Vår ref:2013/h75
Deres ref: 2013/3303 ART-BI-DHT

Direktoratet for naturforvaltning
Tungasletta 2
7485 Trondheim
Dato: 19.04.2013

Vedlagt er inspill fra GenØk – Senter for Biosikkerhet om høringer **EFSA/GMO/NL/2009/75** for oljeraps MS8xRF3xGT73

Vennligst ta kontakt hvis du har noe spørsmål.

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Vår ref:2013/h75
Deres ref: 2013/3303 ART-BI-DHT

Assessment of the technical dossier submitted under EFSA/GMO/NL/2009/75 for approval of MS8xRF3xGT73 oilseed rape from Bayer CropScience AG and Monsanto Company

Submitted to

Direktoratet for Naturforvaltning

by

Rosa Binimelis, Marek Cuhra, Lise Nordgård, Arinze Stanley Okoli, Vinicius Vilperte

Centre for Biosafety – GenØk
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KONKLUSJON PÅ NORSK

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en konklusjon om sikker bruk, samfunnsnytten og bidrag til bærekraftighet av oljeraps MS8xRF3xGT73.

Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytten og bærekraftighet til oljeraps MS8xRF3xGT73 som kreves i den norske genteknologiloven (Appendix 4) for godkjenning i Norge.

Hovedkonklusjon og anbefalinger
Genøk –Senter for Biosikkerhet viser til brev fra Direktoratet for naturforvaltning (DN) angående høring som omfatter oljeraps MS8xRF3xGT73 for bruksområdet mat, fór, import og prosessering.

Oljeraps MS8xRF3xGT73, er en stablet hybrid med ulike herbicid-kodende gener innebygd. Stablede hybridplanter har generelt en mer kompleks genetisk sammensetning og derfor større potensiale for opp- og nedregulering av plantens egne gener. En grundig testing før evt markedsadgang vil derfor være nødvendig. Søker bør fremskaffe eksperimentelle bevis som viser at kombinasjonen ikke er skadelig og ikke bare vise til antagelser basert på vurderinger gjort av disse proteinene hver for seg.

CP4 EPSPS-proteinet gjør maisplantene tolerante overfor ugrasmidler med virkestoffet glyfosat. I den senere tid har laboratorie forsøk vist at glyfosat kan føre til celleskader, blant annet i humane embryoceller. Undersøkelser har også vist en skadelig effekt på vassdrag og vannorganismer. I tillegg forstyrrer glyfosat næringsstoffomsetninga i jord. Søker bør utføre analyser av viktige kjemiske prosesser som erfaringsmessig vites å være aktuelle problemstillinger for denne type genmodifiserte planter (herbicid toleranse medfører akkumulering av pågjeldende stoffer).

I tillegg er plantevermidlet glyfosat-ammonium som MS8xRF3xGT73 bl.a.er genmodifisert til å gi plantene resistens mot, ikke lovlig i Norge eller EU (med unntak av begrenset bruk på epler). Vi mener en godkjennelse av MS8xRF3xGT73 vil skade grunnleggende etiske og sosiale kriterier for bruk, som omtalt i den norske Bioteknologiloven

Å tillate genmodifiserte planter som lages for å tåle større mengder giftige sprøytemidler er ikke bærekraftig. På lang sikt vil det redusere forsyningssikkerheten og skade jordsmonnet.

Transport, lagring og prosessering av importerte partier av oljeraps til fôr vil kunne medføre utilsiktet frøspill og tap av spiredyktige frø og dermed representere et potensiale for utkryssing og spredning av transgener til dyrkede sorter og viltvoksende populasjoner i Norge

Selv om søker konkluderer med at det ikke er behov for en overvåkningsplan på bakgrunn av tidligere godkjenning av oljeraps MS8xRF3xGT73 for mat og fór, er vår vurdering at det er behov for en overvåkningsplan.

Søker gir ikke opplysninger som adresserer vurderingskriteriene bærekraft, samfunnsnytte og etiske aspekter som forutsettes anvendt i den norske genteknologiloven. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke vedlagt søknaden om omsetting av mat produsert fra MS8xRF3xGT73 eller inneholdende ingredienser produsert fra MS8xRF3xGT73

Vår konklusjon er at norske myndigheter ikke godkjenner bruk av MS8xRF3xGT73 for bruksområdene mat, fór, import og prosessering som det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2009/75

As a designated National Competence Center for Biosafety, our mission at GenØk in advice giving is to provide independent, holistic and useful analysis of technical and scientific information/reasoning in order to assist authorities in the safety evaluation of biotechnologies proposed for use in the public sphere.

