Vår ref:2017/H_138
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Høringsuttalelse av søknad om markedsføring av genmodifisert raps MS11

EFSA/GMO/BE/2016/138

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
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Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
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Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/BE/2016/138**, genmodifisert oljeraps MS11, fra Bayer CropScience LP, under EU forordning 1829/2003. Søknaden gjelder bruksområdene mat, fòr, import og prosessering.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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Innhold/Content

Høringsuttalelse – genmodifisert oljeraps MS11 (EFSA/GMO/BE/2016/138), under EU forordning 1829/2003.

Søknad EFSA/GMO/BE/2016/138 omhandler genmodifisert oljeraps linje til bruksområdene mat, for, import og prosessering.

Den genmodifiserte oljerapsen har toleranse mot sprøytemidlet glufosinat ammonium via det innsatte genet *pat*, samt uttrykk av proteinet Barnase som bryter ned RNA i pollen og gjør planten hannsteril. I tillegg utrykkes proteinet Barstar som gir økt transformasjons effektivitet og er en hemmer av Barnase. Dette gjør plantene fertile igjen, ved uttrykk.

Oljeraps linje MS11 er ikke godkjent for noen av bruksområdene i Norge eller EU.

Oppsummering

GenØk–Senter for biosikkerhet, viser til høring av søknad EFSA/GMO/BE/2016/138 om oljeraps MS11 som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra denne oljerapsen.

Vi har gjennomgått de dokumenter som vi har fått tilgjengelig, og nevner spesielt følgende punkter vedrørende søknaden:

- Genmodifisert oljeraps MS11 er ikke godkjent i Norge eller EU for noen av de omsøkte bruksområdene.
- MS11 er tolerant mot sprøytemidler som inneholder glufosinat - ammonium som har ulike grader av helse-og-miljø fare ved bruk.
- Glufosinat ammonium er ikke tillatt brukt i Norge.
- Søknaden om oljeraps MS11 mangler data og informasjon som er relevant for å kunne vurdere kriterier rundt etisk forsvarlighet, samfunnsnytte og bærekraft.

Summary

GenØk-Centre for biosafety refers to the application EFSA/GMO/BE/2016/138 on MS11 oilseed rape for import, processing, food and feed or ingredients thereof.

We have assessed the documents available, and highlights in particular the following points for the current application:

- The gene modified oilseed rape event MS11 is not approved for any application in Norway or the EU.
- Oilseed rape event MS11 is tolerant to herbicides containing gluphosinate ammonium that has distinct health and environmental dangers upon use.
- It is not allowed to use gluphosinate ammonium in Norway.
- The application on oilseedrape event MS11 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

Application on EFSA/GMO/BE/2016/138

The event oilseed rape (OSR) MS11 contains genes providing herbicide tolerance (*pat*) as well as providing male sterility and increased transformation frequency (*barnase and barstar).*

Oilseed rape (OSR)

Oilseed rape (OSR), *Brassica napus,* (also referred to as canola, rape, rapeseed etc.) is a plant with wild relatives in Norway, harboring an estimated number of 49 species. These wild relatives are able to grow throughout the country and as far north as Finnmark (1) and could be potential hybridization partners for gene modified OSRs. Although there are challenges when it comes to the cultivation due to geography, climate, insects and also fungus-related pathogens, the trend has been towards increasing the cultivation of OSR in Norway.

As Norway is not able to keep up with the domestic needs of OSR, most of it is imported.

For more information on the OSR situation in Norway, we refer to the report written by GenØk in 2015 (1).

From this report, we highlight the following:

"*The risk for spread of the transgenes are also highly present. Reports show that "unintentional stacking" of herbicide resistance genes in B. napus has taken place in the volunteers due to intraspecific pollen flow in and from the cultivation areas (2). This means that the volunteers detected have multiple herbicide resistant traits present in the same plant. Also spread of transgenes to wild relatives takes place naturally (3). Spread of transgenes will thus not only happen through spillage of OSR seeds but also along transport routes to and from cultivation areas, transport from the machinery involved in harvesting and by other routes (4).*"

OSR seeds are small and can potentially live for many years in the soil after harvest. In addition, pollen from OSRs can travel over long distances with wind and insects. There is thus a potential for genes from gene modified OSR to spread over distances and to wild relatives, as well as to non-modified OSR crops.

