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Assessment of genetically modified maize Bt11 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-016)

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Abstract

Following the submission of application EFSA-GMO-RX-016 under Regulation (EC) No 1829/2003 from Syngenta the Panel on Genetically Modified Organisms of the European Food Safety Authority was asked to deliver a scientific risk assessment on the data submitted in the context of the renewal of authorisation application for the insect-resistant and herbicide-tolerant genetically modified maize Bt11, for food and feed uses, excluding cultivation within the European Union. The data received in the context of this renewal application contained post-market environmental monitoring reports, a systematic search and evaluation of literature, updated bioinformatic analyses, and additional documents or studies performed by or on behalf of the applicant. The GMO Panel assessed these data for possible new hazards, modified exposure or new scientific uncertainties identified during the authorisation period and not previously assessed in the context of the original application. Under the assumption that the DNA sequences of the event in maize Bt11 considered for renewal is identical to the sequence of the originally assessed events, the GMO Panel concludes that there is no evidence in renewal application EFSA-GMO-RX-016 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on maize Bt11.

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Summary

Following the submission of application EFSA-GMO-RX-016 under Regulation (EC) No 1829/2003 from Syngenta, the Panel on Genetically Modified Organisms of the European Food Safety Authority (GMO Panel) was asked to deliver a scientific risk assessment on the data submitted in the context of the renewal of authorisation application for the insect-resistant and herbicide-tolerant genetically modified maize Bt11. The scope of application EFSA-GMO-RX-016 is for the renewal of the placing on the market of products containing, consisting of, or produced from maize Bt11, excluding cultivation within the European Union (EU).

In delivering its scientific opinion, the GMO Panel took into account application EFSA-GMO-RX-016, additional information provided by the applicant, scientific comments submitted by the EU Member States and relevant scientific publications. The data received in the context of the renewal application EFSA-GMO-RX-016 contained: post-market environmental monitoring reports, an evaluation of the literature retrieved by a systematic search, updated bioinformatics analyses and additional documents or studies performed by or on behalf of the applicant. The GMO Panel assessed these data for possible new hazards, modified exposure or new scientific uncertainties identified during the authorisation period and not previously assessed in the context of the original application.

Under the assumption that the DNA sequences of the events in maize Bt11 considered for renewal is identical to the sequences of the originally assessed events, the GMO Panel concludes that there is no evidence in the renewal application EFSA-GMO-RX-016 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on maize Bt11 (EFSA, 2009).

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1. Introduction

1.1. Background

On 11 October 2018, the European Food Safety Authority (EFSA) received from the European Commission (EC) application EFSA-GMO-RX-016 for the renewal of the authorisation of maize Bt11 (Unique Identifier SYN-BTØ11-1), submitted by Syngenta (hereafter referred to as 'the applicant') according to Regulation (EC) No 1829/2003.¹

Following receipt of application EFSA-GMO-RX-016 EFSA informed the Member States (MS) and made the summary of the application available to the public on the EFSA website.²

EFSA checked the application for compliance with the relevant requirements of Regulation (EC) No 1829/2003 and Regulation (EU) No 503/2013³ and, when needed, asked the applicant to supplement the initial application. On 26 November 2018, EFSA declared the application valid and made the valid application available to the MS and the European Commission.

Following the submission of application EFSA-GMO-RX-Bt11 and the publication of the EFSA scientific opinion (EFSA, 2009), the placing on the market of maize Bt11 for products containing, consisting of, or produced from this GM maize, excluding cultivation in the EU, was authorised by Commission Decision 2010/419/EU⁴. A copy of this authorisation was provided by the applicant.⁵

From the validity date, EFSA and its scientific Panel on Genetically Modified Organisms (hereafter referred to as 'the GMO Panel') endeavoured to respect a time limit of 6 months to issue a scientific opinion on application EFSA-GMO-RX-016. Such time limit was extended whenever EFSA and/or its GMO Panel requested supplementary information to 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 MS and European Commission (for further details, see the section 'Documentation', below).

