Vår ref:2016/H_130
Deres ref: 2016/4313

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 22.06.16

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på høringen av søknad **EFSA/GMO/DE/2016/130**, genmodifisert mais **VCO-Ø1981-5**, fra Genective 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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Vår ref:2016/H_130
Deres ref: 2016/4313

Assessment of the technical dossier submitted under EFSA/GMO/DE/2016/130 for approval of VCO-Ø1981-5 maize

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
June 2016

ANBEFALING

Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående høring som omfatter **VCO-Ø1981-5 mais** for bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **VCO-Ø1981-5 mais**.

Vi trekker frem mangler i dossieret som ikke gir grunnlag for en anbefaling om sikker bruk, samfunnsnytte og bidrag til bærekraftighet av **VCO-Ø1981-5** mais. Søker har ikke inkludert noe av den informasjonen omkring samfunnsnytte og bærekraftighet til **VCO-Ø1981-5 mais** som kreves i den norske genteknologiloven (NGTA) (Appendix 4) for godkjenning i Norge. I denne sammenheng er det viktig å få dokumentert erfaringer med hensyn på effekter på miljø, helse og samfunnsaspekter. Denne type dokumentasjon er ikke tilstrekkelig i søknaden om omsetting av **VCO-Ø1981-5 mais** til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **VCO-Ø1981-5 mais.**

Vår anbefaling er at norske myndigheter ikke godkjenner bruk av **VCO-Ø1981-5 mais** til import og prosessering og til bruk i fòr og mat som det søkes om.

SUMMARY OF THE ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/DE/2016/130

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 event **VCO-Ø1981-5 maize**, setting out the risk of adverse effects on the environment and health, including other consequences of proposed release under the pertinent Norwegian regulations.

Specific recommendations

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

- Maize event VCO-Ø1981-5 should be checked for level of accumulation (level of herbicide residues) of glyphosate prior to use in food and feed
- The Applicant should include high quality data to prove size and copy number of all detectable inserts.
- The Applicant should provide additional data using a comprehensive set of smaller probes in the Southern blot studies in order to evaluate the genetic stability of the event; longer exposure times for Southern Blots are recommended if indicated sample or control bands are not clearly distinguishable;
- The Applicant should include one more diet group (maize sprayed with glyphosate) in the 90-day feeding experiment.
- An untreated conventional maize should be used as a control to make a negative control from plant derived tissue.
- To get proper comparisons between field trials, the so called "conventional maize herbicide control" treatment should be the same in all trials to avoid potential for deviations and make real replicates .
- It is recommended that the Applicant use plant derived proteins in studies performed and in the development of antibodies for analysis of plant derived proteins.
- It is recommended that the ORFs leading to detected potential allergens should be analyzed further.
- The regulator is encouraged to ask the Applicant to provide data for the assumption that processed products from VCO-Ø1981-5 maize, here understood as modified form of EPSPS, is no different from other EPSPS.
- The Applicant should specify whether the antibodies raised for detection of EPSPS ACE5 in plant tissues were made by original exposure to the bacterial or plant version of the protein. This is not clear from the methodology part described in the Application.
- The Applicant should use proper size markers on western blots and re-do membranes when markers are too weak or invisible.
- The regulator should encourage the Applicant to explain better why there is a difference in enzymatic activity when the same substrate was used during analysis of activity of bacterially produced and plant derived EPSPS ACE5 enzyme.
- The Applicant should better explain whether it was the plant or the bacterial version of the EPSPS ACE5 protein that was used during analysis of heat stability.
- It is recommended to perform proteolytic protein stability assays over a broader pH range to better mimic the situation in the gastric system.
- The regulator is encouraged to ask the Applicant to include an additional group of animals in the feeding study to analyze already sprayed maize for comparison with the unsprayed one.
- We recommend the Applicant to perform serum studies of the EPSPS ACE 5 protein.
- 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 social utility of the

VCO-Ø1981-5 maize and its contribution to sustainable development. The information provided by the Applicant must be relevant for the agricultural context in the producing country/countries. The information should include issues such as: changes in herbicide use and herbicide resistance in weed populations, co-existence consequences and possible impacts among poor and/or small-scale farmers in producing countries and share of the benefits among sectors of the society.

Overall recommendation

In our assessment of maize event VCO-Ø1981-5, we find that the information provided in the summary of the technical dossier does not provide enough data to support claims of safe use, social utility and sustainable development.

We therefore comment that the Applicant has not provided the information required under Norwegian law to warrant approval in Norway at this time.

Especially, the Applicant has not included information which is required to assess social utility and sustainability as required by the Norwegian Gene Technology Act (Appendix 4) for consideration of approval in Norway.

