Annex to: Scientific opinion on the assessment of genetically modified maize DP51291 (application GMFF-2021-0071) doi:10.2903/j.efsa.2024.9059

Annex 8 Comments and opinions submitted by Member States during the three-months consultation period of application GMFF-2021-0071 (maize DP51291)

Country	Organization	Reference	Comment	GMO Panel responses
Belgium	Sciensano	1.3.7 Summary of comparative analysis including conclusions	It would be more scientifically correct to state "the compositional characteristics of DP51291 maize are not identical but quite similar compared to those of the conventional counterpart and commercial reference maize lines, taking into account biological variation. "	The GMO Panel thanks Belgium for the comment. Quantitative results for the compositional endpoints showing significant differences between maize DP51291 and its conventional counterpart and falling under category III/IV for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain are given in Section 3.4.6 of the Scientific Opinion. These differences were further assessed.
Belgium	Sciensano	1.4.1 Testing of newly expressed proteins	Concerning the PAT protein, which is an acetylating enzyme, Christ et al. (2017) (https://doi.org/10.1038/s41477-017-0061-1) showed that the closely related BAR protein, due to a certain enzyme promiscuity, also acetylates other amino acids. The EFSA (2018) rebuttal of the concern that arises due to the findings of Christ et al. (2017) is certainly reasonable, but not entirely convincing in relation to a	The GMO Panel thanks Belgium for the comment. The study by Christ et al. (2017) has been previously assessed by EFSA in the context of a mandate from the European Commission on public comments on genetically modified oilseed rape Ms8, Rf3 and Ms8×Rf3 under application EFSA-GMO-RX-004 (question number EFSA-Q-2018-00138). EFSA is of the opinion that the results reported in this publication cannot be at present placed in the context of the risk assessment of PAT/bar-expressing genetically modified plants.

			phenomenon that concerns a massive use of food products.	
Belgium	Sciensano	2.1 Dietary role in food and feed	In reading the application, our expert has the impression that the applicant tries to minimize the estimated exposure. Could EFSA comment on this?	In line with Regulation (EU) No 503/2013 the applicant provided dietary exposure estimates (Section 3.5.4.1 of the Scientific Opinion). 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).
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	1. Hazard identification and characterisation	The scope of application GMFF-2021-0071 covers the import, processing and all uses of maize DP51291 as any other maize but excludes cultivation. The Federal Office of Consumer Protection and Food Safety (BVL) as German CA is of the opinion that the data provided by the applicant on molecular characterization as well as on comparative, allergenic and toxicological assessment do not indicate that maize DP51291 has any adverse effects on human and animal health or on the environment in the context of its intended use. However, completion and/or clarification on further points of the dossier are recommended. In addition, the provided monitoring plan needs further elaboration.	The EFSA GMO Panels thanks Germany for the provided comments. The EFSA GMO Panel requested the needed additional information in order to perform the risk assessment. The GMO Panel thanks Germany for this comment, which was taken into account. Indeed, a set of recommendations for the preparation of PMEM plans in order to provide more detail on the measures proposed for the implementation of General Surveillance was proposed for applicant's consideration (see Annex I of the minutes of the CompERA WG of January 2024). EFSA reminds that monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.
Germany	Bundesamt für Verbraucherschutz und	5. Environmental	The import documents should indicate that maize DP51291 has not been approved for cultivation	The GMO Panel thanks Germany for this comment and reminds that labelling is outside the remit of EFSA.

	Lebensmittelsicherheit	Risk Assessment	by the EC. Furthermore, appropriate measures have to be taken during transport, storage, and processing to avoid unintended release of viable maize seed into the environment. In this context, the applicant should inform all parties involved in the handling and processing of maize DP51291 about avoidance and control of spillage.	The applicant indicates in the PMEM plan for this application that procedures will be put in place by the companies involved in the import, handling, processing or transport of the GM material to limit losses and avoid spillage of viable maize DP51291 grains. Furthermore, the PMEM plan states there will be annual communication by the applicant and the parties involved in the PMEM activities to remind of the need to implement these measures.
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	6. Environmental Monitoring Plan	The monitoring plan is acceptable, but needs further elaboration for implementation. Therefore, the applicant is recommended to revise the monitoring plan during the initial implementation phase (after consent is given) and present this revised monitoring plan together with a first report one year after consent is given to be reassessed.	The GMO Panel thanks Germany for this comment, which was taken into account. Indeed, a set of recommendations for the preparation of PMEM plans in order to provide more detail on the measures proposed for the implementation of General Surveillance was proposed for applicant's consideration (see Annex I of the minutes of the CompERA WG of January 2024). EFSA reminds that monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	1.2.2 Information relating to the genetically modified plant	In plasmid PHP74638 there are three sequences for recombination sites of the Lambda-Integrase-Recombinase (attB1, attB2, attB3) located within the T-DNA-fragment reported to be inserted into the genome of maize DP51291. The applicant shall clarify the function of these sites. The applicant does not present raw data for phenotyping, RT-qPCR and qualitative PCR of the segregation analysis (PHI-2018-064). Thus, the	The GMO Panel has reviewed all the elements contained in the event. The presence of these sequences, usually used for cloning purposes, does not raise safety concerns. The segregation analysis is reported in study PHI-2018-035. In all the tested plants the genotypic result matched the phenotypic result (herbicide tolerance), demonstrating co-segregation between the inserted DNA and the trait.

			applicant should be asked to provide the missing raw data and clarify which dataset is summarized in Table 3 (PHI-2018-064).	
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	1.3.4 Comparative analysis of composition	In order to discuss the biological relevance of nutritional components present in the category 5-7 of the equivalence testing in study PHI-2022-175, the applicant refers to the tolerance interval as established in study PHI-R144-Y21. This study is not available and should be supplied.	The GMO Panel assessed all the significant differences between maize DP51291 and the conventional counterpart. 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 forage and grain composition between maize DP51291 and its conventional counterpart needs further assessment regarding food and feed safety except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain, which were further assessed in section 3.5 of the Scientific Opinion. The GMO Panel did not use the information on tolerance interval for the assessment of the outcomes of the statistical analysis. Hence, report PHI-R144-Y21 was not considered necessary.
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	1.4.1 Testing of newly expressed proteins	Maize DP51291 expresses the previously unevaluated protein IPD072Aa with an insecticidal activity against certain coleopteran species. However, the underlying mechanism is not presented by the applicant. The applicant refers to application EFSA-GMO-NL-2019-163 which applies for placing on the market of maize DP23211 expressing the identical protein. Nevertheless, data on the mode of action of protein IPD072Aa are	The GMO Panel has previously assessed IPD072Aa, including data on stability, and no safety concerns for humans and animals have been identified (EFSA GMO Panel, 2024). Furthermore, the publication of Jiménez-Juárez et al. (2023), describing the mode of action of the IPD072Aa protein has also been considered and the GMO Panel concluded that it does not add new information that would raise concerns for safety (EFSA GMO Panel, 2024).

