Vår ref:2017/H_135
Deres ref: 2017/1018

Høringsuttalelse av søknad om markedsføring av genmodifisert soya MON87708 x MON89788 x A5547-127

EFSA/GMO/NL/2016/135

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
Mars 2017

GenØk – Senter for biosikkerhet
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Vår ref:2017/H_135
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Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 24.03.2017

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2016/135**, genmodifisert, stablet soya MON87708 x MON89788 x A5547-127, fra Monsanto Europe S.A/N.V, 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:2017/H_135
Deres ref: 2017/1018

Høringsuttalelse – genmodifisert, stablet soya, MON87708 x MON89788 x A5547-127, EFSA/GMO/NL/2016/135, under EU forordning 1829/2003.

Søknad EFSA/GMO/NL/2016/135 omhandler genmodifisert, stablet soyalinje til bruksområdene mat, for, import og prosessering.

Den genmodifiserte soyaen har toleranse mot herbicider som inneholder glyfosat via det innsatte genet *cp4 epsps* , mot glufosinat ammonium via det innsatte genet *pat*, og mot dicamba via det innsatte genet *dmo*.

Hverken den stablete soya linjen eller dens foreldrelingjer er godkjent for noen av bruksområdene i Norge.

I EU er samtlige foreldrelinjer godkjente for de omsøkte bruksområder, men ikke den stablete soyalinjen denne søknaden omhandler.

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OPPSUMMERING

GenØk–Senter for biosikkerhet, viser til høring av søknad EFSA/GMO/NL/2016/135 om MON87708 x MON89788 x A5547-127 soya som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra denne soyaen.

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

- Genmodifisert soya linje MON87708 x MON89788 x A5547-127 er ikke godkjent i Norge eller EU for noen av de omsøkte bruksområdene.
- MON87708 x MON89788 x A5547-127 er tolerant mot sprøytemidler som inneholder glyfosat, glufosinat - ammonium og dicamba som har ulike grader av helse-og-miljø fare ved bruk.
- Glufosinat ammonium er ikke tillatt brukt i Norge.
- Søknaden om soya linje MON87708 x MON89788 x A5547-127 mangler data og informasjon som er relevant for å kunne vurdere kriterier rundt etisk forsvarlighet, samfunnsnytte og bærekraft.

SUMMARY

GenØk-Centre for biosafety refers to the application EFSA/GMO/NL/2016/135 on MON87708 x MON89788 x A5547-127 soy for import, processing, food and feed or ingredients thereof.

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

- The gene modified soy event MON87708 x MON89788 x A5547-127 is not approved for any application in Norway or the EU.
- Soy event MON87708 x MON89788 x A5547-127 is tolerant to herbicides containing glyphosate, gluphosinate ammonium and dicamba that has distinct health and environmental dangers upon use.
- It is not allowed to use gluphosinate ammonium in Norway.
- The application on soy event MON87708 x MON89788 x A5547-127 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

Application on EFSA/GMO/NL/2016/135

The stacked event Mon87708 x MON89788 x A5547-127 soy contains genes providing herbicide tolerance *(cp4 epsps, pat and dmo*).

Previous evaluations

The Norwegian Scientific Committee for Food Safety (VKM) has commented on the application for the parental, single event soy MON87708 (EFSA/GMO/NL/2011/93) (1) with the following issues:

- There is no increased probability for spread, establishment and invasion of this soy event in natural habitats caused by seed spillage during transport and processing. It has no wild relatives in Norway.
- More information on residues of herbicides used and metabolites DCSA, DCGA and 5-OH-dicamba is wanted and they should be analysed. Residue levels are important to know due to increased herbicide resistance provided by the inserted transgenes and thus more intensive use of herbicides on the plants.
- Herbicide treated soya should have been included in the animal experiments.
- Feeding studies on fish should be performed due to soy being a natural ingredient in fish feed.
- Some nutrients have not been analyzed. Consensus documents/ guidelines should be followed.
- No health related effects were found in the performed feeding experiments.
- The DMO protein is most probably not allergenic based on performed studies.

VKM has evaluated the parental, single event MON89788 (EFSA/GMO/NL/2006/36) (2) in 2015 in a final health and environmental risk assessment where they commented on the following issues:

- The molecular characterization did not reveal any safety concerns.
- The soy event MON89788 is equivalent to its conventional counterpart, with the exception of the introduced trait.
- There is no increased likelihood of establishment of this event in Norway and do not represent any environmental risk.

