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Testbiotech comment on EFSA's assessment of genetically engineered oilseed rape GT73 for renewal authorisation under Regulation (EC) No 1829/2003 (application EFSA-GMO-RX-026/1) by Bayer

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Introduction

The EFSA GMO panel assessed GT73 oilseed rape for renewal of authorisation. GT73 expresses the proteins GOXv247 and CP4 EPSPS conferring resistance to herbicides containing glyphosate (EFSA, 2022a).

1. Systematic literature review

A systematic literature review as required in Regulation (EU) No 503/2013 was provided. However, this review only considered a fraction of the relevant studies. According to comments submitted by experts from Member States (EFSA, 2022b), even highly relevant studies, e.g. Swiss studies on spillage and persistence of transgenic oilseed rape, were excluded from the literature research simply because Switzerland is outside the EU.

Testbiotech agrees with Member States' experts that the *"applicant's exclusion criteria are neither appropriate nor comprehensible since these publications provide new data and information that need further consideration."*

Studies that should have been considered include i.a. Hecht et al., 2014; Laforest et al., 2022; Nishizawa et al., 2016; Pandolfo et al., 2016, 2018; Schoenenberger et al., 2012; Schulze et al., 2014, 2015; or Sohn et al., 2021.

It is in general necessary to also review literature which might indicate indirect, delayed or cumulative long-term risks, including interaction with other genetically engineered plants, which might occur due to spillage and further crossings. Literature research should, therefore, take particular account of potential persistence, spread and crossings with other transgenic plants already in the environment due to spillage along transport routes etc. The biological characteristics of potential offspring are also relevant for the application. Literature research should further include all relevant publications concerning the crop species and relatives. Any environmental risk assessment should take indirect, unintended, delayed and long-term cumulative effects of animal excretions into account.

Interactions with other genetically engineered plants which might be mixed into the diet are a further aspect of food and feed safety which needs to be considered. Implementing Regulation 503/2013 (3.2.3) requests that "*the applicant shall evaluate the data generated to estimate possible short-term and long-term risks to human or animal health associated with the consumption of genetically modified food or feed with respect to the expression of new proteins/metabolites, as well*

as significantly altered levels of original plant proteins/metabolites." Apparently, this legal request is not limited to the specific event. It requires that the risk assessment of mixed diets must be equivalent to the risk assessment of stacked events, as the risks are equivalent. This would require a much more comprehensive literature review of potential interactions with other regulated GMOs.

There is also no information in the literature review on studies investigating the unexpected effects (e.g. higher fitness) of transgenic plants containing epsps genes (see chapter on environmental risk assessment).

The systematic literature review provided by the applicant and accepted by EFSA is completely unacceptable.

2. Molecular characterisation and gene expression

There is a complete lack of more recent data on genetic stability and gene expression in the context of ongoing climate change. Regulation 503/2013, however, requests data on changes in gene expression. Therefore, experiments must be conducted under controlled and defined conditions in order 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 higher and/or repeated dosages of herbicide applications will also influence gene expression. Regulation 503/2013 requests data from realistic agronomic practices. Therefore, data should have been requested which take all relevant patterns of complementary herbicide application and the highest dosage of glyphosate that can be tolerated by the plants into account, including repeated spraying.
The generation of data on meteorological and agronomic conditions should also take a number of different genetic backgrounds into account, and also represent a broad range of the relevant varieties. The data should further include so-called 'Omics'.

3. Comparative assessment of plant composition and agronomic and phenotypic characteristics

According to the requirements set out in Implementing Regulation 503/2013, there should have been a request for recent data on genetic stability and gene expression under ongoing climate change. Experiments under controlled and defined conditions should also have been conducted in order to expose the plants to 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 increasing problems with herbicide-resistant weeds, it is probable that the dosages applied to the plants are now much higher compared to agricultural practice ten or twenty years ago. It is also to be expected that higher and/or repeated herbicide application dosages will 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, including the highest dosage of glyphosate that can be tolerated by the plants and 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 include so-called 'Omics'.

It is known that the genomic background of the varieties can influence both the expression of the inserted genes and plant metabolism (see, for example, Lohn et al., 2020; Trtikova et al., 2015). Therefore, EFSA should also have requested additional data on transgenic plant varieties.

Such data were not made available, therefore no conclusion can be drawn from the comparative assessment.

