 proteins in soybean MON 94637 do not raise safety concerns for human and animal health. No interactions between newly expressed Cry1A.2 and Cry1B.2 proteins relevant for food and feed safety were identified. Moreover, the GMO Panel does not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed Cry1A.2 and Cry1B.2. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of soybean MON 94637, as food and feed. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of soybean MON 94637 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that soybean MON 94637, as described in this application, is as safe as the conventional counterpart and the non-GM reference varieties tested, and no post-market monitoring of food/feed is considered necessary.

## 3.6 | Environmental risk assessment and monitoring plan[29]

### 3.6.1 | Environmental risk assessment

Considering the scope of this application, which excludes cultivation, the environmental risk assessment (ERA) mainly takes into account: (1) the exposure of microorganisms to recombinant DNA in the gastrointestinal tract of animals fed with GM material and of microorganisms present in environments exposed to faecal material of these animals (manure and faeces); and (2) the deliberate or accidental release into the environment of GM material, including spillage of viable soybean MON 94637 grains during transportation and/or processing (EFSA GMO Panel, 2010a).

#### 3.6.1.1 | *Persistence and invasiveness of the GM plant*

Cultivated soybean (*Glycine max* (L.) Merr.) is a species in the subgenus *Soja* of the genus *Glycine*. The species originated from eastern Asia and is a highly domesticated crop, generally unable to survive in the environment without proper management (Lu, 2005).

Occasional feral GM soybean plants may occur outside cultivation areas, but survival is limited mainly by a combination of low competitiveness, absence of a dormancy phase and susceptibility to plant pathogens (OECD, 2000). Additionally, soybean is a sub-tropical species susceptible to cold climatic conditions (Bramlage et al., 1978; Staniak et al., 2021; Szczerba et al., 2021; Tyagi & Tripathi, 1983), although cold tolerance varies across maturity groups and among cultivars (Alsajri et al., 2019; Wang et al., 2023). Soybean can grow as volunteers, and the presence of volunteers of *G. max* was occasionally reported in some areas of Italy where soybean is intensively cultivated (Celesti-Grapow et al., 2010). However, as for the same reasons mentioned above, soybean seeds usually do not survive during cold winters (Matsushita et al., 2020;

[29]Dossier: Part II – Section 5; additional information: 5/6/2024, 2/10/2024.

Owen, 2005), and any soybean volunteers can be effectively controlled by mechanical methods or appropriate chemical control (Bond & Walker, 2009; Jhala et al., 2013; Soltani et al., 2019). Owing to this, soybean plants are often not considered a problematic volunteer in temperate climates (Jhala et al., 2021). Thus, the establishment and survival of feral and volunteer soybean in the EU is currently limited and transient.

It is unlikely that the intended trait of soybean MON 94637 will provide a selective advantage to soybean plants, except when they are infested by insect pests that are susceptible to the Cry1A.2 and/or Cry1B.2 proteins. However, if this was to occur, this fitness advantage will not allow the GM plant to overcome other biological and abiotic factors (described above) limiting the plant's persistence and invasiveness. Therefore, the presence of the intended trait will not affect the persistence and invasiveness of the GM plant.

In conclusion, the GMO Panel considers that it is very unlikely that soybean MON 94637 will differ from conventional soybean varieties in their ability to survive until subsequent seasons, or to establish occasional feral plants under European environmental conditions in case of accidental release into the environment of viable soybean MON 94637 seeds.

#### 3.6.1.2 | *Potential for gene transfer*

A prerequisite for any gene transfer is the availability of pathways for the transfer of genetic material, either through horizontal gene transfer (HGT) of DNA or through vertical gene flow via cross-pollination from feral plants originating from spilled grains.

##### *Plant-to-microorganism gene transfer*

Genomic DNA can be a component of food and feed products derived from soybean. It is well documented that such DNA becomes substantially degraded during processing and digestion in the human or animal gastrointestinal tract. However, bacteria in the digestive tract of humans and animals, and in other environments, may be exposed to fragments of DNA, including the recombinant fraction of such DNA.

Current scientific knowledge of recombination processes in bacteria suggests that horizontal transfer of non-mobile, chromosomally located DNA fragments between unrelated organisms (such as from plants to bacteria) is not likely to occur at detectable frequencies under natural conditions (for further details, see EFSA, 2009).

Homologous recombination is known to facilitate horizontal transfer of non-mobile, chromosomal DNA fragments to bacterial genomes. This requires the presence of at least two stretches of DNA sequences that are similar in the recombining DNA molecules. In the case of sequence identity with the transgene itself, recombination would result in gene replacement. In the case of identity with two or more regions flanking recombinant DNA, recombination could result in the insertion of additional DNA sequences in bacteria and thus confer the potential for new properties.

In addition to homology-based recombination processes, at a lower transformation rate, the non-homologous end-joining and microhomology-mediated end joining are theoretically possible (EFSA, 2009; Hülter & Wackernagel, 2008). Independently of the transfer mechanism, the GMO Panel did not identify a selective advantage that a theoretical HGT would provide to bacterial recipients in the environment.

Bioinformatic analysis of event MON 94637 revealed that there are no elements providing sufficient similarity to bacterial DNA which would facilitate homologous recombination including the sequences of bacterial origin encoding for Cry1A.2 and Cry1B.2 proteins that were all plant codon-optimised (see Section 3.3.1).

In summary, there is no indication of an increased likelihood of horizontal transfer of DNA from soybean MON 94637 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

##### *Plant-to-plant gene transfer*

The potential for occasional feral soybean MON 94637 plants originating from seed import spills to transfer recombinant DNA to sexually compatible plants and the environmental consequences of this transfer was considered.

For plant-to-plant gene transfer to occur, imported GM soybean seeds need to germinate and develop into plants in areas containing sympatric wild relatives and/or cultivated soybean with synchronous flowering and environmental conditions favouring cross-pollination. It must be noted that most soybean MON 94637 seeds are processed in the countries of production or in ports of importation.

Vertical gene transfer from soybean (*G. max*) is limited to the species of the subgenus *Soja* to which *G. max* belongs, as well as the wild relatives *G. soja* and *G. gracilis* (Zhang et al., 2023). Although wild relatives exist elsewhere, no wild relatives of the subgenus *Soja* have been reported in Europe so far (Dorokhov et al., 2004; Lu, 2005). Therefore, vertical gene transfer from GM soybean is restricted to cultivated soybean (*G. max*).

Soybean is an annual, almost completely self-pollinating crop with a percentage of cross-pollination usually below 1% (Abud et al., 2007; Lu, 2005; OECD, 2000; Ray et al., 2003; Yoshimura et al., 2006), although natural cross-pollination rates can fluctuate significantly among different soybean varieties under particular environmental conditions, such as favourable climate for pollination and an abundance of pollinators (Ahrent & Caviness, 1994; Caviness, 1966; Gumisiriza & Rubaihayo, 1978; Kikuchi et al., 1993; Lu, 2005; Ray et al., 2003).

The potential of spilled soybean seeds to establish, grow and produce pollen is extremely low and transient (see Section 3.6.1.1). Therefore, the likelihood/frequency of cross-pollination between occasional feral GM soybean plants resulting from seed spillage and weedy or cultivated soybean plants is also considered extremely low. Even if cross-pollination would occur, the GMO Panel is of the opinion that the likelihood of environmental effects as a consequence of the spread of genes from occasional feral GM soybean plants in Europe will not differ from that of conventional soybean varieties for the reasons given in Section 3.6.1.1.

#### 3.6.1.3 | *Interactions of the GM plant with target organisms*

Taking the scope of application GMFF-2023-21116 into account (no cultivation), potential interactions of occasional feral soybean MON 94637 plants arising from seed import spills with the target organisms are not considered a relevant issue.

