EFSA is working to advance the environmental risk assessment of genetically modified crops to better protect butterflies and moths

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The cultivation of genetically modified (GM) crops is subject to a prospective risk assessment and regulatory approval in most jurisdictions worldwide. In the risk analysis process, the role of risk assessors such as the European Food Safety Authority (EFSA) and its Panel on Genetically Modified Organisms (GMO Panel) is to assess any risk that the cultivation of a GM crop may pose to human and animal health and the environment, and recommend options for risk mitigation, if necessary. Decisions to approve the cultivation of a GM crop are taken by risk managers, i.e. the European Commission and Member States of the European Union (EU).

Risk to butterflies and moths

The EFSA GMO Panel has performed pan-European environmental risk assessments (ERAs) for the cultivation of several GM maize events that express an insecticidal protein from the biocontrol agent
Bacillus thuringiensis (Bt) (i.e. Cry1Ab for MON810 and Bt11, and Cry1F for 1507). The Bt-protein expressed in maize MON810/Bt11 and 1507 confers protection against lepidopteran maize insect pests (i.e. target organisms) such as the European corn borer (Ostrinia nubilalis) and the Mediterranean corn borer (Sesamia nonagrioides). However, the potential exposure of non-target (NT) butterflies and moths (Lepidoptera) through the ingestion of harmful amounts of Bt-maize pollen deposited on their host plants in or near Bt-maize fields has been identified as a concern associated with the cultivation of lepidopteran-active Bt-maize.

Modelling

Quantifying the risk to NT Lepidoptera arising from the ingestion of Bt-maize pollen at pan-European level can be challenging. This is primarily due to the heterogeneity and complexity of receiving environments, which may cover different scenarios in terms of pedo-climatic zones, agricultural systems, landscape structures, exposure to Bt-maize pollen and NT lepidopteran species (including their habitat use, body size and larval susceptibility to the Bt-protein) (Lang et al., 2020; Arpaia, 2021). Therefore, modelling approaches are followed to predict outcomes (i.e. risks to NT Lepidoptera) from data and understand how complex systems work (Topping et al., 2020). Models can provide a valuable contribution to the weight of scientific evidence considered in prospective ERAs and complement the need to gather additional data in relevant receiving environments.

Risk characterisation

Since 2009, the risk to NT Lepidoptera due to ingestion of Bt-maize pollen has been quantified by the EFSA GMO Panel through estimates of larval mortality generated by the models developed by Perry et al. (2010, 2012; see also Perry et al. (2011, 2013). These models integrate a mortality–dose relationship based on laboratory bioassays, with a dose–distance relationship from a maize crop based on field measurements. Mortality is estimated within a Bt-maize field and at various distances from it. Perry et al. (2012) extended the initial model to: (1) differentiate between small-scale, local mortality and global mortality allowing for exposure effects at larger scales; (2) account for the between-species variability in lepidopteran susceptibility to Bt-proteins; (3) assess the efficacy of various risk mitigation measures; and (4) study different host plant densities in crops and field margins.

In its 2009 Scientific Opinion, the EFSA GMO Panel used the Perry et al. (2010) model to estimate the risk to NT Lepidoptera following ingestion of maize MON810 pollen. The model generated estimates for three widespread European species (Vanessa atalanta, Inachis io and Plutella xylostella) in 11 representative maize ecosystems in four European countries. Based on the model predictions, the GMO Panel recommended risk managers to mitigate the possible exposure of NT Lepidoptera to maize MON810 pollen. Subsequently, the GMO Panel recalibrated the aforementioned model to simulate and assess potential adverse effects resulting from the exposure of NT Lepidoptera to maize 1507 pollen under representative EU cultivation conditions. A similar exercise was carried out for maize MON810/Bt11. In the 2015 Scientific Opinion of the GMO Panel, calculations were further refined to provide updated quantitative estimates of exposure levels, accounting for new information on maize pollen deposition over long distances.

EFSA procurement

The EFSA GMO Panel acknowledged several types of uncertainties including: (1) uncertainties pertaining to the structure of the Perry et al. (2010, 2012) models, mostly caused by the lack of data...
from bioassays estimating the susceptibility of a wider range of ‘real’ NT Lepidoptera for most assessed Bt-maize events; and (2) uncertainties contributing to the variability in exposure of NT Lepidoptera to Bt-maize pollen. Moreover, none of the models that have been developed for assessing risks associated with the cultivation of lepidopteran-active Bt-maize on NT Lepidoptera at that time (e.g. Holst et al., 2013; Lang et al., 2015; Fahse et al., 2018) did explicitly take the landscape structure and crop management into account. Consequently, uncertainty remained about the actual risk to NT Lepidoptera at the landscape scale and risk mitigation measures to recommend. To address these uncertainties, EFSA outsourced the development of a spatially and temporally explicit model that would account for landscape structure, crop management, sublethal effects and weather.

