TESTBIOTECH Background 30 - 8 - 2020

Testbiotech comment on EFSA GMO Panel´s Scientific Opinion on the assessment of genetically modified oilseed rape GT73 for renewal of authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-002) from Bayer/ Monsanto

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Christoph Then & Andreas Bauer-Panskus

Introduction

The genetically engineered, herbicide-tolerant (glyphosate) oilseed rape, GT73, produces two different enzymes that confer herbicide resistance (CP4 EPSPS and GOX proteins). The assessment for renewal of authorisation was carried out within the framework of EU Implementing Regulation 503/2013 (EFSA, 2020a).

Molecular characterisation

There is a complete lack of more recent data on genetic stability and gene expression in the context of ongoing climate change. Such data on changes in gene expression are requested by Regulation 503/2013. Experiments should have been performed under controlled and defined conditions to expose the plants to all relevant biotic or abiotic stressors, and to gather sufficiently reliable data on gene expression and functional genetic stability.

Herbicide-resistant oilseed rape is known to tolerate extremely high concentrations of glyphosate (Nandula et al., 2007). Due to increasing pressure from herbicide-resistant weeds, it is likely that the dosages of glyphosate currently applied are much higher compared to agricultural practice ten or twenty years ago. It is probable that these high and/or repeated dosages of herbicide applications will also influence gene expression. Regulation 503/2013 requests data on realistic agronomic practices, but these are absent from the application. Therefore, data should have been requested which take into account all relevant patterns of application of the complementary herbicide, also taking into account the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.

The generation of data on meteorological and agronomic conditions should also take into account a number of different genetic backgrounds and represent a broad range of the relevant varieties. The data should also include so-called ‘omics’ (transcriptomics, proteomics, metabolomics).

Moreover, it should also be considered that the genetic engineering process can give rise to open reading frames (ORFs) from which biologically active molecules may emerge. All potential gene products emerging from the genetic changes should have been subjected to detailed assessment, including molecules besides proteins, such as dsRNA. Since detailed assessment is missing, too many uncertainties remain about the risks due to biologically active substances arising from the introduction of the gene constructs.

In conclusion, the data provided by the applicant do not allow reliable conclusions to be drawn on gene expression and molecular risk assessment.

Comparative analysis
In regard to the compositional analysis, agronomic traits and the characteristics of the GE phenotype, Implementing Regulation 503/2013 requests assessment of whether the expected agricultural practices influence the outcome of the studied endpoints. According to the Regulation, this is especially relevant for herbicide-resistant plants. Furthermore, the different sites selected for the field trials should reflect the different meteorological and agronomic conditions under which the crop is to be grown.

Therefore, recent data should have been requested on genetic stability and gene expression under ongoing climate change. Furthermore, experiments under controlled and defined conditions should have been performed, exposing the plants to all biotic or abiotic stressors representative of the full range of expected agricultural and bioclimatic conditions.

Herbicide-resistant oilseed rape is known to tolerate extremely high concentrations of glyphosate (Nandula et al., 2007). Due to the increasing problem of herbicide-resistant weeds, it is probable that the dosages applied to the plants are much higher compared to agricultural practice ten or twenty years ago. It is further to be expected that high and/or repeated herbicide application dosages will also influence gene expression, plant composition and phenotypical characteristics (for comparison see Miyazaki et al., 2019). Therefore, data should have been requested which take all relevant patterns of application of the complementary herbicide into account, also taking into account the highest dosage of glyphosate that can be tolerated by the plants, including repeated spraying.

The generation of data on meteorological and agronomic conditions should also take into account a number of different genetic backgrounds, representing a broad range of the relevant varieties. The data should also include so-called ‘omics’ (transcriptomics, proteomics, metabolomics).

However, no such data were made available. Therefore, no conclusion can be drawn on the comparative analysis.

Toxicology
As shown in previous comments, the scientific standards applied in the whole food and feed studies carried out for the original application were seriously deficient (Testbiotech 2013). Therefore, EFSA should have requested further whole food and feed feeding studies, including data on the reproduction and immune system as well as for several generations.

Further, there is no mention in the application that the source of the GOX protein is an opportunistic human pathogen (see Chain et al., 2011). In response, further data should have been requested to demonstrate the safety of the GT73 event. New data from a 28-day feeding study with the isolated protein were presented. However, this study did not demonstrate the equivalence between the test substance and the oilseed rape GOXv247 protein, i.e. the functionality of the E. coli-produced GOXv247 protein was not tested (ESFA, 2020c). Therefore, the data are inconclusive. Further, the health effects of the toxin present in the matrix of the plants was not examined. This is a problem since the plant compounds might interfere with the biological characteristics and potential health effects of the protein. Finally, no study was performed to study the impact of ingestion of the plants on the intestinal microbiome of animals and humans. Therefore, further experiments should have been conducted, including data with and without the residues from spraying.
Since these data are missing, major uncertainties remain about risks of the protein produced in the plants when they are consumed.