The following information is respectfully submitted for consideration in the evaluation of product safety and corresponding impact assessment of MS8xRF3xGT73, setting out the risk of adverse effects on the environment, including other consequences of proposed release under the pertinent Norwegian regulations.

In this joint Application from Bayer and Monsanto, the Applicant is referring to the molecular data presented in H_81 and H_87 that has already been considered (application **EFSA/GMO/BE/2011/81** and **EFSA/GMO/NL/2010/87**).

Specific recommendations

Based on our findings, we propose a few specific recommendations, summarized here and detailed in the critique below.

- The regulator is encouraged to ask the Applicant to demonstrate the lack of interactive effects between transgenic proteins in this stacked event through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

- The regulator is encouraged to ask the Applicant to include long term exposure-/feeding studies in a risk assessment before a GM plant product is released on the marked for food/feed consumption

- The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of this herbicide domestically as a health concern, but support its use in other countries.

- The regulator is encouraged to ask the Applicant to provide clear information on the source of GOXv247.

- The regulator is encouraged to ask the Applicant to provide information on the 2 other amino acid substitutions in GOXv247 as well as its implications on environment and human health.

- The regulator is encouraged to ask the Applicant to consider recent scientific findings, meaning that the Applicant should extend the molecular characterization of the event by examining the possibility of partial expression of P6.

- The regulator is encouraged to ask the Applicant to provide additional data order to evaluate the genetic stability of the event.

- The regulator is encouraged to ask the Applicant to extend the molecular characterization of the event by examining the possibility for different RNA variants and fusion proteins.

- The regulator is encouraged to ask the Applicant to provide detailed metabolomics data showing non effect of the modification on different biosynthetic pathways of the GM plants.

- The regulator is encouraged to ask the Applicant to compare the amino acid sequences of the expressed transgenic proteins between the single and stacked events. Additionally, analyses of potential posttranslational events in the transgenic proteins of stacked events versus single events are recommended. Where differences are identified, the Applicant should relate the implication to environment and human health.

- The regulator is encouraged to ask the Applicant to provide a revised monitoring plan.

- The regulator is encouraged to ask the Applicant to consult relevant peer-reviewed literature on the key subjects of the monitoring plan, notably the established evidence of globally occurring feral populations of GMO oil seed rape and other commercial varieties of *Brassica*. The Applicant should plan for monitoring of such occurrence and present realistic strategies for remediation.

- The regulator is encouraged to ask the Applicant to justify the sustainability of the product by providing information on the length of time (e.g. number of planting seasons) required before the MS8xRF3xGT73 GM plants develop sensitivity to the combined glufosinate-ammonium and glyphosate herbicides.

- The regulator is encouraged to ask the Applicant to submit required information on the social utility of MS8xRF3xGT73 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Overall recommendation

From our analysis, we find that the deficiencies in the dossier do not support claims of safe use, social utility and contribution to sustainable development of **MS8xRF3xGT73**. **Critically, the Applicant has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway.** Hence at minimum, the dossier is deficient in information required under Norwegian law. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of MS8xRF3xGT73, we conclude that based on the available data supplied by the Applicant, the Applicant has not substantiated claims of environmental safety satisfactorily or provide the required information under Norwegian law to warrant approval in Norway at this time.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/NL/2009/75

About the event

The genetically modified MS8xRF3xGT73 oilseed rape was obtained by conventional crossing between three genetically modified oilseed rape events: MS8, RF3 and GT73 oilseed rape. The methods used for genetic modification in the different events are described in application **EFSA/GMO/BE/2011/81** and **EFSA/GMO/NL/2010/87.**

The event MS8 has the ***barnase* gene** that results in lack of viable pollen and male sterility and the ***bar* gene** (from *Streptomyces hygroscopicus*) encoding for a phosphinothricin acetyl transferase (PAT) that confers tolerance to herbicides containing glufosinate-ammonium.
The event RF3 has the ***barstar* gene** that inhibits activity of the Barnase protein and therefor restores fertility in addition to the ***bar* gene**.
The event GT73 contains one intact copy of the ***goxv247*** and ***cp4 epsps*** expression cassettes encoding the GOXv247 and CP4 EPSPS proteins, which confer tolerance to glyphosate.