#### 3.6.1.4 | *Interactions of the GM plant with non-target organisms*

The environmental risk assessment considers potential effects of the GM plant on populations of non-target organisms, defined as all those species directly or indirectly exposed to the GM plant and which are not targets of the newly expressed metabolite(s) it expresses. The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure of non-target organisms to spilled GM soybean material or occasional feral GM soybean plants arising from spilled soybean MON 94637 seeds will be limited. Additionally, ingested proteins are typically degraded before entering the environment through manure and faeces of animals fed with GM soybean (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2.3) supports that the NEPs will also be degraded. As compared to non-GM soybean, the GMO Panel considers that potential interactions of soybean MON 94637 with non-target organisms do not raise any safety concerns. Interactions that may occur between the insecticidal proteins will not alter this conclusion.

#### 3.6.1.5 | *Interactions with biogeochemical cycles*

Biogeochemical cycles encompass the microbiologically mediated movement, transformation and storage of carbon, nitrogen and other compounds in the soil that are considered here for the receiving environments. The GMO Panel evaluated the potential hazards of the NEPs and considered that the environmental exposure to spilled GM soybean material or occasional feral GM soybean plants arising from spilled soybean MON 94637 seeds will be limited, whereas exposure to manure and faeces of animals fed with soybean MON 94367 material is expected to be higher. Additionally, ingested proteins are typically degraded before entering the environment through manure and faeces of animals fed with GM soybean (Harmon & Swanson, 2020; Miner-Williams et al., 2014; Mok & Urschel, 2020; Santos-Hernández et al., 2018; van Bruchem et al., 1985), and the data provided for the assessment of protein stability (see Section 3.5.1.2.4) support that the NEPs will also be degraded. As compared to non-GM soybean, the GMO Panel considers that potential interactions of soybean MON 94637 with biogeochemical cycles do not raise any environmental safety concern.

### 3.6.2 | Post-market environmental monitoring

The objectives of a post-market environmental monitoring (PMEM) plan, according to Annex VII of Directive 2001/18/EC, are: (1) to confirm that any assumption regarding the occurrence and impact of potential adverse effects of the GMO, or its use, in the ERA is correct; and (2) to identify the occurrence of adverse effects of the GMO, or its use, on human health or the environment that were not anticipated in the ERA.

Monitoring is related to risk management, and thus, a final adoption of the PMEM plan falls outside the mandate of EFSA. However, the GMO Panel gives its opinion on the scientific rationale of the PMEM plan provided by the applicant (EFSA GMO Panel, 2011b).

As the ERA did not identify potential adverse environmental effects from soybean MON 94637, no case-specific monitoring is required.

The PMEM plan proposed by the applicant for soybean MON 94637 includes: (1) the description of a monitoring approach involving operators (federations involved in import and processing), reporting to the applicant via a centralised system any observed adverse effect(s) of GMOs on human health and the environment; (2) a coordinating system established by CropLife Europe for the collection of information recorded by the various operators; and (3) the review of relevant scientific publications retrieved from literature searches (Lecoq et al., 2007; Windels et al., 2008). The applicant proposes to submit a PMEM report on an annual basis for the duration of the authorisation period.

The GMO Panel considers that the scope of the PMEM plan provided by the applicant is consistent with the intended uses of soybean MON 94637. The GMO Panel agrees with the reporting intervals proposed by the applicant in its PMEM plan.

#### 3.6.2.1 | *Conclusion of the environmental risk assessment and monitoring plan*

The GMO Panel concludes that it is unlikely that soybean MON 94637 would differ from conventional soybean varieties in its ability to persist under European environmental conditions. Considering the scope of application GMFF-2023-21116, interactions of occasional feral soybean MON 94637 plants with the biotic and abiotic environment are not considered to be relevant issues. The analysis of HGT from soybean MON 94637 to bacteria does not indicate a safety concern. Therefore, considering the introduced trait, the outcome of the agronomic and phenotypic analysis, and the routes and levels of exposure, the GMO Panel concludes that soybean MON 94637 would not raise safety concerns in the event of the release of processed GM soybean MON 94637 or the accidental spillage of viable GM soybean seeds into the environment.

The scope of the PMEM plan provided by the applicant and the reporting intervals are in line with the intended uses of soybean MON 94637.

## 4 | OVERALL CONCLUSIONS

The GMO Panel was asked to carry out a scientific assessment of soybean MON 94637 for import, processing and food and feed uses in accordance with Regulation (EC) No 1829/2003. The molecular characterisation data establish that soybean MON 94637 contains a single insert consisting of one copy of the *cry1A.2 and cry1B.2* expression cassettes. The quality of the sequencing methodology and datasets was assessed by the EFSA GMO Panel and is in compliance with the requirements listed in the EFSA Technical Note. Bioinformatic analyses of the sequences encoding the NEPs, the sequences corresponding to ORFs within the insert or spanning the junctions between the insert and genomic DNA, as well as the flanking regions, do not raise any safety concern. The stability of the inserted DNA was confirmed over several generations. The methodology used to quantify the levels of the Cry1A.2 and Cry1B.2 proteins is considered adequate. Considering the selection of test materials, the field trial sites and the associated management practices and the agronomic-phenotypic characterisation as an indicator of the overall field trial quality, the GMO Panel concludes that the field trials are appropriate to support the comparative analysis. None of the identified differences in the agronomic/phenotypic and compositional characteristics tested between soybean MON 94637 and its conventional counterpart needed further assessment. The GMO Panel does not identify safety concerns regarding the toxicity and allergenicity of the Cry1A.2 and Cry1B.2 proteins as expressed in soybean MON 94637. The GMO Panel finds no evidence that the genetic modification impacts the overall safety of soybean MON 94637, as food and feed. In the context of this application, the consumption of food and feed from soybean MON 94637 does not represent a nutritional concern in humans and animals. The GMO Panel concludes that soybean MON 94637, as described in this application, is as safe as the conventional counterpart and non-GM soybean varieties tested, and no post-market monitoring of food/feed is considered necessary.

The GMO Panel concludes that there is a very low likelihood of environmental effects resulting from the accidental release of viable seeds from soybean MON 94637 into the environment. The PMEM plan and reporting intervals are in line with the intended uses of soybean MON 94637. The literature searches did not identify any relevant publications on soybean MON 94637; therefore, the GMO Panel does not identify any safety issue pertaining to the intended uses of soybean MON 94637. The GMO Panel concludes that soybean MON 94637 is as safe as its conventional counterpart and the tested non-GM soybean reference varieties with respect to potential effects on human and animal health and the environment.

## 5 | DOCUMENTATION AS PROVIDED TO EFSA

The documentation as provided to EFSA is available on OpenEFSA.[30]

### ABBREVIATIONS

| | |
|---|---|
| BBCH | Biologische Bundesanstalt, Bundessortenamt and CHemical industry |
| bp | base pair |
| bw | body weight |
| DM | dry matter |
| dw | dry weight |
| ELISA | Enzyme-Linked Immunosorbent Assay |
| ERA | environmental risk assessment |
| FOB | functional observational battery |
| fw | fresh weight |
| GLP | good laboratory practice |
| GM | genetically modified |
| GMO | genetically modified organisms |
| GMO | Panel on Genetically Modified Organisms |

[30] https://open.efsa.europa.eu/dossier/GMFF-2023-21116.

| | |
|---|---|
| HGT | horizontal gene transfer |
| HR | homologous recombination |
| MS | mass spectrometry |
| NDF | neutral detergent fibre |
| NEP | newly expressed protein |
| OECD | Organisation for Economic Co-operation and Development |
| ORFs | open reading frames |
| PCR | polymerase chain reaction |
| PMEM | post-market environmental monitoring |
| SDS-PAGE | sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| SES | standardised effect sizes |
| T-DNA | transfer-deoxyribonucleic acid |

## ACKNOWLEDGEMENTS

The Panel wishes to thank the members of the Working Groups on Molecular Characterisation, Food and Feed Safety Assessment and Working Group On Comparative Analysis and Environmental Risk Assessment for the preparatory work on this scientific output and EFSA staff member Silvija Kolosevska for the support provided to this scientific output.

