European Journal of Nutrition & Food Safety

11(1): 51-55, 2019; Article no.EJNFS.2019.008
ISSN: 2347-5641

Environmental Risk Assessment of Glufosinate-Tolerant Genetically Modified Oilseed Rape MS8, RF3 and MS8 x RF3 for Import, Processing and Feed Uses under Directive 2001/18/EC (Notification C/BE/96/01)

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Authors' contributions

This work was carried out in collaboration among all authors. The opinion has been assessed and approved by the Panel on Genetically Modified Organisms of VKM. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2019/v11i130130

Grey Literature

Received 19 July 2019
Accepted 24 October 2019
Published 19 November 2019

ABSTRACT

In preparation for a legal implementation of EU-regulation 1829/2003, the Norwegian Scientific Committee for Food Safety (VKM) has been requested by the Norwegian Directorate for Nature Management to conduct final environmental risk assessments for all genetically modified

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organisms (GMOs) and products containing or consisting of GMOs that are authorized in the European Union under Directive 2001/18/EC or Regulation 1829/2003/EC. The request covers scope(s) relevant to the Gene Technology Act. The request does not cover GMOs that VKM already has conducted its final risk assessments on. However, the Directorate requests VKM to consider whether updates or other changes to earlier submitted assessments are necessary.

The genetically modified, glufosinate-tolerant oilseed rape lines MS8, RF3 and MS8 x RF3 (Notification C/BE/96/01) are approved under Directive 2001/18/EC for import and processing for feed and industrial purposes since 26 March 2007 (Commission Decision 2007/232/EC). In addition, processed oil from genetically modified oilseed rape derived from MS8, RF3 and MS8 x RF3 were notified as existing food according to Art. 5 of Regulation (EC) No 258/97 on novel foods and novel food ingredients in November 1999. Existing feed and feed products containing, consisting of or produced from MS8, RF3 and MS8 x RF3 were notified according to Articles 8 and 20 of Regulation (EC) No 1829/2003 and were placed on the market in January 2000.

An application for renewal of the authorisation for continued marketing of existing food, food ingredients and feed materials produced from MS8, RF3 and MS8 x RF3 was submitted within the framework of Regulation (EC) No 1829/2003 in June 2007 (EFSA/GMO/RX/MS8/RF3). In addition, an application covering food containing or consisting of, and food produced from or containing ingredients produced from oilseed rape MS8, RF3 and MS8 x RF3 (with the exception of processed oil) was delivered by Bayer CropScience in June 2010 (EFSA/GMO/BE/2010/81).

The VKM GMO Panel has previously issued a scientific opinion related to the notification C/BE/96/01 for the placing on the market of the oilseed rape lines for import, processing and feed uses (VKM 2008). The health and environmental risk assessment was commissioned by the Norwegian Directorate for Nature Management in connection with the national finalisation of the procedure of the notification C/BE/96/01 in 2008. Due to the publication of updated guidelines for environmental risk assessments of genetically modified plants and new scientific literature, the VKM GMO Panel has decided to deliver an updated environmental risk assessment of oilseed rape MS8, RF3 and MS8 x RF3.

A scientific opinion on an application for the placing on the market of MS8/RF3 for food containing or consisting of, and food produced from or containing ingredients produced from MS8/RF3 (with the exception of processed oil) (EFSA/GMO/BE/2010/81) have also been submitted by the VKM GMO Panel (VKM 2012).

The environmental risk assessment of the oilseed rape MS8, RF3 and MS8 x RF3 is based on information provided by the notifier in the applications EFSA/GMO/RX/MS8/RF3, EFSA/GMO/BE/2010/8, the notification C/BE/96/01, and scientific comments from EFSA and other member states made available on the EFSA website GMO Extranet. The risk assessment also considered other peer-reviewed scientific literature as relevant.

The VKM GMO Panel has evaluated MS8, RF3 and MS8 x RF3 with reference to its intended uses in the European Economic Area (EEA), and according to the principles described in the Norwegian Gene Technology Act and regulations relating to impact assessment pursuant to the Gene Technology Act, Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms, and Regulation (EC) No 1829/2003 on genetically modified food and feed. The Norwegian Scientific Committee for Food Safety has also decided to take account of the appropriate principles described in the EFSA guidelines for the risk assessment of GM plants and derived food and feed (EFSA 2006, 2011a), the environmental risk assessment of GM plants (EFSA 2010), the selection of comparators for the risk assessment of GM plants (EFSA 2011b), and for the post-market environmental monitoring of GM plants (EFSA 2006, 2011c).