In accordance with Regulation (EC) No 1829/2003, EFSA consulted the nominated risk assessment bodies of the MS, including national Competent Authorities within the meaning of Directive 2001/18/EC⁶. The MS had 3 months to make their opinion known on application EFSA-GMO-RX-016 as of 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 maize Bt11 for the renewal of authorisation for placing on the market of products containing, consisting of, or produced from GM maize Bt11 in the context of its scope as defined in application EFSA-GMO-RX-016.

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 including the opinions of the nominated risk assessment bodies of the MS.⁷

In addition to the present scientific opinion on maize Bt11, EFSA and its GMO Panel were also asked to report on the particulars listed under Articles 6(5) and 18(5) of Regulation (EC) No 1829/2003. The relevant information is made available in the EFSA Register of Questions,⁸ including the information required under Annex II to the Cartagena Protocol, a labelling proposal, a post-market environmental

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, p. 1–23.

² Available online: <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2018-00799>

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 L 157, 8.6.2013, p. 1–48.

⁴ Commission Decision of 28 July 2010 renewing the authorisation for continued marketing of products containing, consisting of, or produced from genetically modified maize Bt11 (SYN-BTØ11-1), authorising foods and food ingredients containing or consisting of field maize Bt11 (SYN-BTØ11-1) pursuant to Regulation (EC) No 1829/2003 of the European Parliament and of the Council and repealing Decision 2004/657/EC. Official Journal of the European Union L 197/11, 29.7.2010.

⁵ Dossier: Maize Bt11 – Appendix 2.1-1.

⁶ 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, p. 1–38.

⁷ Opinions of the nominated risk assessment bodies of EU Member States can be found at the EFSA Register of Questions, <http://registerofquestions.efsa.europa.eu/roqFrontend/login>, querying the assigned Question Number.

⁸ <http://registerofquestions.efsa.europa.eu/roqFrontend/questionDocumentsLoader?question=EFSA-Q-2019-00524>

monitoring (PMEM) plan as provided by the applicant; the method(s), validated by the Community reference laboratory, for detection, including sampling, identification of the transformation event in the food-feed and/or foods-feeds produced from it and the appropriate reference materials.

2. Data and methodologies

2.1. Data

The data for application EFSA-GMO-RX-016 provided by the applicant at the time of submission, or in reply to requests for additional information, are specified below.

In the context of this renewal application, a new sequencing study was submitted among the additional documents or studies performed by or on behalf of the applicant (Appendix B). In accordance with the GMO Panel guidelines for renewal of applications of GM food and feed authorised under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015), the GMO Panel evaluated the data provided in the context of this maize Bt11 renewal application under the assumption that Bt11 event sequences is identical to the sequences of the originally assessed event (EFSA, 2009).

2.1.1. Post-market monitoring reports⁹

Based on the outcome of the initial food and feed risk assessment, a post-market monitoring plan for monitoring of GM food and feed was not required by the authorisation decision. The implementation of a PMEM plan, consisting of a general surveillance plan to check for any adverse effects on the environment arising from maize Bt11, was a condition for the authorisation. As no potential adverse environmental effects were identified in the environmental risk assessment of maize Bt11 (EFSA, 2009), case-specific monitoring was not considered necessary by the GMO Panel.

The applicant provided nine annual PMEM reports covering a reporting period from July 2010 till June 2018. The annual PMEM plans submitted by the applicant included (1) commodity crop (GM and non GM) imports into the EU by country of origin and destination; (2) the description of a centralised system established by EuropaBio for the collection of information recorded by various operators (federations involved in maize grains import and processing) on any observed adverse effect(s) on human health and the environment arising from handling of maize possibly containing maize Bt11; (3) the reports of the surveillance activities conducted by such operators; and (4) the review of relevant scientific peer-reviewed studies retrieved from literature searches.

