Vår ref:2017/H_132
Deres ref:2017/2793

Høringsuttalelse av søknad om markedsføring av genmodifisert soya DAS-81419-2 x DAS-44406-6

EFSA/GMO/NL/2016/132

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
Mai 2017

Vår ref:2017/H_132
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Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 10.05.2017

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2016/132**, genmodifisert, stablet soya DAS-81419-2 x DAS-44406-6, fra Dow AgroSciences Europe, 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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Søknad EFSA/GMO/NL/2016/132 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 *2mepsps*, mot glufosinat ammonium via det innsatte genet *pat*, og mot 2, 4-D via det innsatte genet *aad-12*.

I tillegg er soyaen resistent mot larver fra Lepidoptera ordenen via insatte gener *cry1Fv3* og *cry1Ac*.

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

Iht «International Service for the Aquisiton of Agri-Biotech Applications» (ISAAA) er følgende regulatoriske godkjennelser gitt for foreldrelinjen DAS-81419-2, DAS-44406-6 og den stablede soyaen i denne søknaden DAS-81419-2 x DAS-44406-6 internasjonalt:

Tabell 1: Regulatoriske godkjennelser for DAS-81419-2.

Country	Food direct use or processing	Feed direct use or processing	Cultivation domestic or non-domestic use
Argentina	2016	2016	2016
Australia	2014		
Brazil	2016	2016	2016
Canada	2014	2014	2014
Japan	2017	2017	2017 *
Mexico	2015		
New Zealand	2014		
South Korea	2016	2016	
Taiwan	2015		
United States of America	2014	2014	2014

* point mouse arrow over year for notes

Last updated: March 30, 2017
(kilde: http://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=339)

Tabell 2: Regulatoriske godkjennelser for DAS-44406-6

Country	Food direct use or processing	Feed direct use or processing	Cultivation domestic or non-domestic use
Argentina	2015	2015	2015
Australia	2013		
Brazil	2015	2015	2015
Canada	2013	2013	2013
Colombia	2016		
Japan	2014	2015	2015 *
Mexico	2014		
New Zealand	2013		
South Africa	2013	2013	
South Korea		2014	
Taiwan	2014 *		
United States of America	2014	2014	2014

* point mouse arrow over year for notes

Last updated: August 11, 2016
(kilde: http://www.isaaa.org/gmapprovaldatabase/event/default.asp?EventID=345)

Tabell 3: Regulatoriske godkjennelser for DAS-81419-2 x DAS-44406-6

Country	Food direct use or processing	Feed direct use or processing	Cultivation domestic or non-domestic use
Argentina	2016	2016	2016
Taiwan	2016		

Last updated: March 1, 2017

Oppsummering

GenØk–Senter for biosikkerhet, viser til høring av søknad EFSA/GMO/NL/2016/132 om DAS-81419-2 x DAS-44406-6 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 DAS-81419-2 x DAS-44406-6 er ikke godkjent i Norge eller EU for noen av de omsøkte bruksområdene.
- DAS-81419-2 x DAS-44406-6 er tolerant mot sprøytemidler som inneholder glyfosat, glufosinat - ammonium og 2, 4-D som har ulike grader av helse-og-miljø fare ved bruk.
- Glufosinat ammonium er ikke tillatt brukt i Norge.
- Søknaden om soya linje DAS-81419-2 x DAS-44406-6 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/2016/132 on DAS-81419-2 x DAS-44406-6 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 DAS-81419-2 x DAS-44406-6 is not approved for any application in Norway or the EU.
- Soy event DAS-81419-2 x DAS-44406-6 is tolerant to herbicides containing glyphosate, gluphosinate ammonium and 2, 4-D that has distinct health and environmental dangers upon use.
- It is not allowed to use gluphosinate ammonium in Norway.
- The application on soy event DAS-81419-2 x DAS-44406-6 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

Application on EFSA/GMO/NL/2016/132

The stacked event DAS-81419-2 x DAS-44406-6 soy contains genes providing herbicide tolerance *(2mepsps, pat and aad-12*). In addition, it contains genes providing resistance to Lepidoptera-insects (*cry1Fv3* and *cry1Ac*).

Previous evaluations

The Norwegian Food Safety Authority (VKM) has not risk assessed this stack previously.

The Norwegian Biotechnology Advisory board has commented on the application for the parental line DAS-44406-6 in 2013 (1) with several questions directed to the Applicant. These are questions, among others, regarding:

- Health related issues related to the use of herbicides.
- If the use of this soy will change the frequency, concentrations and time of spraying
- If the proteins used for the toxicity studies are from the event itself or from the bacteria they originally were produced in
- Resistance development issues in the areas were the soy is to be produced and what measures are made to slow down resistance development in other plants than the soy itself.
- Questions related to the farmers cultivating it (economy, health, systems present to prevent spread of the soy to non-modified soy etc),

These are questions we find relevant for the present application on the stacked soy event DAS-81419-2 x DAS-44406-6.