VKM also evaluated the single, parental soy A5547-127 (EFSA/GMO/NL/2008/52) (3) in 2015 in a final health and environmental risk assessment with the following comments:

- The molecular characterizations performed gave expected results and did not reveal any safety concerns.
- Soy event A5547-127 is equal to its conventional counterpart, with the exception of the introduced trait.
- There is no increased likelihood of spread to the environment and establishment. Soy has no wild relatives in Norway, thus hybrid formation with spread of transgenes is not an issue. This soy do not represent an environmental risk in Norway.

- More research is needed when it comes to the application of glyphosate and residue and metabolite levels.

The Norwegian Biotechnology Advisory Board has recently assessed the soy event MON87708 x MON89788 x A5547-127 (4) and advised the Norwegian authorities to prohibit this soy for food, feed, import and processing based on the issue of the plant being tolerant to herbicides that are harmful to health and environment.

GenØK has previously commented on some of the parental, single events or combinations of these in previous hearings (http://genok.no/radgiving/horingsuttalelser/).

In the assessment EFSA/GMO/NL/2012/108 (2013) on MON87708 x MON89788, we had these comments to the Application:

- *Data should be provided for evidence of lack of combinatorial effects arising from the expression of stacked proteins.*
- *Evaluate the environmental consequences from the use of multiple herbicides in the same plant.*
- *Evaluate the potential of the herbicide dicamba on food-webs.*
- *Long term feeding studies of the whole plant before release on the market.*
- *What is the fate of the herbicide residues?*
- *The antibodies used should be specified in order to detect all in-planta forms.*
- *Microbial versions of the proteins were used for the safety assessments.*
- *Toxic potential should be analysed by repeated dose toxicity studies.*
- *Data from the stack itself should be the basis of identification of the transgenic proteins, rather than conclusions made in the single, parental lines.*
- *Data on glycosylation status of the transgenic proteins should be provided for the allergenic risk assessment.*

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Social utility and sustainability issues on the stacked soy event MON87708 x MON89788 x A5547-127EFSA/GMO/NL/2016/135

In addition to the EU regulatory framework for GMO assessment, an impact assessment in Norway follows the Norwegian Gene Technology Act (NGTA) (5). In accordance with the aim of the NGTA, production and use of the GMO needs to be *ethically justifiable*, demonstrate a *benefit to society* and contribute to *sustainable development*. This is further elaborated in section 10 of the Act (approval), where it is stated that: "*significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development*" (See section 17 and annex 4 for more detail on the regulations on impact assessment). Recent developments within European legislation on GMOs allow Member States to restrict the cultivation of GMOs on their own territory based on socio-economic impacts, environmental or agricultural policy objectives, or with the aim to avoid the unintended presence of GMOs in other products (Directive 2015/412) (6). Additionally, in recent years, attention increased within academic and policy spheres to broaden the assessment of new and emerging (bio) technologies to include issues that reach beyond human and environmental health. (7-12).

With the assessment of *ethically justifiability, benefit to society* and *sustainability* as in the NGTA, significant dedication is demanded as it covers a wide range of aspects that need to be investigated (e.g. Annex 4 within the NGTA, or 13). Nevertheless, the applicant has currently not provided any information relevant to enable an assessment of these criteria. Therefore, this section will highlight some areas that are particularly relevant to consider for soy MON 87708 x MON 89788 x A5547-127 and where the applicant should provide data for in order to conduct a thorough assessment according to the NGTA.

Sustainability

The soy MON 87708 x MON 89788 x A5547-127 contains particular events that confer tolerance to herbicides containing glyphosate, gluphosinate ammonium 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 (for further elaboration and references on this issue see section on Herbicides), as well as in villages in areas where glyphosate is used systematically as part as the GM crops tolerant to glyphosate (14). Consequently, glyphosate is now increasingly recognized as more toxic to the environment and human health than what it was initially considered to be. This is particularly a concern as the introduction of glyphosate tolerant GM plants has led to an increase in the use of glyphosate (15, 16). As soy MON 87708 x MON 89788 x A5547-127 is genetically modified to possess genes that provide glyphosate tolerance, as well as gluphosinate ammonium and dicamba, it is likely to assume that this GM crop is tolerant to higher doses of these herbicides and could potentially further increase the use of them.

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 (17).

Therefore, considerations of the co-products also warrant an evaluation of safe use and data required for such an assessment is not provided by the Applicant.