2.1.2. Systematic search and evaluation of literature¹⁰

In addition to the separate searches provided as part of the annual PMEM reports, the applicant performed three systematic literature searches covering the period from January 2008 till July 2020, in accordance with the recommendations on literature search outlined in EFSA (2010, 2017a).

Searches in electronic bibliographic databases and in websites of relevant organisations were performed to identify relevant publications. Altogether, 6,059 publications were identified (after removal of duplicates). After applying the eligibility/inclusion criteria defined *a priori* by the applicant, 61 publications were identified as relevant for food and feed safety assessment or molecular characterisation. The list of relevant publications is provided in Appendix A.

2.1.3. Updated bioinformatic data¹¹

At the time of submission of the renewal dossier, the applicant provided a complete bioinformatic dataset for maize Bt11 event including an analysis of the insert and flanking sequences, an analysis of the potential similarity to allergens and toxins of the newly expressed proteins and of all possible open reading frames (ORFs) within the insert and spanning the junction sites, an analysis of possible horizontal gene transfer (EFSA, 2017b), and a safety assessment of the newly expressed proteins Cry1Ab and PAT regarding their capacity to trigger celiac disease (EFSA GMO Panel, 2017). The outcome of the updated bioinformatic analyses is presented in Section 3.3.

⁹ Dossier: Maize Bt11 – Appendices 2.2-01 to 2.2-08b.

¹⁰ Dossier: Maize Bt11 – Folder 2.3.1-01; additional information: 20/6/2019, 28/10/2019; spontaneous information 7/9/2020.

¹¹ Dossier: Maize Bt11 Part II – Section 2.3.2; spontaneous information 16/4/2020.

2.1.4. Additional documents or studies provided by the applicant¹²

In line with the renewal guidance requirements (EFSA GMO Panel, 2015), the applicant provided an overview on the worldwide approvals of maize Bt11 and searched for any available full reports of studies performed by or on behalf of the applicant over the course of the authorisation period and not previously submitted to the EU (Appendix B).

The relevance of the listed studies for molecular characterisation, human and animal safety and the environment was assessed by the applicant.

2.1.5. Overall assessment as provided by the applicant¹³

The applicant provided an overall assessment concluding that information provided in the application for renewal of authorisation of maize Bt11 for food and feed uses in the EU does not change the outcome of the original risk assessment (EFSA, 2009).

2.1.6. Monitoring plan and proposal for improving the conditions of the original authorisation¹⁴

The applicant indicated in the dossier that the environmental post-market monitoring plan is appropriate and does not need any changes.

2.2. Methodologies

The GMO Panel assessed the application for renewal of the authorisation of maize Bt11 for food and feed uses in accordance with Articles 11 and 23 of Regulation (EC) No 1829/2003. The GMO Panel took into account the requirements described in its guideline for the risk assessment of renewal applications of GM food and feed authorised under Regulation (EC) No 1829/2003 (EFSA GMO Panel, 2015). The comments raised by the nominated risk assessment bodies of EU Member States were taken into consideration during the scientific risk assessment.

3. Assessment

3.1. Evaluation of the post-market monitoring reports

During the general surveillance activities covering the authorisation period of maize Bt11, no adverse effects were reported by the applicant.

3.2. Evaluation of the systematic search and evaluation of literature

The GMO Panel assessed the applicant's literature searches on maize Bt11 and the newly expressed proteins Cry1Ab and PAT. The overall quality of the performed literature searches is acceptable.

The GMO Panel acknowledges that no publications raising a safety concern for human and animal health and the environment which would change the original risk assessment conclusions on maize Bt11 (EFSA, 2009) have been identified by the applicant.

3.3. Evaluation of the updated bioinformatic data

The results of the updated bioinformatic analyses to assess the interruption of maize endogenous genes confirm previous results indicating that no endogenous genes were interrupted (EFSA, 2009).