The Norwegian Biotechnology Advisory Board has also commented on the application for the parental line DAS-81419-6 in 2014 (2), where they refer to their report on "Herbicide resistant genetically modified plants and sustainability" (3) for issues to consider for the applicant. In this report, they highlight the following issues for decisions on contributions to sustainability:

- Environmental/ecological issues: effect on non-target organisms, additive/synergic effects of many herbicides applied at the same time and in the same area, development of resistant weed, if antibiotic resistance genes are present etc.
- Economic and societal issues: prohibition of the herbicide used, long-term effects on health of farmers using herbicides, farmer training in use of protective equipment, issues related to replanting of seed, freedom of choice for cultivation in the future etc.
- Serious issues grounding rejection in cases of: presence of antibiotic resistance genes, not possible to perform independent risk research, herbicide used is prohibited in Norway, international treaties deciding on prohibiton of herbicide(s) etc.

These are issues that also should be highlighted for the present application.

EFSA has previously evaluated the parental line DAS-81419-2 for food and feed uses, import and processing (4). The same has been done for parental line DAS-44406-6 (5). They have concluded that the soy events DAS-81419-2 and DAS-44406-6 is as safe and nutritious as conventional soy.

GenØk- Centre for biosafety has previously commented on DAS-81419-2 in 2014 and on DAS-44406-6 in 2013.

For the application on DAS-81419-2 (EFSA/GMO/NL/2013/116), the following points were highlighted from the application:

- *The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically challenging and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.*
- *The regulator is encouraged to address the potential of non-target effects of Bt toxins*
- *The regulator is encouraged to ask the Applicant to demonstrate the lack of interactive effects between transgenic proteins through proper scientific testing and evidence gathering.*
- *The regulator is encouraged to ask the Applicant to state the minimum level above which the expressed proteins are undesirable and what comparators are used.*
- *The regulator is encouraged to ask the Applicant to explain the implications of the Cry1Ac partial fragments and the deletion of parental locus in the light of the assumed substantial equivalence to the parental comparator.*
- *The regulator is encouraged to ask the Applicant to use the real plant versions of the proteins for the safety assessments as plants and bacteria differ in their post-translational processing of proteins. This should be considered and further analysed.*
- *The regulator is encouraged to ask the Applicant to analyze for other meaningful post-translational modifications. If glycosylation is the only PTM relevant for risk assessment, it should be clearly stated in the dossier.*
- *The regulator is encouraged to ask the Applicant to also analyze the entire soybean proteome for PTMs.*
- *The regulator is encouraged to ask the Applicant to also include molecular weight markers on gels for size determination.*
- *The regulator is encouraged to ask the Applicant to provide more recent/updated data for the proteolytic cleavage of synpro Cry1Ac and Cry1F proteins.*
- *The regulator is encouraged to ask the Applicant to perform repeated dose toxicity studies with the exact versions of the synpro proteins applied for in this application and not refer to data from old and sequencence wise potentially different Cry proteins.*
- *The regulator is encouraged to ask the Applicant to perform acute oral toxicity studies with the actual synpro proteins in combination and also a whole GM plant feeding study as these proteins are expressed in a new context*
- *The regulator is encouraged to ask the Applicant to be clear on whether the homology to known allergens are checked for the Cry protein parts derived from the subspecies of Bacillus Thuringiensis.*

- *The regulator is encouraged to ask the Applicant to submit required information on the social utility of **DAS-81419-2 soybean** and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*
- *The regulator is encouraged to ask the Applicant to submit required information on the social utility of **DAS-81419-2 soybean** and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*

For the application on DAS-44406-6 (EFSA/GMO/NL/2012/106), the following points were highlighted from the application:

- *The regulator is encouraged to ask the Applicant to extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.*
- *The regulator is encouraged to ask the Applicant to re-design the probes in order to have a set of smaller ones and re-design the strategy for the restriction enzymes.*
- *The regulator is encouraged to ask the Applicant to conduct generational sequencing studies.*
- *The regulator is encouraged to ask the Applicant to specify whether it is the plant or the microbially derived protein that is used in the analysis.*
- *The regulator is encouraged to ask the Applicant to use newly expressed proteins from real field studies and clarify whether the soy was sprayed or not.*
- *The regulator is encouraged to ask the Applicant to provide western blots with visible standard so that it is possible to interpret size data. Also, some of the blots should have been exposed more to visualize additional bands better.*
- *The regulator is encouraged to ask the Applicant to include herbicide treated soya in the animal experiments, and analyze the residue level of the herbicides and their metabolites.*
- *The regulator is encouraged to ask the Applicant to consider that we find that it would be ethically incongruous and a double standard of safety for Norway to ban the use of these herbicides domestically as a health concern, but support its use in other countries.*
- *The regulator is encouraged to ask the Applicant to submit required information on the social utility of DAS-444Ø6-6 and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*

Social utility and sustainability issues on the stacked soy event DAS-81419-2 x DAS-44406-6 (EFSA/GMO/NL/2016/132)

In Norway, an impact assessment follows the Norwegian Gene Technology Act (NGTA) (6) in addition to the EU regulatory framework for GMO assessment. In accordance with the aim of 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 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) (7). 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 (8-13).

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 14). 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 soy DAS-81419-2 x DAS-44406-6 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.

Sustainability

The soy DAS-81419-2 x DAS-44406-6 contains a modified *2mepsps* gene that confers increased tolerance to herbicides that contain 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 pages 26-31) as well as in villages in areas where glyphosate is systematically used as part of the GM crops tolerance to glyphosate (15). 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 (16-19). As soy DAS-81419-2 x DAS-44406-6 is genetically modified to possess 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 soy DAS-81419-2 x DAS-44406-6. An increase in the resistance and use of glyphosate is in contrast to a contribution to sustainable development and therefore an important aspect the applicant should provide information on, for example by mentioning the current use of glyphosate in the sites of cultivation and what approaches are used to minimize the use of glyphosate.

