Vår ref:2017/H_136
Deres ref: 2017/5067

Høringsuttalelse av søknad om markedsføring av genmodifisert bomull DAS-81910-7

EFSA/GMO/NL/2016/136

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
Juni 2017

Vår ref:2017/H_136
Deres ref: 2017/5067

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 27.06.2017

Vedlagt er innspill fra GenØk – Senter for biosikkerhet på offentlig høring av søknad **EFSA/GMO/NL/2016/136**, genmodifisert bomull DAS-81910-7, fra Dow AgroSciences LLC, under EU forordning 1829/2003. Søknaden gjelder bruksområdene mat, fôr, import og prosessering.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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Innhold/Content

Vår ref:2017/H_136
Deres ref: 2017/5067

Høringsuttalelse – genmodifisert bomull DAS-81910-7 (EFSA/GMO/NL/2016/136) under EU forordning 1829/2003.

Søknad EFSA/GMO/NL/2016/136 omhandler genmodifisert bomull til bruksområdene mat, for, import og prosessering.

Den genmodifiserte bomullen har toleranse mot herbicider som inneholder glufosinat ammonium via det innsatte genet *pat*, og mot 2,4-D via det innsatte genet *aad-12*.

Bomull DAS-81910-7 er ikke godkjent for noen av bruksområdene i Norge eller EU.

Oppsummering

GenØk–Senter for biosikkerhet, viser til høring av søknad EFSA/GMO/NL/2016/136 om DAS-81910-7 bomull som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra denne bomullen.

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

- Genmodifisert bomull DAS-81910-7 er ikke godkjent i Norge eller EU for noen av de omsøkte bruksområdene.
- Genmodifisert bomull DAS-81910-7 er tolerant mot sprøytemidler som inneholder 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 bomull linje DAS-81910-7 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/2016/136 on DAS-81910-7 cotton 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 cotton event DAS-81910-7 is not approved for any application in Norway or the EU.
- Cotton event DAS-81910-7 is tolerant to herbicides containing glufosinate ammonium and 2, 4-D that has distinct health and environmental dangers upon use.
- It is not allowed to use glufosinate ammonium in Norway.
- The application on cotton event DAS-81910-7 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

Application on EFSA/GMO/NL/2016/136

Through *Agrobacterium tumefaciens* mediated plant transformation, cotton (*Gossypium hirsutum*) has been inserted with genes providing multiple herbicide tolerance. The resulting event, DAS-81910-7 contains genes that provides tolerance to herbicides 2,4-D (*aad-12* gene) and glufosinate-ammonium (*pat* gene).

Previous evaluations

Cotton event DAS-81910-7 has not been evaluated in Norway or the European Union previously.

Food Standards Australia New Zealand has evaluated cotton event DAS-81910-7 for food purposes (1, 2) with no concerns relating consumption as compared to conventional cotton.

GenØk has not evaluated this cotton event previously.

Social utility and sustainability issues on the cotton event DAS-81910-7, EFSA/GMO/NL/2016/136

In Norway, an impact assessment follows the Norwegian Gene Technology Act (NGTA) (3) 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) (4). 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 (5-10).

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 11). 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 cotton DAS-81910-7 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

Cotton DAS-81910-7 contains a modified *aad-12* gene that confers increased tolerance to herbicides that contain 2,4-D. When an herbicide 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, as is the case with DAS-81910-7, 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. 12), 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 cotton DAS-81910-7 could affect the emergence of herbicide resistance in weeds, nor if there are cases of this in the requested areas for cultivation of the variety, which are also important aspect to evaluate the ethical justifiability.

Furthermore, the applicant aims to cultivate DAS-81910-7 in the USA, Canada, Mexico, Australia, New Zealand, Korea and Brazil. In all these countries, herbicide resistant weeds has been located and the amount of herbicide resistant weeds has increased significantly[1]. Although the Applicant claims that the location of the six field trials in the US provide a variety of environmental conditions, no argumentation or justification is documented how this may suffice, differ and / or relate to the potential sites of cultivation. 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. The Applicant considers information on this not relevant as DAS-81910-7 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

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 (13, 14). 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 DAS-81910-7 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 criterion 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 cotton DAS-81910-7 would be 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 in relation to the field trials. The difference between the field trials and the sites where DAS-81910-7 will eventually be cultivated can affect the adequacy of the evaluation of DAS-81910-7. It is therefore important that the Applicant provides information on how the difference between the site of field trials and future cultivation sites may affect the evaluation of DAS-81910-7.