Analyses of the amino acid sequence of the newly expressed Cry1Ab and PAT proteins reveal no significant similarities to toxins, allergens or immunogenic gluten-related epitopes. In addition, bioinformatic analyses of the newly created ORFs within the insert or spanning the junctions with genomic DNA confirm the previous conclusions indicating that the expression of ORFs showing significant similarities to toxins or allergens in maize Bt11 is highly unlikely (EFSA, 2009).

The updated bioinformatic analysis for event Bt11 did not reveal any DNA sequence that could provide sufficient length and identity which could facilitate horizontal gene transfer (HGT) by double homologous recombination, confirming previous conclusions (EFSA, 2009). Given the results of this analysis and that the recombinant DNA in maize Bt11 does not confer selective advantages to

¹² Dossier: Maize Bt11 Part II – Section 2.3.3.

¹³ Dossier: Maize Bt11 Part II – Section 3.

¹⁴ Dossier: Maize Bt11 Part II – Section 4.

microorganisms, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

3.4. Evaluation of the additional documents or studies provided by the applicant

The GMO Panel evaluated the full study reports of the additional studies provided (Appendix B, C¹⁵). This new information does not raise any concern for human and animal health and the environment, which would change the original risk assessment conclusions on maize Bt11 (EFSA, 2009).

3.5. Evaluation of the overall assessment as provided by the applicant

The GMO Panel evaluated the overall assessment provided by the applicant and confirms that there is no evidence in renewal application EFSA-GMO-RX-016 – indicating new hazards, relevant changes in exposure or scientific uncertainties that would change previous conclusions on maize Bt11.

3.6. Evaluation of the monitoring plan and proposal for improving the conditions of the original authorisation

The PMEM plan covers general surveillance of imported GM plant material, including maize Bt11. This general surveillance is coordinated by EuropaBio and implemented by selected operators (federations involved in maize grains import and processing). In addition, the applicant reviews relevant scientific publications retrieved from literature searches on an annual basis. The GMO Panel is of the opinion that the scope of the plan provided by the applicant is consistent with the scope of application EFSA-GMO-RX-016, but reminds that monitoring is related to risk management, and thus the final adoption and implementation of the PMEM plan falls outside the mandate of EFSA.

4. Conclusions

Under the assumption that the DNA sequence of the event in maize Bt11 considered for renewal is identical to the sequence of the originally assessed event, the GMO Panel concludes that there is no evidence in renewal application EFSA-GMO-RX-016 for new hazards, modified exposure or scientific uncertainties that would change the conclusions of the original risk assessment on maize Bt11 (EFSA, 2009).

5. Documentation as provided to EFSA

- 1) Letter from the European Commission to EFSA received on 11 October 2018 concerning a request for the continued marketing of food and feed containing, consisting of or produced from genetically modified maize Bt11 and products other than food and feed containing or consisting of it with the exception of cultivation, authorised under Regulation 1829/2003 (commission Decision 2010/419/EU) by Syngenta Crop Protection NV/SA.
- 2) Application EFSA-GMO-RX-016 validated by EFSA, 26 November 2018.
- 3) Request for supplementary information to the applicant, 19 December 2019.
- 4) Receipt of supplementary information from the applicant, 20 June 2019.
- 5) Request for supplementary information to the applicant, 25 July 2019.
- 6) Request for supplementary information to the applicant, 06 August 2019.
- 7) Receipt of supplementary information from the applicant, 28 October 2019.
- 8) Receipt of supplementary information from the applicant, 03 April 2020.
- 9) Receipt of spontaneous information from the applicant, 16 April 2020.
- 10) Receipt of spontaneous information from the applicant, 07 September 2020.

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¹⁵ Appendix C contains the GMO Panel assessment of a 90-day study on Bt11 maize provided by the applicant in the context of this application.