## REQUESTOR

Competent Authority of the Netherlands

## QUESTION NUMBER

EFSA-Q-2024-00054



## PANEL MEMBERS

Josep Casacuberta, Francisco Barro, Albert Braeuning, Ruud de Maagd, Michelle M. Epstein, Thomas Frenzel, Jean-Luc Gallois, Frits Koning, Antoine Messéan, F. Javier Moreno, Fabien Nogué, Giovanni Savoini, Alan H. Schulman, Christoph Tebbe and Eve Veromann.

## REFERENCES

Abud, S., de Souza, P. I. M., Vianna, G. R., Leonardecz, E., Moreira, C. T., Faleiro, F. G., Júnior, J. N., Monteiro, P. M. F. O., Rech, E. L., & Aragão, F. J. L. (2007). Gene flow from transgenic to nontransgenic soybean plants in the Cerrado region of Brazil. *Genetics and Molecular Research*, *6*, 445–452.

Ahrent, D. K., & Caviness, C. E. (1994). Natural cross- pollination of 12 soybean cultivars in Arkansas. *Crop Science*, *34*, 376–378.

Alsajri, F. A., Singh, B., Wijewardana, C., Irby, J. T., Gao, W., & Reddy, K. R. (2019). Evaluating soybean cultivars for low- and high- temperature tolerance during the seedling growth stage. *Agronomy*, *9*, 13. https://doi.org/10.3390/agronomy9010013

Bond, J. A., & Walker, T. W. (2009). Control of volunteer glyphosate- resistant soybean in rice. *Weed Technology*, *23*, 225–230. https://doi.org/10.1614/WT-08-156.1

Bramlage, W. J., Leopold, A. C., & Parrish, D. J. (1978). Chilling stress to soybeans during imbibition. *Plant Physiology*, *61*, 525–529.

Breiteneder, H., & Mills, E. N. (2005). Molecular properties of food allergens. *Journal of Allergy and Clinical Immunology*, *115*, 14–23.

Caviness, C. E. (1966). Estimates of natural cross- pollination in Jackson soybeans in Arkansas. *Crop Science*, *6*, 211–212.

Celesti-Grapow, L., Pretto, F., Carli, E., & Blasi, C. (Eds.). (2010). *Flora vascolare alloctona e invasiva delle regioni d'Italia*. Casa Editrice Università La Sapienza.

Codex Alimentarius. (2009). *Foods derived from modern biotechnology*. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme. https://www.fao.org/docrep/011/a1554e/a1554e00.htm

Costa, J., Bavaro, S. L., Benede, S., Diaz-Perales, A., Bueno-Diaz, C., Gelencser, E., Klueber, J., Larre, C., Lozano-Ojalvo, D., Lupi, R., Mafra, I., Mazzucchelli, G., Molina, E., Monaci, L., Martın-Pedraza, L., Piras, C., Rodrigues, P. M., Roncada, P., Schrama, D., … Holzhauser, T. (2021). Are physicochemical properties shaping the allergenic potency of plant allergens? *Clinical Reviews in Allergy and Immunology*, *62*, 37–63. https://doi.org/10.1007/s12016-020-08810-9

Dorokhov, D., Ignatov, A., Deineko, E., Serjapin, A., Ala, A., & Skryabin, K. (2004). *Introgression from genetically modified plants into wild relatives* (H. C. M. den Nijs, D. Bartsch, & J. Sweet, Eds.). CAB International.

EFSA (European Food Safety Authority). (2009). Consolidated presentation of the joint Scientific Opinion of the GMO and BIOHAZ Panels on the "Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants" and the Scientific Opinion of the GMO Panel on "Consequences of the Opinion on the Use of Antibiotic Resistance Genes as Marker Genes in Genetically Modified Plants on Previous EFSA Assessments of Individual GM Plants". *EFSA Journal*, *7*(6), 1108. https://doi.org/10.2903/j.efsa.2009.1108

EFSA (European Food Safety Authority). (2010). Application of systematic review methodology to food and feed safety assessments to support decision making. *EFSA Journal*, *8*(6), 1637. https://doi.org/10.2903/j.efsa.2010.1637

EFSA (European Food Safety Authority). (2014). Explanatory statement for the applicability of the guidance of the EFSA Scientific Committee on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/ feed for GMO risk assessment. *EFSA Journal*, *12*(10), 3871. https://doi.org/10.2903/j.efsa.2014.3871

EFSA (European Food Safety Authority), Gennaro, A., Gomes, A., Herman, L., Nogue, F., Papadopoulou, N., & Tebbe, C. (2017). Technical report on the explanatory note on DNA sequence similarity searches in the context of the assessment of horizontal gene transfer from plants to microorganisms. *EFSA Supporting Publications*, *14*(7), EN-1273. https://doi.org/10.2903/sp.efsa.2017.en-1273

EFSA (European Food Safety Authority), Paraskevopoulos, K., Ramon, M., Dalmay, T., du Jardin, P., Casacuberta, J., Guerche, P., Jones, H., Nogué, F., Robaglia, C., & Rostoks, N. (2018). Explanatory note on the determination of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation. *EFSA Supporting Publications*, *15*(8), EN-1466. https://doi.org/10.2903/sp.efsa.2018.EN-1466

EFSA (European Food Safety Authority), Gomez Ruiz, J. A., Bresson, J.-L., Frenzel, T., & Paoletti, C. (2019a). Statement on the human dietary exposure assessment to newly expressed proteins in GM foods. *EFSA Journal*, *17*(7), 5802. https://doi.org/10.2903/j.efsa.2019.5802

EFSA (European Food Safety Authority), Devos, Y., Guajardo, I. M., Alvarez, F., & Glanville, J. (2019b). Explanatory note on literature searching conducted in the context of GMO applications for (renewed) market authorisation and annual post-market environmental monitoring reports on GMOs authorised in the EU market. *EFSA Supporting Publications*, *16*(4), EN-1614. https://doi.org/10.2903/sp.efsa.2019.en-1614

EFSA (European Food Safety Authority). (2021a). Administrative guidance for the processing of applications for regulated products (update 2021). *EFSA Supporting Publications*, *18*(3), EN-6471. https://doi.org/10.2903/sp.efsa.2021.EN-6471

EFSA (European Food Safety Authority). (2021b). Administrative guidance for the preparation of applications on genetically modified plants. *EFSA Supporting Publications*, *18*(3), EN-6473. https://doi.org/10.2903/sp.efsa.2021.EN-6473

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010a). Guidance on the environmental risk assessment of genetically modified plants. *EFSA Journal*, *8*(11), 1879. https://doi.org/10.2903/j.efsa.2010.1879

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010b). Statistical considerations for the safety evaluation of GMOs. *EFSA Journal*, *8*(1), 1250. https://doi.org/10.2903/j.efsa.2010.1250

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010c). Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011a). EFSA Panel on Genetically Modified Organisms (GMO); Scientific Opinion on guidance for risk assessment of food and feed from genetically modified plants. *EFSA Journal*, *9*(5), 2150. https://doi.org/10.2903/j.efsa.2011.2150

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2011b). Scientific Opinion on guidance on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. *EFSA Journal*, *9*(8), 2316. https://doi.org/10.2903/j.efsa.2011.2316