Herbicide-resistant genes

When an 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. The development of management strategies to make sure that this does not create (more) resistant weed is 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. 20), 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 soy DAS-81419-2 x DAS-44406-6 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 aspects to evaluate the ethical justifiability. Furthermore, this soy is cultivated in Argentina, where glyphosate resistant weeds have increased significantly[1]. However, the field trials of the soy have taken place in the USA, not Argentina. 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 in Argentina. 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 in the future is assessed, as the applicant considers information on this not relevant because soy DAS-81419-2 x DAS-44406-6 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.

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 (21). 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 DAS-81419-2 x DAS-44406-6 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 DAS-81419-2 x DAS-44406-6 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

[1] http://weedscience.org/Summary/Country.aspx Status of Herbicide Resistance in Argentina, accessed on May the 5th 2017.

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 soy are not in country as its planned cultivation.

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 soy DAS-81419-2 x DAS-44406-6 needs to demonstrate how it will benefit Norway. However, no information on this part is provided by the applicant. It is important to evaluate how GM crops in general, GM soy in particular, and the use of GM soy 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 soy. 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 (22). Nevertheless, the empirical data available on the attitude of Norwegian citizens towards GM approaches and applications remain limited (e.g. 23, 24) and more empirical research on this is warranted 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 are 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 (11, 25) as a way to reflect on what kind of society we want, and then assess how certain (biotechnological) developments may or may not contribute to shaping a desired future. Thus, in order to evaluate whether soy DAS-81419-2 x DAS-44406-6 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 (26). 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. (27) 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. Although this study focused on Bt-maize, 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.

Even though more empirical information is warranted, there is some information available about the socio-economic aspects of GM cultivation in Argentina, where soy DAS-81419-2 x DAS-44406-6 is cultivated. An article by Leguizamón in 2014 (28) analysing the contribution of GM soy in Argentina on socio-economic aspects (i.e. labour and rural depopulation, agricultural deskilling, distribution of land, protection of indigenous and small peasant communities, increase of violence related to landgrabs, herbicide-sprays over rural populations or food sovereignty) and environmental aspects (i.e. expansion of the agrocultural frontier, deforestation, biodiversity, nutrient depletion and soil structure degradation), concludes that although the massive adoption of GM soy has provided important economic revenues, *"the GM soy-based agro-export model as currently configured in Argentina is a socially and ecologically unsustainable model of national development"* (28). Although there is an important controversy, similar conclusions have been also reached by other authors for the case of Argentina and Brazil (e.g. 29, 30-35). Given this information and the increasingly recognized importance of socio-economic aspects of GM crops, it is striking that none of the above mentioned points is recognized or 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 soy DAS-81419-2 x DAS-44406-6 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 for co-existence in the countries intended for cultivation of soy DAS-81419-2 x DAS-44406-6 and minimize the likelihood for gene flow to wild relatives, or contamination during transport or processing. Currently, the applicant describes no strategies to prevent contamination. Indeed, they stress that soy DAS-81419-2 x DAS-44406-6 *"is as safe and nutritious as conventional soy"* and that therefore the GM soy *"will be packaged, transported, handled and used in the same manner as the commercial soybean products"* (page 4 of the summary of the application). However, even if this soy is as safe and nutritious as conventional soy that does not mean that contamination would not matter as this could be a significant problem for non-GM farmers. Furthermore, legal information and clarity could provide 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). 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 as soy DAS-81419-2 x DAS-44406-6 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. 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.

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 justifiable, benefit to society and sustainability assessment, as well as a correction of the outdated information. An important part that is lacking is information about the consequences of the cultivation of soy DAS-81419-2 x DAS-44406-6 for the producing countries and how the sites of field trials relate to the sites of cultivation. Furthermore, the information provided by the Applicant must be relevant for the specific agricultural context of these countries and should also stress the need for information on integrated weed management strategies (43). Moreover, the information should contain 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, share of the benefits among sectors of the society and as explained, effects of co-existence of different agricultural systems. Furthermore, soy DAS-81419-2 x DAS-44406-6 is tolerant to gluphosinate-ammonium which

is banned for use in Norway. Banning the use of gluphosinate-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. Additionally, the applicant does not attempt to demonstrate a benefit to society, a reference of the consumers' attitude on GM soy, or the demand within Norway for soy DAS-81419-2 x DAS-44406-6 and does therefore not provide sufficient information as required by the NGTA.