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.

ASSESSMENT OF THE TECHNICAL DOSSIER RELATED TO EFSA/GMO/DE/2016/130

About the event

The **VCO-Ø1981-5** maize is a GM maize that is produced by *Agrobacterium tumefaciens* mediated transformation.

This maize event contains a modified (also called synthetic) *EPSPS* gene from the bacterium *Arthrobacter globiformis* called *epsps grg23ace5* (original gene is called *epsps grg23*). This gene encodes an EPSPS protein called EPSPS ACE5, which confers tolerance to glyphosate containing herbicides. This modified form of EPSPS tolerate to higher temperatures and glyphosate containing herbicides than the unmodified form of the protein.

One must expect that this single event with a modified form of EPSPS will be used in stacks in the future to improve glyphosate tolerance.

Assessment findings

Herbicides

Herbicides as co-products

Herbicide tolerant (HT) plants are specifically designed to be used in combination with herbicides, and will always be sprayed with the intended herbicide. Without spraying, the introduction of HT plants would be useless. Surprisingly, these herbicides are often not tested as part of the assessment and risk evaluation of HT plants. In feeding studies with HT GM plants for quality assessment the herbicide is systematically overlooked, which represents a serious flaw in the testing and risk evaluation. Viljoen et al. (2013) found that in 13 out of 16 published feeding studies with HT GM crops the plant material used had not been sprayed with the intended co-technology herbicide. There is also a gap in knowledge regarding herbicide accumulation and residues, including metabolic pathways and metabolites thereof. Bøhn et al. (2014) documented high levels of glyphosate residues in HT GM soybeans grown in the USA, and the same research group have published papers showing that such residues negatively affect the feed quality of HT GM soybeans (Cuhra et al., 2015). Moreover, safety testing (in relation to health and environmental issues) has focused on the active ingredient in the co-technology herbicides, and not the commercial formulations actually used, providing unrealistic and possibly misleading results (Mesnage et al., 2014). Stacked HT GM plants are tolerant to one or more agrochemicals, allowing for combinatory and alternating use of several herbicides. Tolerance to multiple herbicides is also often combined with multiple other proteins (like Cry toxins) that could have additive or even synergistic effects on non-target species and the environment.

In the toxicology assessment of a gene modified plant used for food or feed the focus is mostly on the resulting protein from the inserted gene, and the potential of herbicide exposure through consumption of herbicide treated maize is not considered. A recent study found that glyphosate and AMPA, constituents of the herbicide Roundup accumulated in soybeans

(Bøhn et al., 2014), highlighting the importance of including the herbicides in the comparative and toxicological assessment of GM crops with herbicidal co-technology.

Glyphosate tolerance
The *epsps grg23ace5* gene present in VCO-Ø1981-5 maize confers tolerance to herbicide products containing glyphosate.

Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS), necessary for production of important amino acids. Some microorganisms have a version of EPSPS that is resistant to glyphosate inhibition.

Glyphosate has been announced as an ideal herbicide with low toxicity for operators, consumers and the environment surrounding agriculture fields (Duke and Powles 2008, Giesy et al 2000). However, it has received more risk-related attention due to its potential for negative effects on both aquatic and terrestrial ecosystems (Blackburn and Boutin 2003,Solomon and Thompson 2003), as well as in studies in animals and cell cultures that have indicated possible negative health effects in rodents, fish and humans (Axelrad et al 2003, Dallegrave et al 2003, Benachour et al 2007).

Recent studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (Benbrook 2009). Large proportions of the GM agricultural system is crops that is tolerant to glyphosate (GT-cultivars) (James 2010).

A restricted number of recent publications indicate unwanted effects of glyphosate on health (Dallegrave et al 2003, Malatesta et al 2002), aquatic (Solomon and Thompson 2003) and terrestric (Ono et al 2002, Blackburn and Boutin 2003) organisms and ecosystems.
A study of Roundup effects on the first cell divisions of sea urchins (Marc et al 2002) is of particular interest to human health. The experiments demonstrated cell division dysfunctions at the level of CDK1/Cyclin B activation. Considering the universality among species of the CDK1/Cyclin B cell regulator, these results question the safety of glyphosate and Roundup on human health. In another study (Axelrad et al 2003) it was demonstrated a negative effect of glyphosate, as well as a number of other organophosphate pesticides, on nerve-cell differentiation. Surprisingly, in human placental cells, Roundup is always more toxic than its active ingredient. The effects of glyphosate and Roundup were tested at lower non-toxic concentrations on aromatase, the enzyme responsible for estrogen synthesis (Richard et al, 2005). The glyphosate-based herbicide disrupts aromatase activity and mRNA levels and interacts with the active site of the purified enzyme, but the effects of glyphosate are facilitated by the Roundup formulation. The authors conclude that endocrine and toxic effects of Roundup, not just glyphosate, can be observed in mammals. They suggest that the presence of Roundup adjuvants enhances glyphosate bioavailability and/or bioaccumulation.