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2015). Guidance on the agronomic and phenotypic characterisation of genetically modified plants. *EFSA Journal*, *13*(6), 4128. https://doi.org/10.2903/j.efsa.2015.4128

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2017). Guidance on allergenicity assessment of genetically modified plants. *EFSA Journal*, *15*(5), 4862. https://doi.org/10.2903/j.efsa.2017.4862

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Casacuberta, J., Nogué, F., Naegeli, H., Birch, A. N., De Schrijver, A., Gralak, M. A., Guerche, P., Manachini, B., Messéan, A., Nielsen, E. E., Robaglia, C., Rostoks, N., Sweet, J., Tebbe, C., Visioli, F., Wal, J.-M., Moxon, S., Schneeberger, K., … Jones, H. (2018). Scientific Opinion on the technical note on the quality of DNA sequencing for the molecular characterisation of genetically modified plants. *EFSA Journal*, *16*(7), 5345. https://doi.org/10.2903/j.efsa.2018.5345

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Naegeli, H., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Mullins, E., Nogue, F., Rostoks, N., Sànchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., & Fernandez Dumont, A. (2021). Statement on in vitro protein digestibility tests in allergenicity and protein safety assessment of genetically modified plants. *EFSA Journal*, *19*(1), 6350. https://doi.org/10.2903/j.efsa.2021.6350

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., George Firbank, L., Guerche, P., Hejatko, J., Naegeli, H., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Fernandez Dumont, A., & Moreno, F. J. (2022). Scientific opinion on development needs for the allergenicity and protein safety assessment of food and feed products derived from biotechnology. *EFSA Journal*, *20*(1), 7044. https://doi.org/10.2903/j.efsa.2022.7044

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Mullins, E., Bresson, J.-L., Dalmay, T., Dewhurst, I. C., Epstein, M. M., Firbank, L. G., Guerche, P., Hejatko, J., Moreno, F. J., Naegeli, H., Nogué, F., Rostoks, N., Sánchez Serrano, J. J., Savoini, G., Veromann, E., Veronesi, F., Dumont, A. F., & Ardizzone, M. (2023). Animal dietary exposure in the risk assessment of feed derived from genetically modified plants. *EFSA Journal*, *21*(1), 7732. https://doi.org/10.2903/j.efsa.2023.7732

EFSA Scientific Committee. (2011). EFSA guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. *EFSA Journal*, *9*(12), 2438. https://doi.org/10.2903/j.efsa.2011.2438

Fernandez, A., Mills, E. N., Lovik, M., Spoek, A., Germini, A., Mikalsen, A., & Wal, J. M. (2013). Endogenous allergens andcompositional analysis in the allergenicity assessment of genetically modified plants. *Food and Chemical Toxicology*, *62*, 1–6.

Foo, A. C. Y., & Müller, G. A. (2021). Abundance and stability as common properties of allergens. *Frontiers in Allergy*, *2*, 769728. https://doi.org/10.3389/falgy.2021.769728

Gumisiriza, G., & Rubaihayo, P. R. (1978). Factors that influence outcrossing in soybean. *Zeitschrift für Acker- und Pflanzenbau/Journal of Agronomy and Crop Science*, *147*, 129–133.

Hammond, B., Kough, J., Herouet-Guicheney, C., & Jez, J. M. (2013). Toxicological evaluation of proteins introduced into food crops. *Critical Reviews in Toxicology*, *43*(2), 25–42. https://doi.org/10.3109/10408444.2013.842956

Harmon, D. L., & Swanson, K. C. (2020). Review: Nutritional regulation of intestinal starch and protein assimilation in ruminants. *Animal*, *14*, S17–S28. https://doi.org/10.1017/S1751731119003136

Helm, R. M. (2001). *Topic 5: Stability of known allergens (digestive and heat stability)*. Report of a joint FAO, WHO expert consultation on allergenicity of food derived from biotechnology, 22–25, January 2001. Food and Agriculture organisation of the United Nations (FAO).

Hülter, N., & Wackernagel, W. (2008). Double illegitimate recombination events integrate DNA segments through two different mechanisms during natural transformation of Acinetobacter baylyi. *Molecular Microbiology*, *67*, 984–995.

Jhala, A. J., Beckie, H. J., Peters, T. J., Culpepper, A. S., & Norsworthy, J. K. (2021). Interference and management of herbicide- resistant crop volunteers. *Weed Science*, *69*, 257–273.

Jhala, A. J., Sandell, L., Kruger, G., Wilson, R., & Knezevic, Z. (2013). *Control of glyphosate- resistant volunteer soybean in corn. Crop watch*. University of Nebraska–Lincoln.

Jurat-Fuentes, J. L., & Crickmore, N. (2017). Specificity determinants for cry insecticidal proteins: Insights from their mode of action. *Journal of Invertebrate Pathology*, *142*, 5–10. https://doi.org/10.1016/j.jip.2016.07.018

Kikuchi, A., Murata, K., Tabuchi, K., & Sakai, S. (1993). Inheritance of seed embryo color and investigation of degree of natural cross- pollination in soybeans. *Breeding Science*, *43*(Suppl. 2), 112.

Koch, M. S., Ward, J. M., Levine, S. L., Baum, J. A., Vicini, J. L., & Hammond, B. G. (2015). The food and environmental safety of Bt crops. *Frontiers in Plant Science*, *6*, 283.

Lecoq, E., Holt, K., Janssens, J., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2007). General surveillance: Roles and responsibilities the industry view. *Journal für Verbraucherschutz und Lebensmittelsicherheit/Journal of Consumer Protection and Food Safety*, *2*(S1), 25–28.

Li, Y., Tran, A. H., Danishefsky, S. J., & Tan, Z. (2019). Chemical biology of glycoproteins: from chemical synthesis to biological impact. *Methods in Enzymology*, *621*, 213–229.

Lu, B. R. (2005). Multidirectional gene flow among wild, weedy, and cultivated soybeans. In J. Gressel (Ed.), *Crop ferality and volunteerism* (pp. 137–147). OECD + Taylor & Francis.

Matsushita, A., Goto, H., Takahashi, Y., Tsuda, M., & Ohsawa, R. (2020). Consideration of familiarity accumulated in the confined field trials for environmental risk assessment of genetically modified soybean (Glycine max) in Japan. *Transgenic Research*, *29*, 229–242. https://doi.org/10.1007/s11248-020-00193-z

Miner-Williams, W. M., Stevens, B. R., & Moughan, P. J. (2014). Are intact peptides absorbed from the healthy gut in the adult human? *Nutrition Research Reviews*, *27*, 308–329. https://doi.org/10.1017/S0954422414000225

Mok, C. H., & Urschel, K. L. (2020). Invited review – Amino acid requirements in horses. Asian- Australasian. *Journal of Animal Science*, *33*(5), 679–695. https://doi.org/10.5713/ajas.20.0050

OECD (Organisation for Economic Co-operation and Development). (2000). *Consensus document on the biology of Glycine max (L.) Merr. (soybean). ENV/JM/MONO(2000)9. Series on harmonization of regulatory oversight in biotechnology no. 15*. Organisation for Economic Co- operation and Development.

OECD (Organisation for Economic Co-operation and Development). (2008). *Test no. 407: Repeated dose 28-day oral toxicity study in rodents, OECD guidelines for the testing of chemicals, section 4*. OECD Publishing. https://doi.org/10.1787/9789264070684-en

OECD (Organisation for Economic Co-operation and Development). (2012). *Revised consensus document on compositional considerations for new varieties of soybean [Glycine max (L.) Merr.]: Key food and feed nutrients, anti-nutrients, toxicants and allergens*.