Table 1: Questions to the applicant

Sustainability	*How does the cultivation of soy DAS-81419-2 x DAS-44406-6 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 a potential increase of resistant weeds distributed and what strategies are in place to compensate this?
	How do the sites of the field trial relate to the proposed sites for cultivation? What are the differences and how may these affect the adequacy of the assessment of the field trials?
Benefit to society	*Is soy DAS-81419-2 x DAS-44406-6 available for further breeding and research? If so, under which circumstances?*
	Is there a demand for soy DAS-81419-2 x DAS-44406-6 in Norway?
	Does soy DAS-81419-2 x DAS-44406-6 contribute to business development and value creation in Norway, including new job opportunities?
Assessing alternatives	*Will soy DAS-81419-2 x DAS-44406-6 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 soy DAS-81419-2 x DAS-44406-6 within Norway and how does the development of soy DAS-81419-2 x DAS-44406-6 relates to these?*
	Does the development, introduction or use of soy DAS-81419-2 x DAS-44406-6 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 soy DAS-81419-2 x DAS-44406-6 and how does this change their autonomy, practice and position compared to other stakeholders?*
	Does soy DAS-81419-2 x DAS-44406-6 change the power dynamic among stakeholders? If so, how?
	Can the development, introduction or use of soy DAS-81419-2 x DAS-44406-6 create significant ruptures or ecological relationships?
Co-existence	*Does the cultivation of soy DAS-81419-2 x DAS-44406-6 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

Soy is not cultivated in Norway due to climate related issues and there are no comparable 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 in Norway.

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

Stacked events

The stacked soy event DAS-81419-2 x DAS-44406-6 contains five inserted transgenes providing herbicide tolerance towards three different herbicides and two transgenes providing resistance towards certain Lepidopteran species. This stack could 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 combination of inserted 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. 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 (44, 45).

Cry proteins

The DAS-81419-2 x DAS-44406-6 soy combines two Bt proteins named Cry1Fv3 and Cry1Ac. These proteins, also called Bt-toxins are claimed to be safe, and the EFSA GMO Panel Working Group on Animal Feeding Trials has gone through a number of studies (published) where GM crops with Bt have been used (ref). In this overview they have concluded that the majority of studies showed no adverse effects (46).

The potential of non-target effects of Bt toxins have also been investigated, including alternative modes of action for Cry toxins that has been addressed previously (47-50).
Two meta-analyses of published studies on non-target effects of Bt-proteins in insects, (Lövei and Arpaia (51) in relation to non-target and environmental effects, documented that 30% of studies on predators and 57% of studies on parasitoids display negative effects to Cry1Ab transgenic insecticidal proteins.
Further, Cry toxins and proteinase inhibitors have shown non-neutral effects on natural enemies, and both negative and positive effects (52).
A review by Hilbeck and Schmidt (50) on Bt-plants, found 50% of the studies documenting negative effects on tested invertebrates.
Additionally, a review by van Frankenhuyzen (53) indicated that several Cry proteins exhibit activity outside of their target orders. This study also found that many Cry proteins only had been tested with a very limited number of organisms: thus, activity outside of the target organisms of many Cry proteins may be undocumented simply because testing has not included sensitive organisms. As not every potentially sensitive species can be tested for sensitivity to Bt toxins, it cannot be excluded that sensitive species have been overlooked in testing until now. The issue is complicated further by the number of variables which can affect toxicity testing, which may include toxin preparation and purification, life stage of the specimens, differences in toxin expression hosts, as well as solubilization (or lack thereof) of the toxin, among other factors (54).

A quantitative review analysis based on 42 field experiments showed that unsprayed fields of Bt-transgenic maize plants have significantly higher abundance of terrestrial non-target invertebrates than sprayed conventional fields (55). Thus, Bt-plants with a single Bt-gene inserted may represent an improvement for non-target organisms in the environment. However, an indication of some negative effects of the Cry1Ab toxin itself, or the Cry1Ab maize plant, on non-target abundance was shown in the same meta-analysis: when conventional (non-GM) fields were not sprayed, the non-target abundance was significantly higher than in the Bt-fields. Research on aquatic environments with emphasis on the impact of Bt-crops on aquatic invertebrates including *Daphnia magna* (47)and caddisflies (56)has also been performed. Given the potential load of Cry toxins (also in combination with herbicides) that may end up in aquatic environments, further studies are warranted. Douville et al (57)presented evidence of the persistence of the *cry1Ab* transgene in aquatic environments: more than 21 days in surface waters, and 40 days in sediments. A follow-up on this study in 2009 indicated possible horizontal gene transfer of transgenic DNA fragments to aquatic bacteria (58). Impacts on soil microflora and fauna, including earthworms (59), mychorizzal fungi (60)and microarthropods in response to Cry endotoxins have also been reported (61-63). The significance of tri-trophic effects of accumulation, particularly of insecticidal Cry toxins (64, 65) is, however, yet to be firmly established. It has been demonstrated that sub-chronic dosages of Cry proteins may affect both foraging behavior and learning ability in non-target bees (66), and may have indirect effects on recipient populations, and, given the key-stone role of bees as pollinators, on both primary production and on entire food-webs.

The use of multiple, related transgenes in a single (stacked) event may accelerate resistance development to both transgene products. This was the experience of Baxter et (67) who tested the effect of using broccoli plants containing Cry1Ac, Cry1C or both, on resistance development in a population of diamondback moths (*Plutella xylostella*). They found that the use of similar Cry proteins in stacks, in close proximity to single gene events led to accelerated resistance development to both traits. Bravo and Soberón (68) commented on this effect, acknowledging that gene stacking is not a universal solution to resistance development towards Cry proteins. Studies such as these ask the question as to whether the stacked use of related Cry proteins, such as Cry1Ab and eCry3.1Ab, in the same event is advisable.

