Vår ref:2017/H_113
Deres ref: 2017/5588

Høringsuttalelse av søknad om markedsføring av genmodifisert mais MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9

EFSA/GMO/NL/2013/113

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
August 2017

Vår ref:2017/H_113
Deres ref: 2017/5588

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 05.08.2017

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2013/113**, genmodifisert, stablet mais linje MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9, 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,

Lise Nordgård
Forsker II
GenØk – Senter for biosikkerhet
lise.nordgard@genok.no

Bidragsyter(e):

Lilian van Hove
Forsker III
GenØk-Senter for biosikkerhet

Idun Merete Grønsberg
Forsker II
GenØk-Senter for biosikkerhet

Odd-Gunnar Wikmark
Forsker II
GenØk-Senter for biosikkerhet

Malachy Okeke
Forsker II
GenØk-Senter for biosikkerhet

Innhold/Content

Vår ref:2017/H_113
Deres ref: 2017/5588

Høringsuttalelse – genmodifisert, stablet mais MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9, EFSA/GMO/NL/2013/113, under EU forordning 1829/2003.

Søknad EFSA/GMO/NL/2013/113 omhandler genmodifisert, stablet maislinje til bruksområdene mat, for, import og prosessering.

Den genmodifiserte maisen har toleranse mot herbicider som inneholder glyfosat, glufosinat-ammonium og 2, 4-D via de innsatte genene *cp4 epsps*, *pat* og *aad-1*.

I tillegg har denne maislinjen resistens mot insekter i *Lepidoptera* og *Coleoptera* ordenen via de 6 innsatte Cry genene: *cry1A.105*, *cry2Ab2*, *cry1F*, *cry3Bb1*, *cry34Ab1* og *cry35Ab1*.

Den stablete maislinjen eller dens foreldrelinjer er ikke godkjent for noen av bruksområdene i Norge.

Foreldrelinje DAS-40278-9 er ikke godkjent for noen av de omsøkte bruksområder i EU.

Import av levende maislinje 1507 ble forbudt av Regjeringen pr 2.Juni 2017. Maislinje 1507 er en av foreldrelinjene i søknaden.

Oppsummering

GenØk–Senter for biosikkerhet, viser til høring av søknad EFSA/GMO/NL/2013/113 om MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 mais som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra denne maisen.

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

- Genmodifisert, stablet mais linje MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 er ikke godkjent i Norge eller EU for noen av de omsøkte bruksområdene.
- MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 er tolerant mot sprøytemidler som inneholder glyfosat, 2, 4-D og glufosinat - ammonium som har ulike grader av helse-og-miljø fare ved bruk.
- Glufosinat-ammonium er ikke tillatt brukt i Norge.
- Det er forbudt å importere levende 1507 til Norge.
- Søknaden om mais linje MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 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/2013/113 on MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 maize 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, stacked maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 is not approved for any application in Norway or the EU.
- The stacked maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 is tolerant to herbicides containing glyphosate, 2, 4-D and glufosinate ammonium that has distinct health and environmental dangers upon use.
- It is not allowed to use glufosinate ammonium in Norway.
- It is not allowed to import living maize 1507 to Norway.
- The application on maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

Application on EFSA/GMO/NL/2013/113

The stacked event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 maize consists of 5 parental single events containing herbicide tolerance or insect resistance genes, or both.

Previous evaluations

Here are some evaluations made by Norwegian and European agencies/committees regarding the events in the present application on event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 maize:

The Norwegian Scientific Committee for Food Safety (VKM) has assessed or commented on the parental, single events MON89034 [1], 1507 [2], MON88017 [3], 59122 [4] and DAS-40278-9 [5] previously.

From these assessments, the following where concluded for the parental lines MON89034, 1507, MON88017 and 59122:

- Based on the current knowledge, the parental event is nutritionally equivalent to conventional maize. It is unlikely that the expressed, transgenic proteins will introduce toxic or allergenic potential in food or feed. Also, based on the current knowledge, there is no environmental risk in Norway with the intended usage.

For the parental event DAS-40278-9 a temporary evaluation concluded that there is no indications for increased spread, establishment and invasion of maize event DAS-40278-9 in natural environments or other areas outside agricultural areas resulting from spillage of seed during transport or processing. There is no natural wild relatives in Norway in which a hybridization would occur and the cultivation of maize is limited.

EFSA commented on the parental line MON89034 in 2008 [6] with the following points:

- Molecular characterization and bioinformatic analysis did not reveal any safety concerns.
- MON89034 is equal to conventional maize in composition.
- Low exposure gives little or no risk to target and non-target organism.
- There is no likelihood for establishment or survival of feral maize plants.
- Based on available information and intended use it is unlikely that maize MON89034 will have adverse effects on humans or animals.

EFSA has also commented on the reapplication on 1507 [7] in 2017 with the following note:

- *"Under the assumption that the DNA sequence of the event in maize 1507 considered for renewal is identical to the corrected sequence of the originally assessed event, the GMO Panel concludes that no new hazards or modified exposure and no new scientific uncertainties were identified for the application for renewal that would change the conclusions of the original risk assessment on maize 1507".*

EFSA has commented on the application for MON88017 [8] with the following issues:

- *"In conclusion, the Panel considers that the information available for Maize MON88017 addresses the scientific comments raised by the Member States and that it is as safe as its non genetically modified counterpart with respect to potential effects on human and animal health or the environment. Therefore the GMO Panel concludes that MON88017 is unlikely to have any adverse effect on human or animal health or on the environment in the context of its intended uses".*

The Norwegian Authorities have, through a Royal Resolution dated on the 2nd of June, 2017 [9] pointed the following regarding maize event 1507:

- Ministry of Climate and Environment (KLD) propose that maize event 1507 is prohibited to be traded in Norway under the Gene Technology Act [10]. This applies to living maize only (dead and processed 1507 is not covered by this prohibition)
- This prohibition applies for the approved areas of use after directive 2001/18/EC (http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32001L0018&from=EN), feed and industrial processes.
- The Ministry base its conclusion on the following: "the use is ethically problematic" and emphasizes that based on the use of glufosinate ammonium where the maize is produced and that Norway has a ban on this herbicide, import of 1507 is evaluated as ethically problematic and not sustainable by consumer organizations in Norway. This is because the cultivation of the maize depends on the use of glufosinate ammonium, a herbicide that is banned in Norway.
- Maize event 1507 have no traits evaluated as useful for Norwegian consumers/users since it is not allowed to use the herbicide that the maize is modified to tolerate.

See also https://www.regjeringen.no/no/aktuelt/regjeringen-sier-nei-til-genmodifiserte-planter/id2555387/

GenØk has commented on several combinations of the 5 parental events since 2010. We will not mention all here. However, recently we commented on the following:

- *2017: EFSA/GMO/NL/2013/112, maize event* ***MON89034*** *x* ***1507*** *x NK603 x* ***DAS-40278-9.***
- *2015: EFSA/GMO/BE/2013/118, maize event MON87427 x* ***MON89034*** *x* ***1507 x 88017*** *x* ***59122***

Social utility and sustainability issues on the stacked maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9

In Norway, an impact assessment follows the Norwegian Gene Technology Act (NGTA) in addition to the EU regulatory framework for GMO assessment. In accordance with the NGTA, the development, introduction and/or use of a GMO needs to be *ethically justifiable*, demonstrate a *benefit to society* and contribute to *sustainable development*. This is further elaborated in section 10 of the Act (approval), where it is stated that: "*significant emphasis shall also be placed on whether the deliberate release represent a benefit to the community and a contribution to sustainable development"* (See section 17 and annex 4 for more detail on the regulation on impact assessment). Recent developments within European regulation 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). Additionally, attention within academic and policy spheres increased in recent years on broadening the scope of the assessment of new and emerging (bio)technologies to include issues that reach beyond human and environmental health [11-17].

To assess the criteria of *ethically justifiable, benefit to society* and *sustainability* as in the NGTA, significant dedication is demanded as it covers a wide range of aspects that need to be investigated (e.g. Annex 4 within the NGTA, or [18]). Nevertheless, the Applicant has currently not provided any information relevant to enable an assessment of these criteria. Therefore, this section will highlight some areas that are particularly relevant to consider with maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 and where the Applicant should provide data for in order to conduct a thorough assessment according to the NGTA. Table 1 offers specific questions connected to the sections below.