OECD (Organisation for Economic Co-operation and Development). (2018). *OECD guideline for the testing of chemicals – Test no. 408: Repeated dose 90-day oral toxicity study in rodents*. OECD Publishing.

Owen, M. D. K. (2005). Maize and soybeans – Controllable volunteerism without ferality? In J. Grassel (Ed.), *Crop ferality and volunteerism* (pp. 149–165). CRC Press.

Polevoda, B., & Sherman, F. (2000). Na-terminal acetylation of eukaryotic proteins. *Journal of Biological Chemistry*, *275*, 36479–36482.

Ray, J. D., Kilen, T. C., Abel, C. A., & Paris, R. L. (2003). Soybean natural cross- pollination rates under field conditions. *Environmental Biosafety Research*, *2*, 133–138. https://doi.org/10.1051/ebr:2003005

Santos-Hernández, M., Miralles, B., Amigo, L., & Recio, I. (2018). Intestinal signaling of proteins and digestion- derived products relevant to satiety. *Journal of Agricultural and Food Chemistry*, *66*(39), 10123–10131. https://doi.org/10.1021/acs.jafc.8b02355

Selb, R., Wal, J. M., Moreno, F. J., Lovik, M., Mills, C., Hoffmann-Sommergruber, K., & Fernandez, A. (2017). Assessment ofendogenous allergenicity of genetically modified plants exemplified by soybean – Where do we stand? *Food and Chemical Toxicology*, *101*, 139–148.

Soltani, N., Shropshure, C., & Sikkema, P. H. (2019). Control of volunteer adzuki bean and soybean in white bean with halosulfuron. *Canadian Journal of Plant Science*, *99*, 375–378. https://doi.org/10.1139/cjps-2018-0261

Staniak, M., Stępień-Warda, A., Czopek, K., Kocira, A., & Baca, E. (2021). Seeds quality and quantity of soybean [*Glycine max* (L.) Merr.] cultivars in response to cold stress. *Agronomy*, *11*, 520. https://doi.org/10.3390/agronomy11030520

Sys, C., Van Ranst, E., Debaveye, J., & Beernaert, F. (1993). *Land evaluation. Part III: Crop requirements. Agricultural publication no. 7*. General Administration for Development Cooperation.

Szczerba, A., Płażek, A., Pastuszak, J., Kopeć, P., Hornyák, M., & Dubert, F. (2021). Effect of low temperature on germination, growth, and seed yield of four soybean (*Glycine max* L.) cultivars. *Agronomy*, *11*, 800. https://doi.org/10.3390/agronomy11040800

Tyagi, S. K., & Tripathi, R. P. (1983). Effect of temperature on soybean germination. *Plant and Soil*, *74*(2), 273–280. https://doi.org/10.1007/BF02143617

van Bruchem, J., Rouwers, S. M. G., Bangma, G. A., Leffering, C. P., & van Adrichem, P. W. M. (1985). Digestion of proteins of varying degradability in sheep. 1. Fermentation in and rate of passage from the reticulorumen. *Netherlands Journal of Agricultural Science*, *33*, 263–272.

Wang, X., Li, X., Zhou, Q., Song, S., & Dong, S. (2023). Comparison and evaluation of low- temperature tolerance of different soybean cultivars during the early- growth stage. *Agronomy*, *13*, 1716. https://doi.org/10.3390/agronomy13071716

Windels, P., Alcalde, E., Lecoq, E., Legris, G., Pleysier, A., Tinland, B., & Wandelt, C. (2008). General surveillance for import and processing: the EuropaBio approach. *Journal of Consumer Protection and Food Safety*, *3*(Suppl. 2), 14–16.

Yoshimura, Y., Matsuo, K., & Yasuda, K. (2006). Gene flow from GM glyphosate- tolerant to conventional soybeans under field conditions in Japan. *Environmental Biosafety Research*, *5*, 169–173. https://doi.org/10.1051/ebr:2007003

Zhang, L., Liu, L., Fang, Z., Shen, W., Dai, Y., Jia, R., Liang, J., & Liu, B. (2023). Fitness changes in wild soybean caused by gene flow from genetically modified soybean. *BMC Plant Biology*, *23*, 424.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms), Casacuberta, J., Barro, F., Braeuning, A., de Maagd, R., Epstein, M. M., Frenzel, T., Gallois, J.-L., Koning, F., Messéan, A., Moreno, F. J., Nogué, F., Savoini, G., Schulman, A. H., Tebbe, C., Veromann, E., Ardizzone, M., De Sanctis, G., Fernandez, A., Ferrari, A., … Raffaello, T. (2025). Assessment of genetically modified soybean MON 94637 (application GMFF-2023-21116). *EFSA Journal*, *23*(10), e9581. https://doi.org/10.2903/j.efsa.2025.9581

# APPENDIX A

## Statistical analysis and statistically significant findings in the 28-day toxicity studies in mice and in the 90-day toxicity study in rats

### A.1 | Statistical analysis of the 28-day toxicity study on the *Bacillus thuringiensis*-produced Cry1A.2 protein in mice

The following endpoints were statistically analysed: mortality, clinical signs, body weights, body weight gains, food consumption, neurobehavioral assessments, motor activity, ophthalmology, clinical pathology parameters (haematology, coagulation, and serum chemistry), organ weights and macroscopic and microscopic examinations.

The main statistical analysis compared rats consuming the test diets (at low dose – 10 mg/kg of body weight/day of Cry1A.2 protein, Group 5, medium dose – 100 mg/kg of body weight/day of Cry1A.2 protein, Group 4 and high dose – 1000 mg/kg of body weight/day of Cry1A.2 protein, Group 3 with those consuming the vehicle diet – 10 mM Sodium carbonate/bicarbonate, pH 10.2, Group 1).

Continuous data were investigated separately for each sex, variable and period or time interval, according to a linear mixed model (factor: diet) then, a pairwise comparisons, between test and vehicle groups (separately for each sex, time interval if necessary, and variable), were defined within the ANOVA and tested using a Dunnett's tests (two-tailed at the significance level of 5%). For the locomotor activity data, a more complex model was set up taking into consideration the time effect and its interaction with the dose fixed effect as additional factors. Only when the interaction of the treatment per time was significant, the comparison of the treated group versus the vehicle group was conducted within each time interval and tested using Dunnett's tests. Binomial category data and unordered multi-category data were analysed by Fisher's exact probabilities test.

For all continuous endpoints, the mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable and period or time interval were reported. Missing data were considered by the Panel and found not to have an impact on the results (Table A.1).

**TABLE A.1** Statistically significant findings in the 28-day toxicity study on *Bacillus thuringiensis*-produced Cry1A.2 protein in mice.

| Statistically significant parameter/endpoint | Finding (vs. control) | GMO panel interpretation |
|---|---|---|
| Body weight (bw) (day 15) | Lower (10%) in top dose males | All values were within those of the control group. Small magnitude. Within normal variation. Not an adverse effect of treatment |
| Body weight gain (cumulative) | Lower at all time points in top dose males | Due to a reduced gain over the first 2 weeks; gains were higher than controls over the last 2 weeks. Within normal variation. No impact on terminal body weight. Not an adverse effect of treatment |
| Body weight gain (d1–29) | Lower in top dose females | Higher over days 15–22. No impact on terminal body weight. Within normal variation. Not an adverse effect of treatment |
| Food consumption (d8–22) | Decreased (20%) in top dose males | Increased 10% (not significant) d22–29. Similar to BSA group. No impact on terminal body weight. Not an adverse effect of treatment |
| Food consumption (d8–15) | Increased in all treated females | No dose response. No impact on terminal body weight. Within normal variation. Not an adverse effect of treatment |
| Forelimb grip strength | Decreased (20%) in top dose females | Not seen in males. Within normal variation. Not an adverse effect of treatment |
| Motor activity (total) | Decreased (37%) in top dose males | All values in the top dose males are within the negative control range. Not significant versus the BSA control group. Not an adverse effect of treatment |
| Neutrophil count | Increased (80%) in top dose females | No other changes in WBC parameters. All values within BSA and low dose group ranges. Within normal variation. Not an adverse effect of treatment |
| Epididymis weight relative to body weight | Increased (10%) in top dose males | Small magnitude. No associated histopathology or changes in related organs. Within normal variation. Not an adverse effect of treatment |
| Brain weight relative to body weight | Increased (7%) in top dose females | Small magnitude. No associated histopathology. Within normal variation. Not an adverse effect of treatment |
| Thymus weight, absolute and relative to body weight | Decreased (20%–30%) in top dose and mid-dose females | No associated histopathology. Similar to BSA group. Within normal variation. Not an adverse effect of treatment |

*Note*: Where changes are given as percentages (e.g. reduced (30%)), this indicates the magnitude of the change relative to the control value (e.g. 30% decrease in mean body weights means a value of 70 g in test group animals vs. 100 g in controls).