A report by the Norwegian Biotechnology Advisory Board in 2013 also describes this (5). Here, they point to data showing that gene modified OSR is growing beside roads and railway tracks, where they have been transported, in USA and Canada. Spread of OSR during handling and transport is thus important and relevant to consider.

Previous evaluations

GenØK-Centre for Biosafety has not previously assessed MS11 or stacks thereof.

Social utility and sustainability issues on the oilseed rape (OSR) event MS11 (EFSA/GMO/BE/2016/138)

In Norway, an impact assessment follows the Norwegian Gene Technology Act (NGTA) (6) in addition to the EU regulatory framework for GMO assessment. In accordance with the aim of the NGTA, the development, introduction and/or use of a GMO needs to be *ethically justifiable*, demonstrate a *benefit to society* and contribute to *sustainable development*. This is further elaborated in section 10 of the Act (approval), where it is stated that: "*significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development*" (See section 17 and annex 4 for more detail on the regulation on impact assessment). Recent developments within European legislation on GMOs allow Member States to restrict the cultivation of GMOs on their own territory based on socio-economic impacts, environmental or agricultural policy objectives, or with the aim to avoid the unintended presence of GMOs in other products (Directive 2015/412) (7). Additionally, attention within academic and policy spheres increased in recent years on broadening the scope of the assessment of new and emerging (bio) technologies to include issues that reach beyond human and environmental health (8-13).

To assess the criteria of *ethically justifiable*, *benefit to society* and *sustainability* as in the NGTA, significant dedication is demanded as it covers a wide range of aspects that need to be investigated (e.g. Annex 4 within the NGTA, or 14). Nevertheless, the applicant has currently not provided any information relevant to enable an assessment of these criteria. Therefore, this section will highlight some areas that are particularly relevant to consider with oilseed rape MS11 and where the applicant should provide data for in order to conduct a thorough assessment according to the NGTA. Table 1 offers specific questions connected to the sections below.

Sustainability

The oilseed rape MS11 contains a *pat* gene that confers increased tolerance to herbicides that contain gluphosinate-ammonium, a class of herbicide banned in Norway (see the section *The ethical issue of glufosinate-ammonium* below). When an herbicide - such as gluphosinate-ammonium – is used in agriculture, it is important to minimize the potential of weeds becoming resistant. Indeed, when crops are engineered to be herbicide tolerant in order to maintain an agricultural practice that uses herbicides, it is essential to remain attentive to the amount of herbicide used, the potential increase of use and the consequences of this for the area in which the crop is cultivated. The development of management strategies to make sure that this does not create (more) resistant weed is warranted to be able to respond to a potential increase in weed-resistance. Moreover, studies have shown increased levels of herbicide residues in herbicide tolerant GM crops (e.g. 15), which could have health impacts on humans and animals consuming food/feed based on ingredients from this type of GM plants. The applicant has not provided information on whether the cultivation of oilseed rape MS11 could affect the emergence of herbicide resistance in weeds, nor if there are cases of this in the areas intended for cultivation of the variety, which are also important aspects to evaluate for the criteria of *ethically justifiable*. Furthermore, the request to cultivate this oilseed rape in the USA is in process, but it should be noted that herbicide resistant weeds have increased significantly in the

USA in recent years[1]. Field trials of the oilseed rape have taken place in both the USA and Canada, but no information is currently provided by the applicant that demonstrates reflection on how the monitoring, assessment or evaluation of the GM crop in countries where the crop will potentially be cultivated in the future is assessed, as the applicant considers information on this not relevant because oilseed rape MS11 will not be cultivated in Europe. However, it remains an important aspect for a sustainability evaluation and thus necessary if the application is to be evaluated according to this criteria in the NGTA.

Impacts of the co-technology: glyphosate

The evaluation of the co-technology, that is, secondary products that are intended to be used in conjunction with the GMO, is also considered important in the risk assessment of a GMO (16). Therefore, considerations of the co-products also warrant an evaluation of safe use and data required for such an assessment is not provided by the Applicant.

Impacts in producer countries

As already stated, the Applicant does not provide data relevant for an environmental risk assessment of oilseed rape MS11 as it is not intended to be cultivated in the EU/Norway. However, this information is necessary in order to assess the sustainability criteria as laid down in the NGTA. This criteria is referring to a global context, including the contribution to sustainable development in the producing countries with a view to the health, environmental and socio-economic effects in other countries, in this case where the oilseed rape MS11 is cultivated.