The ban on maize 1507

Norwegian authorities have banned the release of Maize 1507 in Norway. After an evaluation, the Norwegian Scientific Committee for Food Safety concluded that this maize is as safe as conventional maize. However, the Norwegian Biotechnology Advisory Board concluded in their assessment that this maize should not be allowed in Norway as it is ethically problematic and does not contribute to sustainable development.

Maize 1507 is developed to be resistant to glufosinat-ammonium. This is a class of herbicide that is banned in Norway (except a limited use on apples) due to the risks to human health and the environment. The NBAB concluded that 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 also troubles the fulfilment of the criteria of *sustainable development*, as this criteria is meant to be considered in a global context. This problem has been previously identified by the Norwegian Biotechnology Advisory Board [19] and GenØk has addressed it multiple times when an applicant seeks approval of a product containing maize 1507 [e.g. 20, 21]. Although the Norwegian Environmental Agency recommended approval of maize 1507, the Ministry of Climate and Environment was oppose to this approval. In the Royal Resolution

of June 2nd 2017, a final decision was made and maize 1507 is prohibited to be traded in Norway. This is the first GM crop to be prohibited in Norway based on ethical considerations only.

As the current application includes maize 1507 and this event is now prohibited in Norway, we consider this application as opposing the aim of the criteria in the NGTA. Approving this application would be against the Royal Resolution. Unless the applicant is able to demonstrate how the combination of maize 1507 with MON89034, MON88017,59122 and DAS40278-9 contains a benefit that could outweigh this decision, we consider a reference to this resolution as sufficient and therefore consider a further elaboration on the evaluation of maize 1507 x NK603 according to the NGTA as superfluous.

Sustainability

The maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 contains a modified *cp4 epsps* gene that confers increased tolerance to herbicides containing glyphosate. 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 later in this document) as well as in villages in areas where glyphosate is systematically used as part of the GM crops tolerance to glyphosate [22]. 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 crops has led to an increase in the use of glyphosate [23-26]. As maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 is genetically modified to possess, among others, a gene that provides glyphosate tolerance, this crop could potentially further increase the use of glyphosate as a higher amount of glyphosate will not affect this maize. It is therefore important that the Applicant provides information on management strategies for dealing with the increased tolerance and how this may be justified in order for us to perform an accurate evaluation. The Applicant could for example provide information on the current use of glyphosate in the sites of cultivation and what approaches are used to minimize the use of glyphosate.

Herbicide-resistant genes

As already became clear in the above mentioned section, when a herbicide - such as glyphosate – is used in agriculture, it is important to minimize the potential of weeds becoming resistant. Indeed, when crops are engineered to be herbicide tolerant in order to maintain an agricultural practice that uses herbicide, it is essential to remain attentive to the amount of herbicide used, the potential increase of use and the consequences of this for the area in which the crop is cultivated. Chemical treatment coupled with the unavoidable resistance development are major blocking factors to a sustainable agriculture [27]. The development of management strategies to make sure that this does not create (more) resistant weed is therefore highly warranted to be able to respond to a potential increase in weed-resistance. Moreover, studies have shown increased levels of herbicide residues in herbicide tolerant GM crops [e.g. 28], which could have health impacts on humans and animals consuming food/feed based on ingredients from this type of GM plants.

The Applicant has not provided information on whether the cultivation of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 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. Indeed, from the application it is not clear where this maize will be cultivated, other than that field trials have been in the USA. Although the Applicant claims that the location of these field trials provide a variety of environmental conditions, no argumentation or justification is documented how this may suffice, differ and / or relate to the sites of cultivation as it is unclear where this maize will be cultivated. For a proper assessment of the criteria in the NGTA it is important to know where the crop will be cultivated, and that information is provided that demonstrates reflection on how the monitoring, assessment or evaluation of the GM crop in sites of cultivation is given assessed. Currently, the Applicant merely states that information on this is not relevant because maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 will not be cultivated in Europe. This is not sufficient and more information on the sites of cultivation and management strategies is needed.

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 [29]. 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 maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 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 maize 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 maize are performed in the USA, while

The Applicant highlights that the appearance of “volunteer” soy in rotational fields following the soy crop from the previous year is rare under European conditions. Still, an evaluation of the occurrence of volunteer plants in the producing countries and suggested control strategies is important for a sustainability assessment. Information about the occurrence of volunteers and

which herbicides that will potentially be used for killing volunteers is required to evaluate potential health and environmental impacts of these.

Benefit to society

The criteria of 'benefit to society' in the NGTA should be interpreted on a national level. That means that the import of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 needs to demonstrate how it will benefit Norway. However, the Applicant provides no information on this part. It is important to evaluate how GM crops in general, GM maize in particular, and Norwegian consumers value the use of GM maize in food and feed. 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 maize. A report published in 2017 on the perceptions among Norwegian citizens on GMOs describes how about half of the respondents expressed that they were negative for sale of GMO-products in Norwegian grocery stores in the future, whereas only 15 percent were positive [30]. Nevertheless, the empirical data available on the attitude of Norwegian citizens towards GM products remains limited [e.g. 31, 32] and more empirical research on this is needed to investigate consumers' attitude, demand and acceptance on different aspects such the cultivation, import and or processing of GM crops within and outside of Norway, as the perspectives on GM food and feed.

Assessing alternatives

When a new (bio-) technology is developed, it is important to reflect on what problem it aims to solve and to investigate whether alternative options may achieve the same outcomes in a safer and / or a more ethically justifiable way. After all, when a crop is genetically modified to tolerate a particular herbicide, it means that the crop is developed for a particular cultivation practice in which these herbicides are to be used. What is meant with alternatives, and what would benefit from being assessed could include alternative varieties (e.g. conventional or organic maize) for import, alternative sources to satisfy the demand, alternative ways of agriculture, or even explore alternative life visions. In fact, this corresponds with the increased trend within research and policy of science and innovation to anticipate impacts, assess alternatives and reveal underlying values, assumptions, norms and beliefs [14, 33] as a way 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 maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 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 maize 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 [34]. Nevertheless, another substantial part of the debate is around the socio-economic impacts of GM productions and many questions for evaluating the above mentioned criteria in the NGTA are based on an assessment of the socio-economic impacts. These impacts can vary and range from seed choice for farmers, co-existence of different agricultural practices,

impacts among poor and/or small-scale farmers in developing countries, share of the benefits among sectors of the society, changing power dynamics among stakeholders, autonomy of farmers, intellectual property right on 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. [35] concerning social implications from cultivating GM crops found that from 2004 – 2015 there have 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 [36] 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 maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 is not planned in Europe/Norway, it is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic maize production and information about consequences for co-existence in the countries intended for cultivation of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 and minimize the likelihood for gene flow to wild relatives, or contamination during transport or processing. Furthermore, legal information and clarity could offer evaluators a more comprehensive understanding of governance strategies and possibilities to ensure co-existence, although it has been noted that this may not suffice as co-existence has become an arena of opposed values and future vision of agriculture, including the role of GM crops within these visions [37]. Indeed, although a framework for maintaining co-existence in Europe was established in 2003 [38] this effectively meant technical measurements and recommendations (e.g. cleaning of sowing machines and transport vehicles) and remains challenging in practice [39, 40]. 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 [41, 42]. For the criteria in the NGTA, information on co-existence is required to enable a coherent analysis.

The ethical issue of glufosinate-ammonium

A significant ethical issue arises with maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 as is meant to be resistant to gluphosinate-ammonium, a class of herbicide that is banned in Norway (except a limited use on apples) due to the risks to human health and the environment. As already stated in the beginning and affirmed by the ban on maize 1507 in

Norway, it seems ethically ambiguous and inconsistent to import a plant that is resistant to this herbicide, thereby allowing the use and development of a harmful herbicide in other countries, while considering the herbicide as too harmful to be used in Norway. Additionally, this troubles the fulfilment of the criteria of *sustainable development*, as this criteria is meant to be considered in a global context. Information on how this can be ethically justified is therefore highly warranted.