## A.2 | Statistical analysis of the 28-day toxicity study on the *Bacillus thuringiensis*-produced Cry1B.2 protein in mice

The objective of this study was to evaluate the potential toxicity of Cry1B.2 protein when administered daily by oral gavage to CD-1 mice for at least 28 consecutive days. The use of oral gavage represents a relevant exposure route for the protein evaluated in this study, as well as the standard method for dosing, as identified in OECD Test Guideline 407 (2008). For each sex, 100 mice were individually housed in cages and assigned to each of the following dose groups at random on body weight stratification into a block design with 20 animals per sex per group: vehicle group (Group 1 at a target dose level of 0 mg/kg/day), control group (Group 2 at a target dose level of 1000 mg/kg/day), test (high) (Group 3 at a target dose level of 1000 mg/kg/day), test (medium) (Group 4 at a target dose level of 100 mg/kg/day), test (low) (Group 5 at a target dose level of 10 mg/kg/day). Animals were assigned to groups by a stratified straight randomisation block design in order to achieve similar group mean body weights. The following endpoints were statistically analysed: body weights, cumulative body weight changes, food consumption, clinical pathology values, absolute and relative organ weights, functional observational battery (FOB) data and locomotor activity data. Summary statistics, including sample mean, standard deviation, median, minimum and maximum, difference, standardised difference, 95% confidence intervals (CI) and standardised 95% CI were provided for each dose group, sex, variable and period. Adjusted p-values were reported for each comparison. The statistical analysis compared mice between each test substance group and the vehicle group. All pairwise comparisons were conducted using two-sided Dunnett's tests at the significance level of 5%. For microscopic findings, a one-sided Fisher's exact test was used to compare the vehicle group to the high-dose test substance-treated group by sex. Linear mixed models (LMMs) were employed to analyse continuous data for each variable and period, followed by pairwise comparisons between each treated group and the vehicle group using Dunnett's tests (at the 5% level of significance). For each LMM, the full output, including the visual examination of residual plots and histograms, the estimated means and standard errors for each fixed effect (i.e. Least-squares (LS) means and standard errors (SE), and degrees of freedom of each fixed effect), and the variance components for the random terms in each mixed model are provided (Table A.2).

**TABLE A.2** Statistically significant findings in the 28-day toxicity study on *Bacillus thuringiensis*-produced Cry1B.2 protein in mice.

| Statistically significant parameter/endpoint | Finding (vs. control) | GMO panel interpretation |
|---|---|---|
| Total bile acids | Increased (threefold) in low dose males | No dose response – not present at high and mid-dose. Within normal variation. Not an adverse effect of treatment |
| Blood urea nitrogen | Increased (30%) in mid-dose males | No dose–response- not seen at high dose. Similar to BSA control group. Within normal variation. Not an adverse effect of treatment |
| Body temperature | Increased (3% (1°C)) in low-dose males | No dose response – not present at high and mid-dose. Within normal variation. Not an adverse effect of treatment |

*Note*: Where changes are given as percentages (e.g. reduced (30%)), this indicates the magnitude of the change relative to the control value (e.g. 30% decrease in mean body weights means a value of 70 g in test group animals vs. 100 g in controls).

## A.3 | Statistical analysis of the 90-day toxicity study on soybean MON 94637 in rats

The following endpoints were statistically analysed: body weights, body weight changes, food consumption, clinical pathology values (as applicable), absolute and relative organ weights, functional observational battery (FOB) data, locomotor activity and histopathological data.

For all continuous endpoints, the mean, standard deviation in terms of the standardised effect sizes (SES) of each dose group for each sex, variable and period or time interval were reported.

The main statistical analysis compared rats consuming the test diets (at low dose – 15% test substance MON 94637 + 15% control substance A3555 in 5002 rodent diet and high dose – 30% test substance MON 94637 in 5002 rodent diet) with those consuming the control diet. Continuous data were analysed separately for each variable and period or time interval, according to a linear mixed model (factor: diet, sex and interaction 'dose-sex'); then, pairwise comparisons, between each test and control group (separately for each sex) were performed using an *F*-test (at the 5% level of significance). For the locomotor activity data, a more complex model was set up taking into consideration the time effect and its interaction with the dose fixed effect as additional factors. Pairwise comparisons were made for the test group with the control group at each time interval through linear contrasts. Binomial category data and unordered multi-category data were analysed by Fisher's exact probabilities test.

Missing data were considered by the Panel and found not to have an impact on the results (Table A.3).

**TABLE A.3** Statistically significant findings in the 90-day toxicity study on soybean MON 94637 in rats.

| Statistically significant parameter/endpoint | Finding (vs. control) | GMO panel interpretation |
|---|---|---|
| Red cell distribution width | Decreased (2%) in top dose groups (sexes combined) | Small magnitude. Within normal variation. No changes in other red cell parameters. Not an adverse effect of treatment |
| ALT | Decreased (15%) in top dose groups (sexes combined) | Small magnitude. Within normal variation. Not adverse in isolation, no other indications of hepatotoxicity. Not an adverse effect of treatment |
| Potassium | Increased (4%–6%) in both dose groups (sexes combined) | Small magnitude. Within normal variation. Not an adverse effect of treatment |
| Total bilirubin | Decreased (15%) in both dose groups (sexes combined) | Small magnitude. Within normal variation. Not adverse in isolation. Not an adverse effect of treatment |
| T3 | Increased in females; low dose (20%) and high dose (40%) | Within normal variation. No changes in T4 or TSH. No pathological findings in the thyroid. Not an adverse effect of treatment |
| Adrenal weight (absolute and relative to body weight) | Mean and median values are identical in test and control groups but flagged as a significant change at the low dose. | Small magnitude, with no dose response. Within normal variation. No associated histopathological findings. Not an adverse effect of treatment. |
| Thymus weight (absolute and relative to body weight) | Reduced (15%) in low dose groups combined | Small magnitude, with no dose response. Within normal variation (only one female outside control range). No associated histopathological findings. Not an adverse effect of treatment |

*Note*: Where changes are given as percentages (e.g. reduced (30%)), this indicates the magnitude of the change relative to the control value (e.g. 30% decrease in mean body weights means a value of 70 g in test group animals vs. 100 g in controls).