In addition to a lack of information, there can also be ambiguity about how scientific conclusions may be achieved. For example, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, genetic and socio-economic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. It can therefore not be expected that the same effects will apply between different environments and across continents.

The applicant highlights that the appearance of “volunteer” oilseed rape in rotational fields following the soy crop from the previous year is rare under European conditions. Still, an evaluation of the occurrence of volunteer plants in the producing countries and suggested control strategies is important for a sustainability assessment. Information about the occurrence of volunteers and which herbicides will potentially be used for killing volunteers is required to evaluate potential health and environmental impacts of these.

Benefit to society

The criteria of ‘benefit to society’ in the NGTA should be interpreted on a national level. That means that the import of oilseed rape MS11 needs to demonstrate how it will benefit Norway. However, no information on this part is provided by the applicant. Furthermore, it is important

[1] http://weedscience.org/Summary/Country.aspx Status of Herbicide Resistance in USA, accessed on May the 7th 2017.

to evaluate how GM crops in general, GM oilseed rape in particular, and the use of GM oilseed rape in food and feed are valued by Norwegian consumers. This information will contribute to anticipate impacts at an early stage, as well as that it may demonstrate a need to assess the alternative options for import of oilseed rape. A report published in 2017 on the perceptions among Norwegian citizens on GMOs describes how about half of the respondents expressed that they were negative for sale of GMO-products in Norwegian grocery stores in the future, whereas only 15 percent were positive (17). Nevertheless, the empirical data available on the attitude of Norwegian citizens towards GM approaches and applications remain limited (e.g. 18, 19) and more empirical research on this is warranted to investigate consumers' attitude, demand and acceptance on different aspects such the cultivation, import and or processing of GM crops within and outside of Norway, as are the perspectives on GM food and feed.

Assessing alternatives

When a new (bio-) technology is developed, it is important to reflect on what problem it aims to solve and to investigate whether alternative options may achieve the same outcomes in a safer and / or a more ethically justifiable way. After all, when a crop is genetically modified to tolerate a particular herbicide, it means that the crop is developed for a particular cultivation practice in which these herbicides are to be used. What is meant with alternatives, and what would benefit from being assessed could include alternative varieties (e.g. conventional or organic maize) for import, alternative sources to satisfy the demand, alternative ways of agriculture, or even explore alternative life visions. In fact, this corresponds with the increased trend within research and policy of science and innovation to anticipate impacts, assess alternatives and reveal underlying values, assumptions, norms and beliefs (11, 20) as a way to reflect on what kind of society we want, and then assess how certain (biotechnological) developments may or may not contribute to shaping a desired future. Thus, in order to evaluate whether oilseed rape MS11 contributes to social utility, it is important to investigate current and future demands and acceptance of this in Norway and if there are alternatives sources for oilseed rape that could be cultivated elsewhere that may satisfy this demand, or are more desirable.

Ethical considerations: socio-economic impacts

As known, GM crops have been, and still are, a topic of debate. A significant amount of this debate focuses on the safety of GMOs and currently no scientific consensus on this topic has been achieved (21). Nevertheless, another substantial part of the debate is around the socio-economic impacts of GM productions and many questions for evaluating the above mentioned criteria in the NGTA are based on an assessment of the socio-economic impacts. These impacts can vary and range from seed choice for farmers, co-existence of different agricultural practices, impacts among poor and/or small-scale farmers in developing countries, share of the benefits among sectors of the society, changing power dynamics among stakeholders, autonomy of farmers, intellectual property right on GMOs, benefit sharing, the decreasing space for regional and local policy, and more organisational work and higher costs for non-GM farmers (e.g. for cleaning of sowing machines or transport equipment to avoid contamination). Although the examples of socio-economic impacts clearly indicate the complexity and extensive list of concerns beyond safety aspects, little empirical investigation on these kind of aspects has been done. For example a study performed by Fischer et al. (22) concerning social implications from cultivating GM crops found that from 2004 – 2015 there has only been 15 studies corning socio-economic implications of cultivating Bt-maize. The study demonstrates that published literature is dominated by studies of economic impact and conclude that very few studies take a comprehensive view of social impacts associated with GM crops in agriculture. Although this study focused on Bt-maize, the amount of research performed in this case and the minimal focus on social impacts strongly indicate a high need for further investigation on how the cultivation of GM crops affects different parties involved.