Impacts in producer countries

As already stated, the Applicant does not provide data relevant for an environmental risk assessment of soy MON 87708 x MON 89788 x A5547-127 as it is not intended to be cultivated in the EU/Norway. However, this information is necessary in order to assess the sustainability criteria as laid down in the NGTA. This criteria is referring to a global context, including the contribution to sustainable development in the producing countries with a view to the health, environmental and socio-economic effects in other countries, in this case where the soy MON 87708 x MON 89788 x A5547-127 is cultivated.

In addition to a lack of information, there can also be ambiguity about how scientific conclusions may be achieved. For example, it is difficult to extrapolate on hazards or risks taken from data generated under different ecological, biological, genetic and socio-economic contexts as regional growing environments, scales of farm fields, crop management practices, genetic background, interactions between cultivated crops, and surrounding biodiversity are all likely to affect the outcomes. It can therefore not be expected that the same effects will apply between different environments and across continents. This is particularly relevant to consider as field trials of the soybean have been in the USA, while the request for cultivation is in Mexico and Canada and no information about how this difference may affect the risk evaluation has been provided.

The applicant highlights that the appearance of “volunteer” soy in rotational fields is not documented and therefore are unlikely to unlikely to pose any threat to the EU environment or to require special measures for its containment. Furthermore, they state that *soybean volunteers can be easily controlled using currently available herbicides (except dicamba, glyphosate and glufosinate) or by mechanical means*. Still, an evaluation of the occurrence of volunteer plants in the producing countries and suggested control strategies is important for a sustainability assessment. Information about which herbicides that will potentially be used for killing volunteers is required to evaluate potential health and environmental impacts of these.

Herbicide-resistant genes

When herbicides are used in agriculture, it is important to minimize the potential of weeds becoming resistant. Indeed, when crops are engineered to be herbicide resistant in order to maintain an agriculture practice that uses herbicide, it is essential to remain attentive to the amount of herbicide used, the potential consequences of this use for the area in which the crop is cultivated and develop management strategies to make sure that this does not create (more) resistant weed. Moreover, studies have shown increased levels of herbicide residues in herbicide tolerant GM soy (e.g. 18), which could have health impacts on humans and animals consuming food/feed based on ingredients from these type of GM plants. The Applicant has not provided information on whether the cultivation of soy MON 87708 x MON 89788 x A5547-127 could affect the emergence of glyphosate resistance in weeds, nor if there are cases of this in the areas intended for cultivation of the variety which are also important aspect to evaluate the ethical justifiability. Furthermore, this soybean is meant to be cultivated in Mexico and Canada, where glyphosate resistant weeds has increased significantly[1]. Additionally, no information is currently provided by the applicant that demonstrates reflection on how the monitoring, assessment or evaluation of the GM crop in countries where the crop will potentially be cultivated is assessed, as the applicant considers information on this not relevant because soy MON 87708 x MON 89788 x A5547-127 will not be cultivated in Europe. However, it remains an important aspect for a sustainability evaluation and thus necessary if the application is to be evaluated according to this criteria in the NGTA.

The ethical issue of glufosinate-ammonium

A significant ethical issue arises due to the fact that soy MON 87708 x MON 89788 x A5547-127 is meant to be resistant to gluphosinate-ammonium, a herbicide that is banned in Norway due to the risks to human health and the environment. It seems ethically ambiguous and inconsistent to import a plant that is resistant to this herbicide, thereby allowing the use and development of a harmful herbicide in other countries, while considering the herbicide as too harmful to be used in Norway. This thereby troubles to fulfil the criteria to contribute to *sustainable development*, the criteria that is meant to be considered in a global context. Information on how this can be ethically justified is therefore highly warranted.

In sum, a proper evaluation of potential impacts that are relevant for the sustainability criteria in the producing country is lacking, and sufficient information in this agricultural context needs to be provided. This should include information from an ERA concerning impacts on cultivation, management and harvesting stages, as well as the post-market environmental monitoring in the producing country.

Benefit to society

The criteria of 'benefit to society' in the NGTA should be interpreted on a national level. That means that the import of soy MON 87708 x MON 89788 x A5547-127 needs to demonstrate how it will benefit Norway. However, no information on this part is provided by the applicant. Indeed, the applicant state that this soy will replace soy in existing food and feed products. It is therefore important to evaluate how GM crops in general, GM soybean in particular, and the use of GM soybean in food and feed are valued by Norwegian consumers. This information will contribute to anticipate impacts at an early stage, as well as that it may demonstrate a need to assess the alternative options for import of soybeans. However, the limited amount of empirical data available on the attitude of Norwegian citizens towards GM (e.g. 19, 20) is outdated and more empirical research on this is warranted to investigate consumers' attitude, demand and acceptance.