# APPENDIX B

## Animal dietary exposure

**TABLE B.1** Dietary exposure to Cry1A.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean full fat seed.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN FULL FAT SEED | Daily dietary intake of SOYBEAN FULL FAT SEED | NEP level Cry1A.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 10 | 3.85 | 24 | 92.31 | 0.09 |
| Beef cattle | 500 | 12 | 10 | 2.40 | 24 | 57.60 | 0.06 |
| Dairy Sheep | 80 | 2.8 | 14 | 4.90 | 24 | 117.60 | 0.12 |
| Dairy Goat | 60 | 3.4 | 11 | 6.23 | 24 | 149.60 | 0.15 |
| Rabbit | 2 | 0.15 | 20 | 15.00 | 24 | 360.00 | 0.36 |
| Fattening pig | 100 | 3 | 20 | 6.00 | 24 | 144.00 | 0.14 |
| Lactating sow | 200 | 6 | 10 | 3.00 | 24 | 72.00 | 0.07 |
| Piglet | 20 | 1 | 30 | 15.00 | 24 | 360.00 | 0.36 |
| Broiler | 2 | 0.158 | 20 | 15.80 | 24 | 379.20 | 0.38 |
| Laying hens | 1.9 | 0.13 | 15 | 10.26 | 24 | 246.32 | 0.25 |
| Turkey | 7 | 0.5 | 15 | 10.71 | 24 | 257.14 | 0.26 |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | NA | NA | NA | NA | NA |
| Dog | 25 | 0.36 | 30 | 4.32 | 24 | 103.68 | 0.10 |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.2** Dietary exposure to Cry1B.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean full fat seed.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN FULL FAT SEED | Daily dietary intake of SOYBEAN FULL FAT SEED | NEP level Cry1B.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 10 | 3.85 | 12 | 46.15 | 0.05 |
| Beef cattle | 500 | 12 | 10 | 2.40 | 12 | 28.80 | 0.03 |
| Dairy Sheep | 80 | 2.8 | 14 | 4.90 | 12 | 58.80 | 0.06 |
| Dairy Goat | 60 | 3.4 | 11 | 6.23 | 12 | 74.80 | 0.07 |
| Rabbit | 2 | 0.15 | 20 | 15.00 | 12 | 180.00 | 0.18 |
| Fattening pig | 100 | 3 | 20 | 6.00 | 12 | 72.00 | 0.07 |
| Lactating sow | 200 | 6 | 10 | 3.00 | 12 | 36.00 | 0.04 |
| Piglet | 20 | 1 | 30 | 15.00 | 12 | 180.00 | 0.18 |
| Broiler | 2 | 0.158 | 20 | 15.80 | 12 | 189.60 | 0.19 |
| Laying hens | 1.9 | 0.13 | 15 | 10.26 | 12 | 123.16 | 0.12 |
| Turkey | 7 | 0.5 | 15 | 10.71 | 12 | 128.57 | 0.13 |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | NA | NA | NA | NA | NA |
| Dog | 25 | 0.36 | 30 | 4.32 | 12 | 51.84 | 0.05 |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.3** Dietary exposure to Cry1A.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean meal.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN MEAL | Daily dietary intake of SOYBEAN MEAL | NEP level Cry1A.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 25 | 9.62 | 31.68 | 304.62 | 0.30 |
| Beef cattle | 500 | 12 | 20 | 4.80 | 31.68 | 152.06 | 0.15 |
| Dairy Sheep | 80 | 2.8 | 20 | 7.00 | 31.68 | 221.76 | 0.22 |
| Dairy Goat | 60 | 3.4 | 25 | 14.17 | 31.68 | 448.80 | 0.45 |
| Rabbit | 2 | 0.15 | 24 | 18.00 | 31.68 | 570.24 | 0.57 |
| Fattening pig | 100 | 3 | 30 | 9.00 | 31.68 | 285.12 | 0.29 |
| Lactating sow | 200 | 6 | 30 | 9.00 | 31.68 | 285.12 | 0.29 |
| Piglet | 20 | 1 | 22 | 11.00 | 31.68 | 348.48 | 0.35 |
| Broiler | 2 | 0.158 | 40 | 31.60 | 31.68 | 1001.09 | 1.00 |
| Laying hens | 1.9 | 0.13 | 25 | 17.11 | 31.68 | 541.89 | 0.54 |
| Turkey | 7 | 0.5 | 45 | 32.14 | 31.68 | 1018.29 | 1.02 |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | 42 | 8.40 | 31.68 | 266.11 | 0.27 |
| Cat | 4 | 0.06 | 29.5 | 4.43 | 31.68 | 140.18 | 0.14 |
| Dog | 25 | 0.36 | 30 | 4.32 | 31.68 | 136.86 | 0.14 |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.4** Dietary exposure to Cry1B.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean meal.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN MEAL | Daily dietary intake of SOYBEAN MEAL | NEP level Cry1B.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 25 | 9.62 | 15.84 | 152.31 | 0.15 |
| Beef cattle | 500 | 12 | 20 | 4.80 | 15.84 | 76.03 | 0.08 |
| Dairy Sheep | 80 | 2.8 | 20 | 7.00 | 15.84 | 110.88 | 0.11 |
| Dairy goat | 60 | 3.4 | 25 | 14.17 | 15.84 | 224.40 | 0.22 |
| Rabbit | 2 | 0.15 | 24 | 18.00 | 15.84 | 285.12 | 0.29 |
| Fattening pig | 100 | 3 | 30 | 9.00 | 15.84 | 142.56 | 0.14 |
| Lactating sow | 200 | 6 | 30 | 9.00 | 15.84 | 142.56 | 0.14 |
| Piglet | 20 | 1 | 22 | 11.00 | 15.84 | 174.24 | 0.17 |
| Broiler | 2 | 0.158 | 40 | 31.60 | 15.84 | 500.54 | 0.50 |
| Laying hens | 1.9 | 0.13 | 25 | 17.11 | 15.84 | 270.95 | 0.27 |
| Turkey | 7 | 0.5 | 45 | 32.14 | 15.84 | 509.14 | 0.51 |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | 42 | 8.40 | 15.84 | 133.06 | 0.13 |
| Cat | 4 | 0.06 | 29.5 | 4.43 | 15.84 | 70.09 | 0.07 |
| Dog | 25 | 0.36 | 30 | 4.32 | 15.84 | 68.43 | 0.07 |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.5** Dietary exposure to Cry1A.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean hull.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN HULLS | Daily dietary intake of SOYBEAN HULLS | NEP level Cry1A.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 10 | 3.85 | 8.16 | 31.38 | 0.03 |
| Beef cattle | 500 | 12 | 10 | 2.40 | 8.16 | 19.58 | 0.02 |
| Dairy Sheep | 80 | 2.8 | 45 | 15.75 | 8.16 | 128.52 | 0.13 |
| Dairy Goat | 60 | 3.4 | 61 | 34.57 | 8.16 | 282.06 | 0.28 |
| Rabbit | 2 | 0.15 | 32.5 | 24.38 | 8.16 | 198.90 | 0.20 |
| Fattening pig | 100 | 3 | 10 | 3.00 | 8.16 | 24.48 | 0.02 |
| Lactating sow | 200 | 6 | 10 | 3.00 | 8.16 | 24.48 | 0.02 |
| Piglet | 20 | 1 | 3 | 1.50 | 8.16 | 12.24 | 0.01 |
| Broiler | 2 | 0.158 | 10 | 7.90 | 8.16 | 64.46 | 0.06 |
| Laying hens | 1.9 | 0.13 | 5 | 3.42 | 8.16 | 27.92 | 0.03 |
| Turkey | 7 | 0.5 | NA | NA | NA | NA | NA |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | 14 | 2.10 | 8.16 | 17.14 | 0.02 |
| Dog | 25 | 0.36 | 7.5 | 1.08 | 8.16 | 8.81 | 0.01 |
| Horse | 450 | 9 | 75 | 15.00 | 8.16 | 122.40 | 0.12 |