The Applicant is requesting the authorization for GM plants for food, feed, and import and processing.

Assessment

Stacked events

A stacked organism has to be regarded as a new event, even if no new modifications have been introduced. The gene-cassette combination is new and only minor conclusions could be drawn from the assessment of the parental lines, since unexpected effects (e.g. synergistic effects of the newly introduced proteins) cannot automatically be excluded.

Stacked events are in general more complex and it has been an increased interest in the possible combinatorial and/or synergistic effects that may produce unintended and undesirable changes in the plant – like the potential for up- and down regulation of the plants own genes. Interactions with stacked traits cannot be excluded that the group of expressed toxins in the plant can give specific immunological effects or adjuvant effects in mammals (Halpin 2005, Schrijver et al, 2006).

MS8xRF3xGT73 oilseed rape combines three genetically modified oilseed rape events: MS8, RF3 and GT73 oilseed rape. Robust data are necessary to identify whether the combined presence of these transgenes influences expression levels.

Recommendation: The Applicant should demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering, rather than justify the lack of testing based on assumptions-based reasoning of no effects.

Glyphosate tolerance
The genetically modified MS8xRF3xGT73 oilseed rape expresses a *CP4EPSPS and a Goxv247* gene that confers tolerance to herbicides products containing glyphosate.

In recent years glyphosate has received more risk-related attention due to negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003, Ono et al 2002, Solomon and Thompson 2003), and also because of constantly increasing number of glyphosate herbicide applications since the introduction of this chemicals in 1971 (Dill et al. 2010, Cuhra et al 2012).

Studies in animals and cell cultures indicate possible health effects in rodents, fish and humans. Glyphosate given in the feed to pregnant female rats resulted in higher embryonic mortality and aberrations in the skeleton (Dallegrave et al. 2003). Nile-tilapia (Oreochromis niloticus) fed sublethal concentration of Roundup (active ingredient: glyphosate) resulted in a number of different histopathological changes in organs (Jiraungkoorskul et al. 2003). Experiments with sea urchins exposed to Roundup influenced early cell divisions (Marc et al 2002), effects that have relevance to potential health effects in many eukaryotic organisms, including domestic animals and humans. Exposure to Roundup affected the CDK1/CyclinB regulator which is nearly identical in sea urchins and humans.

Glyphosate has also been shown to negatively affect the differentiation of nerve cells (Axelrad et al. 2003). In human placenta cells, Roundup is more toxic than the active ingredient glyphosate (Richard et al. 2005). The authors concluded that additional components of Roundup increase the biological availability and accumulation in organisms.

In a recently published study by Seralini et al (Seralini et al. 2012) the authors concludes that long term exposure of lower levels of complete agricultural glyphosate herbicide formulations, at concentrations well below officially set safety limits, induce severe hormone-dependent mammary, hepatic and kidney disturbances in rats.

Recommendation: The Applicant should include long term exposure-/feeding studies should in a risk assessment before a GM plant product is released on the marked for food/feed consumption.

Glufosinate-ammonium tolerance
The genetically modified MS8xRF3xGT73 oilseed rape expresses a *bar* gene that confers tolerance to herbicides containing glufosinate-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. Studies have shown that glufosinat ammonium is harmful by inhalation, swallowing and by skin contact and 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 glufosinate ammonium is used (Hung 2007; Matsumura et al. 2001; Schulte-Hermann et al. 2006; Watanabe and Sano 1998). According to EFSA, the use of glufosinate ammonium will lead to exposures that exceed acceptable exposure levels during application.

Recommendation: The Applicant should consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of this herbicide domestically as a health concern, but support its use in other countries.

Information relating to the genetic modification (p.25)
Molecular characterization

The applicant states that "*A detailed and complete description of the genetic modification of the single parental events MS8, RF3 and GT73 oilseed rape has been previously provided in the EFSA-GMO-RX-MS8-RF3 and EFSA-GMO-RX-GT73*." Therefore, the comments about the molecular characterization for the Oilseed Rape MS8XRF3XGT73 are based on the two single parental events (MS8XRF3 and GT73).