Short summary of the evaluations

We have pointed out that information was lacking to enable a fruitful evaluation of the criteria in the NGTA. More information is required on the following key issues:

- *Herbicide resistant genes*; when crops are engineered to be herbicide tolerant (such as maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9) in order to maintain an agricultural practice that uses herbicide, information is warranted on the amount of herbicide used, the potential increase of use and what management strategies are in place to avoid weed resistant.
- *Impact in producer countries*; some products may not directly affect Norway, but will have a (potential negative) impact in producer countries. Currently, this and previous applicants provide no information on this as the product will not be cultivated in Norway. However, to be able to evaluate the criteria of 'sustainable development' and 'ethically justifiable', information on the effect of cultivation on producing countries is warranted. The ground on which maize 1507 is now prohibited in Norway is a suiting example of this.
- *Benefit to society*; this criteria refers to a national context. Hence, it asks the question how the import of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 will benefit Norway, which is not made clear by the applicant. Furthermore, more empirical research is needed to investigate consumers' attitude, demand and acceptance on different aspects such the cultivation, import and or processing of GM crops within and outside of Norway, as the perspectives on GM food and feed.
- *Co-existence*; the cultivation of GM plants in general is causing problems with regard to co-existence. It is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic maize production at the sites of cultivation. The applicant should provide information on this to enable an accurate evaluation of the criteria in the NGTA.
- *The ethical issue of glufosinat-ammonium;* maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 is tolerant to glufosinate-ammonium by including the event 1507. Approving this application would be against the Royal Resolution that is released in June, 2017. Unless the applicant is able to demonstrate how the combination of maize 1507 with MON89034, MON88017,59122 and DAS40278-9 contains a benefit that could outweigh this decision, we consider the acceptance of this application as a breach with this resolution.

Table 1: Questions to the Applicant

Sustainability	*How does the cultivation of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 affect the use of glyphosate?*
	How is the current use of glyphosate in the sites of cultivation and what approaches are used to minimize the use of glyphosate?
Herbicide-resistant weed	*What kind of management strategies are taken to prevent the increase of herbicide-resistant weed?*
	Who will be affected if the amount of resistant weeds increases?
	How is the burden of increase of resistant weeds distributed and what strategies are in place to compensate this?
Benefit to society	*Is maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 available for further breeding and research? If so, under which circumstances?*
	Is there a demand for maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 in Norway?
	Does maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 contribute to business development and value creation in Norway, including new job opportunities?
Assessing alternatives	*Will maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 benefit Norwegian consumers more than the other alternatives available from conventional or organic agricultural practices? If so, how?*
Ethically justifiable	*What are the different public values and visions on the development, introduction or use of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 within Norway and how does the development of this maize relates to these?*
	Does the development, introduction or use of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 contradict ideas about solidarity and equality between people, such as the particular consideration of vulnerable groups in the population?
Socio-economic impacts	*Which parties will be affected by the development, introduction or use of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 and how does this change their autonomy, practice and position compared to other stakeholders?*
	Does maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 change the power dynamic among stakeholders? If so, how?
	Can the development, introduction or use of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 create significant ruptures or ecological relationships?
Co-existence	*Does the cultivation of maize MON89034 x 1507 x MON88017 x 59122 x DAS40278-9 affect other types of agricultural practices in the nearby areas? If so, how?*
	Is there a system in place for keeping GMO and non-GMO crops separate in the production and transport line? If so, who pays for this system?

Environmental risk issues in a Norwegian context

The level of maize production is very low in Norway and only some varieties can grow in the southern part due to climate conditions. There are also no wild populations of maize in Norway.

These limitations lead to minimal possibilities for establishment of maize outside agricultural practices. Loss of gene modified maize seed through storage or transport would therefore not involve great risk for spread into the wild or spread of transgenes to wild relatives.

Environmental effects of herbicides

The use of herbicides like glyphosate also has the potential to affect ecosystem, animal and human health. The massive use of glyphosate, totaling 852 million kg globally by 2014 [25], which directly or indirectly will expose non-target biodiversity in terrestrial, soil and aquatic communities [43], represent a major source of environmental pollution.

Herbicide use on GM plants

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

Total use of herbicides

HT GM plants are documented to be a strong driver of increased use of glyphosate-based herbicides (the dominant herbicide tolerance trait until now). From 1995 to 2014 the global agricultural use of glyphosate rose 14.6 fold, from 51 million kg to 747 million kg and HT GM crops have been a major driver for this change. Moreover, by 2016, about 56 % of the global use of glyphosate was related to the use of HT GM crops [25]. The massive use of herbicides like glyphosate has the potential to affect ecosystem, animal and human health. Non-target biodiversity will be exposed both directly and indirectly, in terrestrial, soil and aquatic ecosystems. This represents a major source of environmental pollution [43] . A similar development as seen for glyphosate can be expected for other herbicides that GM plants are made tolerant to, i.e. glufosinate ammonium, 2,4-D, dicamba and others. Bio-active herbicides ultimately get into soil and water systems through processes such as drifting, leaching and surface runoff [44].

Modeling studies have shown that long-term implications of large scale bioenergy crops can surpass toxicity thresholds for fish (bluegill) and humans in significant parts of relevant watersheds, particularly because of glyphosate, and thus negatively impact aquatic life and drinking water [45]. Given that glufosinate, 2,4-D, dicamba and other herbicides may replace the role of glyphosate, such modeling studies may have to be re-calibrated with a new attention to the concentration of these chemicals.

Combinatorial effects of multiple herbicides

Stacked GM plant have the combination of two or more genes of interest in one single plant. Some of these have "gene pyramiding" of several herbicide tolerance traits. Today there is a clear trend towards combining two or more transgenic traits present in single events through conventional breeding.

Stacked events are in general more complex than the single events as there are more genes involved. There has been an increased interest in the possible combinatorial and/or synergistic effects of stacked traits [46-48]. Stacking may critically influence the bioactivity and hence the potential for unintended effects. Therefore, robust data are necessary to identify whether the combined presence of transgenes and multiple co-technology herbicides may influence the quality of the plant or harm the environment.

At present, many stacked events combine different classes of herbicide traits. For example, the maize hybrid MON 89034 x 1507 x MON 88017 x 59122, from Monsanto and Dow AgroSciences, is tolerant to both glyphosate and glufosinate ammonium (in addition to insect resistance). Co-exposure of multiple herbicides may trigger combinatorial effects in non-target organism in the environment. However, this represents a major knowledge gap in the scientific literature.

The development towards stacked events as the norm will arguably lead to increased doses/more applications of herbicides per season. Since effects of these chemical may interact with each other, eventually with other stressors in the environment, the co-exposure and potential combinatorial effects need to be studied [49, 50] .

Increased use and resistance evolution

Specific for the HT GM plants is that herbicides can be sprayed in higher doses than before, and repeatedly during the growth season of the plants. The increased use must be linked to resistance evolution in weeds.

At present, 37 species of weeds are documented to be glyphosate resistant on a global scale [51]. Such development may lead to a 'treadmill' where resistance triggers more applications/higher doses, which leads to stronger selection pressure for resistance, etc. and eventually the use of additional herbicides like atrazine, 2,4-D or others [52]. Crop and herbicide monoculture makes the agroecosystem more vulnerable to further resistance development [53]. It is already clear that glyphosate tolerance will be replaced or combined with other herbicides, of which 2,4-D and dicamba are likely candidates to take important market shares. Given such development, the toxicity and non-target effects of herbicides that eventually replace glyphosate becomes more important. For 2,4-D, 33 species of weeds are shown to be resistant, and six of these (18%) were documented after 2015 [51]. Thus, the use of 2,4-D may be expected to increase as seen for glyphosate.

For glufosinate ammonium, six species of weeds are shown to be resistant and 50 % of these were discovered after 2015 [51].

Accumulating herbicide residues and health effects

Glyphosate accumulates in HT soybeans, more when the plant is sprayed later in the season [54]. This may bring significant amounts of glyphosate into the food and feed chain. Bøhn and colleagues measured on average 9.0 mg/kg of glyphosate in HT GM soybeans grown in Iowa [55]. If 9.0 mg/kg is representative for all glyphosate tolerant soy produced globally, then about 2000 tons of pure glyphosate is brought into the food and feed chains per year.

The issue of accumulation of herbicides in the HT plants, including metabolites, is not regularly tested as part of the risk assessment of HT plants. However, it is documented that most feeding studies to test HT GM plant material have very limited relevance as the GM plant test material was not sprayed with the relevant herbicide [56]. In *D. magna* it is shown that residues of glyphosate negatively affect the feed quality of HT GM soybeans [28, 57].

The increased awareness of glyphosate toxicity, coupled with the increased volume used, should lead to stronger restrictions, for example lower acceptance level for glyphosate residues in food and feed [58]. But the opposite has happened, the maximum residue level (MRL) for glyphosate has been raised 200-fold from 0.1 to 20 mg/kg in Europe,, and to 40 mg/kg in the US. This set of events has been termed "The Glyphosate Paradox" [58]. The WHO/IARC categorization of glyphosate as *probably carcinogenic to humans*[59], while EFSA EFSA concluded with the opposite [60], is underlining the significance of the controversy around the glyphosate-based herbicides.