In relation to health impacts, a publication by Dona and Arvanitoyannis (69) reviews the potential health implications of GM foods for humans and animals, including incidences and effects of increased immunogenicity, amounts of anti-nutrients, possible pleiotropic and epigenetic effects, including possible reproductive and developmental toxicity. They conclude that while there is evidence for health concerns on many fronts, the exposure duration have not been long enough to uncover important effects.
A study in mice showed that exposure to purified Cry1Ab resulted in specific anti-Cry1Ab IgG1 and IgE production, indicating inherent immunogenicity and allergenicity. Further, mice exposed to leaf extracts from both MON810 and unmodified maize demonstrated influx of lymphocytes and eosinophils in the broncho-alveolar lavage,and increased cytokine release in mediastinal lymph node cells (70). Further studies should also include animals with immune-deficiencies and/or animals exposed to other stress agents simultaneously.

Molecular characterization

For a full description of the molecular characterization of DAS-81419-2 and DAS-44406-6 the applicant refers to the applications for authorization in the EU of DAS-81419-2 (EFSA-GMO-NL-2013-116) and DAS-44406-6 (EFSA-GMO-NL-2012-106). GenØk has previously commented on DAS-81419-2 in 2014 and on DAS-44406-6 in 2013.

The key findings on DAS-81419-2 (H_116, 2014)

Conclusions (Page 76) and Table 5 (Page 70)

(1) The conclusion on the bioinformatics analysis of the flanking boarder sequences is limited to linear sequence comparisons. This will not reveal all possible potential similarity in structure and function known allergenic or toxic proteins because only sequence identity and not similarity was reported.

(2) The conclusion that "Based on the above, no unintended changes were identified" (second paragraph, Page 76), overlooks that inserted partial fragments of Cry1Ac as well as the deletion of 57 bp of parental locus (see Page 69), can constitute unintended effects.

Summary:

- Sequence similarity data should also be reported alongside sequence identity data
- Applicant should explain the implications of the Cry1Ac partial fragments and the deletion of parental locus in the light of the assumed substantial equivalence to the parental comparator.

The key findings from 2013 on DAS-44406-6 (H-106)

Information relating to the GM plant

1) The size of some probes used in the Southern Blot analysis is considered too long (RB7 probe – 1010bp; 2mEPSPS probe – 1712bp; Histone promoter probe – 1516bp; AtUbi10 Promoter probe – 1313bp; aad-12 probe- 882bp; AtuORF1 UTR probe – 799bp; Ori-Rep probe – 1087; Backbone 2 probe – 1714bp; Backbone 1 probe- 1254bp; SpecR probe – 795bp; and also all the probes used for the southern blot studies covering the small gaps (Poorbaugh,J. 2011,Study ID#101947, Dow AgroSciences, unpublished)). The size of probes can have an effect of the detected result and lead to false negative results since the strength of the interaction between probe and target is based on the number of bonds that form between the single strand od DNA (probe) and the matching recombinant DNA (target). A long probe that binds perfectly to a short fragment will not bind strongly and might be washed of depending on the stringency of the wash.
2) Most of the Southern blot results showed clear results and with a molecular weight marker visible. However, some of the blots had very weak bands, which could be explained by the use of long probes. The best probe is one that approximates the size of the target sequence and does not exceed approximately 500 nucleotides in length.
3) For southern blot studies, the probes were designed to bind in only a single fragment generated by the restriction enzymes. The probes could have been designed to bind also in the restriction site, allowing it to bind in two different fragments. Thus, this strategy would be able to confirm the strength of interaction between the probe and the target. A set of different restriction enzymes could have been used.
4) The promoter used for the *pat* gene expression cassette is the viral sequence from the Cassava vein Mosaic Virus (CsVMV), a virus from the genus Caulimovirus, the same genus as the Cauliflower Mosaic Virus (CaMV). Scientists recently reported the overlap between CaMV 35S promoter regions (P35S) and the viral gene VI (71). The authors state that some P35S variants contain open reading frames that when expressed could lead to "unintended phenotypic changes. In light of these new findings, the present viral sequence should be examined carefully to exclude possible overlaps with other viral genes.
5) The sequencing studies were conducted only with plants from one generation. Since this analysis is not able to detect small rearrangements, sequencing analysis should have been conducted as well.
6) The electropherograms for the sequencing studies are not available therefore is not possible to check the quality of the sequences.

Summary:

- Extend the molecular characterization of the event by examining the possibility for different RNA variants, fusion proteins and partial expression of P6.
- Re-design the probes in order to have a set of smaller ones and re-design the strategy for the restriction enzymes.
- Include generational sequencing studies.

The applicant claims that there is a low likelihood of molecular interactions between the different inserts and, therefore, low likelihood of any changes in the molecular characteristics of the inherited inserts in **DAS-81419-2xDAS-44406-6** soybean (e.g. copy number, insert number, absence of backbone DNA and integrity of the individual inserts).

The applicant choose to not repeat the laboratory analysis of the full stack, which makes it difficult to say something about any unintended effects in this part.

An analytical confirmation of the presence of the two inserts in the combined product **DAS-81419-2xDAS-44406-6)** was performed by Southern blot analysis (1.2.2.2 p. 26 and appendixes). Here, the quality of the SB are good.