Assessing alternatives

When a new (bio-) technology is developed, it is important to reflect on what problem it aims to solve and to investigate whether alternative options may achieve the same outcomes in a safer and ethically justified way. After all, when a crop is genetically modified to tolerate a particular herbicide, it means that the crop is developed for a particular cultivation practice in

[1] http://weedscience.org/Summary/Country.aspx Status of Herbicide Resistance in Mexico and Canada, Accessed on 16 March 2017.

which these herbicides are to be used. What is meant with alternatives, and what would benefit from being assessed could include alternative varieties (e.g. non-GM) for import, alternative sources to satisfy the demand, alternative ways of agriculture, or even explore alternative life visions. In fact, this corresponds with the increased trend within research and policy of science and innovation to anticipate impacts, assess alternatives, reveal underlying values, assumptions, norms and beliefs (10, 21) in order to reflect on what kind of society we want, and assess how certain (biotechnological) developments may or may not contribute to shaping a desired future. Thus, in order to evaluate whether soy MON 87708 x MON 89788 x A5547-127 contributes to social utility, it is important to investigate current and future demands and acceptance of this in Norway and if there are alternatives sources for soy that could be cultivated elsewhere that may satisfy this demand, or are more desirable.

Ethical considerations: socio-economic impacts

As known, GM crops have been, and still are, a hot topic for debate. A significant amount of this debate focuses on the safety of GMOs and currently no scientific consensus on this topic has been achieved (22). Nevertheless, another substantial part of the debate is around the socio-economic impacts of GM productions and many questions for evaluating the above mentioned criteria in the NGTA are based on an assessment of the socio-economic impacts. These impacts can vary and range from seed choice for farmers, co-existence of different agricultural practices, changing power dynamics among stakeholders, new dependencies of farmers, intellectual property right on seeds, benefit sharing, the decreasing space for regional and local policy, and more organisational work and higher costs for non-GM farmers (e.g. for cleaning of sowing machines or transport equipment to avoid contamination). Although the examples of socio-economic impacts clearly indicate the complexity and extensive list of concerns beyond safety aspects, little empirical investigation on these kind of aspects has been done. For example a study performed by Fischer et al. (23) concerning social implications from cultivating GM crops found that from 2004 – 2015 there has only been 15 studies corning socio-economic implications of cultivating Bt-maize. The study demonstrates that published literature is dominated by studies of economic impact and conclude that very few studies take a comprehensive view of social impacts associated with GM crops in agriculture. The amount of research performed in this case and the minimal focus on social impacts strongly indicate a high need for further investigation on how the cultivation of GM crops affects different parties involved. It is therefore striking that no information on any of the above mentioned points is discussed by the applicant.

Co-existence

The cultivation of GM plants in general is causing problems with regard to co-existence, an important socio-economic impact. For instance, Binimelis (24) has investigated consequences on co-existence of Bt maize in Spain among small-scale farmer and has found that co-existence is very difficult and that farmers in some areas have given up growing non-GM maize. Even though the cultivation of soy MON 87708 x MON 89788 x A5547-127 is not planned in Europe/Norway, it is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic soy production and information about consequences on co-existence in the countries intended for production of soy MON 87708 x MON 89788 x A5547-127 and minimize the likelihood for gene flow to wild relatives. Legal information could inform assessing organs on the governance strategies and possibilities to ensure co-existence, although it has been noted that this may not suffice, as co-existence has become an arena of opposed values and future vision of agriculture and the role of GM crops within these visions (25). Indeed, although a framework for maintaining co-existence in Europe was established in 2003 (26) this effectively meant technical measurements and recommendations (e.g. cleaning of sowing machines and transport vehicles) and remains challenging in practice (27, 28). Moreover, this framework arguably reduced the significance of the issue of co-existence to questions concerning economic aspects for individuals (e.g. farmers), rather than recognizing that agricultural practices are interwoven in dynamic social, economic and political systems (29, 30). For the criteria in the NGTA, information on co-existence is required to enable a coherent analysis.

