Vår ref: 2016/H_126
Deres ref: 2016/1908

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 18.04.16

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2015/126**, genmodifisert soya MON87705 x MON87708 x MON89788, fra Monsanto Company 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_126
Deres ref: 2016/1908

Assessment of the summary of the technical dossier of EFSA/GMO/NL/126 soy event MON87705 x MON87708 x MON89788 under EC regulation1829/2003.

Sent to

Norwegian Environment Agency

by

GenØk- Centre for Biosafety
April 2016

KONKLUSJON PÅ NORSK

Hovedkonklusjon og anbefalinger:
Genøk–Senter for Biosikkerhet viser til brev fra Miljødirektoratet angående offentlig høring i EU for **genmodifisert soya MON87705 x MON87708 x MON89788** i bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **MON87705 x MON87708 x MON89788** soya.

Ut ifra vurderingskriteriene for bærekraft, samfunnsnytte og etiske aspekter, gir ikke søker opplysninger som belyser disse i henhold til det som forutsettes anvendt i den norske genteknologilovens (Appendix 4).

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 den oppsummerte søknaden om omsetting av **MON87005 x MON87008 x MON89788** mais til import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra **MON87005 x MON87008 x MON89788** mais

Vi anbefaler at norske myndigheter ikke godkjenner bruk av **MON87005 x MON87008 x MON89788** mais til import og prosessering og til bruk i fòr og mat basert på dette.

Assessment of the summary of the technical dossier of EFSA/GMO/NL/126 soy event MON87705 x MON87708 x MON89788 under EC regulation1829/2003.

As a designated National Competence Center for Biosafety, GenØk aims to provide advice giving which is independent and holistic and with a 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 assessment of product safety and corresponding impact assessment of event **MON87705 x MON87708 x MON89788 soy**, 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 some specific recommendations, summarized here and detailed in the go-through below.

- The Authorities are recommended to look into the recommendations made for the combinations of parental lines of MON87705 x MON87708 x MON89788 that has been subjected to previous assessments. Our recommendations on previous combinations of the parental lines are given on p. 7-10
- The applicant should include a full evaluation of the co-technology intended to be used with MON87705 x MON87708 x MON89788, namely glyphosate-based and dicamba - herbicide. Particular focus should be given to the level of accumulation of herbicides in the plants, particularly the parts used in food and feed production, and whether or not these levels of exposure could cause acute and/or chronic health issues. This needs to be tested in animal and feeding studies, separating the effects of the plant and the herbicide(s) by using both sprayed and unsprayed plant samples.
- The Applicant should look into and compare the levels of herbicide residues in the plants in order to provide an improved comparative assessment. The health implications (if any) of the herbicide residue exposure to humans and animals should subsequently be discussed in the toxicological assessment. The toxicological assessment should also include a section on farm worker exposure to the herbicide.
- The Applicant should use herbicide treated, as well as untreated plant material in long-term chronic exposure feeding studies.
- The environmental risk assessment should include a section on the potential environmental effects of the herbicide (monitoring changes in use, potential drift into surrounding areas and ecosystems, leaching to aquatic environments, potential effects on wildlife).
- We encourage the applicant to investigate the deletions and insertions in the transgenic stacks insertion sites, to verify potential changes by using sequence alignment analysis.
- The Applicant is encouraged to analyze level of interaction of dsRNAs as regulators, and if it will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in parental line MON87705xMON89788 will likewise be safe but should be tested and demonstrated to be safe.
- The applicant is asked to provide data for dsRNA effectivity.
- We encourage the Applicant to specify the source of DMO and EPSPS proteins used for safety analysis, also in the summary of the technical dossiers.
- We encourage the Applicant to perform toxicity studies using the whole, fatty acid changed stack MON87705 x MON87708 x MON87988.
- We encourage the Applicant to perform allergenicity analysis of proteins isolated from the whole stack.
- 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 MON87705 x MON87708 x MON89788 soy 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: 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 **soy event MONMON87705 x MON87708 x MON89788** 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.