The soy stack DAS-81419-2xDAS-44406-6

There is no scientific literature available on the genetic construct and genetic stability of the stacked event in question in order to make an appropriate scientific evaluation. The applicant should therefore provide information on the stability of the insert over multiple generations as well as compositional data and expression analyses over all growing seasons

We expect that the analyzes performed by the applicant should be of high scientific quality which also could meet the requirements for publication in peer reviewed and well-known international journals
This application reflects the trend with stacked events with tolerance against several selective herbicides, which means that besides evaluating the potential risks arising from the genetic modification, it is also important to address possible concerns when it comes to changes in herbicide/pesticide management.

Other comments relevant for the assessment of the current application

This soy stack contains two *pat* genes. One from each parental line. According to the Applicant, *pat* is used as a selection marker. However, the plants can be sprayed with the intended herbicide gluphosinate-ammonium, as the gene is present in the stack also after the selection procedure.

As expected, the level of pat expression in the stack DAS-81419-2 x DAS-44406-6 is higher than in the parental events (Technical dossier, p.50-51), although the Applicant says they are similar (Techical dossier, p.52):

"Based on the results of the study, it is concluded that expression of the AAD-12, PAT and 2mEPSPS proteins in the single events DAS-81419-2 and DAS-44406-6 is similar to expression in the stacked event DAS-81419-2 × DAS-44406-6".

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

The key findings from our previous risk assessment of parental event DAS-81419-2 in 2014:

Expression levels of inserted sequences of *Cry1Ac*, *Cry1Fv3* and *PAT* were analyzed in soy grain using ELISA. The applicant is not stating whether the level of expression is good / sufficient for the different proteins.
Applicant states "In addition, expression levels of the newly expressed proteins, *Cry1Ac*, *Cry1F* and *PAT*, were characterized and presented a relatively low SD across sites" (page 76), but did not state level of the insert gene product that is undesirable or the standard used as a comparator.

Summary: The minimum level above which the expressed proteins of are undesirable should be clarified; the Applicant should also state what comparators are used.

Expression levels in the soy stack DAS-81419-2 x DAS-44406-6

For the soy stack DAS-81419-2 x DAS-44406-6 the Applicant has provided data for protein expression levels in grain, as the Application in the EU area not is for cultivation.

Soy plants subjected to expression analysis were cultivated in 10 field sites across US representing diversity in environmental as well as agronomic conditions/practices. Sprayed and unsprayed parental lines (DAS-81419-2 and DAS-44406-6) as well as the stack itself (DAS-81419-2 x DAS-44406-6), were cultivated in the 2012 season together with a non-transgenic control line.

The measured levels of Cry1F, Cry1Ac, AD-12, PAT and 2mEPSPS were within the expected sample-sample/site-site variability according to the Applicant.

Effects of processing

As soy is processed into a range of different food and feed products, it was investigated whether there was a difference between processed DAS-68416-4 x DAS-44406-6 and control soybean.
According to the Applicant, there is no difference in composition between these two.
The proteins Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS were investigated during industrial processing and the proteins tertiary structure were degraded during this process.
The proteins were found to be heat labile.
Thus, further toxicology analysis was not performed due to this.

Microbial versus plant derived proteins

The Applicant refers to analysis performed in the single, parental lines of the stack Das-81419-2 x DAS-44406-6 for safety evaluations of the expressed proteins. Thus, we have gone through these as well as we refer to our previous comments in our assessments of the parental lines.

Equivalence of microbially-derived proteins to DAS-81419-2 Soybean expressed proteins.

The statement in the 2nd paragraph, line 6 "There was no evidence of any post-translational modifications (PTMs) (i.e. glycosylation) of the DAS-81419-2 Soybean-derived Cry1F protein" is misleading because only glycosylation was determined. Applicant did not analyze

for other post-translational modifications. In addition, only the expressed protein was checked for PTMs. The entire proteome of the plant was not analyzed for potential PTMs.
This applies to the Cry1Ac and PAT proteins also.
The SDS-PAGE gel on p.161 in the dossier with microbial and plant version of the *Cry1F* protein lack molecular weight marker for the glycoprotein stained gel. This is also the case for the SDS-PAGE gel with the different versions of *Cry1Ac* (p.179). It is thus difficult to interpret sizes of proteins.

Also, the Applicant refers to old data (Study ID#GH-C 5508, Dow AgroSciences, 2006) for the data on Western blot analysis. Data newer than the ones generated in 2006 should preferably be presented to support risk assessment of a transgenic plant meant for human consumption.

The unidentified peptides found in the MS spectra for *Cry1F* and *Cry1Ac* protein should have been discussed for potential biological relevance. The Applicant states that these peptides do not indicate that the protein is different from the predicted amino acid sequence: however, no data are provided to support this statement.

The *PAT* protein has been assessed at several occasions previously. The figure text of figure 56 in the dossier (p.194) states that the molecular weight markers used in the western blot with microbial and plant version of the protein was applied AFTER the development of the film. This can cause mistakes and it is recommended to use pre-stained/labeled markers that are following the whole development process. The bands with nonspecific binding should have been further analyzed by MS for protein identification.

Summary:

- The Applicant should analyze for other meaningful post-translational modifications. If glycosylation is the only PTM relevant for risk assessment, it should be clearly stated in the dossier.
- The Applicant should also analyze the entire soybean proteome for PTMs.
- The Applicant should include molecular weight markers on gels for size determination.
- The Applicant should use plant version of the protein for the risk assessments.