Co-existence

The cultivation of GM plants in general is causing problems with regard to co-existence. For instance, Binimelis (23) has investigated consequences on co-existence of Bt maize in Spain among small-scale farmer and has found that co-existence is very difficult and that farmers in some areas have given up growing non-GM maize. Even though the cultivation of oilseed rape MS11 is not planned in Europe/Norway, it is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic oilseed rape production and information about consequences for co-existence in the countries intended for cultivation of oilseed rape MS11.

Another socio-economic challenge related to co-existence and biodiversity is the effect that GM production may have one bees and beekeepers. Oilseed rape is a great food source for honeybees, offering both nectar and pollen. As a food source for honeybees, GM oilseed rape can still affect the honey produced by bees and thus beekeepers and the marketability of their honey or other bee products as non-GM (24). To properly assess the criteria of sustainability and ethical justifiability, it is also important that the applicant provides information on the potential implications oilseed rape MS11 has on bees (i.e. biodiversity) (25) and the practice and products of beekeepers.

Furthermore, legal information and clarity could provide evaluators a more comprehensive understanding of governance strategies and possibilities to ensure co-existence, although it has been noted that this may not suffice as co-existence has become an arena of opposed values and future vision of agriculture, including the role of GM crops within these visions (26). Although a framework for maintaining co-existence in Europe was established in 2003 (27) this effectively meant technical measurements and recommendations (e.g. cleaning of sowing machines and transport vehicles) and remains challenging in practice (28, 29). Moreover, this framework arguably reduced the significance of the issue of co-existence to questions concerning economic aspects for individuals (e.g. farmers), rather than recognizing that agricultural practices are interwoven in dynamic social, economic and political systems (30, 31). For the criteria in the NGTA, information on co-existence is required to enable a coherent analysis.

The ethical issue of sterilizing a crop

MS11 contains the *barnase/barstar* gene system, which consist of a blocking sequence (encoding a Barnase) linked to the gene of interest and a recovery sequence (encoding a Barstar) (32). Hence, the *barnase* gene makes the plant male sterile and the *barstar* gene is able to recover fertility. There remain some concerns around this type of technology, though, that are important to consider in evaluating MS11 to the criteria of the NGTA. Firstly, a concern that rises by sterilizes a plant, is the effect this has on farmers, namely offspring seeds that a farmer cannot use again because the seeds do not germinate. This would make the farmers more dependent as they would have to buy new seeds every year. In case of unintended contamination, it can be problematic as well. When a field is unknowingly contaminated and the non-GM farmer keeps a part of its production to reuse the next year, it could cause economic losses to the farmer as it does not know that part of the farm-saved seeds are male sterile. This was and still is a major concern with all the 'genetic use restrictions technologies' (GURT), which got a global moratorium that is still in place (33). Even though the *barstar* gene is meant to make the seed fertile again, it is arguably uncontrollable whether this works completely (34). Moreover, there could be concerns that reach beyond the effect that the use of this technology could have on farmers. An ethical issue that arises the moment a technology interferes with the reproducibility of a plant is on the autonomy of a plant. One of the characteristics of a living organism is it's ability to grow, develop and reproduce itself. These characteristics, that make the plant a *living* organism, also make the plant to be more than its non-living parts (e.g. genes) (35). Although it could be argued that in the case of MS11 this should not be an issue as the *barstar* gene could undo the male sterility, it is important to remain attentive to differences in the means and purposes of genetic engineering. There is a difference in using a technique to 'add' a particular gene (in order to express a desired trait), and using a technique to prohibit an organism to perform one of its core characteristics (i.e. reproducibility). This different kind of purpose used can raise different concerns within society and this should be further explored in public debate before approving any application using this technology.

The ethical issue of gluphosinate-ammonium

A significant ethical issue arises as oilseed rape MS11 is meant to be resistant to gluphosinate-ammonium, a class of herbicide that is banned in Norway (except a limited use on apples) due to the risks to human health and the environment. It seems ethically ambiguous and inconsistent to import a plant that is resistant to this herbicide, thereby allowing the use and development of a harmful herbicide in other countries, while considering the herbicide as too harmful to be used in Norway. Additionally, this troubles the fulfilment of the criteria of *sustainable development*, as this criteria is meant to be considered in a global context. Information on how this can be ethically justified is therefore highly warranted.