The scientific risk assessment of oilseed rape MS8, RF3 and MS8 x RF3 include molecular characterisation of the inserted DNA and expression of target proteins, comparative assessment of agronomic and phenotypic characteristics, unintended effects on plant fitness, potential for horizontal and vertical gene transfer, and evaluations of the post-market environmental plan.

In line with its mandate, VKM emphasised that assessments of sustainable development, societal utility and ethical considerations, according to the Norwegian Gene Technology Act and Regulations relating to impact assessment pursuant to the Gene Technology Act, shall not be carried out by the Panel on Genetically Modified Organisms.

The genetically modified oilseed rape lines MS8 and RF3 were developed to provide a pollination control system for production of F1-hybrid seeds (MS8 x RF3). Oilseed rape is a crop capable of undergoing both self-pollination (70%) as well as cross-pollination (30%). Therefore a system to ensure only cross-pollination is required for producing hybrids from two distinct parents. As a result of hybrid vigor cross-pollinated plants produce higher yield as compared to self-pollinating rape.

The hybrid system is achieved using a pollination control system by insertion and expression of barnase and barstar genes derived from the soil bacterium Bacillus amyloliquefaciens into two separate transgenic oilseed rape lines. The barnase gene in the male sterile line MS8 encode a ribonuclease peptide (RNase), expressed in the tapetum cells during anther development. The RNase effect RNA levels, disrupting normal cell function, arresting early anther development, and results in the lack of viable pollen and male sterility.

The fertility restoration line RF3 contains a barstar gene, coding for a ribonuclease inhibitor (Barstar peptide) expressed only in the tapetum cells of the pollen during anther development. The peptide specifically inhibits the Barnase RNase expressed by the MS8 line. The RNase and the ribonuclease inhibitor form a stable one-to-one complex, in which the RNase is inactivated. As a result, when pollen from the receptor line RF3 is crossed to the male sterile line MS8, the MS8 x RF3 progeny expresses the RNase inhibitor in the tapetum cells of the anthers allowing hybrid plants to develop normal anthers and restore fertility.

The barnase and barstar genes in MS8 and RF3 are each linked with the bar gene from Streptomyces hygroscopus. The bar gene is driven by a plant promoter that is active in all green tissues of the plant, and encodes the enzyme phosphinothricin acetyltransferase (PAT). The PAT enzyme inactivates phosphinothricin (PPT), the active constituent of the non-selective herbicide glufosinate-ammonium. The bar gen were transferred to the oilseed rape plants as markers both for use during in vitro selection and as a breeding selection tool in seed production.

Molecular characterization:

The oilseed rape hybrid MS8xRF3 is produced by conventional crossing. The parental lines MS8 and RF3 are well described in the documentation provided by the applicant, and a number of publications support their data. It seems likely that MS8 contains a complete copy of the desired T-DNA construct including the bar and barnase genes. Likewise, the event RF3 is likely to contain complete copies of the bar and barstar genes in addition to a second incomplete non-functional copy of the bar-gene. The inserts in the single events are preserved in the hybrid MS8xRF3, and the desired traits are stably inherited over generations.

Oilseed rape MS8, RF3 and MS8xRF3 and the physical, chemical and functional characteristics of the newly expressed proteins have previously been evaluated by the VKM Panel on Genetically Modified Organisms, and considered satisfactory (VKM 2008, 2012). The GMO Panel finds the characterisation of the physical, chemical and functional properties of the recombinant inserts in the oilseed rape transformation events MS8, RF3 and MS8xRF3 to be satisfactory. The GMO Panel has not identified any novel risks associated with the modified plants based on the molecular characterisation of the inserts.

Comparative assessment:

Based on results from comparative analyses of data from field trials located at representative sites and environments in Europe and Canada, it is concluded that oilseed rape MS8, RF3 and MS8 x RF3 is agronomically and phenotypically equivalent to the conventional counterpart, except for the newly expressed barnase, barstar and PAT proteins.