Additionally, the International Agency for Research on Cancer (IARC) recently released a report concluding that glyphosate is "probably carcinogenic to humans" (Fritschi et al., 2015).

Recommendation:

- Maize event VCO-Ø1981-5 should be checked for level of accumulation (level of heribicide residues) of glyphosate prior to use in food and feed.

Information relating to the genetic modification (p.32 in Application)

1.2. Molecular characterization

1.2.1 .Information relating to the genetic modification.

To test for the numbers of all detectable inserts, both complete and partial, the Applicant used Southern blot analysis. In general, the southern membranes provided in the dossier lack labeled markers and some seem pasted in from another source (example on p.69 with different backgrounds between samples and size markers)

Further, Southern blot analysis was also conducted to verify the absence of the plasmid backbone components. Here, the probes used in these studies were ranging in size from 280-2001 bp (listed in Table 7, p.50). Figure 22 on .p 62 gives an overview of the probes and Table 10 p. 67, which shows a list of long probes and figures without a molecular weight ladder.

Southern blot analyses in Figure 21 indicates the integrity of the expression cassette and copy number using a full T-DNA probe. The predicted size for the hybridization products are difficult to see (lane 5). It is recommended that gels are run for a longer time to make the bands clearly distinguishable. A properly labeled size marker should also be included in the run.

The southern blot analyses in Figure 26 indicates the absence of the plasmid backbone components in the VCO-Ø1981-5 maize genome. However, some of the probes (BP3, BP29, BP11 and BP20) give weak signals in the plasmid vector control and the predicted band in BP 28 cannot be seen. Longer exposure times are recommended to make the bands clearly visible. Washing that is more stringent is also recommended to make less background noise.

The southern blot analyses in Figure 39-41 is supposed to show genotypic stability from five consecutive generations. Figure 40, lower part, give no visible signals for several plant samples. The quality of this blot can be better; longer exposure times and more stringent washing to remove background is suggested.

In general, we have the following comments:

- Lack of ladders: Fig. 17,18,19 and 21, p. 54-60 in the dossier

- Lack of ladders: Fig. 23, Fig 24 and Fig. 26 p. 68-70
- Lane in Figure 18, lane 1 is very weak without explaining probable reason.
- Figure 26: bad quality of membrane with background noise.
- Figures and tables should be improved on heading and labelling.

The use of long probes to detect recombinant DNA can lead to false negative results. The strength of the interaction between probe and target is based on the number of bonds that form between the single strand of DNA that is the probe and the matching recombinant DNA that is the target. A long probe that binds perfectly to a short insertion will not be strongly bound and may be washed off depending on the stringency of the wash. Probes that are > 500bp means that point mutations, small deletions and rearrangements that might occur will possible not be detected (Fagard andVauvheret 2000, de Schrijver et al 2007).

Recommendation:

- The Applicant should include high quality data to prove size and copy number of all detectable inserts.
- The Applicant should provide additional data using a comprehensive set of smaller probes in the Southern blot studies in order to evaluate the genetic stability of the event; longer exposure times for Southern Blots are recommended if indicated sample or control bands are not clearly distinguishable;
- The Applicant should include one more diet group (maize sprayed with glyphosate) in the 90-day feeding experiment.

1.2.2. Information relating to the genetically modified plant (p.42 in Application)

1.2.2.3 Information on the expression of the inserted/modified sequences (p.86)

Expression analysis of the EPSPS ACE5 protein was conducted after field trials with VCO-Ø1981-5 maize using ELISA (Enzyme-linked immune-sorbent assay) from Envirologix (Quantiplate EPSPS ACE5 ELISA) on various tissues (leaf, pollen, root and grain). Plants were treated with a “conventional maize herbicide weed control” or “glyphosate” treatment prior to protein isolation and analysis of expression.

Comments:

- There is no mention of untreated conventional maize used as an negative control in addition.
- The “conventional maize herbicide control” treatment varied between countries conducting the field trial.
- The reference protein EPSPS ACE5 used during analysis was a microbial version produced in *E.coli*.
- Antibodies used for detection of the EPSPS ACE5 protein in the Envirologix kit is probably developed using the bacterial version of the protein (not specified in the Application).

The highest expression of EPSPS ACE5 was found in leaf during the vegetative stages and there was no difference between the conventionally treated vs glyphosate treated VCO-Ø1981-5 maize samples.