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 cotton DAS-81910-7 needs to demonstrate how it will benefit Norway. However, no information on this part is provided by the Applicant. Indeed, the Applicant state that this cotton will replace cotton in existing food and feed products. It is therefore important

[1] http://weedscience.org/Summary/Country.aspx Status of Herbicide Resistance, accessed on 18 May 2017.

to evaluate how GM crops in general, GM cotton in particular, and the use of GM cotton in food and feed are valued by Norwegian consumers. This information will contribute to the anticipation of impacts at an early stage, as well as that it may demonstrate a need to assess the alternative options for import of cotton. Although the empirical data available on the attitude of Norwegian citizens towards GM is limited (e.g. 15, 16) and more empirical research on this is warranted to investigate consumers' attitude, demand and acceptance, a report 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, and only 15 percent were positive (17). Furthermore, if there are any beneficial characteristics for consumers that would prioritize this cotton over non-GM cotton, it would be useful if the applicant elaborates on that.

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 cotton) for import, alternative sources to satisfy the demand, alternative ways of agriculture, or even explore alternative life visions. In fact, this corresponds with the increased trend within research and policy of science and innovation to anticipate impacts, assess alternatives, reveal underlying values, assumptions, norms and beliefs (8, 18) 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 DAS-81910-7 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 cotton 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 (19). 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 organizational 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. (20) concerning social implications from cultivating GM crops found that from 2004 – 2015 there has only been 15 studies corning socio-economic implications of cultivating Bt-maize. The study demonstrates that published literature is dominated by studies of economic impact and conclude that very few studies take a comprehensive view of social impacts associated with GM crops in agriculture. The amount of research performed in this case and the minimal focus on social impacts strongly indicate a high need for further investigation on how the cultivation of GM crops affects different parties involved. It is therefore striking that no information on any of the above mentioned points is discussed by the Applicant.

Co-existence

The cultivation of GM plants in general is causing problems with regard to co-existence, an important socio-economic impact. For instance, Binimelis (21) investigated consequences on co-existence of Bt maize in Spain among small-scale farmer and has found that co-existence is very difficult. Farmers in some areas have even given up growing non-GM maize. Even though the cultivation of cotton DAS-81910-7 is not planned in Europe/Norway, it is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic production. Information about consequences for co-existence in the countries intended for cultivation of DAS-81910-7 should be provided. This information should demonstrate how the Applicant aims to minimize the likelihood for gene flow to wild relatives, or contamination during transport or processing.

Additionally, legal information and clarity could provide evaluators with a more comprehensive understanding of governance strategies and possibilities to ensure co-existence. However, it could be 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 (22). Indeed, although a framework for maintaining co-existence in Europe was established in 2003 (23) this effectively meant technical measurements and recommendations (e.g. cleaning of sowing machines and transport vehicles) and remains challenging in practice (24, 25). 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 (26, 27). 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 cotton DAS-81910-7 is meant to be resistant to glufosinate-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. This also 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. An important part that is lacking is information about the consequences of potential cultivation of cotton DAS-81910-7 for the producing countries. The information provided by the Applicant must be relevant for the specific agricultural context of these country and should also stress the need for information on integrated weed management strategies in those countries (28). 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, cotton DAS-81910-7 is tolerant to glufosinate-ammonium which is banned for use in Norway. Banning the use of glufosinate-ammonium based herbicides domestically due to health and environmental concerns, while indirectly supporting its use in other countries would be ethically ambiguous and goes against the criteria of sustainable development. Additionally, the Applicant does not attempt to demonstrate a benefit to society, a reference of the consumers' attitude on GM cotton, or the demand within Norway for cotton DAS-81910-7 and does therefore not provide sufficient information as required by the NGTA.

Table 1: Questions to the Applicant

Sustainability	*How does the cultivation of DAS-81910-7affect the use of herbicide?*
	How is the current use of herbicide in requested sites of cultivation and what approaches are used to minimize the use of herbicide?
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 DAS-81910-7 available for further breeding and research? If so, under which circumstances?*
	Is there a demand for DAS-81910-7 in Norway?
	Does DAS-81910-7 contribute to business development and value creation in Norway, including new job opportunities?
Assessing alternatives	*Will DAS-81910-7 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 DAS-81910-7 within Norway and how does the development of DAS-81910-7 relates to these?*
	Does the development, introduction or use of DAS-81910-7 contradict ideas about solidarity and equality between people, such as the particular consideration of vulnerable groups in the population?
Socio-economic impacts	*Does DAS-81910-7 affect the seed choice of farmers?*
	Which parties will be affected by the development, introduction or use of DAS-81910-7 and how does this change their autonomy, practice and position compared to other stakeholders?