As shown for glyphosate-tolerant GM plants, HT GM plants with tolerance to glufosinate ammonium, 2,4-D, dicamba and other herbicides may serve as a vector for these chemicals into the global food and feed chains.

EFSA confirms that the parent compound of 2,4-D can be found in GM maize that is resistant to 2,4-D but see no need to change the current maximum residue level (MRL) of 2,4-D. For Europe, the MRL is set at 0.05 mg/kg, which is near the limit of quantification (LOQ) [61]. EFSA argues that there is no need to perform specific studies on the nature and magnitude of 2,4-D residues in processed commodities since "significant residues are not expected in the raw agricultural commodities" [61]. This is to base conclusion on assumptions rather than on data. Given the fact that glyphosate accumulates in orders of magnitude higher concentration in HT GM soy (see above), EFSA should not assume that the concentrations of 2,4-D will be lower than accepted levels. What is needed are measurements and data of 2,4-D residues in relevant products.

EFSA show that 2,4-D is relatively stable in the plant matrix for 12-18 months [62]. The residues of glyphosate in GM soybeans found by Bøhn et al. [28] also confirmed that glyphosate was stably incorporated into the soybean product for several years.

Studies in Daphnia

In *Daphnia magna*, the LC50/EC50 acute toxicity is shown in the range 144 – 248 mg/L for 24 h, and 25 mg/L for 48 h, respectively [63, 64].

However, the issue on accumulation of herbicides in the HT plants, including metabolites, are not regularly tested as part of the risk assessment of HT plants. Bøhn et al. [55] 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 [57, 65]. 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.

Glyphosate tolerance

The *cp4 epsps* gene present in MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 maize confers tolerance to herbicides containing glyphosate.

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

Glyphosate has previously been announced as an herbicide with low toxicity for users and consumers as well as the environment surrounding agricultural fields [54, 66]. However, glyphosate has recently received more risk-related attention due to its potential for negative effects on both aquatic and terrestrial ecosystems [67], as well as from studies in animals and cell cultures that have indicated possible negative health effects in rodents, fish and humans [68-70].

It has also been shown that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture [71].

A number of publications indicate unwanted effects of glyphosate on health [70, 72], aquatic [73] and terrestric [67, 74] organisms and ecosystems. Also, a study of Roundup (containing glyphosate as the active ingredient) effects on the first cell divisions of sea urchins [75] is of particular interest to human health. The experiments demonstrated dysfunctions of cell division at the level of CDK1/Cyclin B activation (these proteins are involved in mitosis). 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 [68] 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 was 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 [76]. 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 the 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 where glyphosate was considered as "probably carcinogenic to humans"[77], an issue which is under debate.

Glufosinate ammonioum tolerance

The stacked maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 contain the *pat* gene from *Streptomyces viridochromogenes* that confers tolerance to herbicides containing glufosinate-ammonium, a class of herbicides that are banned in Norway and in EU (except a limited use on apples) due to both acute and chronic effects on mammals including humans. Glufosinate ammonium is harmful by inhalation, swallowing and by skin contact. 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) [78, 79]. Chronic exposure in mice has been shown to cause spatial memory loss, changes to certain brain regions, and autism-like traits in offspring [80, 81](. According to EFSA, the use of glufosinate-ammonium will lead to exposure to farm workers that exceed acceptable exposure levels during application.
Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where glufosinate ammonium is used [78, 79, 82, 83]. EFSA has concluded on the risk of glufosinate ammonium, as especially harmful to mammals [84].

2, 4-D (Dichlorophenoxyacetic acid)

Tolerance to 2,4-D in GM plants come from the insertion of the *aad-12* gene from *Sphingobium herbicidovorans.* 2,4-D is a systemic herbicide that leads to uncontrolled growth and death in broad leaf plants. Grasses and cereals like corn, oat, rice and wheat have relatively high tolerance to 2,4-D, giving the option of using 2,4-D as a post emergence herbicide on selected crops.

2,4-D and can be found in different chemical forms: as acid (basic form), inorganic salts, amines or esters [85]. Plants absorb 2,4-D through roots and leaves within 4-6 hours, the chemical follows the phloem of the plant and mimics the role of auxins (plant hormones) leading to disturbances, abnormal growth and eventually death [86].
Technical grade 2,4-D acid, esters and salts show similar toxicity in rats, with some effects on liver and kidney at doses 15 mg/kg/day and higher [87]. A study requested by the Industry Task Force II on 2,4-D, report a no observed effect level (NOEC, 13 weeks) in dogs on 1.0 mg/kg/day [88].

In humans, use or mixing of 2,4-D is linked to cancers Non-Hodgkins's Lymphoma (NHL) and Soft Tissue Sarcoma (STS), although co-exposure to 2,4,5-T and TCDD in some cases make cause of disease difficult [88]. 2,4-D was by WHO/IARC in 2015 classified as a *possible carcinogen to humans* [89].

The interdisciplinary field of toxicogenomics may be helpful to understand gene-environment interactions and pathways that are affected by specific chemicals. For example, yeast cells exposed to 2,4-D re-model cell walls that are important in the protection of membranes with multiple functional roles [90]. Further, 2,4-D is shown to give several stress responses in yeast, including signaling pathways, cell growth, nutritional regulation, amino acid depletion and oxidative stress [91].

The 2,4-D has relative low toxicity in aquatic systems. For example, the EC50 for the cyanobacteria *Anabaena* CPB4337 was 25.23 mg/L. When this cyanobacteria was pre-exposed to the surfactant perfluorooctanic acid (PFOA), the toxicity of 2,4-D increased, illustrating the important topic of interacting multiple stressors [92]. In *Daphnia magna*, the LC50/EC50 acute toxicity is relatively low, i.e. in the range 144 – 248 mg/L for 24 h, and 25 mg/L for 48 h, respectively [63, 64].

From the homepage of the Norwegian government (https://www.regjeringen.no/no/sub/eos-notatbasen/notatene/2015/okt/plantevernmiddel---24-d/id2469257/) the following is noted:

"Commission Implementing Regulation (EU) 2015/2033 of 13 November 2015 renewing the approval of the active substance 2,4-D in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011".

Thus, 2.4D is approved for use in Norway.

Summary:

- Three herbicide genes are inserted into stacked maize MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9
- Glufosinate ammonium is banned in Norway due to health and environmental issues.
- Glyphosate, glufosinate ammonium and 2, 4-D have and increased focus due to potential health related effects.

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

Stacked events

The stacked maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 contains nine inserted transgenes providing herbicide tolerance towards three different herbicides, and 6 transgenes providing resistance towards certain Lepidoptera and Coleoptera insect species.

Stacking, in this context, means the process performed to obtain certain combinations of genes into ONE single plant. This is done to obtain beneficial traits for use in agriculture, such as pest management, weed management and to avoid disease.

Stacks are combinations of several, single parental events and should be regarded as new events, as the combination itself in the stack is unique. The combinations of the gene-cassettes are 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. The potential for synergistic effects of transgenic proteins has also been described by Kramer et al [93] from Syngenta where they look at new approaches for risk assessment of stacked events.

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 [46, 47]. There has also been investigations of whether stacking have effects on the maize proteome [94]. Here, results indicate that the levels of transcripts were affected by the stacking per se (seemingly), with changes in protein profiles that needs further investigation.

Molecular characterization (Section 2. p20 and onwards).

The stacked event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 has been made by traditional breeding methods. Thus, MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 contains all the inserted transgenes from each of the parental events.

Sequence information

The applicant states that no new genetic modifications have been inserted in the stacked variant, because it has been produced by traditional breeding techniques. They verify the presence of the genes from the individual events, by using southern blot analysis with the same probes as in the single events. The positive control for the probes are plasmids and not the appropriate single event.

There is no evidence to support the claim that the events in the stacks are identical in the single events. Southern blot probes only prove the presence of parts of the inserted genes. There is no sequence information of the stacked events provided/or that easily can be analyzed.

To verify that the inserted genes are identical to the parental GM variant, the applicant used restriction enzyme analysis and southern blots. While southern blots are a great start, they do not give information on the genes, their sequence information or how the final GM variant differs or not in sequence composition or layout from the starting varieties.

The applicant also provides sequence data from parental events, but in form of blast alignments and not as fasta files or similar file formats. Fasta files are necessary to use as input in bioinformatics analysis to compare sequences from parental and final GM variant. The applicant does not provide any sequence date for the final GM stacked variant, hence no comparison is possible and the claim that the flanking regions and sequence integrity is identical in parent-offspring plants, is unsubstantiated.