The so called "FAO/WHO decision tree" which include identical peptide matching (mentioned in FAO report 2001 on Evaluation of Allergenicity of Genetically modified Foods) is thought to also be applicable to ORFs (EFSA Journal 2010). This method identifies if an ORF has identical aa (7-8) with any allergen,and then must be considered for further analysis.

Presence of ORFs from VCO-Ø1981-5 maize were detected. A total number of 12+234 (T-DNA junctions and inserts) were found (p.84 of dossier). Of these, two had sequence similarities to known allergens. It is not clear from this part of the Application, what kind, if any; further analysis of allergenicity is performed.

Recommendation:

- An untreated conventional maize should be used as a control to make a negative control from plant derived tissue.
- To get proper comparisons between field trials, the so called "conventional maize herbicide control" treatment should be the same in all trials to avoid potential for deviations and make real replicates .
- It is recommended that the Applicant use plant derived proteins in studies performed and in the development of antibodies for analysis of plant derived proteins.
- It is recommended that the ORFs leading to detected potential allergens should be analyzed further.

1.3.6 Effects of processing (p.158)

According to the Applicant, the assumption is that there are no indications that processed products from VCO-Ø1981-5 maize will be different from products from any other conventional processed maize.
The Applicant has however not provided data for this claim. We recommend that the Applicant provide data that support this claim.

Recommendation:

- The regulator is encouraged to ask the Applicant to provide data for the assumption that processed products from VCO-Ø1981-5 maize, here understood as the modified form of EPSPS, is no different from other EPSPS.

1.4. Toxicological assessment

Accordingly, the Codex (2003) assessment is utilized for evaluation of toxicity. Here the "weight of evidence" approach is used. These evidences include:

- Characterization of the newly expressed protein
- Bioinformatics sequence analysis to search for similarities to toxins, anti-nutrients, allergens, etc.
- Stability of protein *in vitro*

- Toxicity studies (acute oral in this case)

Based on the long history of safe use of EPSPS proteins, and analysis performed on sequence homology, biochemical characterization, stability during temperature and simulated gastric/intestinal fluid treatment, the Applicant consider the protein as non –toxic.

1.4.1 Testing of newly expressed proteins

The EPSPS ACE5 protein from maize leaves were tested for molecular weight, peptide mass identity, immunoreactivity, glycosylation profile, sequence and biochemical reactivity as compared to the bacterial version of EPSPS ACE5 isolated from *E.coli* (Table 40, p.159 in the Application).
The proteins were found equivalent according to the performed tests. No glycosylation was detected for either of the two proteins analyzed with the chosen method.

However, it is not clear if the antibody used during western blot analysis was raised against the bacterial or the plant version of the EPSPS ACE5 protein. Plant and bacterial proteins can potentially have distinct epitopes and thus give variable reactions to the protein (as glycosylation patterns etc.).

In addition, the image of the blotted gel is not good (Figure 60, p.163). The "Novex Sharp Prestained Protein Marker" seems to be drawn on the membrane and not blotted onto it. This gel should be re-done with proper size markers.

The activity of the purified EPSPS ACE5 enzymes from maize and bacteria were not comparable upon presence of the substrate used. The activity of the plant-derived enzyme was 1.35x higher than the bacterially derived enzyme when analyzed under the same test conditions. The Applicant states that the activity is not comparable in this kind of assay (see Table 43, p.166 for details).

The EPSPS ACE5 protein was also subjected to processing and storage conditions and tested for stability. This was done through a heat stability test (invalidated method according to Applicant). The protein was tested at different temperatures for 25 minute each. The protein had up to 100% activity at 37 °C, which dropped to 35-40% at 55°C. Rising the temperature to 75°C resulted in an enzymatic activity that was below level of detection. Data support the issue of degraded protein after heat treatment, and seemingly the process of heating/processing.

It is not clear if it is the bacterial or the plant version of the protein that is used for this testing.

In addition, a quantitative activity assay was performed. This time on a bacterial version of the EPSPS ACE5 enzyme.

A standard pepsin digestion method was used to check the bacterial version of EPSPS ACE5 for resistance to proteolytic degradation. This was done at one pH (1.2) and to simulate human gastric proteolytic degradation of proteins.
An activity assay at pH 7.5 with pancreatin was also performed where the protein also was rapidly degraded.

The pH in the human digestive tract varies greatly. It ranges from 1.5 to 8.5 depending on how long time it was since food was eaten, disease state, where in the stomach the measure is made and several other issues. This can indicate that a proteolytic degradation assay should be performed over a pH range to look at stability of proteins over pH range, and also over time.