			missing in this application as well. The applicant refers to Schellenberger et al., 2016, who, however, does not conclude on the mode of action, specificity or the LD50 of target organisms. The data in Jiménez-Juárez et al. (2023) suggest an action of IPD072Aa on the brushborder vesicles of the insect gut. Yet, the mode of action is presumably different as IPD072Aa is effective in killing WCR larvae that are resistant to Bt proteins produced by currently available transgenic corn (Schellenberger et al., 2016). Although the data on protein IPD072Aa submitted by the applicant do not give any indication of a possible adverse health effect, the applicant is requested to provide a full description of the function and mode of action of the protein IPD072Aa according to Regulation (EU) 503/2013. Depending on its function, further characterisation of the protein might be necessary (e.g. in case of enzymatic activity). The applicant showed identity of IPD072Aa protein preparations derived from maize DP23211 (EFSA-GMO-NL-2019-163) and maize DP51291. On these grounds, he referred back to toxicological characterisation of IPD072Aa in application EFSA-GMO-NL-2019-163. Therefore, we may recall the following comments of the German CA on this part of	

			application EFSA-GMO-NL-2019-163: The IPD072Aa protein is heat stable (95°C) and no data on the stability of the protein at different pH-values are presented. A description of the stability of the protein IDP072Aa under relevant processing and storage conditions of maize DP51291 and the expected treatment of the derived food and feed is missing. The history of save use of the source organism (Pseudomonas chlororaphis) as a bio-pesticide presented by the applicant is not sufficiently transferable to the risk assessment of the newly expressed protein IPD072Aa within the scope of this application for authorisation of genetically modified food and feed.	
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	6.2 Case specific monitoring (strategy method and analysis)	According to the risk assessment, no adverse effects on the environment or human health were identified or were expected. Therefore, there is no necessity for a case-specific monitoring.	The GMO Panel thanks Germany for this comment and takes note of the comment.
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	6.3 General Surveillance (strategy, method)	The monitoring plan does not relate the monitoring activities to relevant protection goals. Even more it is not described which routine observations (including parameters or monitoring characters) are carried out in relation to the protection goals. Only reporting on 'any unanticipated effect' is solely not an appropriate parameter,	The GMO Panel thanks Germany for the comment, which was taken into account. Indeed, a set of recommendations for the preparation of PMEM plans in order to provide more detail on the measures proposed for the implementation of General Surveillance was proposed for applicant's consideration (see Annex I of the minutes of the CompERA WG of January 2024). EFSA reminds that monitoring is related to risk management, and thus a final

			because it already anticipates an evaluation. This evaluation process should be based on a distinct set of parameters and a scientific sound data analysis. It is requested that the applicant specifies in detail, how and which information will be pro-actively queried, gathered, and how they will be evaluated. In addition, it might be useful to integrate information about the use of the product in food and feed to deliver supplementary helpful data (of exposure to consumers and animals) for general surveillance. Therefore, the applicant should specify monitoring activities in the field of human and animal health. He should describe in detail how animal and human health surveillance is integrated in the monitoring plan. The strategy of General Surveillance is mainly based on the involvement of importers, traders, silo operators and processors coordinated by CropLife Europe. The applicant will inform the selected networks of operators about market release of GM plant products and will remind them to report on 'any unanticipated adverse effect'. He stated that these third parties have to follow legal obligations of food and feed hygiene (HACCP). Nevertheless, the role and interplay of all actors on behalf of recording, analysis and evaluation of monitoring data needs more	adoption of the PMEM plan falls outside the mandate of EFSA.

			transparency. The applicant should consider whether other existing monitoring networks might be used in particular in the field of human and animal health. In such a case, the selection and evaluation process should be described in detail. In general, other sources of information e.g. peer-reviewed publications or ongoing research should be taken into account. However, the applicant should describe in detail how he would consider this information within General Surveillance.	
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	6.4 Reporting results of PMEM	A report on GS activities on an annual basis is sufficient. Reporting should refer to the format introduced by the Commission Decision 2009/770/EC. The applicant is requested to state how the monitoring results will be published.	The GMO Panel thanks Germany for the comment and takes note of the comment.
Germany	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit	5.3.4 Interactions of the GM plant with non-target organisms (NTOs)	No comments by BVL, please see comments by BfN attached as a file	
Germany	BfN	II.1 Hazard identification and characterization	The Federal Agency for Nature Conservation (BfN) considers that further information should be presented before the risk assessment of GMFF-2021-0071 can be finalised. No history of safe use can be assumed per se for the newly expressed insecticidal	The GMO Panel concluded that IPD072Aa (as well as PAT and PMI) newly expressed in maize DP51291 do not raise safety concerns for human and animal health. Moreover, the GMO Panel did not identify indications of safety concerns regarding allergenicity or adjuvanticity related to the presence of the newly expressed proteins in maize DP51291.

			protein IPD072Aa. Basic information and data on the toxicity, ecotoxicology and specificity of the toxins are missing. The molecular characterization of event DP51291 revealed several shortcomings and should be improved by the applicant to be able to finalize the risk assessment. The comparative analysis of event DP51291 revealed non-equivalent changes in metabolite composition. Conclusions on the food and feed safety of DP51291 maize based on this information are premature	The GMO Panel finds no evidence that the genetic modification impacts the overall safety of maize DP51291. Based on the outcome of the comparative assessment and the nutritional assessment, the GMO Panel concludes that the consumption of maize DP51291 does not represent any nutritional concern, in the context of the scope of this application. The GMO Panel concludes that maize DP51291, 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.
Germany	BfN	II.1.2 Molecular characterization II. 1.2.2. Information relating to the genetically modified plant	Information on the sequences actually inserted/ deleted: From CBI: The applicant performed Southern-by-Sequencing to determine the number of transgene copies and the structure of the actual insert in event DP51291 (PHI-2022-120). Presented results were mostly conclusive, detecting only two junctions between genomic and insert sequences, as expected for a single insertion. However, there is residual mapping to backbone sequences at a low level (Fig. 16 - 18; plasmids PHP16072, PHP5096, PHP46438 or PHP21139, PHP31729 respectively). The number of reads corresponding to the displayed alignments cannot be deduced from the data provided. In addition to the	Residual mapping to backbone sequences is due to coverage for the endogenous elements that are identical to sequences in the DP51291 maize insertion. It is considered below the threshold of significance.