Summary

In order to meet the requirements for the NGTA, the regulator is encouraged to ask the Applicant to submit information relevant for the assessment of the criteria of ethically justifiability, benefit to society and sustainability assessment. The information provided by the Applicant must be relevant for the agricultural context in the producing country/countries. The information should also include issues such as: Changes in herbicide use, development of herbicide resistant weed, potential for gene flow and possible socio-economic impacts such as poor and/or small-scale farmers in producing countries and share of the benefits among sectors of the society. It is also important to stress the need for (information on) integrated weed management strategies in those countries (31). Furthermore soy MON 87708 x MON 89788 x A5547-127 is tolerant to glufosinate-ammonium which is banned for use in Norway. Banning the use of glufosinate-ammonium based herbicides domestically due to health and environmental concerns, while indirectly supporting its use in other countries would be ethically ambiguous and goes against the criteria of sustainable development. Moreover, the applicant does not attempt to demonstrate a benefit to the community or any reference on the consumer attitude and demand within Norway soy MON 87708 x MON 89788 x A5547-127 and does therefore not provide sufficient information as required by the NGTA.

Environmental risk issues in a Norwegian context

Soy is not cultivated in Norway and there is no wild relatives in the Norwegian environment. There are some varieties of soy that is cultivated in the south of Sweden and in Denmark .

Loss of gene modified soy seed through storage or transport would therefore not involve great risk for spread into the wild or spread of transgenes to wild relatives.

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

Stacked events

The stacked soy event MON87708 x MON89788 x A5547-7 contains three inserted transgenes providing herbicide tolerance towards three different herbicides. This stack should be regarded as a new event, even if no new modifications have been introduced, as the combination itself in the stack is unique for that event. 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 within stacked traits cannot be excluded and whether or not the expressed proteins in the plant can give specific immunological effects or adjuvant effects in mammals has been discussed previously (32, 33).

Molecular characterization

The stacked soy event MON87708 x MON89788 x A5547-127 have the following inserted genes:

- The *cp4 epsps* gene (source:*Agrobacterium tumefaciens*) providing glyphosate tolerance.
- The *pat* gene (source: *Streptomyces viridochromogenes*) providing tolerance towards gluphosinate ammonium.
- The *dmo* gene (source: *Stenotrophomonas maltophilia*) providing tolerance towards dicamba containing herbicides.

According to the Applicant, all the inserted genes in soy event MON87708 x MON89788 x A5547-127 has been thoroughly described before.

The molecular description in the dossiers is however not included as the final GMO is a stack produced by conventional breeding methods of other GM events. In order to asses the final product, it is necessary with molecular data on how the genomic localization of the individual GM elements relate to each other.

The individual molecular description is not public available and thus difficult to obtain. GenØk has responded previously to either the independent events or previous stacks where some of the events are present, and because the applicant states that no new transformation events have been performed, the molecular points to consider already raised, still apply.

Comments relevant for the assessment of the current application

In general there is a lack of available information on the integration of the events. This is either due to restricted information, restricted access or due to lacking characterizations by the applicant in the original events. No new molecular data is given in the current application.

From previous comments by GenØk:

- 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 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.
- Incomplete detection of the organization and sequence adjacent to the introduced DNA in MON 87708
- Incomplete detection of absence of backbone vector DNA/unintended transgenes in event MON 87708

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

The soy event MON87708 x MON89788 x A5547-127 expresses the three proteins EPSPS, PAT and DMO from the genes *cp4 epsps*, *pat* and *dmo*.

Analysis of the expression of the inserts were performed on forage and seed tissue samples from distinct field sites that were sprayed with the actual herbicides, according to the Applicant. The levels of the proteins were analysed using ELISA on protein extracts and detected levels were as expected.

Toxicity and allergenicity

Toxicity

Both EPSPS, PAT and DMO proteins have been evaluated by EFSA in several applications previously and considered to be safe.

The Applicant refer to previously obtained toxicity data from the single, parental events when it comes to molecular and biochemical characterization, stability during processing and storage (through expected treatment of food and feed: temperature, resistance to proteolytic enzymes, pH), history of safe use, source, low exposure level etc.

Since then, toxin, allergen and protein databases have been renewed and analysis reconducted in 2016 (p21, Technical dossier). Data for these analysis are not available, but the Applicant state that the results confirm the initial analysis. Based on this, a 28 day oral toxicity study is found unnecessary.

A 90 day feeding study is not performed and the Applicant refers to OECD reports concluding that whole food feeding studies are unnecessary on GM crops that already have been demonstrated not to be biologically different to its conventional counterparts (34).