Test protein was rapidly degraded in the simulated gastric and intestinal fluid activity tests used. This test is also a test used in the process of evaluating potential for allergenicity of proteins. Based on this test the Applicant claim that it is unlikely that the protein will cause any harm.

A 14 day single dose acute oral toxicity study was conducted in mice with microbially produced EPSPS ACE5 protein. We do not have experience enough to evaluate if 14 days and one single exposure is enough to see any change during gross necropsy.

Recommendation:

- The Applicant should specify whether the antibodies raised for detection of EPSPS ACE5 in plant tissues were made by original exposure to the bacterial or plant version of the protein. This is not clear from the methodology part described in the Application.
- The Applicant should use proper size markers on western blots and re-do membranes when markers are too weak or invisible.
- The regulator should encourage the Applicant to explain better, why there is a difference in enzymatic activity when the same substrate was used during analysis of activity of bacterially produced and plant derived EPSPS ACE5 enzyme.
- The Applicant should better explain whether it was the plant or the bacterial version of the EPSPS ACE5 protein that was used during analysis of heat stability.
- It is recommended to perform proteolytic protein stability assays over a broader pH range to better mimic the situation in the gastric system.

1.4.4. Testing of the whole genetically modified food and feed

1.4.4.1 90-day feeding study in rodents with whole genetically modified food/feed (p.178 in the dossier)

A 90-day feeding study in rodents was performed to determine the potential toxic effect of VCO-Ø1981-5 maize. Since this maize variety is modified to tolerate higher amount of glyphosate an additional diet group with already sprayed maize should be included.

Recommendation:

- The regulator is encouraged to ask the Applicant to include an additional group of animals in the feeding study to analyze already sprayed maize for comparison with the unsprayed one.

1.5 Allergenicity assessment

Amino acid similarity between newly introduced proteins and known allergens/toxins is one of the criteria that used for protein safety assessment and is from Codex Alimentarius (2003). This is done through an evaluation after *in silico* (computer) analysis of the protein(s). The protein(s) were analysed

No hits using 80 AA sliding window or 8-mer exact match were found, meaning that there were no similarity to known toxins or allergens detected with the analysis performed.

Serum screening of the protein was not found relevant according to the Applicant as very low levels of protein was expressed in all parts of plant. Therefore, the Applicant did not consider further allergenicity studies, also because no similarity to known allergen was revealed through the previous analysis in this study.
However, it must be emphasized that even small amounts of proteins can pose allergenic reactions. In addition, that the amount of protein alone is not enough to conclude that a protein is allergenic or not (Breiteneder and Mills, 2005). Thus, we encourage the Applicant to perform serum-screening studies using relevant sera.

The microbially derived EPSPS ACE5 protein was rapidly degraded in simulated gastric fluid with pepsin at pH 1.2 (Figure 64, p.190) already after 30 seconds. The protein digestion was not checked at other pH values. See section 1.4.1 for further elaboration on this issue.

The protein was also tested for stability in pancreatin at pH 7.5 in human simulated intestinal fluid (SIF). After 5 min at 37°C the protein band was very weak (Figure 67, p.195). One must rely on the Applicant according to what size is present on the visible bands, as the size marker of the presented western blot is absent.

Recommendation:

- We recommend the Applicant to perform serum studies of the EPSPS ACE 5 protein.

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 (NGTA). In accordance with the aim of the NGTA, 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 elaborated in the regulations relating to impact assessment pursuant to the NGTA, section 17 and its annex 4.

Sustainability, environmental and biodiversity friendly development, is an important aspect of future national and global agriculture strategies in order to meet the need for food with an increasing world population. The worst type of agricultural production systems, e.g. as seen in parts of the green revolution after the second World war until today, has to change. Examples of these types of production systems are many, e.g. the intensive use of pesticides like DDT, dioxin and other very problematic pesticides. It should be expected that reports like; "late

lessons from early warnings" (EEA Report No 1/2013, and Environmental issue report No 22/2001), the International Assessment of Agriculture Knowledge, Science and Technology for Development (IAASTD), would guide governments, authorities and decision takers in directions that are more environmental friendly, healthy and sustainable. Pesticide tolerant GM plants like the VCO-Ø1981-5 maize, is utilizing strategies that clearly are not sustainable, and which is an inheritance of the worst-case practice from the green revolution (Jacobsen et al 2013). In order for countries and the world to reach the UN Sustainable development goals[1] and the Aichi biodiversity targets[2], where Norwegian Governments have endorsed both goals and targets, it is a huge need to change agricultural practices towards an environmental and biodiversity friendly practice in the future in order to reach these goals and targets.

In the following, we identify issues that are relevant to consider when assessing social utility and contribution to sustainable development of the VCO-Ø1981-5 maize and highlight knowledge gaps and areas that need further investigation.