			relative (logarithmic) scale and in order to enable better assessment, the number of reads (in relation to the scale) should be presented in the study (either in the text or the graph). From CBI: The characterisation of genomic borders of DP51291 revealed inconclusive results regarding the insertion site (PHI-2022-205/ 230). This is likely caused by non-homologous regions between the public maize B73 reference genome assembly used for BLAST searches and the actual genomic background PHR03 of DP51291, as also discussed by the applicant. Some of the potential insertion sites, as identified by BLAST of 3' border, are in close proximity (0.4 – 3.8 kb) to annotated endogenous maize genes, which may affect their expression. Therefore, the applicant should further characterise the insertion site of the event DP51291 within the actual genomic background PHR03 to clearly exclude that no endogenous element (coding or regulatory sequence) was disrupted by the insertion. Ideally, the applicant should describe the genomic neighbourhood of the insertion site also considering the adjacent up and downstream genes (that might not be present in current public databases). Genetic stability	Regarding the characterisation of genomic borders of DP51291, EFSA requested bioinformatic update in additional data request on 08/05/2024 (ADR-7) which was provided by the applicant on 06/08/2024. The updated bioinformatic data package contained an updated analysis for the identification of possible interruption of maize endogenous genes. The analysis using the maize reference genome confirmed that no endogenous genes were interrupted by the insert. The choice of the 3 probes, with the restriction pattern based on *Sac*I digestion allows to cover the entire insert sequence. The GMO Panel considers the approach used compliant to EFSA guidelines and sufficient both in terms of coverage and sensitivity.

			of the insert and phenotypic stability of the genetically modified plant Minor comment; from CBI: The applicant presents Southern Blot and segregation analyses in five consecutive generations to show that the event DP51291 is genetically and phenotypically stable (PHI-2022-064, PHI-2018-035). The results of the analysis indicate the genetic stability of the insert. However, the experimental design of the Southern Blot analysis has shortcomings, e.g. probes used do not cover the entire insert sequence (PHI- 2022-064). Besides, this analysis is not state-of-the-art and better methods are available, e.g. southern-by-sequencing as performed by the applicant for insertion analysis (PHI-2022- 120)	
Germany	BfN	II.1.3. Comparative analysis II.1.3.4 Comparative analysis of composition	The compositional analyses demonstrated an upregulated amino acid profile for maize DP51291 compared to control and reference lines, with 9 (IHT), respectively 11 (CHT) amino acids classified in outcome category 5-7. Hence, equivalence is challenged for a key metabolic pathway. Similar changes in amino acid profile have been observed for maize event DP23211 (EFSA-GMO-NL-2019-163), which also expresses IPD072Aa, PAT and PMI driven by same promoters as in	The GMO Panel assessed all the significant differences between maize DP51291 and the conventional counterpart. 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 forage and grain composition between maize DP51291 and its conventional counterpart needs further assessment regarding food and feed safety except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain, which were further assessed in section 3.5 of the Scientific Opinion.

			DP51291. Therefore, it is likely that the shared genetic modifications of DP51291 and DP23211 cause unintended effects on the plant's metabolism. The applicant discussed the biological relevance of the observed changes in maize DP51291 for each analyte separately, but failed to touch on the potential impact of the changes for plant metabolism as a whole. However, the assessment of biological relevance needs a more holistic approach. The comparative assessment indicates a lack of equivalence in amino acid metabolism for maize DP51291 and hence the impact of these key metabolic changes on other plant metabolism pathways needs further assessment. We suggest a step-by-step omics analysis based on systems biology approach as outlined in Benevenuto et al. (2023) Benevenuto et al. (2023). Integration of omics analyses into GMO risk assessment in Europe: a case study from soybean field trials. Environmental Sciences Europe, 35 (14). doi: 10.1186/ s12302-023-00715-6	
Germany	BfN	II.5 Environmental Risk Assessment	Import and processing of insect resistant maize are usually considered to have less environmental impact than cultivation. However, the fate and	The GMO Panel thanks Germany for this comment. Given that environmental exposure of non-target organisms to spilled GM material or

		II.5.2.4 Interactions of the GM plant with non-target organisms (NTO)	exposure scenarios of the insecticidal IPD072Aa protein from maize DP51291 should be considered in the assessment of environmental effects, similar to other insecticidal toxins. Literature indicates a fate of Bt-toxins into the environment via feed and manure of livestock fed with Bt-Maize (Campos et al. 2018, Gruber et al. 2011; Guertler et al. 2010, Paul et al. 2010). This exposure scenario is also relevant for the intended uses of DP51291 in the EU. Particularly as it was shown that IPD072Aa is stable and biologically active after heat treatment up to 95°C (Carlson et al. 2019). Exposure of the environment via waste material from processing therefore needs to be anticipated. Gastric fluids in mammals are likely to degrade the toxin but studies with a qualitative proof (i.e. bioassays) are missing. Also, the information on non-target soil organisms, which would be the main group affected by waste and manure containing IPD072Aa, is insufficient and not provided by the applicant. As the mode of action of IPD072Aa is not fully known (Jiménez-Juárez et al. 2023), a specificity only to WCR is highly unlikely given the biology of Pseudomonas chlororaphis. In general, available data on non-target organisms (Boeckman et al.	occasional feral GM maize plants arising from spilled maize DP51291 grains is limited, and because ingested proteins are degraded before entering the environment through faecal material of animals fed GM maize, the GMO Panel considers that potential interactions of maize DP51291 with non-target organisms do not raise any environmental safety concern. The protein IPD072Aa newly expressed in maize DP51291 has been previously assessed by the GMO Panel (EFSA GMO Panel, 2024) and no safety concerns for humans and animals have been identified. As stated in EFSA GMO Panel (2024), the results of the assays described in Section 1.4.1 of the dossier (Testing of newly expressed proteins) relevant to that application confirm that the insecticidal toxin IPD072Aa is digested in simulated gastric and intestinal fluid after less than 30 seconds and 20 minutes, respectively. Thus, the exposure of target and non-target soil organisms to the insecticidal protein through manure and faeces of animals fed with the GM maize is likely to be very low. Given that the scope of the present application excludes cultivation, no bioassays to evaluate potential negative effects on non-target organisms are required. Additionally, the CompERA WG discussed the publication Boeckman et al 2019 (see minutes of the CompERA WG of 28 November 2023) and did not identify any information that raised safety concerns. Furthermore, the publication of Jiménez- Juárez et al. (2023), describing the mode of action of the IPD072Aa protein has also been considered and the GMO Panel concluded that it does not add new information

			2019, 2021) are insufficient to finalise risk assessment. Therefore, the applicant should provide 1) data about the concentration of IPD072Aa in manure of livestock fed with DP51291, b) data about the distribution of IPD072Aa in the environment via wastewater and manure into soil and waterbodies, c) data about the effect of IPD072Aa on non-target organisms, especially soil and water organisms, including additive and synergistic effects on lethal and sub-lethal fitness parameters. References: Boeckman, C.J. et al. (2019). Characterization of the Spectrum of Insecticidal Activity for IPD072Aa: A Protein Derived from Psuedomonas chlororaphis with Activity Against Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). Journal of economic entomology 112 (3): 1190–1196. doi: 10.1093/ jee/ toz029. Boeckman, C.J. et al. (2021). Environmental risk assessment of the DvSSJ1 dsRNA and the IPD072Aa protein to non-target organisms, GM Crops & Food, 12 (1): 459-478. doi: 10.1080/ 21645698.2021.1982348. Campos, R.C. et al. (2018). Indirect exposure to Bt maize through pig faeces causes	that would raise concerns for safety (EFSA, 2024).