Allergenicity
There is not sufficient data available to assess the impact of whole food and feed on the immune system or the reproductive system.

Environmental risk assessment
Spillage of whole seeds can lead to the unintended cultivation of rapeseed along transport lines and has to be expected. If spillage occurs, it can give rise to spontaneous GE plant populations, and GE seeds can remain dormant for more than ten years. Under these conditions, gene flow between *Brassica napus* to closely related species and/ or other populations of oilseed rape (cultivated or feral) is known to occur, giving rise to viable GE hybrid populations and further GE seeds which can remain dormant for more than ten years.

The emergence of spontaneous and persisting populations of GE oilseed rape as well as the introgression of the transgenes into other plant populations has been already been confirmed in several publications (for overview, see Bauer-Panskus et al., 2013; for more recent findings and reviews see: Hecht et al., 2014; Schulze et al., 2014; Franzaring et al., 2016; Nishizawa et al., 2016; Pandolfo et al., 2016; Pascher et al., 2017.). However, no specific data on spillage were presented by the applicant.

In cases where the plants manage to propagate in the environment, data from the original event are not sufficient to predict the biological characteristics of subsequent spontaneous hybrid populations (see Bauer-Panskus et al., 2020). However, empirical data on potential next generation effects are absent from the application. No case specific monitoring was performed to trace spillage and spontaneous plant populations.

In awareness of the deficits in risk assessment and risk management, the import of viable kernels has to be discontinued and the application for renewal is to be rejected.

Monitoring
The applicant carried out a literature review which resulted in more than 200 findings. Only two of them were mentioned in the EFSA opinion. Recent findings regarding spontaneous populations of GE oilseed rape emerging from spillage, very often including the GT73 event, were not considered. These unintended occurrences of GE plants may give rise to persistent populations, including gene flow to wild relative populations (for overview see Bauer-Panskus et al., 2013; more recent findings and reviews see: Hecht et al., 2014; Schulze et al., 2014; Franzaring et al., 2016; Nishizawa et al., 2016; Pandolfo et al., 2016; Pascher et al., 2017).

The monitoring report does not include any specific data on spillage, gene flow or waste from production and feeding GT73. Further, there are no data on potential next generation effects if the plants manage to propagate in the environment. Such data are needed since data from the original event are not sufficient to predict the biological characteristics of subsequent hybrid populations (see Bauer-Panskus et al., 2020).

There is no mention of further whole food feed studies being in accordance with the standards for 90-day feeding studies, even though the quality of the original studies were heavily criticised by experts from Member States as well as others (see Testbiotech, 2013).

Furthermore, in the context of increasing herbicide-resistant weed pressure, it is likely that the

dosages applied to the plants are much higher compared to agricultural practice ten or twenty years ago. However, there are no data on how the application of the complementary herbicide (glyphosate) has changed within the last ten years in regard to the number of applications, dosages and resulting residues from spraying. Such changes in agricultural practice can also impact plant composition and food safety. Therefore, potential negative health impacts via direct ingestion or the gut microbiome cannot be excluded. This is a major problem since herbicide-resistant GE oilseed rape is known to tolerate extremely high concentrations of glyphosate (Nandula et al., 2007).

The monitoring report did not include any specific observations on potential health impacts at the stage of consumption. The report did not include any combinatorial or accumulative effects in cases where the plants are mixed with other GE plants in the diet.

All in all, the monitoring data provided by the applicant do not allow any conclusions to be drawn on the health and environmental safety of import, processing and consumption of GT73. Experts from several Members States (Austria, France, Germany, Italy, Netherlands) as well as from Norway made it clear that monitoring should be expanded (EFSA, 2020b). Most of the experts requested case specific monitoring of spillage, occurrence of spontaneous populations and potential gene flow.

In their response to Member States, EFSA also sees the need for further amendments, stating: *"Although the final adoption of PMEM plans fall outside the remit of EFSA, the GMO Panel considers that further discussion with applicants and risk managers is needed on the practical implementation of the PMEM for GM plants for import and processing (e.g. actual data gathered on exposure and/or adverse effects as implemented in existing monitoring systems).*" (EFSA 2020b). Nevertheless, EFSA accepted the data for renewal.

In conclusion, the monitoring data and the literature review are not sufficient to demonstrate the safety of GT73 and cannot be accepted as a basis for renewal of authorisation.

Conclusion

The application for renewal of authorisation has to be rejected. There seems to be a tendency within the EFSA GMO panel and industry to reduce the mandatory process of risk assessment, monitoring and renewal of authorisation to a formality. This in contradiction to the EU regulation which requires the highest scientific standards of risk assessment to be applied in order to exclude risks to health and the environment. It further requires renewal applications to be based on sufficiently reliable monitoring data. If the Commission issues renewal of authorisation based on the poor data presented by the applicant, this would send a wrong and potentially fatal signal to industry and EFSA.