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.

We therefore consider that the information provided in the summary of the technical application not has provided the information required under Norwegian law to warrant approval in Norway at this time.

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 SUMMARY OF THE TECHNICAL DOSSIER OF EFSA/GMO/NL/2015/126 SOY UNDER EU REGULATION 1829/2003.

About the event

Soy event MON87705 x MON87708 x MON89788 was made through traditional breeding of two gene modified parental soy lines, events MON87708 and MON87705 x MON89788.

The gene modified parental lines were all obtained through *Agrobacterium tumefaciens* mediated transformation of distinct soy tissues.

Parental line, soy event MON87708 expresses a gene that encodes an enzyme enabling demethylation of the herbicide dicamba. The resulting soy event is tolerant to dicamba.

Parental soy event MON87705 encodes partial sequences of two genes, FATB1-A and FAD1-A enabling downregulation of key enzymes FATB and FAD2 in the fatty acid synthesis pathway.
In addition, it contains a CP4-EPSPS gene, providing tolerance to the herbicide glyphosate. This gene is used as a selection marker according to the Applicant.

Parental soy event MON89788 contains the same gene, and confers tolerance to glyphosate, also.

Soy event MON87705 x MON87708 x MON89788 is not approved for any applications in Norway or EU.

Applications for approval has been sent to Canada. Applications are also planned to be sent to countries importing high amounts of soy for food and feed use and that have a regulatory approval system.

We have previously assessed the following combinations of gene modified events of this stack:

- EFSA/GMO/NL/2011/100 , transgenic soy MON87705 x MON89788
- EFSA/GMO/NL/2011/93, transgenic soy MON87708
- EFSA/GMO/NL/2002/108, transgenic soy MON87708 x MON89788

ASSESSMENT FINDINGS

The assessment finding are based on the summary of the technical dossier, previous assessment of parental lines and single events of the stack, as well as other per reviewed data, if available.

EFSA have previously commented the following on these two subcombinations of the stack MON87705 x MON87708 x MON87988:

- MON87705 x MON87988 (Scientific report, EFSA, June 2015 on EFSA/GMO/NL/2011/100): The stack is as safe as conventional soy, but requires labelling due to changed nutritional values.

- MON87708 x MON87988 (Scientific report, EFSA, EFSA/GMO/NL/2012/108): The stack is as safe as conventional soybean. Dicamba residues/metabolites in soybean and potential consumer health risk lies under EFSA pesticide unit.

From previous assessments

Event MON87708 (EFSA/GMO/NL/2011/93) was assessed by GenØk in 2011. This soy is genetically engineered to tolerate the herbicide dicamba and also contains a selection marker (PMI) which is expressed. From this assessment we had the following recommendations

- *The Applicant should be required to provide a post-release plan that provides certainty to the regulator on:*
  a. intended and maximum levels of dicamba applications per season per locality;
  b. ability of the Applicant or adopters of dicamba-tolerant soybeans to detect the emergence of dicamba-tolerant weeds with a sensitivity that would allow them to be controlled without resort to higher levels of dicamba application or alternative herbicides.
- *The Applicant should provide information on*
  c. intended and possible maximum dicamba residues on dicamba-tolerant plant materials at various stages in the production chain;
  d. intended and possible maximum dicamba metabolite residues on dicambatolerant plant materials at various stages in the production chain;
  e. non-target effects on microorganisms including those that could select for cross-resistance to clinical or veterinary antibiotics at possible maximum frequencies and doses of application;
  f. effects on nitrogen-fixing microorganisms at both intended and possible maximum dicamba application levels.
- *The Applicant should supply evidence about the substrate specificity of DMO by testing substances more relevant to the safety assessment, using the in-planta produced DMO proteins.*
- *The Applicant should be required to submit data from field trials covering more than one field season in order to allow adequate exposure to the variety of conditions met in nature (Codex, 2003).*
- *The Applicant should the clarify functional status of the transgenic protein after processing with properly designed experiments, and further test the effects of MON 87708 inhalation*

in animals that are used as models of acute respiratory syndrome, compared with inhalation of the proper conventional comparator. This should include an analysis of allergenicity and toxicity.