Impacts in producer countries

The NGTA, with its clauses on societal utility and sustainable development, comes into play with a view to health, environmental and socio-economic effects in other countries, such as where the GMOs are grown.

Social impacts relevant for sustainability

Published reviews on social impacts from cultivating GM crops relevant to assess sustainability (e.g. impacts among poor and/or small-scale farmers in developing countries, share of the benefits among sectors of the society) indicate that these impacts are very complex, mixed and dependent on the agronomic, socio-economic and institutional settings where the technology is introduced (Glover, 2010, Fisher et al. 2015).

Additionally, regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all factors that are likely to affect the performance of GM plants and their potential impacts. Therefore, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, genetic and socio-economic contexts.

A literature review of empirical studies (published between 2004- 2015) on social implications from cultivating GM crops, reveals that in this period only15 studies on social impacts from cultivating Bt-maize have been published, of which most investigate economic impacts only (Fisher et al., 2015). The authors conclude that very few studies that take a comprehensive view of social impacts associated with GM crops in agriculture.

[1] http://www.un.org/sustainabledevelopment/sustainable-development-goals/

[2] https://www.cbd.int/sp/targets/

In order to meet the requirements in the NGTA, comprehensive analysis of social implications (e.g. economic, distribution of benefits, access to seeds and wellbeing) in producer countries of the VCO-Ø1981-5 maize are needed.

Co-existence management

The cultivation of GM plants in general is causing problems with regard to co-existence. For instance, Binimelis (2008) have 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 has given up growing non-GM maize. Information about the strategies adopted to ensure co-existence with conventional and organic maize production and information about consequences on co-existence in the countries intended for production of maize VCO-Ø1981-5 is required.

Environmental and health 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 (Dolezel et al., 2009). Therefore, considerations of the co-products also warrant an evaluation of safe use.

The VCO-Ø1981-5 maize confers tolerance to herbicides containing glyphosate. According to the dossier the gene conferring glyphosate resistance (*epsps grg23ace5*) is more stable than other EPSPS genes and the VCO-Ø1981-5 maize does therefore tolerate increased doses of glyphosate and the glyphosate resistance remains stable at higher temperatures.

See section on herbicide tolerance and glyphosate for further elaboration on this issue (p. 7-9).

The authorization for the use of glyphosate in the EU expires in July 2016, and no decision has yet (as of June 2016) been reached on whether or not the authorization for glyphosate should be extended (European Comission 2016; Banks, 2016).

It is documented that the introduction of glyphosate tolerant GM plants has led to an increase in the use of glyphosate (Dill et al. 2010). There is a gap in knowledge regarding herbicide accumulation and residues, including metabolic pathways and metabolites thereof. Bøhn et al. (2014) documented high levels of glyphosate residues in HT GM soybeans grown in the USA, and the same research group have published papers showing that such residues negatively affect the feed quality of HT GM soybeans (Cuhra et al., 2015). It is reasonable to assume that glyphosate tolerant maize will also have higher levels of glyphosate residues, particularly the VCO-Ø1981-5 maize which is modified to tolerate increased doses of glyphosate. This could have health impacts on humans and animals consuming food/feed based on ingredients from the VCO-Ø1981-5 maize. The herbicides are however often not tested as part of the assessment and risk evaluation of HT plants.

Increasing the plants tolerance level might be an attempt to combat the increasing level of glyphosate tolerance in weeds, meaning that higher doses and more repeated applications during the growing season can be used. Emergence of herbicide resistant weeds in maize is vastly documented globally, particularly for glyphosate[3]. The Applicant should provide

[3] http://weedscience.org/summary/crop.aspx

information on the contribution of the VCO-Ø1981-5 maize to the emergence of glyphosate resistance in weeds, management strategies to prevent herbicide resistance development in weeds, and if there are already cases of this in the producer country. In order to evaluate changes in the use of glyphosate after the introduction of VCO-Ø1981-5 maize, more information about the use of these herbicides in the producing countries are needed.

Assessment of alternatives

It is also important to evaluate whether alternative options (e.g. the parental non-GM version of the VCO-Ø1981-5 maize) may achieve the same outcomes in a safer and more ethically justified way. Furthermore, in order to evaluate whether the VCO-Ø1981-5 maize contributes to social utility, it is important to consider current and future demand for this GM maize product for food, feed and processing purposes in Norway and to what extent this demand is/can be satisfied by existing sources. GM maize accounts for approximately 30% of the current global maize production (www.GMO-compass.org). Non-GM maize is therefore abundant for importation to the Norwegian market and maize VCO-Ø1981-5can therefore not be considered to meet a societal need or demand. Reluctance towards GM food tends to be the dominant attitude among Norwegian consumers (Hviid Nielsen, 2012). Norwegian agricultural interest organisations have developed a common policy document on their attitudes to GM food, which states that the agricultural industry in Norway does not want to use or grow GM crops for food or feed (Norsk landbrukssamvirke 2005). Additionally, the Network for GMO free Food and Feed in Norway works for a restrictive approach to GM in Norway and represents a broad range of interest organizations, including farmers unions and one of the largest food chains in Norway (Coop Norge) (http://gmofrimat.no/).