The proteins are subjected to heat and pH treatment and proteolytical cleavage. For the proteolytical cleavage data the Applicant refer to data from 2001. Newer data should have been provided with the synpro proteins used in this event of soy. It cannot be assumed from the text whether this is the case.
Synergistic effect of microbial version of *Cry1Ac* and *Cry1F* was not found by the analysis performed.
No repeated dose toxicity studies were performed due to the data provided on equivalence, history of safe use, no additive/synergistic/antagonistic effects or structural similarities to proteins with adverse effect on health. However, this should have been done due to the old references used on other versions of these proteins (seemingly) and the fact that the Cry proteins are made with sequences from different subspecies *of Bacillus Thuringiensis*.

Acute oral toxicity data lacks the combination of the transgenic proteins for evaluation of acute oral toxicity and a whole food/feed study with the whole GM plant is not provided as the Applicant does not find it necessary. This should have been performed as this stacked soy event with the synpro Cry proteins are meant for human as well as animal consumption.

Summary:

- The Applicant should provide more recent/updated data for the proteolytic cleavage of synpro *Cry1Ac* and *Cry1F* proteins.
- The Applicant should perform repeated dose toxicity studies with the exact versions of the synpro proteins applied for in this application and not refer to data from old and sequencence wise potentially different Cry proteins.
- The Applicant is encouraged to perform acute oral toxicity studies with the actual synpro proteins in combination and also a whole GM plant feeding study as these proteins are expressed in a new context.

Toxicity and allergenicity

Toxicity

The soy stack DAS-68419-6 x DAS-44406-6 expresses the proteins Cry1F, Cry1Ac, AAD-12, PAT and 2mEPSPS.
The toxicology assessment of these proteins are based on their previous history of safe use, similarity to known toxins and potential to exert acute toxicity on mammals, low concentration and rapid digestion in simulated digestive fluids.

No new toxicological tests were warranted by the Applicant based on previous risk assessments of the expressed proteins in other occasions (Technical report, p.134).

Allergenicity

Proteins Cry1Ac, Cry1F, 2mEPSPS, PAT and AAD-12 have been tested for their allergenic potentialand not considered to be allergenic based on the following:

- There are no indications of altered levesl of allergens or expression of new allergens.
- The proteins are from non allergenic sources, lack structural similarity to known allergens, are present in small amounts and are rapidly digested in simulated gastric fluids

It is however not clear from the technical dossier if the proteins have been analysed as they are exressed in the stack DAS-81419-4 x DAS-44406-6 or if this is based on analysis of microbially expressed proteins analyzed during assessments of the single parental events.

Adjuvancy effects

In the adjuvancy evaluation (section 1.5.3, p.170 in Technical dossier), the Applicant write that there is no sequence similarities to known protein adjuvants and that none of the newly expressed proteins are expected to act as such.

There is also no references to Cry proteins as potential adjuvants. However, the potential adjuvancy of Cry proteins has previously been addressed by the GMO Panel of the Norwegian

Scientific Committee for Food Safety (72). In addition, scientific studies have shown that the Cry1Ac protein is a potent systemic and mucosal adjuvant (73). In the evaluation of a GM maize, MIR604 x GA21, the panel found that it was difficult to evaluate if kernels from this stack would cause more allergenic reactions than kernels from unmodified maize. The Panel continues with: *"As the different Cry proteins are closely related, and in view of the experimental studies in mice, the GMO Panel finds that the likelihood of an increase in allergenic activity due to Cry1Ab and mCry3A proteins in food and feed from maize Bt11 x MIR604 x GA21 cannot be excluded. Thus, the Panel's view is that as long as the putative adjuvant effect of Cry1Ab and mCry3A with reasonable certainty cannot be excluded, the applicant must comment upon the mouse studies showing humoral antibody response of Cry1A proteins and relate this to a possible adjuvant effect of the Cry1Ab and mCry3A proteins expressed. Furthermore, although Cry1Ab and mCry3A proteins are rapidly degraded in gastric fluid after oral uptake, there is also the possibility that the protein can enter the respiratory tract after exposure to e.g. mill dust. Finally, rapid degradation is no absolute guarantee against allergenicity or adjuvanticity" (Norwegian Scientific comitee for Food Safety (2013), Evaluation of EFSA/GMO/UK/2007/48).*

We also agree with these concerns and highlight them for the present stack of soy event DAS-81419-2 x DAS-44406-4 and that this potentially might be the case for the Cry proteins expressed in this stack.

Summary:

- There is a potential for non-target effects by cry proteins that needs to be addressed, especially in the context of their combined use in a stacked event.
- The regulator is encouraged to ask the Applicant to consider the possibility of cross-resistance development to multiple Cry proteins due to the use of stacked events,a s well as the potential for cry proteins as adjuvants.

Hazard identification

No hazards detected by the Applicant in either assessment (health, environment).

Herbicides

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 (18).

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 is linked to resistance evolution in weeds. At present, 36 species of weeds are documented to be glyphosate resistant on a global scale (74). 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 (30). Crop and herbicide monoculture makes the agroecosystem more vulnerable to further resistance development (75).