- *The Applicant should be requested to investigate the differences in composition that may be directly attributed to the treatment with dicamba and the relevance of these for the risk assessment.*
- *The Norwegian Environment Agency should request data from proper immunostimulation and allergenicity testing of MON 87708 including tests from diet and inhalation exposures.*
- *The Applicant should report the DMO concentration of feed used in the feeding trials at the beginning and the end of the studies.*
- *The Applicant should provide feeding data obtained with MON 87708 that has been grown under the relevant agronomic conditions, i.e. in the presence of dicamba.*
- *The Applicant should provide evidence that the effect of MON 87708 on spleen parameters in the rat feeding study was indeed incidental or experimentally determine the cause for the variation in spleen size of female rats fed with 15% MON 87708.*
- *The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.*
- *The Applicant should report detection limits for all methods.*
- *The Applicant should comply with EFSA and Codex guidelines and provide evidence that all isoforms of the newly expressed proteins are not post-translationally modified.*
- *The Applicant should provide data to substantiate claims of specificity, either by using the in-planta produced proteins or by demonstrating equivalence between the test protein and the in-planta produced form.*
- *Given the deletion and insertions reported after integration of the transgenic DNA into the host genome, the Applicant should provide a survey of the actual RNAs produced or absent at the integration junctions and in the DNA surrounding the insert, preferably using high throughput transcriptome sequencing techniques (Heinemann et al., 2011).*
- *The Applicant should submit required information on the social utility of MON 87708 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*

Event MON87705 x MON87798 (EFSA/GMO/NL/2011/100) was assessed by GenØk in 2013. This is one of the parental lines of the stack in the current application. This stacked event is tolerant to glyphosate by expressing EPSPS and the fatty acid biosynthesis pathway is changed due to downregulation of two key enzymes FATB and FAD2.

From this assessment we had the following recommendations:

- *The regulators are encouraged to fill the research gaps*
- *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.*
- *Most of the information submitted in this safety assessment is derived from previous finding with the single lines. Stacked events should not be approved based on the information on the single events but on the actual event.*

- *Clearly MON 87705 contains already the cp4 epsps gene; thus, the need for the present stacked event is merely for an increased expression level of the protein. The Applicant should thus provide good scientific evidence to justify the safety of the expected increased dietary intake of CP4 EPSPS. The data provided in sections 3 & 4 lack relevant scientific rigors.*
- *The stacked event of MON 87705 x MON 89788 does increase the level of CP4 EPSPS; however, it does not add any value to the food because the fatty acid quality remains unaffected. Given that MON 87705 has already been approved, there is no intuitive reason to approve a stacked event that merely increases the level of non-essential enzyme thus increasing the level of health risks. Besides, the application is not for cultivation, thus, the EU does not need an event with increased resistance to glyphosate. This should be explained by the Applicant.*
- *The Applicant should provide data, for further examination, on the unintended effects on the plants of increased expression of the CP4 EPSPS proteins, which potentially can have implications on metabolite expressions by the plants, some of which can be anti-nutrients or toxins.*
- *The Applicant should identify or analyze off-target effects of the novel dsRNAs expressed in soybean MON87705 x MON89788, or other unintended metabolic changes.*
- *When a small RNA molecule will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in MON87705 x MON89788 will likewise be safe but should be tested and demonstrated to be safe.*
- *The Applicant should submit required information on the social utility of MON87705xMON89788 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*

In addition, GenØk assessed the stacked event **MON87708 x MON89788 (EFSA/GMO/NL/2002/108) in 2013.**
The following recommendations were given then:

- *The regulator is encouraged to ask the Applicant to provide direct evidence of the lack of combinatorial effects arising from the expression of the stacked proteins in the plant, instead of relying on the assessment of non-harm of the target genes existing independently, before a conclusion of safety can be scientifically justified.*
- *The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides/pesticides on the same plant.*
- *The regulator is encouraged to ask the Applicant to address the potential influence of dicamba on food-web dynamics.*
- *The regulator is encouraged to ask the Applicant to address potential environmental consequences and combinatorial effects by using multiple herbicides on the same plant.*
- *Long term exposure-/feeding studies should be included 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 comment on the fate of potential herbicide residues.*

- *The Applicant should provide additional data using comprehensive set of smaller probes in order to evaluate the genetic stability of the event.*
- *The Applicant should provide the electropherograms for the sequence analysis in order to be able to check the quality of the sequencing.*
- *The Applicant should provide evidence that the antibodies used in the protein characterization would detect all novel in-planta produced isoforms.*
- *The Applicant should provide data to substantiate claims of specificity; either by using the in-planta produced proteins or by demonstrating equivalence between the test protein and the in-planta produced form.*
- *The Applicant should supply evidence about the substrate specificity of DMO by testing substances more relevant to the safety assessment, using the in-planta produced DMO proteins.*
- *The Applicant should use plant version of the protein(s).*
- *The Applicant should include a chapter on identification of the transgenic proteins in the stack and not base conclusion of analysis made in single parental lines.*
- *The Applicant should perform analysis on the combined event (MON87708 x MON87798) and base conclusions on that rather than on the single events separately.*
- *The Applicant should perform repeated dose studies for analysis of transgenic proteins in combination for analysis of toxicological potential.*
- *The Applicant should provide data on the glycosylation status of the proteins to the allergenic risk assessment.*
- *The regulator is encouraged to ask the Applicant to submit required information on the social utility of MON 87708 × MON 89788 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*

Lack of access to the full technical dossier, allows us not to get insight into whether newer data is provided for the stack in questions. The rest of the assessment is based on the data given in the summary of the Application (part II).

Stacked events

A stacked event 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, de Schrijver et al, 2007).

MON87705 x MON87008 x MON87988 soy combines expression of distinct proteins connected to herbicide tolerance (dicamba and glyphosate) as well as dsRNAs suppressing the expression of specific proteins.

The potential for unintended effects due to the change in the genome of these stacked soy events must be considered carefully, especially considering whether or not these new proteins are able to influence the expression levels of the naturally occurring proteins in soy, or change the level of allergenicity and toxicity of the plants.

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 been 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 that could have additive or even synergistic effects on non-target species and the environment.

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. In the toxicology assessment in the summary of the application the potential of herbicide exposure through consumption of herbicide treated soy is not fully considered.

Dicamba

Dicamba is presumed to act as a plant growth hormone. When the herbicide reaches an effective concentration, plants are stimulated to grow without reference to their nutrient limitations and subsequently die. It is likely that the incorporation of dicamba tolerance on a scale necessary to compensate for the loss of glyphosate tolerance as a specific weed control strategy in soybeans will result in the same herbicide “treadmill” that is rapidly senescing glyphosate as a commercial option (Binimelis et al., 2009). Indeed, dicamba tolerance in wild plants has been reported (Cranston et al., 2001, Jasieniuk et al., 1995). As with glyphosate, weed control using dicamba and dicamba-tolerant crops will involve multiple applications during the growing season at ever higher doses as the agroecosystem becomes more welcoming to weeds less

susceptible to dicamba, or traditionally susceptible but newly arising resistant variants of current weeds.

Dicamba and its normal metabolites (e.g. 3,6-dichlorosalicylic acid which is similar to 3,5-dichlorosalicylic acid) have structural similarity to classes of salicylic acid-based compounds with antimicrobial activity (Gershon and Parmegiani, 1962). There is very little information about the antimicrobial activities, if any, of dicamba metabolites.