Summary

In order to meet the requirements for the NGTA, the regulator is encouraged to ask the Applicant to submit information relevant for the assessment of the criteria of ethically justifiable, benefit to society and sustainability development. An important part that is lacking is information about the consequences of the cultivation of oilseed rape MS11 for the potential producing countries. Furthermore, the information provided by the Applicant must be relevant for the specific agricultural context of these countries and should also stress the need for information on integrated weed management strategies (36). Moreover, the information should contain issues such as changes in herbicide use, development of herbicide resistant weed, potential for gene flow and possible socio-economic impacts such as poor and/or small-scale farmers in producing countries, share of the benefits among sectors of the society and as explained, effects of co-existence of different agricultural systems. As addressed, two ethical concerns are highlighted. Firstly, the use of the *barnase* gene for male sterility, which requires further exploration of public perception on this type of genetic engineering. Secondly, the use of the *pat* gene to make the plant tolerant to gluphosinate-ammonium, which is banned for use in Norway. Banning the use of gluphosinate-ammonium based herbicides domestically due to health and environmental concerns, while indirectly supporting its use in other countries would be ethically ambiguous and goes against the criteria of sustainable development. Additionally, the applicant does not attempt to demonstrate a benefit to society, a reference of the consumers' attitude towards GM oilseed rape, or the demand within Norway for oilseed rape MS11 and does therefore not provide sufficient information as required by the NGTA.

Table 1: Questions to the applicant

Sustainability	*How does the cultivation of oilseed rape MS11 affect the use of glyphosate?*
	How is the current use of glyphosate in the sites of cultivation and what approaches are used to minimize the use of glyphosate?
Herbicide-resistant weed	*What kind of management strategies are taken to prevent the increase of herbicide-resistant weed?*
	Who will be affected if the amount of resistant weeds increases?
	How is the burden of a potential increase of resistant weeds distributed and what strategies are in place to compensate this?
	How do the sites of the field trial relate to the proposed sites for cultivation? What are the differences and how may these affect the adequacy of the assessment of the field trials?
Benefit to society	*Is oilseed rape MS11 available for further breeding and research? If so, under which circumstances?*
	Is there a demand for oilseed rape MS11 in Norway?
	Does oilseed rape MS11 contribute to business development and value creation in Norway, including new job opportunities?
Assessing alternatives	*Will oilseed rape MS11 benefit Norwegian consumers more than the other alternatives available from conventional or organic agricultural practices? If so, how?*
Ethically justifiable	*What are the different public values and visions on the development, introduction or use of oilseed rape MS11 within Norway and how does the development of oilseed rape MS11 relates to these?*
	What are the public values and visions on (temporary) sterilizing a plant?
	Does the development, introduction or use of oilseed rape MS11 contradict ideas about solidarity and equality between people, such as the particular consideration of vulnerable groups in the population?
Socio-economic impacts	*Which parties will be affected by the development, introduction or use of oilseed rape MS11 and how does this change their autonomy, practice and position compared to other stakeholders?*
	Does oilseed rape MS11 change the power dynamic among stakeholders? If so, how?
	Can the development, introduction or use of oilseed rape MS11 create significant ruptures or ecological relationships?
Co-existence	*Does the cultivation of oilseed rape MS11 affect other types of agricultural practices in the nearby areas? If so, how?*
	Is there a system in place for keeping GMO and non-GMO crops separate in the production and transport line? If so, who pays for this system?

Environmental risk issues in a Norwegian context

OSRs produces many small seed. These seeds can potentially live for many years in the soil after harvest. In addition, pollen from OSRs can travel over long distances with wind and insects. There is thus a potential for genes from gene modified OSRs to spread over distances and to wild relatives, as well as to non-modified oilseed rape crops. This is thoroughly described by the Norwegian Biotechnology Advisory Board in their report of 2013 (5).

Another issue is also described by COGEM (Commissie Genetische Modificatie, Netherlands) in their report on import and processing of **another** gene modified OSR, GT73 (37), where they recommend that there must be a post monitoring plan involved along railways in order to monitor the occurrence of potential GM OSR.

In the case of the OSR MS11, spraying with herbicides containing gluphosinate ammonium on stacks of MS11, could have a selection pressure and could be of interest to follow further if spillage occurs during handling and transport.