4. Toxicity

Implementing Regulation 503/2013 asks applicants to perform 90-day subchronic studies with genetically engineered plants for reliable toxicological hazard identification and characterization. However, as yet, no 90-day feeding study in rodents using whole feed or feed from GT73 oilseed rape has been conducted or provided (EFSA, 2022b). Therefore, no final conclusion can be drawn on the food and feed safety of oilseed rape GT73.

Effects of residues from spraying with complementary herbicides specific to GE plants and their mixed toxicity

The residues from spraying were (again) considered to be outside the remit of the GMO Panel. However, without detailed assessment of these residues, no conclusion can be drawn on the safety of the imported products: due to specific agricultural management practices in the cultivation of the herbicide-resistant plants, there are, for example, specific patterns of spraying, exposure and occurrence of specific metabolites that require special attention.
Both EU pesticide regulation and GMO regulation require a high level of protection for health and the environment. Therefore, in regard to herbicide-resistant plants, specific assessment of residues from spraying with complementary herbicides must be considered a prerequisite for granting (renewal) authorisation.

The described effects, which may enhance the uptake of DNA from the transgenic plants into gut bacteria, are not assessed under pesticide regulation, they have to be assessed within GMO risk assessment. The reason: the effects are highly dependent on the specific dosages of applications on the GE plants, as well as their metabolism and the resulting pattern of exposure in food and feed. Cumulative effects (mixtures of GE plants in a diet) may also play a decisive role. Under Directive 2001/18/EC, such effects could be considered to be indirect effects which may be immediate, delayed or cumulative. Implementing Regulation 503/2013 (1.4.2) requires "*testing of new constituents other than proteins*". It is our opinion that this requirement also includes the assessment of residues from the complementary herbicides, which necessarily become constituents of all genetically engineered plants with this resistance.

As far as food and feed safety is concerned, EFSA (2020) considers microbiomes to be highly relevant to the health status of their hosts. Therefore, it is desirable to understand the importance of their role in risk assessment. EFSA expects that gut microbiome research (not in relation to GE plants) will play a relevant role in regulatory science with potential implications for future risk assessment and predictive risk models. As EFSA states: "*considering that the gut microbiome is a biological component directly and indirectly involved in the metabolism of food/feed components and chemicals and in the protection of the host against adverse environmental exposure, it would be useful to establish criteria on how to evaluate the potential adverse impacts of perturbators on this defensive barrier, and consequently, on human/animal health.*"

In general, antibiotic effects and other adverse health effects might occur from exposure to a diet containing these plants, as they were not assessed under pesticide regulation. These adverse effects

on health could be triggered by residues from spraying with the complementary herbicide. Further attention should be paid to the specific toxicity of the metabolites of the active ingredients in the pesticide, which might occur specifically in the GE plants, and therefore might escape pesticide regulation.

However, no attempts have been made to integrate the microbiome into the risk assessment of food and feed derived from the GE plants. This is in direct contradiction to Regulation 1829/2003 which requests "*genetically modified food and feed should only be authorised for placing on the Community market after a scientific evaluation of the highest possible standard, to be undertaken under the responsibility of the European Food Safety Authority (Authority), of any risks which they present for human and animal health and, as the case may be, for the environment.*" (Recital 9).

It should not be overlooked that Implementing Regulation 503/2013 (point 3.2.3) requires that "*the applicant shall evaluate the data generated to estimate possible short-term and long-term risks to human or animal health associated with the consumption of genetically modified food or feed with respect to the expression of new proteins/metabolites, as well as significantly altered levels of original plant proteins/metabolites.*" We conclude that this requirement, which, for example, also comprises long-term accumulated effects, is not fulfilled and safety was not demonstrated.

5. Environmental risk assessment

During the consultation process, several experts voiced concerns about spillage and the persistence of GE oilseed rape. For example, the Finnish authorities demanded more data and stricter measures, as recent studies had confirmed feral and persistent GM oilseed rape populations, including in Europe (EFSA, 2022b).