> "*Even though some soil bacteria are able to tolerate or degrade some pesticides by using them as their sole carbon or nitrogen source, bacteriostatic and lethal effects can also occur*" (p. 780 Drouin et al., 2010).

However, it is known that salicylic acid-based compounds with antimicrobial activities can create a selection for bacteria likely to be resistant to antibiotics (Heinemann et al., 2000). As bacteria throughout the production chain, from soil through to processing and on to the gut of consumers and wild and domestic animals, will be exposed to intended higher levels of dicamba and its metabolites, the effects on microorganisms should be determined before approval is granted.
Although dicamba is presumed to act as a plant growth hormone, it is a genotoxin and a potential carcinogen (Knopper and Lean, 2004, Kovalchuk and Kovalchuk, 2008). Thus, the herbicide has the potential to select for a variety of novel phenotypes in microbes and in plants, as well as to accelerate the evolution of resistance. Other antibiotics with DNA damaging activites, e.g. bleomycin, have been known to select for resistance and resistance has been beneficial to potential pathogens even in the absence of the antibiotic (Heinemann et al., 2000).

Information of this kind should be required for:
· dicamba;
· 3,6-dichlorosalicylic acid;
· 6-dichlorosalicylic acid; and
· 5-hydroxy-2-methoxy-3,6-dichlorobenzoic acid (Casida and Lykken, 1969).

The unintended antimicrobial activities may also have an adverse effect on soil productivity. Of special significance would be an effect on nitrogen fixation, since soybeans are used as an important source of fixed nitrogen in mixed cropping agroecosystems.

> "*The effect of pesticides on rhizobia and their symbiosis with legume, will vary according to the rhizobial species, the rhizobial strains within a given species, the type of pesticide involved, and the pesticide concentration*" (p. 780 Drouin et al., 2010).

Reductions in fixation would have to be supplemented using fertilizers produced at high fossil fuel costs. Holst et al. (1982) found that lower levels (0.1-1 ppm) of dicamba stimulated growth of *Anabaena azollae*, the nitrogen-fixing symbiont of *Azolla mexicana*, but higher concentrations inhibited growth. Concentrations of 1-10 ppm inhibited nitrogen fixation and reduced chlorophyll levels (Holst et al., 1982). Reported effects of dicamba on *Rhizobium* and *Bradyrhizobium* have been concentration and strain-dependent. Two studies reported strains that were inhibited by dicamba. 5% and 3% of *Rhizobium* and *Bradyrhizobium* strains, respectively, surveyed by Drouin et al. (2010) were inhibited by 450 µg of dicamba. While

reassuring that so few responded to dicamba, and then only at concentrations that would be relevant to seed treatment rather than current soil application concentrations, this study did not examine susceptibility in the field under field conditions, leaving some uncertainty as to actual environmental impact of dicamba use. More importantly, given the mode of action of dicamba, current application concentrations may not be predictive of future concentrations and therefore the effects on these symbionts. Finally, again it should be noted that even in this limited survey there were strain-specific differences in susceptibility to dicamba and thus any environmental risk assessment should be conducted on local soil and nodule isolates. Nitrogen-fixing bacteria of four different genera were isolated from soil that originated from a single soybean farm in Argentina (Zabaloy and Gomez, 2005). Of the 76 strains isolated, only 1 (a strain of *Bradyrhizobium*) demonstrated sensitivity to dicamba. Again, this study is reassuring in that a minority of strains surveyed appeared susceptible to dicamba. However, it is concerning that a general prediction about dicamba's effects on important soil microorganisms cannot be reached, and emphases the need for agroecosystem-specific sampling and large surveys. Moreover, this study did not measure sub-lethal effects on nodule formation and fixation, which are important variables for any comprehensive assessment on soil microorganisms.

Glyphosate

In MON87705 x MON87708 x MON89788, both parental events MON87705 and MON89788 expresses the gene CP4 EPSPS that provide glyphosate tolerance.