The importance of sequence information cannot be stressed enough. For instance, the well documented events of MON810 and GTS 40-3-2, both show unexpected sequence variations (deletions, duplications, insertions etc) from the original plasmid used in the transformation [95, 96].

MON89034 also has, by the applicants own account, a 10 bp insertion and a 57 bp deletion when inserted into the single event background. By crossing, there is no guarantee that there are not any additional changes as the applicant only has verified that the size of the inserted genes are similar to similar genes residing on plasmids. By the applicant own account, these genes are not 100% identical (see technical dossier MON89034).

Analysis of the flanking regions

The flanking regions are key to detection and to ORF prediction. Loss of stop signals or alternative starting signals may indicate that unintentional ORFs may be expressed. The applicant indicates that the parental strains are unlikely to produce any new proteins from ORFs, but no analysis on the stacked GM maize is included. Combined with no provided sequence information, it is impossible to draw any conclusions on the appearance of any potential ORFs.

Southern blot analysis of the stacked trait was compared to those of the single parental events to analyze their structure and organization.
Results from each single parental event indicated that they were the same as for the stacked trait.

In the dossier the probe size ranges from 359bp to 1977 bp. 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. The best probe is one that approximates the size of the target sequence and does not exceed approximately 500 nucleotides in length. Probes that are > 500bp means that point mutations, small deletions and rearrangements that might occur during breeding will possible not be detected [46, 97]. This means that in this case, the applicant failed to account for potential inserts that are only partial, either smaller than the probes or with rearrangements, both of which could prevent binding of the probe and therefor detection of rDNA integrated elsewhere in the genome [98].

Cauliflower Mosaic Virus (CaMV) 35S promoter

At least three of the parental events (1507, MON88017 and 59122) have a 35S CaMVpromoter present to drive the transgene expression. This promoter is commonly used for driving transgene expression in the genetically engineered (GE) crop plants that have been commercialized so far [99-101]. Questions related to the use of the CaMV 35S promoter (P35S) in GM plants has been discussed in an article from Podevin and Du Jardin [102]. In the article, the authors state that some P35S variants contain open reading frames (ORFs) that when expressed could lead to "unintended phenotypic changes". Gene VI encodes the multifunctional P6 protein that can be divided into four domains [103]. Functions of P6 include nuclear targeting [104], viral particle binding and assembly [105], si- and ds-RNA interference and interference suppression [106] and transcriptional transactivation [107, 108]. The main debate when it comes to the use of this promoter is that it may not only be active in plants, but may confer activity with respect to gene expression in lower and higher vertebrates such as mammals and fish. Today there are reports that conclude that the 35S CaMV promoter is active in several eukaryotic cell lines after transfection [99, 101], as well as that the promoter is able to drive expression of a transgene in fish as demonstrated recently by Seternes et al [100]. The potential risk connected to the use of this promoter in GM food/feed cannot be excluded.

Another important issue connected to the use of this promoter, is the issue of transgene silencing. The hypermethylation of CaMV 35S has been investigated by among others Weingold in 2013 [109] and connected to this mechanism. Here, they found that the 35S promoter was hypermethylated in some of the vegetative phases of the plants investigated (here *N.attenuata*) and influencing transgene expression. Thus, the stable and inheritable phenoptypes that is of major importance for the GM plant developers are challenged by epigenetic mechanisms that acts during plant development.

Information on the expression of the inserted/modified sequence (section 2.2.3, p73 and onwards)

Expression of transgenic proteins were investigated in the following tissues: leaf, root, grain, pollen, forage and whole plant. However, only expression data from grain is summarized in the application as it is for import of grain to EU.

Field studies in 10 filed sites made the basis for analysis of transgene expression of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1.
Field were treated with herbicides.

Enzyme-linked immunosorbent assay (ELISA) was used for analyszing each of the expressed transgenes.

All expressed protein levels were as expected across treatments and as compared to the controls (analysis of table 11, p. 77).

Potential interactions between newly expressed proteins/Assessment of potential for interaction between the inserts

Conclusions made of interactions for proteins expressed in MON89034 x 1507 x MON88017 x 59122 [110] is also transferable to the event in this application according to the applicant. This is based on the different mode of action between the proteins.

Bioinformatic analysis of MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 maize grain

Analysis of flanking, junction and insert DNA sequences

To demonstrate that no new genetic modification (in sequence and orientation) was introduced in the stacked trait line obtained from traditional breeding crosses of independent, genetically modified single event lines, the applicant provided the restriction map of single insertion sites present in the genomes of MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 (figures 16, 8, 14, 10 and 12; dossier pp 48- 56). Using southern blot analysis, the authors show that the restriction pattern for each of the independent single events is the same as what was obtained in the stacked trait line (figures 17 – 27; dossier pp 57 – 67). Further, the insert and flanking DNA sequences and the six in frame translation of the single trait expression cassette were queried against DNA and protein databases using BLASTn, BLASTnEST and BLASTx, The sequence analysis showed that there is no disruption of known endogenous genes or regulatory elements due to DNA integration in MON 89034, 1507, MON 88017, 59122 and DAS-40279-9. Based on the result of the southern blot hybridization patterns and sequence analysis of flanking/insert regions in the single trait lines, the applicant surmised that no new genetic modification occurred in the combined trait product and "the *conclusions of the single lines can be directly extrapolated to the conclusions on the stack and all its sub-combination, independently of their origin"* (dossier p.73).

Comment

The applicant did not provide the sequence data of the flanking, junction and insert sequences of the stacked events (five in total) present in the genome of the combined trait line (MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9). The MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 maize is the maize line for which authorization is being sought for and not the single event lines used in producing it by conventional breeding. Although the hybridization patterns from the southern blot suggest that the structure and orientation of the single line events are similar to the stacked, southern blot does not provide sequence

information, thus it is incorrect to use hybridization patterns as surrogate for sequence data. The sequence data that were provided and used for analysis were from the five single event lines but not from the stack. Just like what was done with the southern blots, the applicant should provide the sequence data of the five stacked events in MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 and compare it to respective, independent single events in the genome of MON 89034, 1507, MON 88017, 59122 and DAS-40278-9. Such analysis is essential in order to confirm that the position and orientation of the insert/flanking sequences in the genomes of the single trait lines is identical to that of the stacked trait maize.

Since the applicant did not provide the sequence data showing that the five stacked events in the genome of MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 is identical to the corresponding single events in the genomes of MON 89034, 1507, MON 88017, 59122 and DAS-40278-9 respectively, "the *conclusions of the single lines can be directly extrapolated to the conclusions on the stack and all its sub-combination, independently of their origin"* is not supported by the data provided. Thus, it is unknown whether new genetic modification was introduced to the genome of the stacked event maize following conventional crosses of five genetically modified single maize lines. We advise that the applicant provide a genetic map of the stacked events in the genome of MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9, and a comparative sequence analysis of each inserted trait in the stacked maize to corresponding inserts (trait) in the single maize lines.

In silico evaluation of expressed proteins for potential toxicity

To evaluate the potential toxicity of expressed proteins, the amino acid sequences of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1 proteins expressed in MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 maize were queried for sequence similarity to know protein toxins using BLASTp search against an up-to-date GenBank non-redundant protein database. All the nine proteins have no significant sequence homology to any known protein toxin that is harmful to human and animals.

Comment

The method used is in accordance with the recommendation of the EFSA GMO panel (ref EFSA 2011a in the dossier). The conclusion by the applicant is supported by the data provided. However, the method employed relies on similarity matches to annotated known proteins and annotation is based on already characterized proteins rather than functional characterization of the query protein [111]. This homology search method should be corroborated with alternative in *silico* prediction methods like the machine learning approaches [112]. Since the nine proteins are expressed in the stacked line (PAT expressed from two independent expression cassettes), the applicant is advised to concatenate the amino acid sequences and query the protein database with the concatenated sequence and subject the concatenated sequence to machine learning approaches. Overall, the *in silico* analysis for potential toxicity for each of the individual proteins is convincing, but the method did not address the potential for toxicity due to the combinatorial effect and the potential interaction between the proteins in double and multiple combinations to each other as well as to other proteins. The applicant may consider an *in silico* analysis of protein-protein interaction (PPI) [113] in order to exclude the possibility that no PPI

relevant to toxicity exists between the nine expressed proteins, and between any of the nine proteins to other proteins present in humans and animals.