Molecular characterization, expressed proteins and herbicide use - special issues to consider in the present application

The genemodified OSR event MS11 contains three genes called *pat*, *barnase* and *barstar* . These genes encode proteins involved in giving the plant tolerance towards gluphosinate-ammonium containing herbicides as well as providing male sterility and lack of viable pollen (Barnase protein), together with an increased transformation frequency through the Barstar protein and restoration of fertility upon expression.

Plants using the Barnase-Barstar system are developed to provide a controlled pollination system.

Molecular characterization

The three inserted genes in the OSR event MS11 has been thoroughly described before. Here is a short description of their source and actions:

- The *pat* gene (source: *Streptomyces viridochromogenes*) encodes Phosphinotricine N-acetyltransferase that inhibits the activity of gluphosinate-ammonium containing herbicides.
- The *pat* gene (source: *Streptomyces viridochromogenes*) encodes Phosphinotricine N-acetyltransferase that inhibits the activity of gluphosinate-ammonium containing herbicides.
- The *barnase* gene (source: *Bacillus amyloliquefaciens*) encodes a ribonuclease that inhibits the maturation of pollen and provides male sterility.
- The *barstar* gene (source: *Bacillus amyloliquefaciens*) encodes an inhibitor of *barnase*, providing increased transformation frequency.

The production of the OSR MS11 (male sterile line) was produced by *Agrobacterium tumefaciens* mediated transformation.

Barnase-Barstar technology

In Brassicas, a Barnase – Barstar system (male sterility (MS) and fertility restorer (FR) technology) can be used to produce hybrids that have viable seeds, used for agricultural purposes. Hybrid crops have higher yields , and the restoration of MS in hybrids are especially important in the cases where seeds are the important agricultural product (38), which is the case for the OSR MS11.

The introduced *barnase* gene is produced at an early stage in a specific cell type called tapetum which is in the pollen bearing part of the flower. This protein prevents the production of pollen and thus is the cause for male sterility.
The Barstar protein produced by the *barstar* gene encodes an enzyme, a ribonuclease, that inhibits the Barnase protein. Crossing two OSR lines with Barnase and Barstar then fully recovers the fertility of the resulting hybrid plants.

A herbicide tolerant trait is used for selection of the Barnase-Barstar protein, and this is often *pat* or *bar* genes providing gluphosinae-ammoium tolerant plants, but other herbicide genes can also be used. This is often used to remove unwanted hybrids, and also control weeds in the actual hybrid crop[2].

According to the Applicant, the recent application on MS11 is a "male sterile line that segregates 50:50 for sterility and fertility and is only used for the production of MS11 x RF3 hybrid seed. It will also not be commercialized as a standalone product".

The absence of the *barstar* gene was confirmed by PCR analysis as part of the vector – backbone analysis, but could not be confirmed by Southern blot as the probes did not cover this area.

According to the Applicant, the "*bar, barnase, barstar*" genes are present in MS11 as a single construct.

The construct also contains an *aadA* gene fragment of around 300bp. A functional *aadA* gene provides expression of aminoglycoside adenyltransferase, an antibiotic resistance marker gene (resistance towards spectinomycin and streptomycin). But according to the Applicant, the MS11 OSR has no antibiotic resistance marker genes. Apparently, Figure 1.2 18 on page 78 in the technical dossier supports this with the probe used. The probe is however 1800 bp a smaller probe for detection of *aadA* specifically could be recommended.

Southern blots

The transgenic insertion locus of OSR MS11 was analysed by restriction enzyme (RE) analysis followed by agarose gel-electrophoresis. DNA fragments were blotted unto nylon membranes for Southern blots and hybridized with dig-labelled probes. This was also done with the conventional counterpart as well as the transformation vectors used for the production of MS11.

Dig labelled probes varied in size from 217bp to 5865bp according to table 1.2.2 in the dossier (p.44). Figure 1.2.3 on page 46 provide a good illustration of where the probes bind, and where the RE cuts.

[2] http://www.fbae.org/2009/FBAE/website/special-topics_views_genetically_engineered_mustard_2.html

The southern blot picture provided for hybridization with the P021 probe (1660bp) has shades on the membrane (suggesting uneven distribution of liquid/buffer during hybridization and handling), as well as small black dots (often caused by antibody used for dig detection if not properly dissolved before use). This small black dots are also present on the membrane for the probe P014 (figure 1.2.5, page 57) and probe P016 (figure 1.2.6, page 58) and all other southerns performed, except for blots on page 65, 68 and 69 (figure 1.2.13, 1.2.16 and 1.2.17) which are blots with good contrast, and low background.

