Vår ref:2017/H_RX_005
Deres ref: 2017/4373

Høringsuttalelse av fornyelsessøknad om markedsføring av genmodifisert mais GA21

EFSA/GMO/RX/005

Under EU forordning 1829/2003

Sendt til

Miljødirektoratet

av

GenØk-Senter for biosikkerhet
Juni 2017

Vår ref:2017/H_RX_005
Deres ref: 2017/4373

Miljødirektoratet
Postboks 5672 Sluppen
7485 Trondheim
Dato: 12.06.2017

Vedlagt er innspill fra GenØk – Senter for Biosikkerhet på offentlig høring av fornyelsessøknad **EFSA/GMO/RX/005**, genmodifisert mais linje GA21 fra Syngenta France SAS under EU forordning 1829/2003. Fornyelsessøknaden gjelder bruksområdene mat, fòr, import og prosessering.

Vennligst ta kontakt hvis det er noen spørsmål.

Med vennlig hilsen,

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Innhold/Content

Vår ref:2017/H_RX_005
Deres ref: 2017/4373

Høringsuttalelse – genmodifisert mais GA21, EFSA/GMO/RX/005, under EU forordning 1829/2003.

Fornyelsessøknad EFSA/GMO/RX/005 omhandler genmodifisert maislinje GA21 til bruksområdene mat, for, import og prosessering.

Den genmodifiserte maisen har toleranse mot herbicider som inneholder glyfosat via det innsatte genet *mepsps*.

Denne maislinjen er ikke godkjent for noen av de omsøkte bruksområdene i Norge.

Oppsummering

GenØk–Senter for biosikkerhet, viser til høring av fornyelsessøknad EFSA/GMO/RX/005 om GA21 mais som omfatter bruksområdet import og prosessering og til bruk i fòr og mat eller inneholdende ingredienser produsert fra denne maisen.

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

- Genmodifisert mais linje GA21 er ikke godkjent i Norge for noen av de omsøkte bruksområdene.
- GA21 er tolerant mot sprøytemidler som inneholder glyfosat som har økt interesse vedrørende potensielle helse-og-miljø farer ved bruk.
- Fornyelsessøknaden om mais linje GA21 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 reapplication EFSA/GMO/RX/005 on GA21 maize for import, processing, food and feed or ingredients thereof.

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

- The gene modified maize event GA21 is not approved for any application in Norway.
- The maize event GA21 is tolerant to herbicides containing glyphosate that has increasing intereset concerning potential health and environmental dangers upon use.
- The reapplication on maize event GA21 lacks data and information relevant for assessment of criteria on ethically justifiability, social utility and sustainability.

Reapplication on EFSA/GMO/RX/005

The event GA21 maize contains a gene providing herbicide tolerance (*mepsps*).

Previous evaluations

The Norwegian Scientific Committee for Food Safety (VKM) has commented on the application for the parental, single event maize GA21 (EFSA/GMO/UK/2005/19) (1) with the following issues:

- Maize event GA21 is considered "compositionally, agronomically and phenotypically equivalent to its conventional counterpart".
- Molecular characterization data show that there is one locus, containing many copies of the GA21 construct that is inherited as a dominant trait. The analysis performed by the Applicant are considered as adequate.
- There is a concern for spillage of viable maize kernels through accidental release upon handling. However, there is no concern for establishment of maize due to the survival, multiplication and dissemination characteristics it has. There is no natural wild relatives present. Thus, hybridization issues are not relevant.
- Gene flow from GM maize to conventional maize in Norway is considered as negligible.
- The EPSPS protein is most probably not allergenic based on performed studies.

GenØK has previously commented on the parental, single event GA21 in stacked combinations in previous hearings (http://genok.no/radgiving/horingsuttalelser/):

- *EFSA/GMO/DE/2011/103: Bt11 x MIR162 x MIR604 x 1507 x 5307 x* ***GA21***
- *EFSA/GMO/DE/2010/86: Bt11 x MIR162 x 1507 x* ***GA21***
- *EFSA/GMO/BE/2011/99: Bt11 x 59122 x MIR604 x 1507 x* ***GA21***

From these hearings, we highlight the following comments:

- *The regulator is encouraged to ask the Applicant to acknowledge the context of use for the stacked event and its complimentary herbicide technologies and test for to test for herbicide residues.*
- *The regulator is encouraged to ask the Applicant to submit required information on the social utility of Bt11xMIR162xMIR604x1507x5307x**GA21** maize and its contribution to sustainable development, in accordance with the Norwegian Gene Technology Act.*
- *The regulator is encouraged to ask the Applicant whether the inclusion of a glyphosate tolerance trait in this event, and implied glyphosate use, can be considered a sustainable weed control solution, given the spread of glyphosate resistant weeds in many cropping systems.*
- *The regulator is encouraged to consider the safety of co-products intended to be used with the GM event in the evaluation of safety.*

Social utility and sustainability issues on the maize event GA21, EFSA/GMO/RX/RX005

In Norway, an impact assessment follows the Norwegian Gene Technology Act (NGTA) (2) 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) (3). 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 (4-9).