Application EFSA-GMO-RX-GT73

- From the information provided by the Applicant it is unclear whether GOXv247 is sourced from the same or different strain of *Ochrobactrum anthropic*, or whether the enzyme is synthetically produced or sourced from a different organism that is not a bacterium (p.61). In addition, the applicant failed to provide information on the implication (or potential implication) of the 3 amino acid substitutions in GOXv247. Further, the report stated that substitution of histidine residue at position 334 with arginine results in enhanced efficiency of glyphosate degradation, but it was silent on the nature of the other two amino acid residue substitutions and the implications.
- The size of some probes used in the Southern Blot analysis is considered too long (CS-*goxv247* probe 1,3kb, CS-*cp4 epsps* probe 1,4kb, T-*E9* probe 0,8kb, OR-*ori V* + CS-*rop* probe 2,7kb and OR-*ori-PBR322* + *aadA* probe 2,8kb). That can lead to false negative results since the strength of the interaction between probe and target is based on the number of bonds that form between the single strand of DNA (probe) and the matching recombinant DNA (target). A long probe that binds perfectly to a short fragment will not bind strongly and might be washed of depending on the stringency of the wash.
- All Southern Blot pictures lack a molecular weight marker and the quality of some of the pictures is not really good. Therefore, it makes the interpretation of the results more difficult.
- Figure 9 (p.53) shows an expected band around 0,4kb, but also an upper band is present. The applicant doesn't explain why the upper band is present in the gel and, since the molecular marker is lacking, it is not possible to predict its size.
- Figure 10 (p.54), both panels A and B, shows only one band present in the GT73 sample. Since probes 2 and 3 have a binding site in the T-*E9* fragment, two other bands should be present in the pictures.
- For the generational studies (p.80), only two generations were used for the Southern Blot analyses. Also, not all the insert was covered, since the P-*FMV* probe was not used.

- For the insert sequence analyses (Monsanto's study conducted by Palmer *et al*, 2003), the electropherograms are not available, therefore is not possible to check the quality of the sequences. Also, the primer used for the PCR reactions are not available.
- The sequencing studies were conducted only with plants from one generation. Since Southern blot analyses for two generation were conduct, and this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.
- Scientists recently reported the overlap between Cauliflower Mosaic Virus (CaMV) 35S promoter regions (P35S) and the viral gene VI. (Podevin and du Jardin, 2012). In the article, the authors state that some P35S variants contain open reading frames that when expressed could lead to "unintended phenotypic changes". In light of the Podevin and du Jardin findings, the viral sequences from the Figwort Mosaic Virus (FMV) present in the promoters of the MS8XRF3XGT73 Oilseed Rape should be examined carefully to exclude possible overlaps with other viral genes.

Recommendation: (1) The Applicant should provide clear information on the source of GOXv247. If GOXv247 is not sourced from Ochrobactrum anthropic, the applicant should give detailed information on the source microoganisms or the industrial process leading to the synthesis of the enzyme. Safety implications of these sources should also be provided in details. (2) The Applicant should provide information on the 2 other amino acid substitutions in GOXv247 as well as its implications on environment and human health. (3) Considering recent scientific findings, the Applicant should extend the molecular characterization of the event by examining the possibility of partial expression of P6. (4) The Applicant should provide additional data using a comprehensive set of smaller probes in order to evaluate the genetic stability of the event; southern blot studies for generational stability should follow the same methodology as the others southern blot analysis (i.e. using the same probes); longer exposure times for Southern Blots are recommended if sample or control bands are not clearly distinguishable; molecular weight marker always be provided; the electropherograms from the sequencing studies should be provided; Generational sequencing studies should have been conducted.

Application EFSA-GMO-RX-MS8-RF3

- A study by Rang *et al*. (2005) revealed the possibility for read-through of the NOS terminator in GTS 40-3-2 soybean resulting in four different RNA variants with the potential to express unknown EPSPS fusion proteins. Since the NOS terminator sequence is present in this Oilseed rape, the possibility for read- through resulting in different RNA variants and potential fusion proteins should be studied carefully.
- The applicant does not show the exactly sizes of the probes used on the Southern Blot analyses. Although the study from De Beuckeleer *et al*. (1995) shows the location of the probes in the figures, it is not possible to determine their sizes.
- Figure 3a (p.76 - De Beuckeleer *et al*., 1995) shows a molecular weight marker in the right side, but it is not possible to determine its size.
- Figure 3b (p.76 - De Beuckeleer *et al*., 1995) has a poor quality and a molecular weight marker is lacking. Therefore, it makes the interpretation of the results more difficult.