Recommendation:

- 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 social utility of the VCO-Ø1981-5 maize and its contribution to sustainable development. The information provided by the Applicant must be relevant for the agricultural context in the producing country/countries. The information should include issues such as: changes in herbicide use and herbicide resistance in weed populations, co-existence consequences and possible impacts among poor and/or small-scale farmers in producing countries and share of the benefits among sectors of the society.

Final recommendation

The applicant does not attempt to identify socio-economic implications, nor demonstrate a benefit to the community and a contribution to sustainable development from the use of the VCO-Ø1981-5 maize and does therefore not provide sufficient information as required by the NGTA.

General comments for follow up

- This new EPSPS protein is from a “new” bacterial source. It is claimed that this is equal to CP4 EPSPS through the fact that the binding site for gly is at the same place in the proteins. Data for this claim should be presented as safety evaluation of EPSPS is based on “history of safe use” of another EPSPS protein.

- Will there be a demand for this type of glyphosate tolerant maize in Europe?
- Temperature stability measurements can be important for the performance of this type of maize (EPSPS ACE5 enzyme) in a more warm climate, but also for consumption and survival of unprocessed maize.

References

Axelrad, JC., Howard, C V and MClean, WG. (2003). The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. Toxicology, 185**,** pp67-78.

Bank, M. (2016). EU member states fail to back re-authorisation of glyphosate. The parliament. Retrieved from https://www.theparliamentmagazine.eu/articles/news/eu-member-states-fail-back-re-authorisation-glyphosate

Benachour N., Sipahutar, H., Moslemi, S., Gasnier, C., Travert, C and Seralini, GE. (2007). Time- and dose-dependent effects of roundup on human embryonic and placental cells. Arch Environ Contam Toxicol, 53**,** pp.126-33.

Benbrook C (2009): Critical issue report. Impacts of genetically engineered crops on pesticide use: The first thirteen years. www.organic-center.org

Binimelis, R. (2008). Coexistence of plants and coexistence of farmers: Is an individual choice possible? Journal of Agricultural and Environmental Ethics 21, pp. 437-457

Blackburn, L. and Boutin, C. (2003). Subtle Effects of Herbicide Use in the Context of Genetically Modified Crops: A Case Study with Glyphosate (Roundup®). Ecotoxicology, 12**,** pp. 271-285.

Breiteneder H and Mills E.N.C (2005). Plant food allergens – structural and functional aspects of allergenicity. Biotech Adv. 23, p. 395-99.

Bøhn T., Cuhra M., Traavik T., Sanden M., Fagan J. and Primicerio R (2014). Compositional differences in soybeans on the market: Glyphosate accumulates in Roundup Ready GM soybeans. Food Chemistry, 153**,** pp.207-215.

Codex (2003). Principles for the risk analysis for foods derived from modern biotechnology. CAC/GL 44-2003.

Cuhra, M. , Traavik, T. , Dando, M. , Primicerio, R. , Holderbaum, D. and Bøhn, T. (2015) Glyphosate-Residues in Roundup-Ready Soybean Impair Daphnia magna Life-Cycle. Journal of Agricultural Chemistry and Environment, **4**, 24-36. doi: 10.4236/jacen.2015.41003

Dallegrave, E., Mantese, F. D., Coelho, R. S., Pereira, J. D., Dalsenter, P. R., Langeloh, A., (2003). The teratogenic potential of the herbicide glyphosate-Roundup (R) in Wistar rats. Toxicology Letters, 142, pp.45-52.

De Schrijver A, Devos Y, Van den Blucke M, Cadot P, De Loose M, Reheul D and Sneyer M (2007) Risk assessment of GM stacked events obtained from crosses between GM Events. Trends in Food and Sci Technol 18, 101-109.

Dill, GM., Sammons, RD., Feng, PC., Kohn, F., Kretzmer, K., Mehrsheikh, A., Bleeke, M., Honegger, JL., Farmer, D.and Wright, D. (2010). Glyphosate: discovery, development, applications, and properties. Glyphosate Resistance in Crops and Weeds: History, Development, and Management, John Wiley and Sons, Inc., Hoboken, pp.1-33.