In silico evaluation of expressed proteins for potential allergenicity

To evaluate the potential allergenicity of expressed proteins, the amino acid sequences of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1 proteins expressed in MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 maize were queried for sequence similarity to know and putative allergens in an up-to-date FARRP allergen databse All the nine proteins have no significant sequence homology to any known and putative protein allergen that is harmful to human and animals.

Comment

Allergens that have not been reported or not yet included in the FARRP allergen database will be missed (see comment on toxicity). *In silico* analysis of the potential allergenicity of the proteins due to combinatorial effect and/or PPI was not presented in the dossier. Thus, potential allegenicity of the proteins due to combinatorial effect and/or PPI cannot be excluded. However, *in silico* analysis for each of the single protein is in accordance with EFSA guidelines and evidence was presented to show that the amino acid sequences of each of the nine proteins have no significant homology with known and putative allergens curated in the FARRP allergen database.

In silico evaluation of expressed proteins for potential adjuvanticity

Bioinformatic analysis of amino acid sequences of Cry1A.105, Cry2Ab2, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, PAT, CP4-EPSPS and AAD-1 proteins expressed in MON 89034 X 1507 X MON 88017 X 59122 X DAS-40278-9 maize returned no significant alignment with any know protein adjuvant.

Comment

As already commented above in case of toxicity and allegenicity, the evidence provided support the conclusion that each of theses individuals proteins are probably not adjuvants in themselves but it is not shown in the dossier whether or not the combinatorial effect of these proteins or their interaction may have adjuvantic effect

Main summary

Maize event MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9 is tolerant to herbicides containing glyphosate, glufosinate ammonium and 2, 4-D that has different 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.

The herbicide glufosinate ammonium is banned for use in Norway.

Living maize lines of event 1507 is prohibited for import to Norway: stacks of events as MON89034 x 1507 x MON88017 x 59122 x DAS-40278-9, containing 1507, would also be expected to be under this prohibition.

References.

1. Safety, N.S.C.f.F., *Final health and environmental risk assessment of genetically modified maize MON89034*, in *VKM report*. 2014, Norwegian Scientific Committee for Food Safety: Oslo. p. 93pp.
2. Safety, N.S.C.f.F., *Food/feed and environmental risk assessment of insect resistant genetically modified maize 1507 for cultivation, import, processing, food and feed uses under Directive 2001/18/EC and Regulation (EC) No 1829/2003 (C/ES/01/01, C/NL/00/10, EFSA/GMO/NL/2004/02)*. 2014, Norwegian Scientific Committee for Food Safety. p. 141pp.
3. (VKM), N.S.C.f.F.S., *Final health and environmental risk assessment of genetically modified maize MON 88017*. 2016, VKM. p. 107.pp.
4. (VKM), N.S.C.f.F.S., *Food/feed and environmental risk assessment of insect-resistant and herbicide-tolerant genetically modified maize 59122 from Pioneer Hi-Bred/Mycogen Seeds for food and feed uses, import and processing under Regulation (EC) No 1829/2003 (EFSA/GMO/NL/2005/12)*, in *Opinion of the Panel on Genetically Modified Organisms of the Norwegian Scientific Committee for Food Safety*. 2014, VKM: VKM. p. 95p.
5. Safety, N.S.C.f.F., *Foreløpig helse- og miljørisikovurdering av genmodifisert mais DAS-40278-9 fra Dow AgroSciences LLC (EFSA/GMO/NL/2010/89)*. 2011, Norwegian Scientific Committee for Food Safety. p. 40pp.
6. European Food Safety, A., *Application (Reference EFSA-GMO-NL-2007-37) for the placing on the market of the insect-resistant genetically modified maize MON89034, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto.* EFSA Journal, 2008. **6**(12): p. 909-n/a.
7. Organisms, E.Panel o.G.M., et al., *Scientific opinion on an application for renewal of authorisation for continued marketing of maize 1507 and derived food and feed submitted under Articles 11 and 23 of Regulation (EC) No 1829/2003 by Pioneer Overseas Corporation and Dow AgroSciences LLC.* EFSA Journal, 2017. **15**(1): p. e04659-n/a.
8. European Food Safety, A., *Application (Reference EFSA-GMO-CZ-2005-27) for the placing on the market of the insect-resistant and herbicide-tolerant genetically modified maize MON88017, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Monsanto.* EFSA Journal, 2009. **7**(5): p. 1075-n/a.
9. Miljødepartementet, K.o., *Endring av forskrift om forbud mot omsetning i Norge av bestemte genmodifiserte produkter*. 2017, Regjeringen.no: Klima og Miljødepartementet. p. 12.
10. Environment, M.o.C.a., *Gene Technology Act*, in *Act of 2 April 1993 No. 38 Relating to the Production and Use of Genetically Modified Organisms, etc. (Gene Technology Act)*, M.o.C.a. Environment, Editor. 1993, Government.no: Oslo.
11. European Commission, *Responsible Research and Innovation. Europe's Ability to Respond to Societal Challenges*. 2012, Available from: ec.europe.eu: KI-31-12-921-EN-C.

12. Hoven, J.v.d., *Options for strengthening Responsible Research and Innovation. Report of the Expert Group in the State of the Art in Europe on Responsible Research and Innovation.* 2013, Available from: ec.europe.eu: KI-NA-25-766-EN-C.
13. Strand, R., et al., *Indicators for promoting and monitoring Responsible Research and Innovation. Report from the Expert Group on Policy Indicators for Responsible Research and Innovation*. 2015, Available from: ec.europe.eu: KI-NA-26-866-EN-N.
14. Hartley, S., et al., *Essential Features of Responsible Governance of Agricultural Biotechnology.* PLoS Biol, 2016. **14**(5): p. e1002453.
15. Pavone, V., J. Goven, and R. Guarino, *From risk assessment to in-context trajectory evaluation-GMOs and their social implications.* Environmental Sciences Europe, 2011. **23**(1): p. 1.
16. Binimelis, R. and A.I. Myhr, *Inclusion and Implementation of Socio-Economic Considerations in GMO Regulations: Needs and Recommendations.* Sustainability, 2016. **8**(1): p. 62.
17. Van Hove, L. and F. Gillund, *Is it only the regulatory status? Broadening the debate on cisgenic plants.* Environ Sci Eur, 2017. **29**(22).
18. Bioteknologirådet, *Herbicide-resistant genetically modified plants and sustainability*. 2014, Bioteknologirådet: Oslo, Norway.
19. Miljødirektoratet, *Genmodifisert mais 1507. Helhetlig vurdering og anbefaling til vedtak. Available at* http://www.miljodirektoratet.no/Documents/Nyhetsdokumenter/Helhetsvurdering%20og%20anbefaling%20til%20vedtak,%20genmodifisert%20mais%201507%20-%204.%20april%202016.pdf. 2016.
20. GenØk, *Høringsuttalelse av søknad om markedsføring av genmodifisert mais MON89034 x 1507 x NK603 x DAS-40278-9*. 2017, GenØk: Tromsø, Norway.
21. GenØk, *Assessment of the summary of the technical dossier of EFSA/GMO/NL/2015/127 maize event 1507 x MIR162 x MON810 x NK603 under EC regulation1829/2003*. 2016, GenØk: Norway.
22. Vazquez, M.A., et al., *Association between Cancer and Environmental Exposure to Glyphosate.* International Journal of Clinical Medicine, 2017. **8**(02): p. 73.
23. Dill, G.M., et al., *Glyphosate: Discovery, Development, Applications, and Properties*, in *Glyphosate Resistance in Crops and Weeds*. 2010, John Wiley & Sons, Inc. p. 1-33.
24. Benbrook, C.M., *Impacts of genetically engineered crops on pesticide use in the US - the first sixteen years.* Environmental Sciences Europe, 2012. **24**(1): p. 24.
25. Benbrook, C.M., *Trends in glyphosate herbicide use in the United States and globally.* Environmental Sciences Europe, 2016. **28**(1): p. 1-15.
26. Freese, B., *Going Backwards: Dow's 2,4-D-Resistant Crops and a More Toxic Future*, in *Food Safety Review*. 2012: Centre for Food Safety. p. 1-4.
27. United Nations, *Report of the Special Rapporteur on the right to food (General Assembly No. A/HRC/34/48)*. 2017.
28. Bøhn, T., et al., *Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans.* Food chemistry, 2014. **153**: p. 207-215.
29. Dolezel M, M.M., Eckerstorfer M, Hilbeck A, Heissenberger A, Gaugitsch H, *Standardising the Environmental Risk Assessment of Genetically Modified Plants in the EU*. 2009, Umweltsbundesamt GmbH: Bonn, Germany. p. 299.