			behavioural changes in dung beetles. J. Appl. Entomol., 57 (117). doi: 10.1111/ jen.12532. Carlson, A.B. et al. (2019). Safety assessment of coleopteran active IPD072Aa protein from Pseudomonas chlororaphis. In: Food and chemical toxicology: an international journal published for the British Industrial Bio- logical Research Association 129: 376–381. doi: 10.1016/ j.fct.2019.04.055. Gruber, H. et al. (2011). Fate of Cry1Ab Protein in Agricultural Systems under Slurry Management of Cows Fed Genetically Modified Maize (Zea mays L.) MON810: A Quantitative Assessment. Journal of Agricultural & Food Chemistry 59 (13), 7135–7144. doi: 10.1021/ jf200854n. Guertler, S.P. et al. (2010). Long-term feeding of genetically modified corn (MON810) - Fate of cry1Ab DNA and recombinant protein during the metabolism of the dairy cow. Livestock Science, 131: 250-259. doi: 10.1016/ j.livsci.2010.04.010. Jiménez-Juárez, N. et al. (2023). IPD072Aa from Pseudomonas chlororaphis Targets Midgut Epithelial Cells in Killing Western Corn Rootworm (Diabrotica virgifera virgifera). Applied and Environmental Microbiology, 89 (3). doi: 10.1128/ aem.01622-22.	

			Paul, V. et al. (2010). Degradation of Cry1Ab protein from genetically modified maize (MON810) in relation to total dietary feed proteins in dairy cow digestion. Transgenic Res. 19 (4). doi: 10.1007/ s11248-009-9339-z.	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	6. Environmental Monitoring Plan	The monitoring plan presented is very general and in principle identical to monitoring plans for other GM maize products submitted previously. Previous recommendations and suggestions for improvements submitted by Austria - based on issues discussed in the scientific literature, in scientific reports of competent authorities from various member states (see e.g. Zúghart et al. (2011)) were not taken into account. [Zúghart W, Raps A, Wust-Saucy A-G, Dolezel M, Eckerstorfer M, 2011. Monitoring of genetically modified organisms. A policy paper representing the view of the National Environment Agencies in Austria and Switzerland and the Federal Agency for Nature Conservation in Germany. Umweltbundesamt Wien, Reports, Volume 0305, ISBN: 978-3-99004-107-9; http://www.umweltbundesamt.at/aktuell/publikationen/publikationssuche/publikationsdetail/?pub_id=1903.]	The GMO Panel thanks Austria for this comment, which was taken into account. Indeed, a set of recommendations for the preparation of PMEM plans in order to provide more detail on the measures proposed for the implementation of General Surveillance was proposed for applicant's consideration (see Annex I of the minutes of the CompERA WG of January 2024). EFSA reminds that monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.

Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.2 Molecular Characterisation	1.2.2.2 Information on the sequences actually inserted or deleted The molecular characterisation of the transgenic insert in GM maize DP51291 was conducted using a novel approach based on Next Generation Sequencing and Southern-by-Sequencing™ (FROM CBI: Annex 5_PHI-2022-120). This approach was developed as a standardised procedure applicable to events generated by the current techniques of genetic modification (Zastrow-Hayes et al. 2015). According to the notifier such a standard procedure offers advantages compared to methods which need to be customised for each transgenic event, like an analysis by Southern Blot hybridisation. Based on the bioinformatic analysis of the integration locus, the notifier concludes that the insertion of T-DNA sequences in the GM maize DP51291 has not disrupted any endogenous maize gene (FROM CBI: Annex 7 PHI-2022-205/230). However, alignments of the 5'- and 3'- insert flanking sequences as well as the pre-insertion site reveal several significant homologies to maize EST sequences indicating the presence of actively transcribed sequences. EFSA is requested to ask the notifier to provide a more detailed scientific explanation for his	EFSA requested bioinformatic update in additional data request on 08/05/2024 (ADR-7) which was provided by the applicant on 06/08/2024. The updated bioinformatic data package contained an updated analysis for the identification of possible interruption of maize endogenous genes. The analysis using the maize reference genome confirmed that no endogenous genes were interrupted by the insert.

			conclusions. Scientific Information 1.2.2; p. 6 4. Subcellular location(s) of insert(s): The applicant reports that "the bioinformatic analysis of the DP51291 insertion flanking borders (Annex 7) against public databases to identify the insertion position are ambiguous and do not allow for conclusive identification of the insertion site." This observation is disconcerting. The applicant reports that more than 1 kb of genomic sequence information on the 5' and 3' regions flanking the transgenic insert is available. This should be sufficiently extensive to find homologous regions in non-transformed maize wildtype genomes stored in sequence databases. As information about the subcellular localisation of the transgenic insert is of crucial relevance for the risk assessment the applicant would have to make additional efforts besides obtaining approx. 1 kb from the left and the right flanking sequence to confirm the exact localisation of the transgenic insert in the maize genome – even if this means that sequencing has to be extended for several kilobase pairs or more using chromosome walking, primer walking, shotgun sequencing or similar approaches for establishing the correct context of the transgenic insert on the chromosome. We would like to ask	

			the EFSA GMO panel to ask the applicant for a more in-depth sequencing approach. Only referring to "ambiguous" or "inconclusive" results and providing assumptions as solution for this problem is insufficient for a conclusive risk assessment. 5. Sequence information on flanking regions at each insertion site: The applicant reports that the 5' and 3' flanking genomic border sequences "…were subjected to BLAST analysis against separate datasets to identify the genomic location of the insert and to determine if any endogenous maize genes were disrupted by the insertion" and concludes that "no alignments indicating the presence of a gene were returned for the 5' or 3' genomic border sequences." That is insufficient for a responsible risk assessment. We would like to ask the EFSA GMO Panel to ask the applicant for more sequence information on the localisation of the transgenic insert. [Zastrow-Hayes GM, Lin H, Sigmund AL, Hoffman JL, Alarcon CM, Hayes KR, Richmond TA, Jeddeloh JA, May GD, Beatty MK, 2015. Southern-by-sequencing: A robust screening approach for molecular characterization of genetically modified crops. The Plant Genome 8(1).]	

Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.2.1 Information relating to the genetic modification	Scientific Information 1.2.1., p. 4 No physical map (nor a table describing the genetic elements contained on the transformation vector) is presented in the Scientific Information. Directing the reader to several study reports (of more than 100 pages in total) for finding this essential information is very user-unfriendly. This crucial information should be presented in the main body of the Scientific Information.	The EFSA GMO Panel would like to thank Austria for this comment.
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.2.2 Information relating to the genetically modified plant	1.2.2.3 Information on the expression of the insert(s) The applicant presents ELISA data for the concentrations of the proteins IPD072Aa, PAT and PMI from various plant tissues (leaf, root, pollen, forage and grain) gathered from field trials conducted at six locations in 2021 in the USA and Canada (Annex 9). Expression of IPD072Aa is highest in root samples (Annex 9, Tab. 4) and concentrations in root samples seem to vary among sites irrespective of the treatment with the complementary herbicide glufosinate. However, it is not easy to clearly identify the trial sites, at which a specific sample was produced from the way the results are presented in table 7 (Annex 9, Tab. 7). The notifier does not discuss the differential expression levels of IPD072Aa in different tissues as demonstrated by his	The EFSA GMO Panel thanks Austria for this comment. The NEP levels across different tissues assessed by the GMO Panel are presented in Table 1 of the scientific opinion. The NEP levels are comparable between treated and untreated samples. The levels of the NEPs for DP51291 on one hand, and DP23211 on the other, cannot be directly comparable as they were generated across different field trials, different growing seasons and sites.

			analysis, nor does he include and discuss further information on the expression characteristics of the particular banana streak virus promoter, i.e. banana streak virus of acuminata Yunnan strain – BSV(AY), used to drive expression of IPD072Aa in GM maize DP51291. Available literature suggests that BSV promoters generally lead to near-constitutive expression of transgenes in vegetative tissues of monocot plants, including maize (Remans et al. 2007). The submitted data, however, would suggest an expression bias with highest levels of expression in root tissues of growing plants. The same promoter was used in another event expressing IPD072Aa protein, i.e. GM maize DP23211. However, in this event far lower concentrations of the transgenic toxin in root samples were detected (19-31 ng IPD072Aa/mg tissue dry weight in DP23211 vs. 76-180 ng IPD072Aa/mg tissue dry weight in GM maize DP51291). The notifier should explain whether the particular promoter was deliberately chosen to establish this expression pattern. He should further explain whether the expression pattern is an unintended, yet advantageous characteristic and he should discuss possible reasons for the elevated expression compared to	The EFSA GMO Panel wishes to thank Austria for this comment and reminds that the justification on the choice of the genetic elements is not a requirement of the regulation. Therefore, the information provided was deemed sufficient. The applicant performed descriptive statistics on the NEP levels reported for all tissues in accordance with the Explanatory Note on the determinations of newly expressed protein levels in the context of genetically modified plant applications for EU market authorisation (EFSA, 2018) EFSA (European Food Safety Authority), Paraskevopoulos K, Ramon M, Dalmay T, du

			event DP23211. We would also appreciate an analysis of variance and a discussion on the expression results taking into account intended and unintended differences in expression in various tissue types as well as potential impacts of the environmental conditions. In general, we recommend that EFSA requests a comparison of expression data based on a more detailed statistical analysis and based on the requirements in Implementing Regulation (EU) No 503/2013 (Annex II, 1.2.2.3.f) (EC 2013). We consider this to be of significant value for the exposure assessment and the toxicological assessment. Further information on promoter characteristics and trial site identification would be appreciated. 2.2.4 Genetic stability of the insert and phenotypic stability of the GM plant The applicant concludes that the insert is stably integrated into GM maize DP51291 from an assessment of plants from 5 generations of GM maize (T1, T2, T3, T4 and T5) by means of Southern blot analysis. However, the method (Southern blot) to characterise the transgenic inserts present in GM maize DP51291 do not detect minor alterations in the inserts, like single nucleotide polymorphisms (SNPs), which can be introduced during breeding processes	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 publication 2018:EN-1466. 13 pp. doi:10.2903/sp.efsa.2018.EN-1466 The applicant has provided Southern analysis of genomic DNA and PCR-based segregation analysis data from several generations to demonstrate the stability. The data provided were considered sufficient by the GMO Panel.

			(Morisset et al. 2009). Additionally, the stability test was conducted on one plant/generation which is considered an insufficient number of test plants to reliably demonstrate genetic stability. The notifier should therefore amend the molecular characterisation with methods and number of tested plants which allow the assessment of the integrity of the transgenic insertions and the flanking sequences to provide a better basis to assess genetic and phenotypic stability of GM maize DP51291. [EC, 2013. 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. Official Journal of the European Union. L 157/1: 1-48. Morisset D, Demšar T, Gruden K, Vojvoda J, Štebih D, Žel J, 2009. Detection of genetically modified organisms - closing the gaps. Nature Biotechnology 27(8): 700-701. Remans T, Iram S, Shuey L, Jaufeerally-Fakim Y, Schenk P, 2007. Banana streak virus: A highly diverse plant pararetrovirus. Plant viruses published by Global science books.]	

Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.2.3 Additional information relating to the genetically modified plant required for the environmental safety aspects	1.2.2.5 Potential risk associated with horizontal gene transfer Scientific Information 1.2.2; p. 12 The applicant maintains that there are "no scientific elements that would suggest horizontal gene transfer is likely to occur from the insert of DP51291 maize." However, in the following sentence the applicant refers to the fact that "gene-sized plant DNA is expected in environments where crops are grown and in gastrointestinal systems after consumption." We would like to indicate that not the likelihood and/or the frequency of horizontal gene transfers in natural environments like soil or the gastrointestinal tract is decisive for long-term adverse effects on human and animal health or the environment, but the selection pressure persisting the bacterial population under exposition (Pettersen et al. 2005). The applicant maintains that "HGT to soil organisms has only been detected with very promiscuous microbes under laboratory conditions designed to favor transfer" and refers to a publication of the US-Environmental Protection Agency as proof of evidence (US-EPA 2010). This 253-page EPA publication refers to HGT with a total of thirteen lines und provides evidence to their conclusions by referring to "...several experiments	The GMO Panel thanks Austria and takes note of these observations. Bioinformatic analysis of event DP51291 revealed that sufficient sequence identity was detected with the *pmi* coding sequence from *E. coli*. No paired alignments and, thus, no potential to facilitate double HR were identified. Gene replacements of *pmi* sequence on natural *E. coli* might potentially occur in the main receiving environments, i.e. the gastrointestinal tract, but this would not confer any new trait or selective advantage to bacterial recipients. The analysis also confirmed that the genetic elements encoding for PAT and IPD072Aa proteins were plant codon-optimised and did not provide sufficient sequence identity to bacterial DNA. There is no indication for an increased likelihood of horizontal transfer of DNA from maize DP51291 to bacteria. Given the nature of the recombinant DNA, the GMO Panel identified no safety concern linked to an unlikely but theoretically possible HGT.