- Figure 3c (p.77 - De Beuckeleer *et al*., 1995) shows only one band when the sample was digested with ApaI/NsiI restriction enzymes. According to Figure 1c (p.72 – De Beuckeleer *et al*., 1995) two bands were expected, since there are two binding fragments for the pSSuara probe. Also, the molecular weight marker is lacking in the Figure 3c.
- Figure 4a (p.78 - De Beuckeleer *et al*., 1995) has a molecular weight marker, but is not possible to determine its size. Also, when the sample was digested with HindIII/EcorI and BamHI/HindIII, the lower expected band (around 800bp) seems to be very weak. Therefore, a longer exposure time on the x-ray film is recommendable.
- Figure 4b (p.78 - De Beuckeleer *et al*., 1995) has a poor quality, which makes the interpretation of the results more difficult.
- Figure 3 (p.85 - De Beuckeleer *et al*., 1995) shows the Southern Blot for the stability of the insert trough different generation. The molecular weight marker is not visible and the quality of the picture is really poor.
- For the generational studies, only three generations were used for the Southern Blot analyses. Also, not all the insert was covered, since only the TA29 probe was used.
- For the insert sequence analyses, the electropherograms are not available, therefore is not possible to check the quality of the sequences
- The sequencing studies were conducted only with plants from one generation. Since Southern blot analyses for two generation were conduct, and this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.

Recommendation: The Applicant should extend the molecular characterization of the event by examining the possibility for different RNA variants and fusion proteins.
The Applicant should provide additional data about the probes used on the Southern Blot analyses; southern blot studies for generational stability should follow the same methodology as the others southern blot analysis (i.e. using the same probes); longer exposure times for Southern Blots are recommended if marker, sample or control bands are not clearly distinguishable; the electropherograms from the sequencing studies should be provided; Generational sequencing studies should have been conducted.

Information relating to the GM plant (p.29)

Description of the trait(s) and characteristics which have been introduced or modified (p.35)

The report states that "The GOXv247 protein produced by GT73 effectively inactivates the herbicide and enables growth when GT73 plants are treated with glyphosate" (p.35). However, it does not preclude negative (and potentially harmful) effects of the herbicides on different biosynthetic pathways of the plants. For example, the Applicant states "The CP4 EPSPS protein produced in glyphosate-tolerant plants is functionally identical to endogenous plant EPSPS enzymes, with the exception that CP4 EPSPS naturally displays reduced affinity for glyphosate…"(p.36). Hence the plant's shikimic acid pathway may be disrupted. Similarly, other biosynthetic pathways of the GM plant may be affected by glyphosate. However, the Applicant has not provided information to the contrary. Disrupted pathways can

produce toxins and anti-nutrients which could impact long term viability of the GM plants as well as have implications on environment and human health.

Recommendation: The Applicant should provide detailed metabolomics data showing non effect of the modification on different biosynthetic pathways of the GM plants.

Information relating to the expression of the insert (p. 46)
In section D3 the Applicant stated that combination of the MS8, RF3 and GT73 oilseed rape in MS8xRF3xGT73 oilseed rape did not have an impact on the PAT, CP4 EPSPS and GOX protein expression levels; together with the compositional analysis described in Section D.7.1, the Applicant concluded that " no further testing of the whole GM food/feed is considered necessary" (Page 17, Section 7.8.4, Part II - Summary) . Comparative protein expression level alone is not sufficient to conclude that there is no impact on PAT, CP4 EPSPS and GOX by the stack events of MS8, RF3 and GT73. It is important to analyze for protein modifications including substitutions in amino acid residues in view of the fact that it is not unreasonable to expect that the stacked events would exert some pressure in the transcriptional and translational machineries of the plant that can result in protein modifications. Such modifications cannot be detected by comparative protein expression alone.

Recommendation: The Applicant should compare the amino acid sequences of the expressed transgenic proteins between the single and stacked events. Additionally, analyses of potential posttranslational events in the transgenic proteins of stacked events versus single events are recommended. Where differences are identified, the Applicant should relate the implication to environment and human health.