One may assume that this increase in CP4 EPSPS levels increases the plants tolerance to glyphosate (i.e. the crop can be sprayed more intensely). However, the summary of the application contains no information concerning the effect on tolerance. 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. Glyphosate has long been promoted as an ideal herbicide with low toxicity and little environmental impact (Duke and Powles, 2008, Giesy et al., 2000). However, in recent years, glyphosate has received a lot of risk-related attention. This is partly due its increased use since the introduction of glyphosate-tolerant GM-plants (Dill et al., 2010, Cuhra et al., 2013), and reports on negative effects in terrestrial and aquatic ecosystems (Blackburn and Boutin, 2003, Solomon and Thompson, 2003). In addition, studies on animals and cell cultures indicate that there might be health implications from exposure to glyphosate (Axelrad et al., 2003, Benachour et al., 2007, Cuhra et al., 2013). Among the health effects observed in animal models are histopathological changes in organs such as the liver, cell-division dysfunction in early embryos, negative impact on nerve-cell differentiation, increased fetal mortality, growth reduction, and skeletal malformation. 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:

- The applicant should include a full evaluation of the co-technology intended to be used with MON87705 x MON87708 x MON89788, namely glyphosate-based and dicamba - herbicide. Particular focus should be given to the level of accumulation of herbicides in the plants, particularly the parts used in food and feed production, and whether or not these levels of exposure could cause acute and/or chronic health issues. This needs to be tested in animal and feeding studies, separating the effects of the plant and the herbicide(s) by using both sprayed and unsprayed plant samples.

- The Applicant should look into and compare the levels of herbicide residues in the plants in order to provide an improved comparative assessment. The health implications (if any) of the herbicide residue exposure to humans and animals should subsequently be discussed in the toxicological assessment. The toxicological assessment should also include a section on farm worker exposure to the herbicide.
- The Applicant should use herbicide treated, as well as untreated plant material in long-term chronic exposure feeding studies.
- The environmental risk assessment should include a section on the potential environmental effects of the herbicide (monitoring changes in use, potential drift into surrounding areas and ecosystems, leaching to aquatic environments, potential effects on wildlife).

Molecular characterization.
The summary of the Application refers to the molecular characterization performed in the parental lines Mon87705, MON87708 and MON89788. The inserted genes are analyzed for their copy number in each of the single events and not in the resulting stack the Application is on (p. 14, part 3.2.2 a).

A 899bp deletion and some insertions (35bp and 128bp) were detected in MON87708 at the site of insertion of the inserted cassette.
Additionally, deletions have been found in parental line MON87705 (36bp) and parental line MON89788 (40bp). There is no information in the summary of the Application whether sequence analysis have been performed for the insertion sites in the stack itself or if the data only are from the single, transgenic, parental lines. According to the summary, this has been performed for the inserted sequences.

Recommendation:

- We encourage the applicant to investigate the deletions and insertions in the transgenic stacks insertion sites, to verify potential changes by using sequence alignment analysis.

Information on the expression of the insert.
DMO and CP4 EPSPS expression were analyzed in forage and seed in field trials (Argentina, 2013-2014). The whole stack was analyzed using enzyme-linked immuno-sorbent assay (ELISA). Eight field sites with replicated plots were analyzed.
Due to the lack of access to full technical dossier/Application, we cannot comment on antigen or antibodies used in the assay for the detection of DMO and EPSPS. We can also not comment on level of expression as compared to the single, parental, transgenic events.

The stack MON87705 x MON87708 x MON89788 has a change in fatty acid composition as well as expression of DMO and EPSPS.

dsRNA
The modification of MON87705, in MON87705 x MON87708 x MON89788, is based on dsRNA silencing to selectively down-regulate two key enzymes involved in the soybean seed fatty acid biosynthetic pathway. This is a type of manipulation that has not benefited from human food safety studies to our knowledge (Heinemann 2009).
When a small RNA molecule will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in MON87705 x MON87708 x MON89788 will likewise be safe. From the literature, it is clear that dsRNA can have significant biological impact. Recent research (Zhang et al 2012, CERA 2011, Baum et al 2007, Gordon and Waterhouse 2007, Mao et al 2007) establishes beyond doubt that novel RNAs of recombinant or synthetic origin cannot be "generally regarded as safe" but must be tested and demonstrated to be safe when consumers or wildlife is exposed through food or inhalation.