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Abbreviations

| | |
|-------|--|
| ALT | alanine aminotransferase |
| APTT | activated partial thromboplastin time |
| ANOVA | analysis of variance |
| BUN | blood urea nitrogen |
| ELISA | Enzyme-linked immunosorbent assay |
| GLP | good laboratory practice |
| GM | genetically modified |
| GMO | genetically modified organism |
| GMO | Panel EFSA Panel on Genetically Modified Organisms |
| HGT | horizontal gene transfer |
| OECD | Organisation for Economic Co-operation and Development |
| ORFs | open reading frames |
| PCR | polymerase chain reaction |
| PMEM | post-market environmental monitoring |
| PT | partial thromboplastin time |

Appendix A – List of relevant publications identified by the applicant through systematic literature searches (1 January 2008–1 July 2020)

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Appendix B – List of additional studies performed by or on behalf of the applicant over the course of the authorisation period and not previously submitted to the EU with regard to the evaluation of the safety of the food and feed for humans, animal or the environment from maize Bt11

| Study identification | Title |
|---|---|
| SSB-140-09 | Analysis of the Transgenic DNA Insertion Site in the Genome of Event Bt11 Maize |
| TK0120925 Amendment 1 | Bt11 Maize Insert and Flanking Sequence |
| SSB-104-06 Amendment 1 | Additional Molecular Characterization of Event Bt11 Maize by Southern Analysis |
| SSB-154-08 | Southern Blot Analysis of Event Bt11 Maize with a Full-Length Insert-Specific Probe |
| TK0022087 | Comparison of Microbially Produced Cry1Ab Protein and Cry1Ab Protein Produced in Maize Plants Derived from Event Bt11 |
| Study Report 502983 (Report Number 38503) | A 13-Week Oral (Dietary) Toxicity study of Bt11 maize grain in rats ^(a) |

(a): Refer to Appendix C for the outcome of the assessment of this study.

Appendix C – Outcome of the assessment of a 90-day oral repeated dose toxicity study in rat with maize Bt11 (study number 502983)¹⁶

In this 90-day study, pair-housed Han Wistar rats (RccHan:WIST) (10 per sex per group; 2 rats per cage) were allocated to four groups using a randomised complete block design with five replications per sex. Groups were fed test or control diets containing 10% or 41.5% (w/w) maize from maize Bt11 plants treated with the intended herbicide glufosinate ammonium (test material), or from a conventional counterpart (non-GM comparator control material), respectively. The studies were adapted from the Organisation for Economic Co-operation and Development (OECD) test guideline 408 (1998), aligned with the guidance of the EFSA Scientific Committee (2011) and comply with the principles of good laboratory practice (GLP) with some deviations not impacting the study results and interpretation (i.e. test item stability, homogeneity and concentration), which are detailed below. Event-specific polymerase chain reaction (PCR) analysis confirmed the presence of the event Bt11 in the GM maize grains and GM diets and excluded the presence of the event in the respective controls. Enzyme-linked immunosorbent assay (ELISA) analyses also confirmed the presence of event Bt11 in the GM maize grains and GM diets. Both GM and control maize grains and diets were analysed for nutrients and potential contaminants (e.g. selected heavy metals, mycotoxins, pesticides and microorganisms). Balanced diets were based on the CT1 diet prepared by Special Diet Services. Compositional analyses of the test and control items were performed after the preparation of the diets. Although this is not in line with EFSA recommendations (EFSA, 2014), the GMO Panel noted that subsequent dietary analyses demonstrated the diets were nutritionally acceptable; moreover, evaluation of the biological parameters in control animals indicated they were consistent with those typically observed in animals of the same strain and age given balanced diets. Therefore, the GMO Panel consider this deviation not to impact the study results. The stability of the test and control materials was not verified in the studies for the duration of the treatment; however, in accordance to product expiration declared by the diet manufacturer, the constituents of the diets are considered stable for the duration of the study. The GMO Panel considered 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. Feed and water were provided *ad libitum*. In-life procedures and observations and terminal procedures were conducted in accordance to OECD TG 408 (1998).