The first spatially explicit model (briskaR) that estimated NT Lepidoptera mortality more realistically at both local/individual level and landscape scale and assessed the effectiveness of risk mitigation measures, was developed by Leclerc et al. (2018) (see also the EU-FP7-research project AMIGA; Walker et al., 2019; Baudrot et al., 2021a). As part of the EFSA procurement, Baudrot et al. (2021b) extended the briskaR model to integrate: (1) a wider range of possible maize pollen dispersal curves; (2) the variability of exposure to Bt-maize pollen over time through toxicokinetic–toxicodynamic models; (3) sublethal effects of Bt-proteins on the reproduction and development of NT Lepidoptera; and (4) multiannual and cumulative effects of chronic exposure.

In addition, Baudrot et al. (2021b) conducted a sensitivity analysis and expert knowledge elicitations (EKE) on identified sources of uncertainty, ran the model using real-world case studies, and developed a user-friendly model interface, which are briefly described below.

- **EKE:** Renowned experts in the field estimated distributions and uncertainties of the most sensitive parameters (for which few data are available) of the model at an EKE workshop, covering pollen deposition, the slope of dose-response mortality, sublethal effects and the interaction with environmental stressors (such as the microsporidian parasite Nosema).

- **Global sensitivity analysis:** Key factors that may affect mortality of NT lepidopteran larvae were addressed in a global sensitivity analysis. This analysis revealed that the variability of landscape-related parameters (such as spatial crop aggregation, distance from Bt-maize fields, pollen dispersal and deposition, exposure patterns) affected larval mortality more than the variability in larval susceptibility to the Bt-protein between individuals (as typically tested and determined in laboratory bioassays).

- **Model implementation:** Two real-world case studies (i.e. Catalonia (North-West Spain) and Baden-Württemberg (South-West Germany)) were used to run the model under contrasting environmental conditions, and identify key factors that adversely affect larvae of *Papilio machaon*. This assessment confirmed the effect of landscape patterns and crop management practices at a local level.

- **User-friendly interface:** A user-friendly model interface (allowing users to perform case-specific assessments) was created to facilitate model uptake and use by risk assessors and risk managers, and thus inform regulatory decision-making.

In the light of the 2018 EFSA Scientific Committee guidance on uncertainty analysis in scientific assessments, an uncertainty analysis of the briskaR model and some of its parameters has been conducted and will be published in the first half of 2021.

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15 https://www.sciencedirect.com/science/article/abs/pii/S0304380012005315
16 http://www.sciencedirect.com/science/article/pii/S0006320715301300
17 https://www.sciencedirect.com/science/article/pii/S0304380018300152
18 https://www.sciencedirect.com/science/article/pii/S0048969717333879
19 Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems: https://cordis.europa.eu/project/id/289706
20 https://onlinelibrary.wiley.com/doi/full/10.1111/risa.12941
21 https://www.sciencedirect.com/science/article/pii/S014765132031054X
22 https://efsa.onlinelibrary.wiley.com/doi/abs/10.2903/sp.efsa.2021.EN-6443
23 Salvatore Arpaia, Ludovit Cagan, Abigail Colson (EKE facilitator), Gema P Farinos, Andy Hart (EKE facilitator), Helen Hesketh, Niels Holst, Andreas Lang, Marina S Lee, Antoine Messaian, Mylene Ogliastro, Mathias Otto, Aslina Pearson, Joe N Perry, Jörn Rombke and Constanti Stefanescu.
24 https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2018.5123
Outlook

Baudrot et al. (2021b) illustrated how modelling approaches such as briskaR can inform prospective ERAs for NT Lepidoptera by providing estimates of mortality at both local/individual level and landscape scale for a wide range of different receiving environments across the EU. Such estimates would enable risk managers to implement possible risk mitigation measures that are adapted to the relevant receiving environments and specific protection goals (including for regionally protected NT lepidopteran species) under their jurisdiction.

However, additional efforts are required to deliver more robust and realistic risk estimates, reduce uncertainties, and transition to more holistic ERAs for butterflies and moths. Such efforts would need to focus on optimising the integration of modelling, empirical data and monitoring (e.g. Streissl et al., 2018; Lee et al., 2021; More et al., 2021), enabling a more dynamic, iterative interplay between risk assessment and risk management (Topping et al., 2020).

- **Modelling:** The extended briskaR model does not consider the various ecological factors (including environmental stressors) that may affect the population dynamics of NT Lepidoptera. Since such factors may influence potential adverse effects caused by Bt-maize pollen, their integration may contribute to the further improvement of current modelling capabilities. This integration will be facilitated by the modularity and the open-source feature of the extended briskaR model.

- **Empirical data:** Current and new empirical data will inform the revision of model components and development of supplementary ones. To fine tune model predictions, more data are needed on: (1) the species-specific susceptibility of NT lepidopteran larvae to Bt-proteins for a broader range of potentially exposed NT lepidopteran species; (2) the occurrence and distribution of host plants in and around maize fields; (3) the deposition and fate of maize pollen on the leaves of specific host plants; and (4) the additive or synergistic effects caused by exposure to additional environmental stressors.