Oilseed rape (*Brassica napus*) can spread via pollen and seeds, and seeds can remain viable in the soil for more than ten years (seed dormancy). Europe is the centre of origin and genetic diversity for the group of *Brassica* plants to which oilseed rape belongs. Some native plant populations, such as *Brassica rapa* (turnip), can hybridise with oilseed rape. *Brassica napus* itself occurs mainly as a cultivated plant, but still maintains significant characteristics of a wild plant. Disturbed soil promotes the establishment of *Brassica napus* beyond the fields, whereas dense vegetation will hinder establishment. However, *Brassica napus* growing in the wild is found primarily in habitats where wild relatives of the *Brassica* genus and related genera grow. In addition, many related species which can hybridise with oilseed rape occur in environments such as road verges, industrial or feral sites. Gene flow to wild relatives is possible and likely to happen, even if *Brassica napus* itself only has a reduced potential to spread in a densely vegetated environment (Bauer-Panskus et al., 2013). A recent publication (Sohn et al., 2021) shows that the uncontrolled spread of genetically engineered (GE) oilseed rape is already happening in 14 countries on five continents. These are countries which either allow the cultivation of GE oilseed rape (such as the USA and Canada), or have tested it in experimental releases (such as Germany), or allow the import of kernels (such as Japan). Moreover, it has to be assumed that there is a high number of undetected cases, as many regions do not have systematic monitoring. In many cases, the plants have persisted for several years in or around the fields and along of transport routes, and have been found to have a higher potential for environmental spread than previously assumed.

In the case of GT73, it is likely that the plants will persist in the environment after spillage and also start to propagate. This would allow next generation effects to emerge that were neither assessed by the applicant nor by EFSA (Bauer-Panskus et al., 2020). They may also cross with other GE oilseed rape that had previously entered the environment via spillage. However, EFSA did not risk assess the direct or indirect effects, which maybe immediate, delayed or accumulated, as set out in Directive 2001/18/EC for environmental risk assessment.

In addition, there is a need to consider other ways of distribution and spread. For example, a recent report (COGEM, 2022) lists several unpublished studies from Switzerland showing that bird feed sold in Europe appears to be contaminated with genetically engineered oilseed rape. Such unforeseen routes of spread are not reflected in the EFSA risk assessment.

Findings in a further recent publication revealed the hybridisation of genetically engineered herbicide-tolerant oilseed rape (GT73) into the related weed species *Brassica rapa* (bird rape mustard) in Canada (Laforest et al. 2022). Oilseed rape and *B. rapa* are intercrossable, and hybrids of genetically engineered (GE) oilseed rape and bird rape mustard have already been detected along transportation routes and near ports in countries that import GE oilseed rape. However, it was assumed that the hybrid plants had reduced fertility and were, therefore, unable to establish in the environment permanently. The current study shows that the genetically engineered trait is now detectable in purebred and weedy *B. rapa* plants in Canada, presumably through multiple backcrosses of the hybrids.

The paper also indicates hybridisation of GE oilseed rape and field radish (*Raphanus raphanistrum*), another wild relative of oilseed rape with weedy characteristics. Although the two species had been crossed in the laboratory, this is likely the first proven case of hybridisation under natural conditions.

There have been various studies in recent years showing that plants engineered to be resistant to glyphosate can exhibit unexpected biological effects. These effects may give the genetically engineered plants a survival advantage even if no glyphosate is sprayed at all (see, for example, Beres, 2018; Beres et al., 2019; Fang et al., 2018; Wang et al., 2014; Yang et al., 2017).

Overall, new evidence presented in COGEM (2022) and Laforest et al. (2022) as well as the unresolved questions regarding unexpected effects caused by epsps genes, should result in much greater scrutiny regarding environmental risk assessment than is presented in the EFSA opinion.

6. Others

If approval for import is given, the applicant has to ensure that post-market monitoring (PMM) is developed to collect reliable information on the detection of indications showing whether any (adverse) effects on health may be related to GM food or feed consumption. Thus, the monitoring report should, at very least, contain detailed information on: i) actual volumes of the GE products being imported into the EU; ii) the ports and silos where shipments of the GE products are unloaded; iii) the processing plants where the GE products are transferred to; iv) the amount of the GE products used on farms for feed and v) transport routes for GE products. Environmental monitoring should be run in regions where viable material of the GE products, such as kernels, are transported, stored, packaged, processed or used for food/feed. Where there are losses or spread of viable material (such as kernels), all receiving environments need to be monitored. Furthermore, environmental exposure through organic waste material, by-products, sewage or faeces containing GE products, during or after the production process, and during or after human or animal consumption, should all be part of the monitoring procedure (see also comments submitted by experts from Member States experts, EFSA, 2022b).

7. Conclusion

Scientific evidence relevant for the environmental risk assessment of GT73 oilseed rape was deliberately not considered by EFSA, thus massively impairing the outcomes of the opinion. Further, no conclusion can be drawn on food and feed safety, as experimental data is still missing. The EFSA opinion should therefore be rejected since the safety of GT73 oilseed rape was not demonstrated.

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