In the statistical analysis, for each of the two inclusion rates, rats consuming the test diet were compared with those consuming the respective control diet.¹⁷ The cage was considered the experimental unit. For continuous parameters, a multi-way analysis of variance (ANOVA) was conducted for the two sexes combined (factors: treatment, sex, block-within-sex and sex-by-treatment interaction); in case a significant sex-by-treatment interaction was identified, a two-way ANOVA (factors: treatment and block) was performed separately for males and females. The two-way ANOVA was also used to analyse sex-specific organ weights.

There were no diet-related incidents of mortality or clinical signs. One male from the high-dose group was euthanised on day 62 when found in poor condition due to liver lobe torsion, which is considered not to be related to treatment with Bt11 maize grain.

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:

- Present at the low dose but not in the high-dose groups;
- Were within the normal variation 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.

Detailed description of statistically significant findings identified in rats given diets containing maize Bt11 is reported in Table C.1.

No gross pathological findings related to the administration of the test diets were observed at necropsy in rats given diets containing maize Bt11, and the microscopic examinations of a wide range of organs and tissues did not identify relevant differences in the incidence and severity of the histopathological findings related to the administration of the test diet.

¹⁶ Dossier: Maize Bt11 – Section 2.3.3; additional information: 1/4/2020.

¹⁷ There was a difference in the preparation of the 10% and 41.5% diets, which prevented a statistical comparison using both groups.

The GMO Panel concludes that this study is in line with the requirements of Regulation (EU) No 503/2013 and that no test diet related adverse effects were observed in rats after feeding diets including maize Bt11 at 10% or 41.5% for 90 days. The GMO Panel notes that the applicant only tested 41.5% dose level with the full set of OECD parameters; this incorporation rate of maize is in line with commercially available rodent diets. It has been recently reported that a diet incorporating 50% maize may be tolerated without inducing nutritional imbalances in rats after 90-day administration (Steinberg et al., 2019), but the GMO Panel considers that further scientific confirmation is needed before this 50% maize incorporation rate is applicable in future studies.

Table C.1: Statistically significant findings in the 90-day toxicity study in rats on the whole food/feed from maize Bt11

| Statistically significant parameter/endpoint | Finding | GMO Panel interpretation |
|--|--|---|
| Food consumption | Increased | Not of toxicological relevance – sporadic (week 13) – did not impact on the mean body weight or body weight gain |
| Motor activity | Increased and decreased | Not of toxicological relevance – sporadic – did not impact on overall activity – within normal variation |
| Foot splay | Increased in high-dose females | Not of toxicological relevance – decreased in top-dose males – value is below that for low-dose control females – did not impact on the mean body weight or body weight gain |
| Alanine aminotransferase (ALT) | Reduced in the high-dose groups | Not of toxicological relevance – small magnitude (< 20%) – an increase is indicative of liver damage but a reduction is not adverse in isolation. There were no pathological changes in the liver |
| Serum calcium | Increased | Not of toxicological relevance – small magnitude (1%) |
| Blood urea nitrogen (BUN) | Reduced in the high-dose group | Not of toxicological relevance – a BUN increase is indicative of kidney damage but a reduction is not adverse in isolation – no pathological changes in the kidney |
| Clotting parameters: partial thromboplastin time and activated partial thromboplastin time (PT & APTT) | Increased in THE low-dose groups | Not of toxicological relevance – not seen in the high dose groups – small magnitude (< 10%) – within normal variation |
| Spleen weight | Increased in low-dose males; decreased in low-dose females | Not of toxicological relevance – not seen in the high dose groups – small magnitude (< 10%) – no associated changes in blood or pathology |
| Liver weight | Reduced in high-dose animals | Not of toxicological relevance – small magnitude (< 10%) – no associated changes in pathology or clinical chemistry |
| Kidney weight relative to body weight | Reduced in high-dose animals | Not of toxicological relevance – small magnitude (< 10%) – no associated changes in pathology or clinical chemistry |