References

Bauer-Panskus, A., Breckling, B., Hamberger, S., Then, C. (2013) Cultivation-independent establishment of genetically engineered plants in natural populations: current evidence and implications for EU regulation. Environ Sci Eur, 25: 34. https://doi.org/10.1186/2190-4715-25-34

Bauer-Panskus, A., Miyazaki, J., Kawall, K., Then, C. (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. Environ Sci Eur, 32: 32. https://doi.org/10.1186/s12302-020-00301-0

Chain, P.S., Lang, D.M., Comerci, D.J., Malfatti, S.A., Vergez, L.M., Shin, M., Ugalde, R.A., Garcia, E., Tolmasky, M.E., (2011) Genome of *Ochrobactrum anthropi* ATCC 49188 T, a versatile opportunistic pathogen and symbiont of several eukaryotic hosts. J Bacteriol, 193(16): 4274-4275. https://doi.org/10.1128/JB.05335-11

EFSA (2020a) Scientific Opinion on the assessment of genetically modified oilseed rape GT73 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-002). EFSA Journal, 18(7): 6199. https://doi.org/10.2903/j.efsa.2020.6199

EFSA (2020b) Comments from the Experts of Member States on the Scientific Opinion on the assessment of genetically modified oilseed rape GT73 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-002). http://registerofquestions.efsa.europa.eu/roqFrontend/login?0

EFSA (2020c) Scientific Opinion on the statement complementing the EFSA Scientific Opinion on application (EFSA-GMO-NL-2009-75) for placing on the market of genetically modified oilseed rape Ms8 x Rf3 x GT73 and subcombinations, which have not been authorised previously (i.e. Ms8 x GT73 and Rf3 x GT73) independently of their origin, for food and feed uses, import and processing, with the exception of isolated seed protein for food, under Regulation (EC) No 1829/2003), taking into consideration additional information. EFSA Journal, 18(7): 6200. https://doi.org/10.2903/j.efsa.2020.6200

Franzaring, J., Wedlich, K., Eckert, S., Zipperle, J., Krah-Jentgens, I., Hünig, C., Züghart W. (2016) Exploratory study on the presence oilseed rape near German oil mills. Environ Sci Pollut Res, 23: 23300-23307. https://doi.org/10.1007/s11356-016-7735-5

Hecht, M., Oehen, B., Schulze, J., Brodmann, P., Bagutti, C. (2014) Detection of feral GT73 transgenic oilseed rape (Brassica napus) along railway lines on entry routes to oilseed factories in Switzerland. Environ Sci Pollut Res, 21(2): 1455-1465. https://doi.org/10.1007/s11356-013-1881-9

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. Environ Sci Eur, 31: 92. https://doi.org/10.1186/s12302-019-0274-1

Nandula, V.K., Reddy, K.N., Rimando, A.M., Duke, S.O., Poston, D.H. (2007) Glyphosate-resistant and -susceptible soybean (*Glycine max*) and canola (*Brassica napus*) dose response and metabolism relation-ships with glyphosate. J Agric Food Chem, 55: 3540-3545. https://doi.org/10.1021/jf063568l

Nishizawa, T., Nakajima, N., Tamaoki, M., Aono, M., Kubo, A., Saji, H. (2016) Fixed-route monitoring and a comparative study of the occurrence of herbicide-resistant oilseed rape (Brassica napus L.) along a Japanese roadside. GM Crops Food 7(1): 20-37. https://doi.org/10.1080/21645698.2016.1138196

Pandolfo, C.E., Presotto, A., Torres Carbonell, F., Ureta, S., Poverene, M., Cantamutto, M. (2016) Transgenic glyphosate-resistant oilseed rape (Brassica napus) as an invasive weed in Argentina: detection, characterization, and control alternatives, Environ Sci Pollut Res, 23(23): 24081-24091. https://doi.org/10.1007/s11356-016-7670-5

Pascher, K., Hainz-Renetzeder, C., Gollmann, G., Schneeweiss, G.M. (2017) Spillage of Viable Seeds of Oilseed Rape along Transportation Routes: Ecological Risk Assessment and Perspectives on Management Efforts. Front Ecol Evol, 5: 104. https://doi.org/10.3389/fevo.2017.00104

Schulze, J., Frauenknecht, T., Brodmann, P., Bagutti, C. (2014) Unexpected diversity of feral genetically modified oilseed rape (*Brassica napus L*.) despite a cultivation and import ban in Switzerland. PLoS One 9(12): e114477. https://doi.org/10.1371/journal.pone.0114477

Testbiotech (2013) Comment on EFSA GMO Panel´s Scientific Opinion on application (EFSA-GMO-NL-2010-87) for the placing on the market of genetically modified herbicide tolerant oilseed rape GT73 for food containing or consisting of, and food produced from or containing ingredients produced from, oilseed rape GT73 under Regulation (EC) No 1829/2003 from Monsanto. Testbiotech Background 18-3-2013, https://www.testbiotech.org/content/testbiotech-comment-efsa-gmo-panel-s-scientific-opinion-application-efsa-gmo-nl-2010-87