Recommendation:

- The Applicant is encouraged to analyse level of interaction of dsRNAs have as regulators and if it will or might not act as a gene regulator is not always known in advance. Therefore, it cannot be assumed that novel small RNAs that might be created in parental line MON87705xMON89788 will likewise be safe but should be tested and demonstrated to be safe.

In the summary of the Application, there is no reference to whether the dsRNA is working 100% (through analysis of target protein levels).

Recommendation:

- The applicant is asked to provide data for dsRNA effectivity.

Toxicology and allergenicity

According to the Applicant, the safety assessment is based on DMO and EPSPS protein assessment as the FAD2-1/FATB1-A is a suppression cassette encoding dsRNA.

The toxicity assessment is based on the following characteristics and comparisons:
DMO and EPSPS are considered as safe due to the long history of safe use, no structural similarity to known toxins or other biologically active proteins, no knowledge of toxic effect in mammals, rapid digestion in simulated gastric fluids (SGF) and low margin of exposure.

There are however issues that are unclear due to inaccessible data:
Is the EPSPS and DMO tested of bacterial of plant origin? This can not be concluded from the summary of the Application.

Also, we can not see that there is a mentioning of toxicological data from studies with the fatty acid changed stack MON87705 x MON87708 x MON87988 and it is therefore unclear if these have been performed.

The allergenicity assessment of MON87705 x MON87708 x MON87988 is based on the following criteria for DMO and EPSPS:

- Source of protein is allergenic or not
- Structural similarity to known allergens
- How rapid the protein is digested in an in vitro assay of the mammalian gastrointestinal system

It is unclear if the proteins are used for allergenicity testing is of bacterial or plant origin
Based on these criteria, the Applicant considers the proteins as having low risk when it comes to allergenicity.

The allergenicity data are based on data obtained from the parental lines and not from the full stack in the Application.

Recommendation:

- We encourage the Applicant to specify the source of DMO and EPSPS proteins used for safety analysis, also in the summary of the technical dossiers.
- We encourage the Applicant to perform toxicity studies using the whole, fatty acid changed stack MON87705 x MON87708 x MON87988.
- We encourage the Applicant to perform allergenicity analysis of proteins isolated from the whole stack.

Environmental risk assessment (ERA) and monitoring plan

We emphasize the crucial role of the agricultural context in which these crops will be grown. There are several risks connected to the cultivation of genetically modified crops, among them gene flow (both to non-modified crops and wild relatives of the crop) and potential impacts on the surrounding ecosystems through affecting insect and plant life, small mammals and birds and aquatic life (i.e. non-target organisms) (Warwick et al. 2009).

Recommendation:

- We emphasize the importance of environmental monitoring plans when it comes to introduction of new genetic traits into the environment.

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. The NGTA, with its clauses on societal utility and sustainable development, comes into play with a view also to health, environmental and socio-economic impacts in other countries, such as where the GMOs are grown. In the following we identify areas that are relevant to consider in order to assess social utility and sustainability aspects, and highlight information needed to properly assess these issues.

Socio-economic impacts
Very few studies take a comprehensive view of social impacts associated with GM crops in agriculture (Fisher et al. 2015). Reviews on social and economic impacts from GM crop cultivation (e.g. issues such as economic gains, distribution of benefits, access to seeds and improved wellbeing) relevant for a sustainability assessment indicate that these effects have been very complex, mixed and dependent on the agronomic, socio-economic and institutional settings where the technology has been introduced (Glover, 2010). Fisher et al. (2015) point to factors such as different political and regulatory contexts when explaining differences reported in distribution of economic gains and farmers' access to seeds. This underlines that it cannot be expected that the same effects will apply between different social and environmental contexts. 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. In order to meet the requirements in the NGTA, further investigations of social implications (e.g. economic, distribution of benefits, access to seeds and wellbeing) in countries where MON87705 X MON87708 X MON89788 soy is intended for cultivation is needed.