	Does DAS-81910-7 change the power dynamic among stakeholders? If so, how?
	Can the development, introduction or use of DAS-81910-7 create significant ruptures or ecological relationships?
Co-existence	*Does the cultivation of DAS-81910-7 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

Cotton is not cultivated in Norway due to climatic conditions. Most cotton is cultivated in India, China, US and Egypt.

Loss of gene-modified cottonseed through storage or transport would therefore not involve great risk for spread into the wild.

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

Molecular characterization

Cotton event DAS-81910-7 was made through *Agrobacterium tumefaciens* mediated transformation.
Here, glufosinate was used for selection of transformed cells. Glufosinate-ammonium is also used to control weeds during cultivation.

The inserted genes are *pat* from *Streptomyces viridochromogenes* and *aad-12* from *Delftia acidovorans* encoding the proteins phosphinotricin N-acetyltransferase (PAT) and aryloxyalkanoate dioxygenase-12 (AAD-12). Expression of these proteins provides glufosinate ammonium and 2, 4-D tolerance.

The spectinomycin resistance gene (an antibiotic resistance gene) sequence is not detectable with the probe used during Southern blot. Thus, it is expected that this fragment is not present in the gene modified plant.

Information on the sequences actually inserted/deleted or altered

DNA was isolated from individual plants in 5 distinct generations and used for molecular characterization by Southern blot.

Comments:

- Southern blots mainly have a technically high standard and molecular weight markers are present on each blot.
- The Soutern blot membrane for *SpecR* probe has many small dots and uneven background. The many dots could be due to antibody used for detection of DIG-labelled probe that is not properly dissolved. The uneven background could be due to unequal distribution of buffer/drying of membrane.
- Probes used range in the size from 552bp to 1782bp. 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 off depending on the stringency of the wash.
- 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
- Obtained results indicate expected copy numbers and sizes of inserts.

Organization and sequence of the inserted genetic material at each insertion site

Based on the performed analysis the applicant comment that the insert is stably integrated over generations.

Identification of ORFs within the insert and spanning the junction site

Flanking sequences were analyzed using BLAST. The obtained results indicate no ORFs.

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.

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

Information on the expression of the inserted(s)

Protein expression levels of inserted transgenes *pat* and *aad12* were analyzed in samples isolated from 6 test field sites in 2012. Plants were sprayed and unsprayed with the actual herbicides.
Isolated samples represented different growth stages. Comparator used was the one used for the initial transformation.

Methods used for expression analysis

Expression of AAD-12

A method developed by Dow AgroSciences was used to analyse the expressed level of AAD-12 protein in the isolated cotton samples. This analysis is based on an enzyme linked immunoabsorbent assay (ELISA), a kit from Acadia Bioscience (Study ID 120040.02. Dow AgroSciences LLC).

Expression levels of AAD-12 were as expected and not present in control samples with the method used.

Expression of PAT

Expression of PAT was analyzed by ELISA using a method developed by Dow AgroSciences and a kit from Envirologix, Inc.

The source of the anti-PAT antibody used is not clear and should be present.

Expression levels of PAT were as expected and not present in control samples by the method used by the applicant.

Bioinformatic analysis of DAS-81910-7 cotton

Analysis of insert and flanking boarder DNA sequences

To characterize the genetic modification(s) resulting from the *Agrobacterium* mediated transformation of cotton (coker 310) with pDAB4468, the insert and flanking DNA sequences and the six frame translations were queried against DNA and protein databases using BLASTn and BLASTx respectively. The searches confirmed the integrity of the insert and showed that no endogenous gene or regulatory network was modified as a result of the transformation event that generated DAS-8191Ø-7 (Study 1D: 160280 Guttikonda 2016b in Technical dossier).