			(published in scientific journals)…" without providing any citations of these scientific journals. The applicant refers to "eukaryotic promoters used to drive expression of the transgenes in DP51291 maize would show limited, if any, activity in bacteria" implying that this would be a prerequisite for an effective HGT. We would like to indicate that HGT by natural transformation of bacteria is not relying on promoter elements on transgenic inserts to be expressed in bacterial cells (Chen and Dubnau 2004). The applicant maintains that "the inserted genes expressed in DP51291 maize would not pose any risk to human and animal health or the environment if expressed in bacteria." This is not correct. Glufosinate, inactivated by the bacterial pat gene, is interfering with bacterial growth and is acting as antimicrobial agent under certain circumstances leading to shifts in bacterial community structures (Calanduoni and Villafranca 1986; Bartsch and Tebbe 1989; Ahmad and Malloch 1995; Sessitsch et al. 2005; Chau-Ling et al. 2007; Pampulha et al. 2007; Tothova et al. 2010; Kopcáková et al. 2015). Deliberate dispersal of transgenic may, thus, have an adverse affect on the environment. We would like to ask the EFSA GMO Panel to take note	

			of these observations. Scientific Information 1.2.2; p. 13 The applicant maintains that "… there are no reports in the literature demonstrating that HGT occurs from plants to animals and humans..." We would like to indicate that there are several peer-reviewed reports available describing exactly this phenomenon (i.e. integration of food/feed/plant-derived DNA into the mammalian genome) (Schubbert et al. 1998), (Mazza et al. 2005), (Deaville and Maddison 2005). Moreover, plant-derived DNA sequences especially from multi-copy (e.g. plastid) genes are detectable in blood and/or tissues after ingestion (Phipps et al. 2003; Deaville and Maddison 2005; Hanusová et al. 2007; Rehout et al. 2008; Bertheau et al. 2009; Spisák et al. 2013). We would like to ask the EFSA GMO Panel to take note of these observations.	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.2.4 Other information (eg. additional info on single events or subcombinations...)	References regarding Comments on Chapter "1.2.2.5 Potential risk associated with horizontal gene transfer" [Ahmad I, Malloch D, 1995. Interaction of soil microflora with the bioherbicide phosphinothricin. Agriculture, Ecosystems and Environment 54(3): 165-174. Bartsch K, Tebbe CC, 1989. Initial steps in the degradation of phosphinothricin (glufosinate) by soil bacteria. Appl	References

			Environ Microbiol 55(3): 711-716. Bertheau Y, Helbling JC, Fortabat MN, Makhzami S, Sotinel I, Audeon C, Nignol AC, Kobilinsky A, Petit L, Fach P, Brunschwig P, Duhem K, Martin P, 2009. Persistence of plant DNA sequences in the blood of dairy cows fed with genetically modified (Bt176) and conventional corn silage. J Agric Food Chem 57(2): 509-516. Calanduoni JA, Villafranca JJ, 1986. Inhibition of Escherichia coli glutamine synthetase by phosphinothricin. Bioorg. Chem. 14: 163-169. Chau-Ling H, Chiu-Chung Y, Ching-Yuh W, 2007. Screening and Identification of Glufosinate-Degrading Bacteria from Glufosinate-Treated Soils. Weed Sci 55(6): 631-637. Chen I, Dubnau D, 2004. DNA uptake during bacterial transformation. Nature Reviews Microbiology 2(3): 241-249. Deaville ER, Maddison BC, 2005. Detection of transgenic and endogenous plant DNA fragments in the blood, tissues, and digesta of broilers. J Agric Food Chem 53(26): 10268-10275. Hanusová L, Vrabcová P, Rehout V, 2007. Detection of DNA fragments from feed containing GM organisms in blood of broilers. Genetics and Animal Breeding, Brno, Mendel University of Agriculture and Forestry Brno. Kopcáková A, Legáth J, Pristaš P,	

			Javorský P, 2015. Already a short-term soils exposure to the field-rate glufosinate concentration significantly influences soil bacterial communities. Soil and Water Research 10(4): 271-277. Mazza R, Soave M, Morlacchini M, Piva G, Marocco A, 2005. Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Research 14(5): 775-784. Pampulha ME, Ferreira MASS, Oliveira A, 2007. Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms. Journal of Basic Microbiology 47(4): 325-331. Pettersen AK, Bohn T, Primicerio R, Shorten PR, Soboleva TK, Nielsen KM, 2005. Modeling suggests frequency estimates are not informative for predicting the long-term effect of horizontal gene transfer in bacteria. Environ Biosafety Res 4(4): 223-233. Phipps RH, Deaville ER, Maddison BC, 2003. Detection of transgenic and endogenous plant DNA in rumen fluid, duodenal digesta, milk, blood, and feces of lactating dairy cows. J Dairy Sci 86(12): 4070-4078. Rehout V, Hanusová L, Čítek J, Kadlec J, Hosnedlová B, 2008. Detection of DNA fragments from Roundup Ready soya in blood of broilers. Journal of Agrobiology 25: 145-148. Schubbert R, Hohlweg U, Renz D, Doerfler W,	

			1998. On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission to the fetus. Molecular and General Genetics 259(6): 569-576. Sessitsch A, Gyamfi S, Tscherko D, Gerzabek M, Kandeler E, 2005. Activity of microorganisms in the rhizosphere of herbicide treated and untreated transgenic glufosinate-tolerant and wildtype oilseed rape grown in containment. Plant Soil 266: 105-116. Spisák S, Solymosi N, Ittzés P, Bodor A, Kondor D, Vattay G, Barták BK, Sipos F, Galamb O, Tulassay Z, Szállási Z, Rasmussen S, Sicheritz-Ponten T, Brunak S, Molnár B, Csabai I, 2013. Complete Genes May Pass from Food to Human Blood. PLoS One 8(7): e69805. Tothova T, Sobekova A, Holovska K, Legath J, Pristas P, Javorsky P, 2010. Natural glufosinate resistance of soil microorganisms and GMO safety. Central European Journal of Biology 5(5): 656-663. US-EPA, 2010. Biopesticides Registration Action Document: Cry1Ab and Cry1F Bacillus thuringiensis (Bt) Corn Plant-Incorporated Protectants. EPA: 94.]	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.3 Comparative analysis	A field trial was conducted at eight to twelve sites, ten sites in the US and two sites in Canada, in 2021 and comprises GM maize DP51291 treated with conventional	The GMO Panel thanks Austria for these considerations.

			herbicides and GM maize DP51291 treated with the intended herbicide glufosinate, a non-GM control and a total of 20 reference varieties, four at each site (Annex 12). We would like to submit the following comments on the comparative analysis. • The notifier demonstrates the diversity of selected sites and presents information on climatic conditions, soil characteristics and planting. However, beside the indication of the comparative relative maturity of the test material and reference varieties and of respective crop maturity zones of the field trial sites (Annex 12, Fig. 1 & Appendix 4) no rationale is presented regarding the use of the chosen parameters in the selection of the test sites. • We appreciate the indication of the target application rate of the applied glufosinate herbicide (Annex 12, Appendix 4, Table 11) and the statement that "the application rate that was used is a labelled rate that is used by farmers" (Annex 2, Appendix 4, p.12). However, a clarification is missing, whether this application rate represents one of many possible rates or an average rate of this herbicide on glufosinate tolerant GM maize crops. It has been shown for example that glyphosate application rates applied on glyphosate resistant (GR) soybeans grown	The EFSA GMO Panel considered that the agro-meteorological variability at the sites selected for the compositional and agronomic/phenotypic characterisation are able to ensure a sufficient range of environmental conditions reflecting those under which the four-event stack maize might be cultivated in practice. The provided information was considered sufficient to assess site representativeness. In relation with the clarification on the application rate of the intended but also the conventional herbicides, the GMO Panel considered adequate the rationale provided in "Appendix C1_Agro-pheno_DP51291".