30. Bugge, A.B. and T.G. Rosenberg, *Fremtidens matproduksjon. Forbrukernes syn på genmodifisert mat: GMO-mat eller ikke?* 2017, Forbruksforskningsinstituttet SIFO: Oslo.
31. Chern, W.S., et al., *Consumer acceptance and willingness to pay for genetically modified vegetable oil and salmon: A multiple-country assessment.* 2003.
32. Grimsrud, K.M., et al., *Consumer attitudes to genetically modified food in Norway.* Journal of Agricultural Economics, 2004. **55**(1): p. 75-90.
33. Stilgoe, J., R. Owen, and P. Macnaghten, *Developing a framework for responsible innovation.* Research Policy, 2013. **42**(9): p. 1568-1580.
34. Hilbeck, A., et al., *No scientific consensus on GMO safety.* Environmental Sciences Europe, 2015. **27**(1): p. 4.
35. Fischer, K., et al., *Social Impacts of GM Crops in Agriculture: A Systematic Literature Review.* Sustainability, 2015. **7**(7): p. 8598.
36. Binimelis, R., *Coexistence of Plants and Coexistence of Farmers: Is an Individual Choice Possible?* Journal of Agricultural and Environmental Ethics, 2008. **21**(5): p. 437-457.
37. Devos, Y., et al., *Coexistence of genetically modified (GM) and non-GM crops in the European Union. A review.* Agronomy for Sustainable Development, 2009. **29**(1): p. 11-30.
38. European Commission, *Commission addresses GM crop co-existence*. 2003, Press Release, IP/03/314: Brussels.
39. Purnhagen, K. and J. Wesseler, *The Principle (s) of Co-existence in the Market for GMOs in Europe: Social, Economic and Legal Avenues*, in *The Coexistence of Genetically Modified, Organic and Conventional Foods*. 2016, Springer. p. 71-85.
40. Herrero, A., R. Binimelis, and F. Wickson, *Just existing is resisting: The everyday struggle against the expansion of GM crops in Spain.* Sociologia Ruralis, 2017.
41. Binimelis, R., F. Wickson, and A. Herrero, *Agricultural Coexistence.* 2016.
42. Herrero, A., F. Wickson, and R. Binimelis, *Seeing gmos from a systems perspective: The need for comparative cartographies of agri/cultures for sustainability assessment.* Sustainability, 2015. **7**(8): p. 11321-11344.
43. Venter, H.J. and T. Bøhn, *Interactions between Bt crops and aquatic ecosystems: A review.* Environmental Toxicology and Chemistry, 2016: p. n/a-n/a.
44. Mensah, P.K., C.G. Palmer, and W.J. Muller, *Lipid peroxidation in the freshwater shrimp Caridina nilotica as a biomarker of Roundup((R)) herbicide pollution of freshwater systems in South Africa.* Water Sci Technol, 2012. **65**(9): p. 1660-6.
45. Love, B.J., M.D. Einheuser, and A.P. Nejadhashemi, *Effects on aquatic and human health due to large scale bioenergy crop expansion.* Science of The Total Environment, 2011. **409**(17): p. 3215-3229.
46. De Schrijver, A., et al., *Risk assessment of GM stacked events obtained from crosses between GM events.* Trends in Food Science & Technology, 2007. **18**(2): p. 101-109.
47. Halpin, C., *Gene stacking in transgenic plants--the challenge for 21st century plant biotechnology.* Plant Biotechnol J, 2005. **3**(2): p. 141-55.
48. Then, C., *Risk assessment of toxins derived from Bacillus thuringiensis—synergism, efficacy, and selectivity.* Environmental Science and Pollution Research, 2010. **17**(3): p. 791-797.

49. Norgaard, K.B. and N. Cedergreen, *Pesticide cocktails can interact synergistically on aquatic crustaceans.* Environ Sci Pollut Res Int, 2010. **17**(4): p. 957-67.
50. Bjergager, M.B., et al., *Synergy in microcosms with environmentally realistic concentrations of prochloraz and esfenvalerate.* Aquat Toxicol, 2011. **101**(2): p. 412-22.
51. Heap, I. *The International Survey of Herbicide Resistant Weeds*. 2017 [cited 2017 15.March]; Available from: http://www.weedscience.org/.
52. Binimelis, R., W. Pengue, and I. Monterroso, *"Transgenic treadmill": Responses to the emergence and spread of glyphosate-resistant johnsongrass in Argentina.* Geoforum, 2009. **40**(4): p. 623-633.
53. Beckie, H.J. and F.J. Tardif, *Herbicide cross resistance in weeds.* Crop Protection, 2012. **35**: p. 15-28.
54. Duke, S.O. and S.B. Powles, *Glyphosate: a once-in-a-century herbicide.* Pest Management Science, 2008. **64**(4): p. 319-325.
55. Bohn, T., et al., *Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans.* Food Chem, 2014. **153**: p. 207-15.
56. Viljoen, C., *Letter to the editor.* Food Chem Toxicol, 2013. **59**: p. 809-10.
57. Cuhra, M., et al., *Glyphosate-Residues in Roundup-Ready Soybean Impair Daphnia magna Life-Cycle.* Journal of Agricultural Chemistry and Environment, 2015. **Vol.04No.01**: p. 13.
58. Cuhra, M., T. Bøhn, and P. Cuhra, *Glyphosate: Too Much of a Good Thing?* Frontiers in Environmental Science, 2016. **4**(28).
59. Guyton, K.Z., et al., *Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate.* The Lancet Oncology, 2015. **16**(5): p. 490-491.
60. European Food Safety, A., *Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate.* EFSA Journal, 2015. **13**(11): p. 4302-n/a.
61. European Food Safety, A., et al., *Setting of an import tolerance for 2,4-D in maize.* EFSA Journal, 2017. **15**(5): p. e04765-n/a.
62. European Food Safety, A., *Reasoned opinion on the review of the existing maximum residue levels (MRLs) for for glufosinate according to Article 12 of Regulation (EC) No 396/2005.* EFSA Journal, 2015. **13**(1): p. 3950-n/a.
63. Lilius, H., T. Hästbacka, and B. Isomaa, *Short Communication: A comparison of the toxicity of 30 reference chemicals to Daphnia Magna and Daphnia Pulex.* Environmental Toxicology and Chemistry, 1995. **14**(12): p. 2085-2088.
64. Toussaint, M. and B. Hanse, *Solid glyphosate compositions and their use*. 1995, Google Patents.
65. Cuhra, M., T. Traavik, and T. Bøhn, *Life cycle fitness differences in Daphnia magna fed Roundup-Ready soybean or conventional soybean or organic soybean.* Aquaculture Nutrition, 2015. **21**(5): p. 702-713.
66. Giesy, J.P., S. Dobson, and K.R. Solomon, *Ecotoxicological Risk Assessment for Roundup® Herbicide*, in *Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews*, G.W. Ware, Editor. 2000, Springer New York: New York, NY. p. 35-120.