Toxicity and allergenicity (p.53)
The Applicant is asked to give: "Information on any toxic, allergenic or other harmful effects on human or animal health arising from the GM food/feed". The Applicant states that MS8xRF3xGT73 oilseed rape is safe as it has been demonstrated that it is composionally and nutritionally equivalent to grain from commercial available oilseed rape grain. Data supporting these statements has not been presented so this claim is unsubstantiated by experimental evidence. In a similar way, the conclusion presented by the Applicant in section 7.10 p.107 regarding potential adverse effects on human health, is not based on scientific evidence. The safety assessment conducted by the Applicant only includes simple animal feeding studies on nutritional quality, with no histological examinations of relevant organs and tissue.

Moreover, the Applicant does not give any information on potential effects on health and environment from the co-technology used MS8xRF3xGT73 oilseed rape: glufosinate and glyphosate. Such potential effects of glyphosate use are not only restricted to the environment and ecosystem where the glyphosate-tolerant varieties are be grown, but also has the potential to affect nutrient composition of the crops (Zobiole et al., 2010; 2011) as well as inducing

substantial levels of pesticide residues and metabolites of pesticides in seed (Duke et al., 2003) thus influencing the quality of the produce.

Recommendation: The Applicant should provide sufficient data supporting their statement that "MS8xRF3xGT73 oilseed rape is safe as it has been demonstrated that it is compositionally and nutritionally equivalent to grain from commercial available oilseed rape grain".

Risk assessment evaluation and monitoring plan of food and feed derived from GM plants (p114)

Accidental spillage and persistence of MS8xRF3xGT73 oilseed rape

The application covers the use as food and feed, as well as import and processing of MS8xRF3xGT73 oilseed rape. Because the size and shape of the oilseed rape seeds, accidental spillages of herbicide tolerant oilseed rape MS8xRF3xGT73, and consequent introduction establishment and persistence of seeds into the wild and cultivated land, can be considered a major risk not only for the existing European cultivation of non-GM varieties but also for the potential increase of herbicides use.

In section 8.d of the application, it is stated that "*no mandatory restrictions for use, storage and handling are proposed as a condition of the authorisation. All standard practices applicable to oilseed rape today remain adequate for the handling of MS8xRF3xGT73 oilseed rape*". However, several studies have shown the magnitude of systematic and routinely spillage along roadsides, on field margins and in other affected habitats during transport, handling and distribution, as material is typically conducted with semi-open systems. Therefore, accidental spillage should be considered as highly likely. Several studies have documented the importance of oilseed rape spillage from grain trailers along roadsides (Crawley and Brown, 1995; 2004; Knispel et al., 2008; Pivard et al., 2008; Schafer et al., 2011), railways (Schoenenberger and D'Andrea, 2012), ports and riverbanks (Saji et al., 2005), even when GM oilseed rape was not approved for import or cultivation in the country, i.e. Switzerland and Japan. More specifically, a recent study by Bailleul et al. (2012) calculated that the number of seed lost from grain tailers along the road verges represented a mean of 404±94 seeds/m^2.

Regarding the significance of the establishment and persistence of feral oilseed rape, in section II of the application (section 11.4 on general surveillance of the impact of the GM plant), it is stated that "*exposure to the environment will be limited to unintended release of MS8xRF3xGT73 oilseed rape, which could occur for example via substantial losses during loading/unloading of the viable commodity including MS8xRF3xGT73 oilseed rape destined for processing into animal feed or human food products. However, such exposure is highly unlikely to give rise to an adverse effect and can be easily controlled by clean up measures and the application of current practices used for the control of any adventitious oilseed rape plants, such as manual or mechanical removal and the application of herbicides (with the exception of glufosinate-ammonium and glyphosate herbicides). Furthermore, unintended*

effect to the unintended release of MS8xRF3xGT73 oilseed rape will be no different than that of other commercial oilseed rape".

In despite of this statement, there is evidence that feral oilseed rape populations can persist many years in self-sustaining populations, even in non-cultivated areas such as communication ways and urban areas such as waste ground and industrial sites (Squire et al., 2013). In that sense, feral oilseed rape has shown a widespread capacity to persist and retain traits from varieties no longer grown (for a period of more than 7-10 years), which supposes an additional challenge for monitoring and redress in case it was needed (Andersen et al., 2010; Pessel et al., 2001). This capacity also provides opportunities for genetic recombinations and staking of transgenes, through the reproduction of feral populations and their establishment and persistence, even in non-crop environments (Knispel et al., 2008; Schafer et al., 2011).