			commercially in North and South America often exceed the application rates used in experimental field trials with (GR) soybeans (Miyazaki et al. 2019). The EFSA guidance documents (EFSA 2010; EFSA 2015) as well as Implementing Regulation (EU) No 503/2013 (EC 2013) state that a justification shall be provided that the sites and conditions are representative of the range of receiving environments, where the crop will be commercially grown, explicitly justifying the choice of sites (EFSA 2010). Additionally, realistic test conditions are an essential element for an adequate ERA. Thus, we request that the notifier is requested to apply the multi-factor approach elaborated by EFSA including the suggested graphical illustration in order to facilitate the appraisal of the representativeness of sites and provide a clarification regarding the application rate of glufosinate during the field trials in relation to application rates used for commercial GM glufosinate tolerant maize production. [EC, 2013. 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	Even though EFSA encourages the applicants to use the multi-factor tools to facilitate the selection of the field trial sites, such use is not mandatory. Please note that the tools are being used by the EFSA GMO Panel to evaluate the representativeness of the field trial sites selected by the applicant.

			amending Commission Regulations (EC) No 641/2004 and (EC) No 1981/2006. Official Journal of the European Union. L 157/1: 1-48. EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111. EFSA, 2015. Guidance on the agronomic and phenotypic characterisation of genetically modified plants. The EFSA Journal 13(6):4128: 1-44. Miyazaki J, Bauer-Panskus A, Bøhn T, Reichenbecher W, Then C, 2019. Insufficient risk assessment of herbicide-tolerant genetically engineered soybeans intended for import into the EU. Environmental Sciences Europe 31(1): 92.]	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.3.4 Comparative analysis of composition	The scope of the comparative analysis concerning food and feed risk assessment is considered too narrow with a view to the specific characteristics of GM maize DP51291 and the assessment is associated with the following shortcomings: - As the GM maize DP51291 is designed for use with the complementary herbicide glufosinate, the residual levels as well as residual amounts of metabolites of this herbicide need to be analysed. - Glufosinate is no longer an approved active substance in the EU (EC 2023). Currently MRLs of 0.1 mg/kg for glufosinate are established for	The GMO Panel took note of the comment and reminds that the assessment of herbicides residues and metabolites is not in the remit of the GMO Panel. This application has been submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed. All matters related to legal limits for pesticide residues in food and feed are covered by Regulation (EC) No 396/2005. The GMO Panel assessed all the significant differences between maize DP51291 and the conventional counterpart. Taking into account the natural variability observed for the set of non-GM reference varieties, the GMO Panel concludes that none

			maize imported from third countries (EC 2016). Therefore, the notifier should be requested to demonstrate that the MRLs established in the EU for glufosinate and its metabolites in maize imported from third countries are not exceeded. We therefore request that the applicant submits further data with respect to the compositional analysis and includes the analysis of residual glufosinate and its metabolites in his compositional assessment. Significant differences Field trials for the comparative assessment of GM maize DP51291 were conducted at 12 sites during 2021 in the US and Canada, and eight sites were chosen for taking samples and performing compositional analysis. The field trial design included the GM maize (test line), a conventional counterpart (control line), and a total of twenty commercial reference varieties. The trials were performed in a randomised complete plot design using data from eight field sites with four blocks at each site. The field design included two different GM maize treatments: • conventional herbicide treated (CHT) DP51291 maize, • intended herbicide treated (IHT) DP51291 maize. The Study Report (Annex 13, p. 23, Tables 4-7) lists details of the comparison: • GM maize DP51291	of the differences identified in forage and grain composition between maize DP51291 and its conventional counterpart needs further assessment regarding food and feed safety except for phosphorus in forage and manganese, proline, oleic acid (C18:1) and linoleic acid (C18:2) in grain, which were further assessed in section 3.5 of the Scientific Opinion. The GMO Panel did not use the information on tolerance interval for the assessment of the outcomes of the statistical analysis. Hence,

			(CHT): 13 of 69 measured analytes were statistically significantly different. • GM maize DP51291 (IHT): 19 of 69 measured analytes were statistically significantly different. The same analytes are significantly different between the GM maize line and the control line in the two treatments: oleic acid, palmitic acid, palmitoleic acid, eicosenoic acid, lignoceric acid, copper, ferulic acid. The relative difference which is a useful value for estimation if there is a large or small deviation seen for a certain analyte is only presented for cases with statistical difference in the Biological Relevance Report (Annex 14). A comparison with the reference range is also presented in the across-site analysis of Annex 14. In most cases a tolerance interval established from the internal composition database of reference maize was used to further evaluate the biological relevance of significant differences. The establishment of the tolerance interval is described in a Study Report PHI R144-Y21 that is an essential part in the line of argumentation by the notifier regarding the safe use of GM maize DP51291. The EFSA GMO Panel is asked to request Study Report PHI R144-Y21 because it is not included in the notification documents. [EC, 2016.	report PHI-R144-Y21 was not considered necessary.

			Commission Regulation (EU) 2016/1002 of 17 June 2016 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for AMTT, diquat, dodine, glufosinate and tritosulfuron in or on certain products. Official Journal of the European Union. L 167: 1-45. EC, 2023. EU Pesticides database; https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/79; (last accessed: 25/07/2023).]	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.4 Toxicology	With respect to the toxicity evaluation of the IPD072Aa protein, the notifier refers to the data submitted in application EFSA-GMO-NL-2019-163 currently under review by EFSA. Important information is available in the scientific literature, but unfortunately not discussed in the dossier: information on the activity spectrum of the IDP072Aa protein and effective doses (Boeckman et al. 2019), data on the mode of action of IPD072Aa protein (Jimenez-Juarez et al. 2023) and calculated margins of exposure (MOEs) based on worst-case environmental exposure concentrations (EECs) and laboratory bioassay results (tier 1) for various non-target species	The GMO Panel has previously assessed IPD072Aa and no safety concerns for humans and animals have been identified (EFSA, 2024). Furthermore, the publication of Jiménez- Juárez et al. (2023), describing the mode of action of the IPD072Aa protein has also been considered and the GMO Panel concluded that it does not add new information that would raise concerns for safety (EFSA, 2024). Regarding the protein equivalence, the data provided by the applicant in the dossier shows that the plant- and microbe-derived IPD027Aa proteins had comparable functional activity, as described in section 3.3.3 of the Scientific Opinion.

			(Boeckman et al. 2021). However, it remains unclear, whether equivalence between plant and microbially expressed proteins was fully established as Boeckman et al. 2021 present bioassay data only for the microbially produced toxin. Thus, the notifier has established the important fact that the microbially produced toxin has insecticidal activity, however the provided data do not allow to conclude on equivalence in our opinion. According to the relevant EFSA Guidance the ERA conducted for GM plants should focus 'on the identification and characterisation of both (i.e. intended and unintended) effects with respect to possible adverse impacts on human and animal health and the environment' (EFSA 2010). In general, we do appreciate scientific literature submitted in support of applications. However, we are of the opinion that specific data on the GMO of an application and its traits, which are highly relevant for the characterisation of the product and the evaluation of the intended effect - and thus also the ERA - should be an integral part of the notification and should be discussed by the notifier in the notification clarifying questions regarding the equivalence of the microbially produced test substance and the toxin as expressed in the GM plant.	