Comment

This study failed to identify the chromosomal location of the insert. The applicant should explain why it was concluded that no gene or regulatory network was modified when the site of integration is unknown. What is the chromosomal location of the 159 bp deletion in the parental locus? The applicant should mention the DNA source for region 4. It is not clear that it was obtained from Coker 310 (the non-transgenic control cotton). In addition, the applicant should explain why region 4 is 1861 bp (631 bp + 159 bp + 1071 bp) instead of 2545 bp (1315 bp +159bp+1071bp) (Study 1D: 160280, Table 1, Figure 1). Why is the 5/ boarder 1315 bp in DAS8191Ø-7 but only 631 bp in the parental locus (region 4)? Granted that mobile genetic elements are not considered endogenous genetic elements, their position and orientation in DAS-8191Ø-7 compared to untransformed Coker 310 will give an indication of non-target genetic modification. Region 1 and region 4 showed significant matches to parts of the EF457754.1 genome that contain mobile genetic elements. Region 1 and region 4 mapped to different locations in EF-457754.1. Why is this so? The applicant should clarify the sequence length difference in the 5/ boarder between DAS-8191Ø-7 and untransformed parental control (presumable Coker 310). In conclusion, this study confirms the identity of the insert but answers to the questions raised need explanation or clarification to validate that no endogenous genes/regulatory sequences and mobile genetic elements were modified because of the transformation event that generated DAS-8191Ø-7.

In silico evaluation of AAD-12 and PAT proteins for potential toxicity

Aryloxyalkanoate dioxygenase 12 (AAD-12) and Phospophinothricin N-acetylytransferase (PAT) proteins are expressed by *aad-12* and *pat* genes inserted into DAS-8191Ø-7. AAD-12 is 293 amino acid in length while PAT contains 183 amino acids. To evaluate the potential toxicity of the expressed proteins, the amino acid sequences of AAD-12 and PAT were queried for sequence similarity to know protein toxins using BLASTp search against non-redundant NCBI database (Study ID: 160291 Guttikonda 2016 c in Technical dossier). Both the AAD-12 and PAT has no significant sequence similarity to any known toxin that is harmful to humans and animals.

Comment

The method used is recommended by EFSA GMO panel (29) and the results should be considered as valid. Although this method is recommended by EFSA, it has some obvious limitation. One such limitation is that it relies on similarity matches to annotated known proteins and annotation is based on already characterized proteins rather than functional characterization of the query protein (30) . Thus, this method is prone to both false positive and negative hits. It is advised that the applicant should mention this limitation in the dossier. To complement the results of the sequence similarity based approach used in the dossier, the applicant should consider using *in silico* prediction methods based on machine learning approaches (31).

In silico evaluation of AAD-12 and PAT proteins for potential allergenicity

The amino acid sequences of AAD-12 and PAT were compared with 1897 known and putative allergens and celiac-induction proteins in the FARRP allergen database (Study ID: 160288 and Study ID: 160290 Guttikonda 2016 e and f in Techincal dossier). The result show that AAD-12 and PAT does not share any significant sequence similarity with known protein allergens.

Comment

Allergens that have not been reported or not in the FARRP databse may be missed (see comment on toxicity). However, taken into consideration that the donor organisms for AAD-12 and PAT have never been shown to be allergenic, it is unlikely that the expressed AAD-12 and PAT in DAS-8191Ø-7 are allergenic. Thus, we agree with the conclusion made by the applicant.

Toxicity and allergenicity

Cottonseed undergoes processing procedures before the intended use. For food and feed purposes, the actual products are oil, meal and linters.

Toxicological assessment (section 1.4 in Technical dossier, p.112 onwards).

The safety of transgenic proteins PAT and AAD-12 was investigated through the following procedures:

- Biochemical characterization (mode of action, heat lability, equivalence to microbial versions of proteins to those expressed in cotton).
- Toxicity assessment (history of safe use of donor, aa sequence analysis of PAT and AAD-12, analysis of flanking and insert sequences through bioinformatics, toxicity assessment to mammals).
- Feeding study, 90 days.

Due to previous evaluations of PAT by EFSA this protein is considered safe.

The AAD-12 protein has not been evaluated by EFSA before, but is under review. It has been evaluated by Food Standards NewZealand Australia (FSANZ) (32) and considered to be safe.

An acute oral toxicity study was performed according to OECD (33) and an 28-day repeated dose according to OECD (34). According to applicant, no adverse effects were detected.

The performed toxicology assessment did not reveal any adverse effect in humans (or animals).