67. Blackburn, L.G. and C. Boutin, *Subtle effects of herbicide use in the context of genetically modified crops: a case study with glyphosate (Roundup).* Ecotoxicology, 2003. **12**(1-4): p. 271-85.
68. Axelrad, J.C., C.V. Howard, and W.G. McLean, *The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon.* Toxicology, 2003. **185**(1-2): p. 67-78.
69. Benachour, N., et al., *Time- and dose-dependent effects of roundup on human embryonic and placental cells.* Arch Environ Contam Toxicol, 2007. **53**(1): p. 126-33.
70. Dallegrave, E., et al., *The teratogenic potential of the herbicide glyphosate-Roundup in Wistar rats.* Toxicol Lett, 2003. **142**(1-2): p. 45-52.
71. Benbrook, C., *Impacts of Genetically Engineered Crops on Pesticide Use in the United States: The First Thirteen Years*, in *Critical Issue Report*, T.O. Center, Editor. 2009, The Organic Center: The Organic Center. p. 69.
72. Malatesta, M., et al., *Ultrastructural morphometrical and immunocytochemical analyses of hepatocyte nuclei from mice fed on genetically modified soybean.* Cell Struct Funct, 2002. **27**(4): p. 173-80.
73. Solomon, K.R. and D.G. Thompson, *Ecological risk assessment for aquatic organisms from over-water uses of glyphosate.* J Toxicol Environ Health B Crit Rev, 2003. **6**(3): p. 289-324.
74. Ono, M.A., et al., *Inhibition of Paracoccidioides brasiliensis by pesticides: is this a partial explanation for the difficulty in isolating this fungus from the soil?* Med Mycol, 2002. **40**(5): p. 493-9.
75. Marc, J., et al., *Pesticide Roundup provokes cell division dysfunction at the level of CDK1/cyclin B activation.* Chem Res Toxicol, 2002. **15**(3): p. 326-31.
76. Richard, S., et al., *Differential Effects of Glyphosate and Roundup on Human Placental Cells and Aromatase.* Environmental Health Perspectives, 2005. **113**(6): p. 716-720.
77. Guyton, K.Z., et al., *Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate.* The Lancet Oncology. **16**(5): p. 490-491.
78. Hung, D.Z., *Diffused Brain Injury in Glufosinate Herbicide Poisoning*, in *North American Congress of Clinical Toxicology Annual Meeting*. 2007, Clinical Toxicology: New Orleans, Lousiana. p. 605-48.
79. Watanabe, T. and T. Sano, *Neurological effects of glufosinate poisoning with a brief review.* Hum Exp Toxicol, 1998. **17**(1): p. 35-9.
80. Calas, A.G., et al., *Chronic exposure to glufosinate-ammonium induces spatial memory impairments, hippocampal MRI modifications and glutamine synthetase activation in mice.* Neurotoxicology, 2008. **29**(4): p. 740-7.
81. Laugeray, A., et al., *Pre- and Postnatal Exposure to Low Dose Glufosinate Ammonium Induces Autism-Like Phenotypes in Mice.* Frontiers in Behavioral Neuroscience, 2014. **8**: p. 390.
82. Matsumura, N., et al., *Glufosinate ammonium induces convulsion through N-methyl-D-aspartate receptors in mice.* Neurosci Lett, 2001. **304**(1-2): p. 123-5.
83. Schulte-Hermann, R., et al., *Analysis of reproductive toxicity and classification of glufosinate-ammonium.* Regul Toxicol Pharmacol, 2006. **44**(3 Suppl 1): p. S1-76.

84. European Food Safety, A., *Conclusion regarding the peer review of the pesticide risk assessment of the active substance glufosinate.* EFSA Journal, 2005. **3**(4): p. 27r-n/a.
85. Munro, I.C., et al., *A Comprehensive, Integrated Review and Evaluation of the Scientific Evidence Relating to the Safety of the Herbicide 2,4-D.* Journal of the American College of Toxicology, 1992. **11**(5): p. 559-664.
86. Mullison, W.R., *Environmental fate of phenoxy herbicides.* Food and Agriculture Organization of the United Nations, 1987.
87. Gorzinski, S.J., et al., *Acute, pharmacokinetic, and subchronic toxicological studies of 2,4-dichlorophenoxyacetic acid.* Fundam Appl Toxicol, 1987. **9**(3): p. 423-35.
88. Garabrant, D.H. and M.A. Philbert, *Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology.* Crit Rev Toxicol, 2002. **32**(4): p. 233-57.
89. Guha, N., et al., *O04-3 IARC working group meta-analysis of 2,4-d exposure and the risk of NHL.* Occupational and Environmental Medicine, 2016. **73**(Suppl 1): p. A8-A8.
90. Viegas, C.A., et al., *Yeast adaptation to 2,4-dichlorophenoxyacetic acid involves increased membrane fatty acid saturation degree and decreased OLE1 transcription.* Biochem Biophys Res Commun, 2005. **330**(1): p. 271-8.
91. Teixeira, M.C., P. Duque, and I. Sa-Correia, *Environmental genomics: mechanistic insights into toxicity of and resistance to the herbicide 2,4-D.* Trends Biotechnol, 2007. **25**(8): p. 363-70.
92. Rodea-Palomares, I., et al., *Effect of PFOA/PFOS pre-exposure on the toxicity of the herbicides 2,4-D, Atrazine, Diuron and Paraquat to a model aquatic photosynthetic microorganism.* Chemosphere, 2015. **139**: p. 65-72.
93. Kramer, C., et al., *Evolution of risk assessment strategies for food and feed uses of stacked GM events.* Plant Biotechnol J, 2016. **14**(9): p. 1899-913.
94. Agapito-Tenfen, S.Z., et al., *Effect of stacking insecticidal cry and herbicide tolerance epsps transgenes on transgenic maize proteome.* BMC Plant Biology, 2014. **14**(1): p. 346.
95. Rosati, A., et al., *Characterisation of 3' transgene insertion site and derived mRNAs in MON810 YieldGard maize.* Plant Mol Biol, 2008. **67**(3): p. 271-81.
96. Windels, P., et al., *Characterisation of the Roundup Ready soybean insert.* European Food Research and Technology, 2001. **213**(2): p. 107-112.
97. Fagard, M. and H. Vaucheret, *(TRANS)GENE SILENCING IN PLANTS: How Many Mechanisms?* Annu Rev Plant Physiol Plant Mol Biol, 2000. **51**: p. 167-194.
98. Kononov, M.E., B. Bassuner, and S.B. Gelvin, *Integration of T-DNA binary vector 'backbone' sequences into the tobacco genome: evidence for multiple complex patterns of integration.* Plant J, 1997. **11**(5): p. 945-57.
99. Myhre, M.R., et al., *The 35S CaMV plant virus promoter is active in human enterocyte-like cells.* European Food Research and Technology, 2006. **222**(1): p. 185-193.
100. Seternes, T., et al., *A plant 35S CaMV promoter induces long-term expression of luciferase in Atlantic salmon.* Scientific Reports, 2016. **6**: p. 25096.
101. Vlasak, J., et al., *Comparison of hCMV immediate early and CaMV 35S promoters in both plant and human cells.* J Biotechnol, 2003. **103**(3): p. 197-202.

102. Podevin, N. and P. du Jardin, *Possible consequences of the overlap between the CaMV 35S promoter regions in plant transformation vectors used and the viral gene VI in transgenic plants.* GM Crops Food, 2012. **3**(4): p. 296-300.
103. Li, Y. and S.M. Leisner, *Multiple domains within the Cauliflower mosaic virus gene VI product interact with the full-length protein.* Mol Plant Microbe Interact, 2002. **15**(10): p. 1050-7.
104. Haas, G., et al., *Nuclear import of CaMV P6 is required for infection and suppression of the RNA silencing factor DRB4.* Embo j, 2008. **27**(15): p. 2102-12.
105. Himmelbach, A., Y. Chapdelaine, and T. Hohn, *Interaction between cauliflower mosaic virus inclusion body protein and capsid protein: implications for viral assembly.* Virology, 1996. **217**(1): p. 147-57.
106. Shivaprasad, P.V., et al., *The CaMV transactivator/viroplasmin interferes with RDR6-dependent trans-acting and secondary siRNA pathways in Arabidopsis.* Nucleic Acids Res, 2008. **36**(18): p. 5896-909.
107. Kobayashi, K. and T. Hohn, *The avirulence domain of Cauliflower mosaic virus transactivator/viroplasmin is a determinant of viral virulence in susceptible hosts.* Mol Plant Microbe Interact, 2004. **17**(5): p. 475-83.
108. Palanichelvam, K. and J.E. Schoelz, *A comparative analysis of the avirulence and translational transactivator functions of gene VI of Cauliflower mosaic virus.* Virology, 2002. **293**(2): p. 225-33.
109. Weinhold, A., M. Kallenbach, and I.T. Baldwin, *Progressive 35S promoter methylation increases rapidly during vegetative development in transgenic Nicotiana attenuata plants.* BMC Plant Biology, 2013. **13**: p. 99-99.
110. Organisms, E.P.o.G.M., *Scientific Opinion on application (EFSA-GMO-CZ-2008-62) for the placing on the market of insect resistant and herbicide tolerant genetically modified maize MON 89034 × 1507 × MON 88017 × 59122 and all sub-combinations of the individual events as present in its segregating progeny, for food and feed uses, import and processing under Regulation (EC) No 1829/2003 from Dow AgroSciences and Monsanto.* EFSA Journal, 2010. **8**(9): p. 1781-n/a.
111. Franceschi, N., et al., *Predictive Protein Toxicity and Its Use in Risk Assessment.* Trends Biotechnol, 2017. **35**(6): p. 483-486.
112. Gupta, S., et al., *In silico approach for predicting toxicity of peptides and proteins.* PLoS One, 2013. **8**(9): p. e73957.
113. Kotlyar, M., et al., *In silico prediction of physical protein interactions and characterization of interactome orphans.* Nat Methods, 2015. **12**(1): p. 79-84.