For 2,4-D, 32 species of weeds are shown to be resistant, and five of these (16%) were documented after 2015 (74).
For gluphosinate-ammonium, six species of weeds are shown to be resistant and 50 % of these were discovered after 2015 (74).

Sustainability

For the farmers, resistant weeds are a difficult obstacle to handle. However, evolution of resistance is the process by which it develops. Therefore, more research should be performed on the plurality of responses that can be done with integrated pest management, not only to delay resistance but to promote alternative and preferably non-toxic pest control systems (UN). Chemical treatment coupled with the unavoidable resistance development are major blocking factors to a sustainable agriculture. The accelerated use seen for glyphosate used on glyphosate tolerant GM plants can be expected to happen for *any* herbicide used as co-technology for HT GM plants, indicating that HT GM plants are not sustainable.

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 (18), which directly or indirectly will expose non-target biodiversity in terrestrial, soil and aquatic communities (76), represent a major source of environmental pollution.

Accumulating herbicide residues and health effects

Glyphosate accumulates in HT soybeans, more when the plant is sprayed later in the season (43). This may bring significant amounts of glyphosate into the food and feed chain. Bøhn and colleagues measured on average 9.0 mg of glyphosate in HT GM soybeans grown in Iowa (77).

Clearly, HT GM plants with tolerance to 2, 4-D, gluphosinate ammonium or other herbicides may serve as a vector for these chemicals into the global food and feed chains.

There is an increased awareness of the potential toxicity of glyphosate. The volume used is also increasing. However, 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 (78). This set of events has been termed "The Glyphosate Paradox" (79). The WHO/IARC categorization of glyphosate as *probably carcinogenic to humans (80)*, although disputed by EFSA (81), is underlining the significance of the controversy around the glyphosate-based herbicides.

2, 4-D was by WHO/IARC in 2015 classified as a *possible carcinogen to humans* (82).

Therefore, what we may see the starting point of is the replacement of glyphosate with other herbicides, of which 2, 4-D and dicamba are likely candidates. Given such development, the toxicity and non-target effects of herbicides that eventually replace glyphosate becomes more important.

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 (83).
Given that 2, 4-D and dicamba (and other herbicides) may replace or add to the role of glyphosate, such modeling studies may have to be re-calibrated with a new attention to the concentration of these chemicals.

The chemical 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 can be found in different chemical forms: as acid (basic form), inorganic salts, amines or esters (84). 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.

Studies of toxicity in aquatic systems/organisms

The herbicide 2, 4-D has relative low toxicity in aquatic systems. For example, the EC_{50} 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 (85).

Studies in Daphnia

In *Daphnia magna*, the LC_{50}/EC_{50} acute toxicity is shown in the range 144 – 248 mg/L for 24 h, and 25 mg/L for 48 h, respectively (86, 87).
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. (77) 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 (78, 88). 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 *2mepsps* gene present in DAS-81419-2 x DAS-44406-6 soy 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 (43, 89). However, glyphosate has recently received more risk-related attention due to its potential for negative effects on both aquatic and terrestrial ecosystems (90), as well as from studies in animals and cell cultures that have indicated possible negative health effects in rodents, fish and humans (91-93).

It has also been shown that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (94).

A number of publications indicate unwanted effects of glyphosate on health (93, 95), aquatic (96) and terrestric (90, 97) organisms and ecosystems. Also, a study of Roundup (containing glyphosate as the active ingredient) effects on the first cell divisions of sea urchins (98) 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 (91) 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 (99). 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 cancer (IARC) released a report indicating that glyphosate is a "probably carcinogenic to humans"(100) an issue that is under debate.

Gluphosinate ammonioum tolerance

The stacked soy event DAS-81419-2 x DAS-44406-6 contains the *pat* gene from *Streptomyces viridochromogenes* from both parental events. This gene provide the soy plant with tolerance to herbicides containing gluphosinate-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. Gluphosinate ammonium is harmful by inhalation, swallowing and by skin contact. Serious health risks may result from exposure over time. Effects on humans and mammals include potential damage to brain, reproduction including effects on embryos, and negative effects on biodiversity in environments where gluphosinate ammonium is used (101-104). EFSA has concluded on the risk of gluphosinate ammonium, as especially harmful to mammals (105).

2, 4-D tolerance

The *aad-12* gene provides 2, 4-D (dichlorophenoxy) and arylphenoxypropionate tolerance in the soy stack DAS-81419-2 x DAS-44406-6. This herbicide has negative effects on the endocrine and immune system, and is thought to might have a role in cancer as well as affecting reproductively (http://www.pesticideinfo.org/Detail_Chemical.jsp) (see also the page 29).

From the homepage of the Norwegian government,[2] 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, 4-D is approved for use in Norway.

Summary:

- Soy event DAS-81419-2 x DAS-44406-6 is tolerant to glyphosate, gluphosinate ammonium and 2, 4-D. These herbicides are damaging to health and environment in different ways.

[2] https://www.regjeringen.no/no/sub/eos-notatbasen/notatene/2015/okt/plantevernmiddel---24-d/id2469257/

- Potential for accumulation of the herbicides should be considered in GM plants used in food and feed.

Main summary

Soy event DAS-81419-2 x DAS-44406-6 is tolerant to herbicides containing glyphosate, gluphosinate ammonium and 2, 4-D 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(6).

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