Environmental and health impacts of the co-technology: glyphosate and dicamba
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 MON87705 X MON87708 X MON89788 soy confers tolerance to herbicides containing glyphosate and dicamba.

Recent studies have shown negative effects from glyphosate, both on species present in terrestrial and aquatic ecosystems and on animals and cell cultures. Consequently, glyphosate is now increasing recognized as more toxic to the environment and human health than what it was initially considered to be (for further elaboration and references on these issue see section on p.14-15).

Dicamba is a synthetic auxine considered as an herbicide with low toxicity, but with high residuality. However, a research has indicated indirect negative effects of dicamba on insects, while highlighing that few research has been conducted on the issue to date in despite dicamba is, along with 2,4-D, causing most herbicide-drift damage to nontarget plants even though present limited agricultural usage (Bohnenblust et al., 2013, (for further elaboration and references on these issue see section on p.12-13).

Glyphosate resistant weeds in soy is vastly documented globally[1], and 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). As we previously have written, studies has shown increased levels of glyphosate residues in glyphosate tolerant GM crops (Bøhn et al. 2014). This could have health impacts on humans and animals consuming food/feed based on ingredients from this type of GM plants.

In regards to the tolerance to dicamba, although it is expected that this modification could provide an alternative for controlling weeds in glyphosate-tolerant fields and for extending the effective lifetime of glyphosate (Behrens et al., 2007), the effectiveness of dicamba for controlling weeds such as waterhemp is lower than glyphosate, which could cause a "treadmill" effect for farmers if control is low. Besides, reduced sensitivity to dicamba has also been recently reported in *Amaranthus* species by Bernard et al (2012), which makes the authors conclude that "The commercialization of soybean, cotton, and corn resistant to 2,4-D and dicamba should be accompanied by mandatory stewardship practices that will minimize the selection pressure imposed on other waterhemp populations to evolve resistance to the synthetic auxin herbicides".

The Applicant should provide information on the contribution of the MON87705 X MON87708 X MON89788 soy to the emergence of glyphosate/dicamba resistance in weeds, management strategies to prevent herbicide resistance development in weeds, and if there are already cases of this in the areas intended for cultivation of the variety. In order to evaluate changes in the use of glyphosate/dicamba, after the introduction of MON87705 X MON87708 X MON89788 soy, more information about the use of these herbicides in the country(ies) intended for cultivation are needed.

Social and economic impacts from gene flow and co-existence management
The cultivation of GM plants in general is causing problems with regard to co-existence. 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 strategies adopted to ensure co-existence with conventional and organic soy production and potential consequences for these production forms in the producing country(ies) is required for an assessment of social and economic impacts in the producer country.

Assessment of alternatives
It is also important to evaluate whether alternative options (e.g. the parental non-GM version of the MON87705 X MON87708 X MON89788 soy) may achieve the same outcomes in a safer and ethically justified way. Furthermore, in order to evaluate whether the MON87705 X MON87708 X MON89788 soy contributes to social utility, it is important to consider current and future demand for this GM soy product for food, feed and processing purposes in Norway and to what extent this demand is/can be satisfied by existing sources. As the fatty acid composition of MON87705 X MON87708 X MON89788 soy is altered, this event could be of better quality as an ingredient in food and feed. The Applicant needs to specify the intended benefits from this trait.

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

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 MON87705 X MON87708 X MON89788 soy 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: 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.

Conclusion

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 MON87705 X MON87708 X MON89788 soy and does therefore not provide sufficient information as required by the NGTA.

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