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 10). Nevertheless, the Applicant has currently not provided any information relevant to enable an assessment of these criteria. Therefore, this section will highlight some areas that are particularly relevant to consider with maize GA21 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

Maize GA21 contains a modified *epsps* 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 17-19), as well as in villages in areas where glyphosate is systematically used as part of the GM crops tolerance to glyphosate (11). 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 (12-14). As maize GA21 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 the cultivation of GA21. 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. 15), which could have health impacts on humans and animals consuming food/feed based on ingredients from this type of GM plants. The Applicant has not provided information on whether the cultivation of maize GA21 could affect the emergence of glyphosate resistance in weeds, nor if there are cases of this in the areas intended for cultivation of the variety, which are also important aspect to evaluate the ethical justifiability. Furthermore, this maize is cultivated in Argentina, Brazil, Canada, Colombia, the Philippines, Paraguay, South Africa, Uruguay, Vietnam and the USA. In all these countries except Vietnam, glyphosate resistant weeds has been located and in most of them the amount of glyphosate resistant weeds has increased significantly[1]. 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 maize GA21 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 (16). Therefore, considerations of the co-products also warrant an evaluation of safe use and data required for such an assessment is not provided by the Applicant.

Impacts in producer countries

As already stated, the Applicant does not provide data relevant for an environmental risk assessment of maize GA21 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 maize GA21 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, accessed on 18 May 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 in relation to the field trials. The difference between the field trials and the sites of cultivation can affect the adequacy of the evaluation of GA21. It is therefore important that the Applicant provides information on how the difference between the site of field trials and of cultivation sites may affect the evaluation of GA21.

According to the Applicant, the unlikely event whereby small amounts of seed from GA21 maize accidentally find their way into the environment would represent extremely low levels of exposure and the survival of these seeds to produce flowering plants would be very unlikely (Summary, page 7). The Applicant states that volunteers could be easily controlled using any of the current agronomic measures. For a sustainability assessment, it remains important to have an evaluation of the occurrence of volunteer plants in the producing countries and suggested control strategies. Information about the occurrence of volunteers and which herbicides may be used for killing volunteers is required to evaluate potential health and environmental impacts of these.

Benefit to society

The criteria of 'benefit to society' in the NGTA should be interpreted on a national level. That means that the import of maize GA21 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 maize will replace maize in existing food and feed products. It is therefore important to evaluate how GM crops in general, GM maize in particular, and the use of GM maize 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 maize. Although the empirical data available on the attitude of Norwegian citizens towards GM is limited (e.g. 17, 18) 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 (19). Furthermore, it should be noted that 29 % of the global maize production is GM. It is therefore not a problem for Norway to import GM free maize and therefore no need to replace current imports. The GM maize in question does also not contain any beneficial characteristics for consumers that would prioritize this maize over non-GM maize.

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, reveal underlying values, assumptions, norms and beliefs (7, 20) as a way to reflect on what kind of society we want, and assess how certain (biotechnological) developments may or may not contribute to shaping a desired future. Thus, in order to evaluate whether maize GA21 contributes to social utility, it is important to investigate current and future demands and acceptance of this in Norway and if there are alternatives sources for maize that could be cultivated elsewhere that may satisfy this demand, or are more desirable.

Ethical considerations: socio-economic impacts

As known, GM crops have been, and still are, a hot topic for debate. A significant amount of this debate focuses on the safety of GMOs and currently no scientific consensus on this topic has been achieved (21). 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. (22) 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 (23) has 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 even have given up growing non-GM maize. Even though the cultivation of maize GA21 is not planned in Europe/Norway, it is important to obtain information about the strategies adopted to ensure co-existence with conventional and organic maize production. Information about consequences for co-existence in the countries intended for cultivation of maize GA21 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. 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 (24). Indeed, although a framework for maintaining co-existence in Europe was established in 2003 (25) this effectively meant technical measurements and recommendations (e.g. cleaning of sowing machines and transport vehicles) and remains challenging in practice (26, 27). 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 (28, 29). For the criteria in the NGTA, information on co-existence is required to enable a coherent analysis.