			[Boeckman CJ, Anderson JA, Linderblood C, Olson T, Roper J, Sturtz K, Walker C, Woods R, 2021. Environmental risk assessment of the DvSSJ1 dsRNA and the IPD072Aa protein to non-target organisms. GM Crops Food 12(1): 459-478. Boeckman CJ, Huang E, Sturtz K, Walker C, Woods R, Zhang J, 2019. Characterization of the Spectrum of Insecticidal Activity for IPD072Aa: A Protein Derived from Psuedomonas chlororaphis with Activity Against Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae). J Econ Entomol 112(3): 1190-1196. EFSA, 2010. Guidance of the GMO Panel on the environmental risk assessment of genetically modified plants. The EFSA Journal 8(11):1879: 1-111. Jimenez-Juarez N, Oral J, Nelson ME, Lu AL, 2023. IPD072Aa from Pseudomonas chlororaphis targets midgut epithelial cells in killing Western Corn Rootworm (Diabrotica virgifera virgifera). Appl Environ Microbiol 89(3): e0162222.]	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.4.1 Testing of newly expressed proteins	The safety of IPD072Aa protein was tested in a 28-day repeated-dose toxicity study in mice (Study Report "previously submitted Annex 23 in AP163_PHI-2018-088_IPD072Aa 28-day". The results indicate histopathologic changes that occurred more	The GMO Panel thanks Austria for the comments. The 28-day study on the IPD072Aa protein has been previously assessed by the GMO Panel as reported in the Scientific Opinion of AP163. For details, please refer to section 3.5.3.1 and Appendix C.

			frequently in the 1000 mg/lg/day IPD072 group females than in the control group concerning liver, axillary lymph node, and pharynx (pages 1291 to 1294). The notifier should carry out a detailed assessment of these endpoints by taken into consideration the individual animal data.	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	1.4.4 Testing of the whole genetically modified food or feed	The notifier, in Annex 15, presents results from a 90-day rat feeding study with grain from GM maize DP51291. In the Results and Discussion section (p. 26) the significant differences are further evaluated. However, we have noticed that some significances are not discussed in this section, e.g. absolute basophil, female high dose group (p. 116), blood urea nitrogen, male high dose group (p. 121), thyroid with parathyroid weight, female high dose group (p. 151). The notifier should present a discussion of all significantly different endpoints in this toxicity study (also those concerning males or females only) supporting the risk assessment. The mean glucose concentration (GLUC) was significantly higher in the combined male and female DP51291 high group. There is a concentration-related trend across low and high groups for both sexes (males, females) and also the combined sexes (Table 10, p. 124). It is true that the	The GMO Panel thanks Austria for the comments. The 90-day feeding study has been assessed by the GMO Panel as reported in the Scientific Opinion. For details, please refer to section 3.5.2.4 and Appendix A.

			magnitudes of the differences are small. However, a concentration-related trend must be addressed and evaluated, even more when all means (of males, females, and combined sex) of the high group exceed the means of all three reference diet groups (P0760, BK5883, P0843).	
Austria	Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	6.3 General Surveillance (strategy, method)	The proposed general surveillance for unanticipated adverse is not sufficiently elaborated and should be amended regarding the following elements: • Elaboration of a detailed monitoring methodology (e.g. parameters, specific information). • Identification of existing national institutions and operators involved in GS in individual Member States and evidence for their commitment to GS activities. • Assignment of clear responsibilities and concrete tasks to each party involved. • Verification of the skills and expertise of the parties involved which are required for the detection of potential adverse environmental impacts. • Taking into account all potential routes of exposure under commercial use, a fundamental requirement of the EU-approach to monitoring (EFSA 2011). (Involvement of operators further down the food and feed chain, e.g. veterinary networks). • Specification of the specific measures based on HACCP	The GMO Panel thanks Austria for this comment, which was taken into account. Indeed, a set of recommendations for the preparation of PMEM plans in order to provide more detail on the measures proposed for the implementation of General Surveillance was proposed for applicant's consideration (see Annex I of the minutes of the CompERA WG of January 2024). EFSA reminds that monitoring is related to risk management, and thus a final adoption of the PMEM plan falls outside the mandate of EFSA.

			principles in order to verify whether they match with the requirements of environmental monitoring. • More specific data on transport and handling of GM maize grain (e.g. actual import volumes, transport routes, processing plants, amounts used for feed) in order to provide a basis for the development and implementation of national monitoring concepts. [EFSA, 2011. Guidance of the GMO Panel on the Post-Market Environmental Monitoring (PMEM) of genetically modified plants. The EFSA Journal 9(8):2316: 1-40.]	
Netherlands	Rijksinstituut voor Volksgezondheid en Milieu	1. Hazard identification and characterisation	The applicant has declared parts of the information in the application that are relevant for the environmental risk assessment, i.e. details regarding the inserted sequences, confidential. This conflicts with the Aarhus Convention that guarantees the right of the public to access environmental information and has been implemented in European legislation. According to Article 30 of Regulation (EC) No 1829/2003 information on, amongst others, the composition of a genetically modified organism (GMO), physico-chemical and biological characteristics, and effects on human and animal health and the environment cannot be declared confidential. On the 27th of March	Confidentiality Requests referring to the inserted DNA sequences were withdrawn by the applicant in Additional Information-7 (Bioinformatics Update data package)

			of 2021, the new Transparency Regulation came into force, which aims to improve transparency and sustainability of risk assessments in the food chain. The application for maize DP910521 was submitted after the Transparency Regulation came into force. The Dutch CA points out that information which is crucial to assess potential risks of a GM crop, such as information on the inserted sequences, should not be declared confidential, because lack of transparency undermines public trust in the risk assessment. The Dutch CA urges EFSA to lift the confidentiality of the parts in the dossier that are relevant for the environmental risk assessment.	
Netherlands	Rijksinstituut voor Volksgezondheid en Milieu	1.4.4 Testing of the whole genetically modified food or feed	In the assessors' opinion, the 90-day rat feeding study and the 42-day study in broiler chicken performed with maize DP51291 would not have been needed to confirm its safety, given that a proper justification for the execution of these studies is lacking since the outcomes of the comparative assessment had raised no concerns over its safety. These views are also in line with guidance for the safety assessment of GM foods as established by the EFSA GMO Panel and Codex Alimentarius (e.g., Codex Alimentarius, 2008; EFSA, 2014). It is recommended	The GMO Panel would like to thank The Netherlands for the comment.

			to emphasize that the provision of such feeding studies is a departure from what is considered sufficient for safety assessment of biotechnology-derived products according to the internationally harmonized approach.	