Testing of newly expressed proteins

Newly expressed microbial versions of PAT and AAD-12 (both expressed in *Pseudomonas fluorescens*) were used for safety assessment studies. The equivalence to transgenic proteins expressed in cotton were tested with SDS-PAGE, western blot, glycoprotein analysis and protein sequence analysis. Data obtained from these studies confirmed that microbial and plant version of the proteins were equal.

It must be emphasized that it is preferable to use plant version of the proteins for the safety analysis due to the difference in modifications between plant and bacterial proteins. Although the analhysis performed by the Applicant indicate that there is no difference, it is well known that for instance the glycosylation patterns (posttranslational modifications) can be different be different between microbes and plants.

Comments

- There is no visible molecular weight marker on the western blot for AAD-12 (p.121, Figure 39).
- There is no visible molecular weight marker on the western blot for PAT (p.140, Figure 47).
- Data obtained are from microbially produced proteins.

Allergenicity assessment (section 1.5 in Technical dossier, p.161 onwards).

The allergenicity assessment was based on the 1) safety of the donor organism, 2) homology with known allergens and 3) digestibility in simulated gastric fluids.

Both PAT and AAD-12 have been evaluated by EFSA and found to be safe (see references in Technical dossier, p.161).

Potential interactions between newly expressed proteins

Not considered by the applicant.

Hazard identification

No hazards were identified by the applicant. Cotton event DAS-819107-7 is considered to be as safe as its conventional counterpart based on the analysis performed by the applicant.

Herbicides

The use of herbicides on GM plants

Gene modified (GM) herbicide tolerant (HT) plants can be sprayed with the herbicides that are relevant according to the inserted transgenes.

There are however several issues to consider when plants are sprayed with herbicides when it comes to the potential for negative effects on environment, as well as humans and animals.

Some of these issues could be:

1. Increased exposure to herbicides
2. Potential for development of weeds that are herbicide resistant
3. Potential for accumulation of herbicides inside plants
4. Combinatorial effects of several herbicides used on one plant/crop at the same time

Total use of herbicides

The commercialization of GM-HT crops have led to an increased use of certain herbicides in agriculture (35). Especially glyphosate, but also the use of other herbicides connected to this has increased the last decades and by 2016, about 56 % of the global use of glyphosate was related to the use of HT GM crops (35).

There has also been an increased use of some “old” herbicides. The so called “synthetic auxins” like 2,4-D and dicamba has got a new usage through the GM-HT crops. Both of these herbicides are far more toxic than glyphosate, and 2,4-D has been classified by IARC as “possible human carcinogen” (36). EFSA have however raised several questions and concerns related to different aspects of the use and assessment of 2,4-D due to lack of information (37).

The use of glufosinate-ammonium use is also increasing due to the increasing challenge with glyphosate resistant weeds.

Increased use and resistance evolution

For 2,4-D, 32 species of weeds are shown to be resistant, and five of these (16%) were documented after 2015 (38).
For glufosinate-ammonium, six species of weeds are shown to be resistant and 50 % of these were discovered after 2015 (38).

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 (35), which directly or indirectly will expose non-target biodiversity in terrestrial, soil and aquatic communities (39), represent a major source of environmental pollution.

Accumulating herbicide residues and health effects

Through the introduction of GM-HT plants, the use of certain herbicides are increasing. The issue on accumulation and potential of health effects are thus issues of major importance when it comes to plants being used as food and feed.

Modeling studies have shown negative impacts on aquatic life and drinking water (40).
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 looked upon with a new attention to the concentration of these chemicals in food and feed.

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 (41).

Accumulation

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. (42) 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 (43, 44). 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.

Glufosinate ammonioum

Cotton event DAS-81910-7 contains the gene *pat* that confers resistance 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. 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 (45-48). EFSA has concluded on the risk of glufosinate ammonium, as especially harmful to mammals (49).

2,4-D

The *aad-12* gene provides 2, 4-D (dichlorophenoxy) and arylphenoxypropionate tolerance in the cotton event DAS-81910-7. 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).

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.

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

Summary:

- Cotton event DAS-81910-7 is tolerant to glufosinate ammonium and 2, 4-D. These herbicides are damaging to health and environment in different ways.
- Potential for accumulation of the herbicides should be considered in GM plants used in food and feed.

Main summary

Cotton event DAS-81910-7 is tolerant to herbicides containing glufosinate 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, glufosinate 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(3).

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