Summary

In order to meet the requirements for the NGTA, the regulator is encouraged to ask the Applicant to submit information relevant for the assessment of the criteria of ethically justifiable, benefit to society and sustainability assessment. An important part that is lacking is information about the consequences of the cultivation of maize GA21 for the producing countries. The information provided by the Applicant must be relevant for the specific agricultural context of this country and should also stress the need for information on integrated weed management strategies in those countries (30). 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. Additionally, the Applicant does not attempt to demonstrate a benefit to society, a reference of the consumers' attitude on GM maize, or the demand within Norway for maize GA21 and does therefore not provide sufficient information as required by the NGTA.

Table 1: Questions to the Applicant

Sustainability	*How does the cultivation of GA21 affect the use of glyphosate?*
	How is the current use of glyphosate in the sites of cultivation and what approaches are used to minimize the use of glyphosate?
Herbicide-resistant weed	*What kind of management strategies are taken to prevent the increase of herbicide-resistant weed?*
	Who will be affected if the amount of resistant weeds increases?
	How is the burden of increase of resistant weeds distributed and what strategies are in place to compensate this?
Benefit to society	*Is GA21 available for further breeding and research? If so, under which circumstances?*
	Is there a demand for GA21 in Norway?
	Does GA21 contribute to business development and value creation in Norway, including new job opportunities?
Assessing alternatives	*Will GA21 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 GA21 within Norway and how does the development of GA21 relates to these?*
	Does the development, introduction or use of GA21 contradict ideas about solidarity and equality between people, such as the particular consideration of vulnerable groups in the population?
Socio-economic impacts	*Does GA21 affect the seed choice of farmers?*
	Which parties will be affected by the development, introduction or use of GA21 and how does this change their autonomy, practice and position compared to other stakeholders?
	Does GA21 change the power dynamic among stakeholders? If so, how?
	Can the development, introduction or use of GA21 create significant ruptures or ecological relationships?
Co-existence	*Does the cultivation of GA21 affect other types of agricultural practices in the nearby areas? If so, how?*
	Is there a system in place for keeping GMO and non-GMO crops separate in the production and transport line? If so, who pays for this system?

Environmental risk issues in a Norwegian context

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

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

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

For a full description of the molecular characterization of GA21 the full technical dossier was submitted with the application for authorization in the EU of maize GA21 (references EFSA-GMO-UK-2005-19 and EFSA-GMO-RX-GA21) for the placing on the market of glyphosate-tolerant genetically modifies maize GA21, for food and feed uses, import and processing.

GenØk has previously commented on maize GA21 in applications regarding stacked events: H-137 (2017), H-103 (2015), H-99 (2012) and H-86 (2012), but not as a single event.

Norwegian Scientific Committee for Food Safety (VKM): comments on the molecular characterization data regarding maize GA21

- The molecular characterization data indicate that several copies of the GA21 construct are integrated at a single locus in the DNA, and that they are inherited as a dominant, single locus trait. Appropriate analyses of the integration site, inserted DNA sequence, flanking regions, and bioinformatics have been performed. The VKM GMO Panel considers the molecular characterization of maize GA21 as adequate.

Molecular characterization

Information on the sequences actually inserted or deleted

The applicant provides a detailed molecular characterization of the DNA sequence of the event that have been inserted during the process of genetic modification leading to maize event GA21. According to the applicant, the entire event GA21 insert and flanking regions have been sequenced. The sequencing analysis demonstrated that there are several copies (1-6) of the restriction fragment used for transformation, inserted. A single base pair change was noted in the NOS terminator in copies 1 and 2. As a follow up, the applicant performed northern and western analysis to investigate the possibility of transcription of the truncated *mepsps* gene and subsequent translation. The applicant could find no evidence for transcripts or truncated protein related to the truncated gene.

In addition, an *in silico* screen for putative ORFs at the junction between the maize genome and the event GA21 insert was performed. This assessment identified two putative ORF at the 5`end of the insert and one putative ORF and 2 other putative ORFs 3´ of the insert in close proximity to the inserted promoter. The putative ORFs were examined for sequence homology to known toxin and allergens *in silico* against a database of 2005. New updated data examining sequence homology to known toxins an allergens should be included in this renewal application with an examination against an updated database from 2017.

Comments relevant for the assessment of the current application

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

Maize tissues in two maize hybrids of GA21 (whorl, anthesis, seed, maturity and sencescence) were analyzed using enzyme-linked immune-sorbent assay (ELISA) for the expression of mEPSPS protein.

Concentrations of mEPSPS were quantifiable in most tissues analyzed.
It was not clear from the dossier if the mEPSPS proteins isolated from GA21 maize, was from both glyphosate treated and untreated plants.