- **Monitoring:** The integration of monitoring approaches (such as post-market environmental monitoring which is mandatory for the cultivation of lepidopteran-active Bt-maize in the EU) is required to cross-validate the outcomes of prospective ERAs for NT Lepidoptera and assess the effectiveness of risk mitigation measures. Thereby, monitoring would serve as an early warning check of divergency from expected results and hence provide feedback on the effectiveness of the model. While monitoring would provide new input data to the model, such data would also be required to support model validation and its calibration for regulatory purposes.

References

Arpaia S, 2021, in press. Environmental risk assessment in agro-ecosystems: revisiting the concept of receiving environment after the EFSA guidance document. Ecotoxicology and Environmental Safety, 208.

Baudrot V, Walker E, Lang A, Stefanescu C, Rey J-F, Soubeyrand S and Mess A, 2021a, in press. When the average hides the risk of Bt-corn pollen on non-target Lepidoptera: application to Aglais io in Catalonia. Ecotoxicology and Environmental Safety, 207.

Baudrot V, Lang A, Stefanescu C, Soubeyrand S and Messéan A, 2021b. Extension of the spatially- and temporally-explicit “briskaR-NTL” model to assess potential adverse effects of Bt-maize pollen on non-target Lepidoptera at landscape level. EFSA supporting publication 2021;EN-6443, 135 pp. https://doi.org/10.2903/sp.efsa.2021.EN-6443

Fahse L, Papastefanou P and Otto M, 2018. Estimating acute mortality of Lepidoptera caused by the cultivation of insect-resistant Bt maize –The LepiX model. Ecological Modelling, 371, 50–59.

Holst N, Lang A, Lövei G and Otto M, 2013. Increased mortality is predicted of Inachis io larvae caused by Bt-maize pollen in European farmland. Ecological Modelling, 250, 126–133.

Lang A, Oehen B, Ross J-H, Bieri K and Steinbrich A, 2015. Potential exposure of butterflies in protected habitats by Bt maize cultivation: a case study in Switzerland. Biological Conservation, 192, 369–377.

Lang A, Dolek M, Lee MS, Freese-Hager A and Otto M, 2020. Selection of non-target Lepidoptera species to test Bt maize effects in the laboratory: which species and how to breed them? BioRisk, 15, 45–65.

Leclerc M, Walker E, Messéan A and Soubeyrand S, 2018. Spatial exposure-hazard and landscape models for assessing the impact of GM crops on non-target organisms. Science of the Total Environment, 624, 470–479.

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25 https://link.springer.com/article/10.1007/s10646-018-1962-0
26 https://www.sciencedirect.com/science/article/pii/S1470160X21000455
27 https://efsajournal.wiley.com/doi/10.2903/j.efsajournal.2021.e190101
Lee S, Ardanuy A, Juárez-Escario A and Albajes R, 2021, in press. Sampling and selection of butterfly indicators for general surveillance of genetically modified maize in north-east Spain. Ecological Indicators, 124.

More SJ, Auteri D, Rortais A and Pagani S, 2021. Editorial: EFSA is working to protect bees and shape the future of environmental risk assessment. EFSA Journal, 19, e190101. https://doi.org/10.2903/j.efsa.2021.e190101

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J and Sweet JB, 2010. A mathematical model of exposure of non-target Lepidoptera to Bt-maize pollen expressing Cry1Ab within Europe. Proceedings of the Royal Society B – Biological Sciences, 277, 1417–1425.

Perry JN, Devos Y, Arpaia S, Bartsch D, Gathmann A, Hails RS, Kiss J, Lheureux K, Manachini B, Mestdagh S, Neemann G, Ortego F, Schiemann J and Sweet JB, 2011. The usefulness of a mathematical model of exposure for environmental risk assessment. Proceedings of the Royal Society B – Biological Sciences, 278, 982–984.

Perry JN, Devos Y, Arpaia S, Bartsch D, Ehlert C, Gathmann A, Hails RS, Hendriksen NB, Kiss J, Messéan S, Mestdagh S, Neemann G, Nuti MP, Sweet JB and Tebbe CC, 2012. Estimating the effects of Cry1F Bt-maize pollen on non-target Lepidoptera using a mathematical model of exposure. Journal of Applied Ecology, 49, 29–37.

Perry JN, Arpaia S, Bartsch D, Birch ANE, Devos Y, Gathmann A, Gennaro A, Kiss J, Messéan A, Mestdagh S, Nuti M, Sweet JB and Tebbe CC, 2013. No evidence requiring change in the risk assessment of Inachis io larvae. Ecological Modelling, 268, 103–122.

Streissl F, Egsmose M and Tarazona JV, 2018. Linking pesticide marketing authorisations with environmental impact assessments through realistic landscape risk assessment paradigms. Ecotoxicology, 27, 980–991.

Topping CJ, Aldrich A and Berny P, 2020. Overhaul environmental risk assessment for pesticides. Science, 367, 360–363.

Walker E, Leclerc M, Rey J-F, Beaudouin R, Soubeyrand S and Messéan A, 2019. A spatio-temporal exposure-hazard model for assessing biological risk and impact. Risk Analysis, 39, 54–70.

Abbreviations

Bt Bacillus thuringiensis
EKE expert knowledge elicitation
ERA environmental risk assessment
GM genetically modified
GMO genetically modified organism
NT non-target