Monitoring plan

The Applicant has submitted a supplementary document entitled "Monitoring plan for MS8x RF3x GT73 oilseed rape conforming with annex VII to Directive 2001/18/EC". We see that this monitoring plan highlights several important issues to be further addressed, such as the documented invasiveness of feral populations of *Brassica* and the persistence of such established volunteers in various cultivated environments and elsewhere.

However, the Applicant does not provide a thorough plan for monitoring, as requested and expected in the definitions of directive 2001/18/EC and other relevant guidelines. The plan presented by the Applicant can rather be interpreted as an formal disclaimer of responsibility, by defining the role of third-parties such as importers/traders, silo operators and processors and thus de-jure transferring responsibility to these third-parties. By defining these third-parties as responsible of monitoring and reporting adverse effects as defined by the Applicant, and additionally by defining the geographical area of responsibility as being limited to the harbor facilities at EU import hubs and the processing facilities (mills), the Applicant demonstrates avoidance of the main issue of concern, which should be correctly identified, described and addressed as the documented risk of spillage during transport and handling, additionally enhanced by biotic and abiotic vectors for further dispersals, such as avifauna and wind. This issue is substantially documented in the scientific evidence which we have referred in the previous chapter(s) and should not be ignored by the Applicant.

Furthermore the statements provided by the applicant section 4.5 of the monitoring plan can be seen as remarkable. The Applicant states that review of relevant scientific literature on issues encompassed by the monitoring plan has been given priority; *"The Applicant will actively screen peer-reviewed publications relevant..."* (MP section 4.5, page 4; "Additional sources of information"). The Applicant even mentions the contributions of independent researchers as valuable source of information, but this is evidently not considered nor incorporated with neither necessary consequence nor stamina. We expect the Applicant to fulfill the requirements of European Commission directive 2001/18/EC and other formal framework defining the intended purpose and scope of the monitoring plan.

Coexistence

Moreover, as the intended use of MS8xRF3xGT73 oilseed rape is that of any other commercial oilseed rape, coexistence measures (which have to be applied from "farm to fork") need to be implemented in order to prevent the unwanted admixture of MS8xRF3xGT73 with conventional and organic oilseed rape. This is especially relevant in this case, as logistic means for handling and transportation are not isolated, and the Applicant presents no requirements for cleaning and separation of GMO biomass from conventional varieties. The issue is enhanced by the specific biotic factors characteristic of *Brassica*, such as small seed size, high persistence and documented fitness of GMO varieties such as the herbicide-tolerant variety in question.

Recommendation: The Applicant should provide a revised monitoring plan. The Applicant should consult relevant peer-reviewed literature on the key subjects of the monitoring plan, notably the established evidence of globally occurring feral populations of GMO oil seed rape and other commercial varieties of *Brassica*. The Applicant should plan for monitoring of such occurrence and present realistic strategies for remediation.

Missing information in relation to requirements under the Norwegian Gene Technology Act

Social utility and sustainability aspects

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act. In accordance with the aim of the Norwegian Gene Technology Act, production and use of the GMO shall take place in an ethically and socially justifiable way, under the principle of 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*".

These issues are further detailed in the regulation on consequence assessment section 17 and its annex 4. The Applicant has not provided relevant information that allows an evaluation of the issues laid down in the aim of the Act, regarding ethical values, social justification of the GMO within a sustainable development. Given this lack of necessary information for such an evaluation, the Applicant has not demonstrated a benefit to the community and a contribution to sustainable development from the use of MS8xRF3xGT73. In fact, there are important doubts regarding the sustainability of the product as the potential transfer of herbicide tolerant genes to wild relatives might create weed problems and thereby increase herbicide use (other than glufosinate-ammonium and glyphosate-based, as recognized by applicant in section 11.4). Further, the principal difference between event MS8xRF3xGT73 under consideration and the approved event MS8xRF3 is additional tolerance to glyphosate-containing herbicides by MS8xRF3xGT73. This indicates that MS8xRF3 GM plants are no longer tolerant (or have reduced tolerance) to glufosinate-ammonium necessitating an additional event, GT73, to boost tolerance to an additional herbicide. There is no guarantee that the new MS8xRF3xGT73 modified plants for which the Applicant seeks approval will not soon develop sensitivity to the combined glufosinate-ammonium and glyphosate herbicides. The

Applicant has not provided information on how long (e.g. number of planting seasons) it will take before the MS8xRF3xGT73 containing plants develop sensitivity to the combined glufosinate-ammonium and glyphosate herbicides. Therefore, it would be incongruent with the principle of sustainable development (see section about ***Risk assessment evaluation and monitoring plan***). The Applicant should thereby provide the necessary data in order to conduct a thorough assessment on these issues.