Expression of potential fusion proteins

BLAST analysis of 3`and 5`junctions and an *in silico* screen for potential ORFs were performed.
Two ORFs were detected but did not reveal any sequence homology to known allergens or toxins.

The sequence analysis revealed the presence of a truncated mEPSPS protein within the insert of GA21 maize. However, no transcripts were detected from this sequence with the northern analysis performed, and no protein from this sequence was detected after performing a western blot. The antibody used only detected the full length protein according to the Applicant.

Toxicity and allergenicity

Toxicity

Safety assessment of newly expressed proteins (p.24-25 of dossier)

According to the history of safe use of maize, and that no pathogenicity has been detected by the performed methods of mEPSPS analysis, the protein is considered as safe for food and feed usage, and thus not toxic.

Allergenicity

Assessment of allergenicity of the newly expressed proteins (p.27-28 of dossier)

The protein mEPSPS has been tested for its allergenic potential through a stepwise approach including 1) source of protein, 2) bioinformatics search of homologies, 3) susceptibility to proteolytic degradation, 4) heat stability tests.
According to the Applicant, mEPSPS expressed in GA21 is not likely to be allergenic due to the results obtained from the analysis performed.

Potential interactions between newly expressed proteins

Comment: Only one transgenic protein expressed.

Hazard identification

Based on the analysis performed on toxicity and allergenicity, and other analysis performed to address any adverse effect on health the Applicant conclude that the maize event GA21 is safe for consumption.

Summary:

- Two ORFs were detected during sequence analysis. No RNA transcripts was detected on northern blot and no proteins were detected after western blot with the antibody used (source of antibody unclear).
- It is unclear if experimental data from both herbicide treated and untreated GA21 maize were analysed during ELISA.
- Explanation for differences in band intensities on Southern blot after *HindIII* digestion of stack GA21 x T25 is lacking.

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 seem to be strong drivers 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 (14).

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 can be linked to resistance evolution in weeds. At present, 36 species of weeds are documented to be glyphosate resistant on a global scale (31). Such development may lead to 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 (32). Crop and herbicide monoculture makes the agroecosystem more vulnerable to further resistance development (33).

Sustainability

For the farmers, resistant weeds are a difficult obstacle to handle. 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 the potential for resistance development are not sustainable in 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.

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 (14), which directly or indirectly will expose non-target biodiversity in terrestrial, soil and aquatic communities (34), 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 (30). 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 (35).

The increased awareness of the potential toxicity of glyphosate, coupled with the increased volume used, should lead to more awareness to the acceptance level for glyphosate residues in food and feed (36). The present situation is that 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 (37). This set of events has been termed "The Glyphosate Paradox" (36). The WHO/IARC categorization of glyphosate as *probably carcinogenic to humans (38)*, although disputed by EFSA (39), is underlining the significance of the controversy around the glyphosate-based herbicides.

Studies in Daphnia

In *Daphnia magna*, the LC50/EC50 acute toxicity of glyphosate is shown in the range 144 – 248 mg/L for 24 h, and 25 mg/L for 48 h, respectively (40, 41).

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. (35) documented high levels of glyphosate residues in HT GM soybeans grown in the USA, and the same research group have published papers showing that such residues have the potential for negatively to affect the feed quality of HT GM soybeans (37, 42). 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 *mepsps* gene present in GA21 maize confers tolerance to herbicides containing glyphosate.

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

Glyphosate has previously been announced as an herbicide with low toxicity for users and consumers as well as the environment surrounding agricultural fields (30, 43). However, glyphosate has recently received more risk-related attention due to its potential for negative effects on both aquatic and terrestrial ecosystems (44), as well as from studies in animals and cell cultures that have indicated possible negative health effects in rodents, fish and humans (45-47).

It has also been shown that agriculture of GM plants is associated with greater overall usage of pesticides than the conventional agriculture (48).

A number of publications indicate unwanted effects of glyphosate on health (47, 49), aquatic (50) and terrestric (44, 51) organisms and ecosystems. Also, a study of Roundup (containing glyphosate as the active ingredient) effects on the first cell divisions of sea urchins (52) 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 (45) 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 (53). 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.

Summary:

- A glyphosate herbicide tolerance gene is inserted into maize event GA21.
- Glyphosate has and increased focus due to potential health related effects.
- Accumulation of herbicides in transgenic plants intended for food and feed should be considered.

Main summary

Maize event GA21 is tolerant to herbicides containing glyphosate that is under debate regarding the potential for different degrees of health and environmental issues upon use.
Thus, the issue on accumulation should be considered for GM plants to be used in food and feed.
The applicant should provide data relevant for assessment of social utility and sustainable development according to the NGTA (2).

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