There are also some additional bands in some of the membranes were the Applicant suggests why they are present (undigested DNA etc). To get a proper verification, sequencing is suggested to be performed for clarification of potentially additional sequences or copies of inserts.

When it comes to the PCR analysis to assess the absence of the *barstar* gene originating from vectors backbone sequence in MS11, the pictures of the gels provided are dark, making it difficult to see the different PCR fragments. In addition, the molecular weight markers are barely visible, making it difficult to interpret the sizes of the bands present.

Comments relevant for the assessment of the current application

The MS11 OSR is not going to be commerzialized as a stand alone product, but as part of MS11 x RF3 stack.
The purpose of this stack would be tolerance to applications of gluphosinate- ammonium based herbicides, seemingly.

Protein expression and characterization of the newly expressed protein(s)

According to the Applicant EFSA guidelines were followed in the comparison of protein expression levels of the proteins Barnase, Barstar and Pat (p.87 in Technical dossier).
Protein levels were determined in tissues (whole plant, root, raceme, grain) cultivated in US and Canada. These cultivars were both treated and untreated with the actual herbicide (Liberty®280 SL).
Protein expression levels were determined using enzyme-linked immuno-sorbent assay (ELISA). Levels were very low:

From page 14 in the technical dossier: "*Expression levels were <LLOQ for Barnase and Barstar in MS11 B. napus grain and all processed fractions. Levels of PAT/bar were similar in grain, press cake and solvent extracted meal and were <LLOQ in toasted meal, crude oil and refined, bleached, and deodorized oil (the only food product consumed by humans)*".

No unintended proteins were found (caused by ORFs) after bioinformatics analysis of inserts and junctions according to the Applicant.
The expression levels of Barnase, Barstar and PAT were similar between MS11 *B. napus* treated with the conventional and the intended herbicides in the samples analyzed.

Toxicity and allergenicity

There are no indications from the previously performed risk assessments of Pat, Barnase or Barstar to be toxic or allergenic. In addition:

- Barnase was subjected to a 28-day repeated dose toxicity study, with no results indicating toxicity.
- The Barstar protein has no sequence homology to known allergens and is rapidly degraded in simulated gastric fluids. No toxicity was revealed in the 28-day repeated toxicity study referred to.
- Pat: no history of allergenicity of toxicity.

It was microbially produced proteins that were used in these studies.

Potential interactions between newly expressed proteins

Hazard identification

Guidelines in Codex (39) were used for food safety evaluations of the expressed proteins. No hazards or health related adverse effects were detected.

Herbicides

Herbicide use on GM plants

Herbicide tolerant (HT) plants are sprayed with one or more of the relevant herbicide(s), which will kill weeds without harming the HT GM plant with the inserted transgenes. The use of HT GM plants may cause negative effects on ecosystem as well as animal/human health. Of particular concern are: 1) increased use of, and exposure to, toxic herbicides; 2) accelerated resistance evolution in weeds; 3) accumulation of herbicides in the plants since they are sprayed in the growing season; 4) combinatorial effects of co-exposure to several herbicides at the same time (relevant for plants with pyramided HT genes); and 5) points 1-4 indicate that the agricultural practice of growing HT GM plants, fails to fulfill the criteria for a sustainable agriculture.

Gluphosinate-ammonium tolerance

The OSR MS11 contains the *pat* gene from *Streptomyces viridochromogenes*. This gene provide the OSR plant with tolerance to herbicides containing gluphosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Gluphosinate ammonium is harmful by inhalation, swallowing and by skin contact. Serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where gluphosinate ammonium is used (40-43). EFSA has concluded on the risk of gluphosinate ammonium, as especially harmful to mammals (44). Although the Applicant has stated that the Pat protein is expressed as a selection marker, this can also be used to control weeds and used for selectable cultivation of the crops expression the gene upon spraying (also a selection, but in field).

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Main summary

The genemodified ORS event MS11 is tolerant to herbicides containing glyphosate gluphosinate ammonium. Thus the issue on accumulation should be considered for GM plants to be used in food and feed.

In addition, gluphosinate ammonium is banned for use in Norway.

The applicant should provide data relevant for assessment of social utility and sustainable development according to the NGTA.

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