The field evaluations support a conclusion of no phenotypic changes indicative of increased plant weed/pest potential of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape. Furthermore, the results demonstrate that in-crop applications of glufosinate herbicide do not alter the phenotypic and agronomic characteristics of event MS8, RF3 and MS8 x RF3 compared to conventional oilseed rape varieties.

Environmental risk:

Considering the scope of the notification C/BE/96/01, excluding cultivation purposes, the environmental risk assessment is limited to exposure through accidental spillage of viable seeds of MS8, RF3 and MS8 x RF3 into the environment during transportation, storage, handling, processing and use of derived products.

Oilseed rape is mainly a self-pollinating species, but has entomophilous flowers capable of both self- and cross-pollinating. Normally the level of outcrossing is about 30%, but outcrossing frequencies up to 55% are reported.

Several plant species related to oilseed rape that are either cultivated, occurs as weeds of cultivated and disturbed lands, or grow outside cultivation areas to which gene introgression from oilseed rape could be of concern. These are found both in the *Brassica* species complex and in related genera. A series of controlled crosses between oilseed rape and related taxa have been reported in the scientific literature. Because of a mismatch in the chromosome numbers most hybrids have a severely reduced fertility. Exceptions are hybrids obtained from crosses between oilseed rape and wild turnip (*B. rapa* ssp. campestris) and to a lesser extent, mustard greens (*B. juncea*), where spontaneously hybridising and transgene introgression under field conditions have been confirmed. Wild turnip is native to Norway and a common weed in arable lowlands.

Accidental spillage and loss of viable seeds of MS8, RF3 and MS8 x RF3 during transport, storage, handling in the environment and processing into derived products is likely to take place over time, and the establishment of small populations of oilseed rape MS8, RF3 and MS8 x RF3 cannot be excluded. Feral oilseed rape MS8, RF3 and MS8 x RF3 arising from spilled seed could theoretically pollinate conventional crop plants if the escaped populations are immediately adjacent to field crops, and shed seeds from cross-pollinated crop plants could emerge as GM volunteers in subsequent crops.

However, both the occurrence of feral oilseed rape resulting from seed import spills and the introgression of genetic material from feral oilseed rape populations to wild populations are likely to be low in an import scenario in Norway.

There is no evidence that the herbicide tolerant trait results in enhanced fitness, persistence or invasiveness of oilseed rape MS8, RF3 and MS8 x RF3, or hybridizing wild relatives, compared to conventional oilseed rape varieties, unless the plants are exposed to herbicides with the active substance glufosinate ammonium. Apart from the glufosinate tolerance trait, the resulting progeny will not possess a higher fitness and will not be different from progeny arising from cross-fertilisation with conventional oilseed rape varieties.

Glufosinate ammonium-containing herbicides have been withdrawn from the Norwegian market since 2008, and the substance will be phased out in the EU in 2017 for reasons of reproductive toxicity.

Overall conclusion:

The VKM GMO Panel concludes that oilseed rape MS8, RF3 and MS8xRF3 are unlikely to have any adverse effect on the environment in Norway in the context of its intended usage.

Keywords: *GMO; oilseed rape; Brassica napus ssp. oleifera (DC.) Metzg.; genetically modified oilseed rape MS8; RF3; MS8 x RF3; hybrid; C/BE/96/01; glufosinate-tolerant; bar; barnase; barstar; PAT protein; environmental risk assessment; import; processing; directive 2001/18/EC.*

Available:https://vkm.no/download/18.175083d415c86c573b59a5a1/1501677722559/9a78b836b8.pdf

ISBN: 978-82-8259-081-5

NOTE:

This work was carried out in collaboration between all authors. The opinion has been assessed and approved by the Panel on Genetically Modified Organisms of VKM. All authors read and approved the final manuscript.

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Suggested citation: Environmental risk assessment of glufosinate-tolerant genetically modified oilseed rape MS8, RF3 and MS8 x RF3 for import, processing and feed uses under Directive 2001/18/EC (Notification C/BE/96/01). Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety, ISBN: 978-82-8259-081-5. Oslo, Norway.

COMPETING INTERESTS

Authors have declared that no competing interests exist.