Dolezel M, Miklau M, Eckerstorfer M, Hilbeck A, Heissenberger A, Gaugitsch H (2009). Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU / Standardisierung der Umweltrisikoabschätzung gentechnisch veränderter Pflanzen in der EU. BfN – pp.259.

Duke SO and Powles SB. (2008). Glyphosate: a once-in-a-century herbicide. Pest Manag Sci, 64**,** pp.319-25.

EEA report No 1/2013: Late lessons from early warnings. ISBN: 978-92-9213-349-8

EFSA Journal (2010). Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. EFSA Journal 8, p.1700

Environmental issue report No 22/2001

European Commission (2016). Press release Glyphosate: Commission proposes the way forward - Statement by Commissioner for Health and Food Safety, Vytenis Andriukaitis. Retrieved from: http://europa.eu/rapid/press-release_STATEMENT-16-2011_en.htm

Fagard, M and Vaucheret H (2000). (Trans)gene silencing in plants: How many mechanisms? Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 51, pp.167-94.

FAO report (2001): Evaluation of Allergenicity of Genetically Modified Foods.

Fisher, K., Ekener-Petersen, E., Rydhmer, L. and Bjornberg, E. K. (2015). Social impacts of GM crops in agriculture: A Systematic literature review. Sustainability, 7, 8598 – 8620.

Fritschi, L., MClaughlin, J., Sergi, C., Calaf, G., Le Cureieux, F., Forastiere, F., Kromhout, H and Egephy, P (2015). Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Red, pp.114.

Giesy J., Dobson S and Solomon K (2000). Ecotoxicological Risk Assessment for Roundup® Herbicide. *In:* WARE, G. (ed.) Reviews of Environmental Contamination and Toxicology. Springer New York.

Glover D (2010). Exploring the Resilience of Bt Cotton's 'Pro-Poor Success Story'. Development and Change, 41, pp. 955–981.

Hviid Nielsen, T (2012). Holdninger til bioteknologi: Nye vinder? *Genialt*, pp. 12-13.

IAASTD (2009) Agriculture at a crossroads. International Assessment of Agricultural Knowledge, Science and Technology for Development (IAASTD), 250 p.

Jacobsen, S.E., Sørensen, M., Pedersen, M. S and Weiner, J (2013). Feeding the world: genetically modified crops versus agricultural biodiversity. INRA and springer-Verlag, France.

James C (2010). A Global overview of biotech (GM) crops . Adoption, impact andf future prospects. GM Crops 1: 8-12.

Malatesta, M., Caporaloni, C., Gavaudan, S., Rocchi, M. B. L., Serafini, S., Tiberi, C. Gazzanelli, G., (2002). Ultrastructural Morphometrical and Immunocytochemical Analyses of Hepatocyte Nuclei from Mice Fed on Genetically Modified Soybean. Cell Structure and Function, 27, pp.173-180.

Marc, J., Mulner-Lorillon, O., Boulben, S., Hureau, D., Durand, G., Belle, R., (2002). Pesticide roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation. Chemical Research in Toxicology, 15, pp.326-331.

Mesnage R., Defarge N., Spiroux deVendemois J and Seralini GE (2014). Major Pesticides Are More Toxic to Human Cells Than Their Declared Active Principles. BioMed Research International, 2014, pp. 1-8.

NGTA: Norwegian Gene Technology Act (1993).

Norsk landbrukssamvirke (2005). Landbrukets holdning til GMO. Retrieved from: file:///C:/Users/frg022/Downloads/Landbrukets+holdning+til+GMO%20(4).pdf

Ono, M. A., Itano, E. N., Mizuno, L. T., Mizuno, E. H. F., Camargo, Z. P., (2002). Inhibition of Paracoccidioides brasiliensis by pesticides: Is this a partial explanation for the difficulty in isolating this fungus from the soil? Medical Mycology, 40, pp.493-499.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G. E., (2005). Differential effects of glyphosate and roundup on human placental cells and aromatase. Environmental Health Perspectives, 113, pp.716-720.

Solomon K. and Thompson D. (2003). Ecological Risk Assessment for Aquatic Organisms from Over-Water Uses of Glyphosate. Journal of Toxicology and Environmental Health, Part B, 6**,** pp. 289-324.

Viljoen, C. (2013). Letter to the editor. Food Chem Toxicol, 59**,** pp.809-10.

Web-pages

http://www.GMO-compass.org

http://gmofrimat.no/

http://weedscience.org/summary/crop.aspx

http://www.un.org/sustainabledevelopment/sustainable-development-goals/

https://www.cbd.int/sp/targets/