**TABLE B.6** Dietary exposure to Cry1B.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean hull.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN HULLS | Daily dietary intake of SOYBEAN HULLS | NEP level Cry1B.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 10 | 3.85 | 4.08 | 15.69 | 0.02 |
| Beef cattle | 500 | 12 | 10 | 2.40 | 4.08 | 9.79 | 0.01 |
| Dairy Sheep | 80 | 2.8 | 45 | 15.75 | 4.08 | 64.26 | 0.06 |
| Dairy Goat | 60 | 3.4 | 61 | 34.57 | 4.08 | 141.03 | 0.14 |
| Rabbit | 2 | 0.15 | 32.5 | 24.38 | 4.08 | 99.45 | 0.10 |
| Fattening pig | 100 | 3 | 10 | 3.00 | 4.08 | 12.24 | 0.01 |
| Lactating sow | 200 | 6 | 10 | 3.00 | 4.08 | 12.24 | 0.01 |
| Piglet | 20 | 1 | 3 | 1.50 | 4.08 | 6.12 | 0.01 |
| Broiler | 2 | 0.158 | 10 | 7.90 | 4.08 | 32.23 | 0.03 |
| Laying hens | 1.9 | 0.13 | 5 | 3.42 | 4.08 | 13.96 | 0.01 |
| Turkey | 7 | 0.5 | NA | NA | NA | NA | NA |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | 14 | 2.10 | 4.08 | 8.57 | 0.01 |
| Dog | 25 | 0.36 | 7.5 | 1.08 | 4.08 | 4.41 | 0.00 |
| Horse | 450 | 9 | 75 | 15.00 | 4.08 | 61.20 | 0.06 |

**TABLE B.7** Dietary exposure to Cry1A.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean protein concentrate.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN PROTEIN CONCENTRATE | Daily dietary intake of SOYBEAN PROTEIN CONCENTRATE | NEP level Cry1A.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | NA | NA | NA | NA | NA |
| Beef cattle | 500 | 12 | NA | NA | NA | NA | NA |
| Dairy Sheep | 80 | 2.8 | NA | NA | NA | NA | NA |
| Dairy Goat | 60 | 3.4 | NA | NA | NA | NA | NA |
| Rabbit | 2 | 0.15 | NA | NA | NA | NA | NA |
| Fattening pig | 100 | 3 | NA | NA | NA | NA | NA |
| Lactating sow | 200 | 6 | NA | NA | NA | NA | NA |
| Piglet | 20 | 1 | 9 | 4.50 | 49.68 | 223.56 | 0.22 |
| Broiler | 2 | 0.158 | NA | NA | NA | NA | NA |
| Laying hens | 1.9 | 0.13 | NA | NA | NA | NA | NA |
| Turkey | 7 | 0.5 | NA | NA | NA | NA | NA |
| Salmon | 5 | 0.03 | 10 | 0.60 | 49.68 | 29.81 | 0.03 |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | 25 | 3.75 | 49.68 | 186.30 | 0.19 |
| Dog | 25 | 0.36 | 18.7 | 2.69 | 49.68 | 133.78 | 0.13 |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.8** Dietary exposure to Cry1B.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean protein concentrate.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN PROTEIN CONCENTRATE | Daily dietary intake of SOYBEAN PROTEIN CONCENTRATE | NEP level Cry1B.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | NA | NA | NA | NA | NA |
| Beef cattle | 500 | 12 | NA | NA | NA | NA | NA |
| Dairy Sheep | 80 | 2.8 | NA | NA | NA | NA | NA |
| Dairy Goat | 60 | 3.4 | NA | NA | NA | NA | NA |
| Rabbit | 2 | 0.15 | NA | NA | NA | NA | NA |
| Fattening pig | 100 | 3 | NA | NA | NA | NA | NA |
| Lactating sow | 200 | 6 | NA | NA | NA | NA | NA |
| Piglet | 20 | 1 | 9 | 4.50 | 24.84 | 111.78 | 0.11 |
| Broiler | 2 | 0.158 | NA | NA | NA | NA | NA |
| Laying hens | 1.9 | 0.13 | NA | NA | NA | NA | NA |
| Turkey | 7 | 0.5 | NA | NA | NA | NA | NA |
| Salmon | 5 | 0.03 | 10 | 0.60 | 24.84 | 14.90 | 0.01 |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | 25 | 3.75 | 24.84 | 93.15 | 0.09 |
| Dog | 25 | 0.36 | 18.7 | 2.69 | 24.84 | 66.89 | 0.07 |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.9** Dietary exposure to Cry1A.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean forage/silage.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN FORAGE/ SILAGE | Daily dietary intake of SOYBEAN FORAGE/ SILAGE | NEP level Cry1A.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 20 | 7.69 | 84 | 646.15 | 0.65 |
| Beef cattle | 500 | 12 | NA | NA | NA | NA | NA |
| Dairy Sheep | 80 | 2.8 | NA | NA | NA | NA | NA |
| Dairy Goat | 60 | 3.4 | NA | NA | NA | NA | NA |
| Rabbit | 2 | 0.15 | NA | NA | NA | NA | NA |
| Fattening pig | 100 | 3 | NA | NA | NA | NA | NA |
| Lactating sow | 200 | 6 | NA | NA | NA | NA | NA |
| Piglet | 20 | 1 | NA | NA | NA | NA | NA |
| Broiler | 2 | 0.158 | NA | NA | NA | NA | NA |
| Laying hens | 1.9 | 0.13 | 10 | 6.84 | 84 | 574.74 | 0.57 |
| Turkey | 7 | 0.5 | NA | NA | NA | NA | NA |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | NA | NA | NA | NA | NA |
| Dog | 25 | 0.36 | NA | NA | NA | NA | NA |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |

**TABLE B.10** Dietary exposure to Cry1B.2 protein (mg/kg bw per day) in farm and companion animals, based on the consumption of soybean forage/silage.

| Animal species | Animal body weight | Total daily intake of FEED | Inclusion rate of SOYBEAN FORAGE/ SILAGE | Daily dietary intake of SOYBEAN FORAGE/ SILAGE | NEP level Cry1B.2 | Daily dietary exposure to NEP | |
|---|---|---|---|---|---|---|---|
| Category | kg | $kg^{DM}$/animal | IR % | $g^{DM}/kg^{BW}$ | $\mu g^{NEP}/g^{DM}$ | $\mu g^{NEP}/kg^{BW}$ | $mg^{NEP}/kg^{BW}$ |
| Dairy cow | 650 | 25 | 20 | 7.69 | 55 | 423.08 | 0.42 |
| Beef cattle | 500 | 12 | NA | NA | NA | NA | NA |
| Dairy Sheep | 80 | 2.8 | NA | NA | NA | NA | NA |
| Dairy Goat | 60 | 3.4 | NA | NA | NA | NA | NA |
| Rabbit | 2 | 0.15 | NA | NA | NA | NA | NA |
| Fattening pig | 100 | 3 | NA | NA | NA | NA | NA |
| Lactating sow | 200 | 6 | NA | NA | NA | NA | NA |
| Piglet | 20 | 1 | NA | NA | NA | NA | NA |
| Broiler | 2 | 0.158 | NA | NA | NA | NA | NA |
| Laying hens | 1.9 | 0.13 | 10 | 6.84 | 55 | 376.32 | 0.38 |
| Turkey | 7 | 0.5 | NA | NA | NA | NA | NA |
| Salmon | 5 | 0.03 | NA | NA | NA | NA | NA |
| Carp | 1 | 0.02 | NA | NA | NA | NA | NA |
| Cat | 4 | 0.06 | NA | NA | NA | NA | NA |
| Dog | 25 | 0.36 | NA | NA | NA | NA | NA |
| Horse | 450 | 9 | NA | NA | NA | NA | NA |