It is also important to evaluate whether alternative options (e.g. the parental non-GM version of this MS8xRF3xGT73) may achieve the same outcomes in a safer and ethically justified way. Further, the Norwegian Gene Technology Act, with its clauses on societal utility and sustainable development, comes into play with a view also to health and environmental effects in other countries, such as where GMOs are grown. For instance, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, and genetic 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. Hence it cannot be expected that the same effects will apply between different environments and across continents.

Recommendation: The applicant should submit required information on the social utility of MS8xRF3xGT73 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.

Ethical considerations

The evaluation of co- products, that is, secondary products that are specifically designed and intended to be used in conjunction with the GMO, is considered important in the risk assessment of a GMO (Dolezel et al, 2009; Graef et al., 2012). Therefore, considerations of the co-products also warrant an evaluation of safe use.

The events RF3 and MS8XRF3 contain the bar gene (from *Streptomyces hygroscopicus*) encoding for a phosphinothricin acetyl transferase (PAT) that confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway. While it is understood that the Applicant has not applied for deliberate release of MS8xRF3xGT73 in Norway, the acceptance of a product in which the intended use includes the use of a product banned in Norway would violate basic ethical and social utility criteria, as laid out in the Act. That is, we find that it would be ethically incongruous to support a double standard of safety for Norway on one hand, and safety for countries from which Norway may import its food and feed on the other. This line of reasoning is consistent with the provisions under the Act to assess ethical, social utility and sustainable development criteria not only for Norway, but for countries from which Norway imports food and feed.

Therefore, we find it difficult to arrive at justified use of this event without engaging in such an ethical double standard. Specifically, this issue is relevant particularly in revised regulations of 2005 Section 17 “Other consequences of the production and use of genetically modified organisms” points 2 and 3 “ethical considerations that may arise in connection with the use of the genetically modified organism(s), and “any favorable or unfavorable social consequences that may arise from the use of the genetically modified organism(s)”, respectively.

Conclusion

Available information for risk assessment evaluation

This evaluation is based on the Applicant's own submitted information, along with our own expertise in related fields. The relevant scientific literature provided in the application is very limited in some cases, yet we have tried to extract information from the peer-reviewed literature that may inform the scientific validity of the information under consideration. In situations where lack of knowledge, complexity and uncertainty are high, particularly in relation to unknown adverse effects that may arise as a result of approval for release of a living modified organism into the environment or food supply, the available information may not be sufficient to warrant approval. Further information may address some of these issues, however an accurate description of uncertainties provided by the applicant would provide a more useful basis for assessing the level of risk that may come with regulatory approval of the GMO, taken on a case-by-case basis.

In all cases, product-related safety testing should have an independent and unbiased character. This goes both for the production of data for risk assessment, and for the evaluation of the data.

The lack of compelling or complete scientific information to support the claims of the Applicant documented here highlights the need for independent evaluation of the dossier as performed here, including the raw data produced by the Applicant. We therefore support better transparency and independent review of information to ensure high standards within the regulatory process. This would include any information provided by the Applicant used to justify confidentiality claims on any scientific data. We encourage the authorities to insist on this level of transparency and accessibility to all scientific data (including raw data) to ensure the scientific validity of the information presented.

Overall recommendation

Above we highlight a number of issues in relation to the questionable safe use of MS8xRF3xGT73 that do not justify a conclusion of safe use, social utility and contribution to sustainable development. Critically, the Applicant's environmental monitoring plan lacks sufficient details and descriptions to support the required monitoring activities, and has not included any of the required information to assess social utility and sustainability as required in Appendix 4 of the Norwegian Gene Technology Act, which would be necessary for consideration of approval in Norway. Taken together, these deficiencies fail to address the necessary safety regulations under Norwegian Law, and thus the application is incomplete and should not be approved. A new application or reapplication should only be reconsidered with the delivery of the information requests recommended here, including any additional information deemed significant by the Norwegian authorities.

Therefore, in our assessment of MS8xRF3xGT73 we conclude that based on the available data, the Applicant has not substantiated claims of safety satisfactorily to warrant approval in Norway at this time.

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