Allergenicity

Proteins EPSPS, PAT and DMO have been tested for their allergenic potential thorugh their assessment of the parental, single events.
Based on expected biochemical characteristics, history of safe use, lack of amino acid homology to known allergens and other biochemical characteristics, these proteins are considered non-allergenic and an assessment of the stack (whole) MON87708 x MON89788 x A5547-127 is not considered necessary.

Potential interactions between newly expressed proteins

Mode of action, molecular analysis of the corresponding genes and activity of proteins of soy event MON87708 x MON89788 x A5547-127 made the basis for the conclusion made by the applicant that there are no indications of potential interactions of safety concern between the traits expressed.

Hazard identification

According to the applicant, it is unlikely that the proteins expressed from the gene modified soy event will be hazardous.

Summary:

- *Evaluations of allergenicity and toxicity of soy event MON87708 x MON89788 x A5547-127 is mainly based on previous assessments of the single, parental events.*
- *Microbial versions of PAT and EPSPS proteins are used for the safety assessments.*
- *Potential interactions of expressed transgenes has not been analyzed.*

Herbicides

The soy event MON87708 x MON89788 x A5547-127 contains a cp4 epsps gene, pat gene and dmo gene providing glyphosate, gluphosinate ammonium and dicamba tolerance.

Herbicide use on GM plants

Herbicide tolerant (HT) plants are sprayed with the actual herbicide(s), leaving the weed to die whereas the plant with the inserted genes will survive. However, the issue on accumulation of herbicides in the HT plants, including metabolic pathways and metabolites of these, are often not tested as part of the risk assessment of HT plants. Bøhn et al. (35) 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 have the potential for negatively to affect the feed quality of HT GM soybeans (36, 37). It is important to look at the potential metabolites of the herbicides in use and if these are documented to have a negative effect on health and environment.

Another issue is the development of resistance towards the herbicides (38) in use that is a relevant issue, but not discussed further here.

Glyphosate tolerance

The *cp4 epsps* gene present in MON87708 x MON89788 x A5547-127 soy 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 (31, 39). However, it has received more risk-related attention due to its potential for negative effects on both aquatic and terrestrial ecosystems (40), as well as in studies in animals and cell cultures that have indicated possible negative health effects in rodents, fish and humans (41-43).

Studies indicate that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (44).

A restricted number of publications indicate unwanted effects of glyphosate on health (43, 45), aquatic (46) and terrestric (40, 47) organisms and ecosystems.

A study of Roundup effects on the first cell divisions of sea urchins (48) 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 (41) 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 (49). 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 Reseach on caner (IARC) released a report concluding that glyphosate is “probably carcinogenic to humans”(50).

Glufosinate ammonioum tolerance

The *PAT* gene confer tolerance to herbicides containing gluphosinate ammonium.

Glufosinate-ammonium belongs to a class of herbicides that is banned in Norway and in EU (except for a limited use on apples) due to both acute and chronic effects on mammals including humans. Studies have shown that glufosinate-ammonium is harmful by inhalation, ingestion and skin contact and that serious health risks may result from exposure over time. Observations of patients poisoned by glufosinate-ammonium have found that acute exposure causes convulsions, circulatory and respiratory problems, amnesia and damages to the central nervous system (CNS) (51). Chronic exposure in mice has been shown to cause spatial memory loss, changes to certain brain regions, and autism-like traits in offspring (52, 53).

Dicamba tolerance

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 (54). Indeed, dicamba tolerance in wild plants has been reported (55, 56). 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 (57). 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 (58).

However, it is known that salicylic acid-based compounds with antimicrobial activities can create a selection for bacteria likely to be resistant to antibiotics (59). 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 (60, 61). 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 (59).

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 (62).

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 (58).

Reductions in fixation would have to be supplemented using fertilizers produced at high fossil fuel costs. Holst et al. (63) 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. 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. (58) 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 (64). 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.

Summary:

- Soy event MON87708 x MON89788 x A5547-127 is tolerant to glyphosate, gluphosinate ammonium and dicamba containing herbicides that damaging to health and environment in different degrees.
- Potential for accumulation of the herbicides should be considered in GM plants used in food and feed.

Main summary

Soy event MON87707 x MON89788 x A5547-127 is tolerant to herbicides containing glyphosate, gluphosinate ammonium and dicamba that has distinct degrees of health and environmental dangers upon use, thus the issue on accumulation should be considered for GM plants to be used in food and feed.

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

The applicant should provide data relevant for assessment of social utility and sustainable development according to the NGTA(5).

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